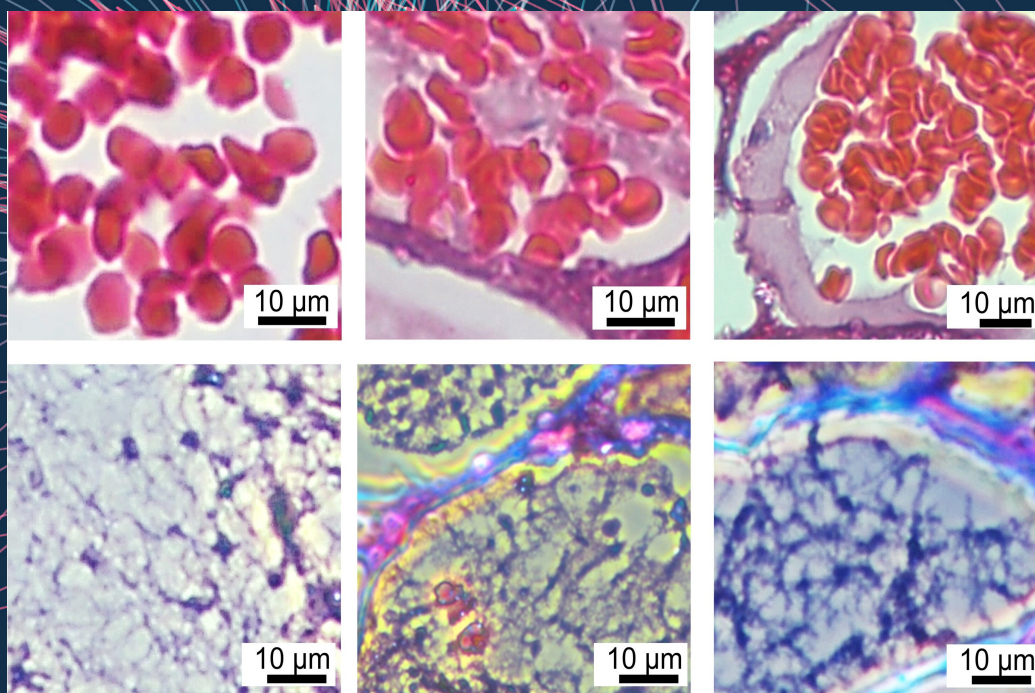


OSR Oral Sciences Reports

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Chiang Mai University's Faculty of Dentistry publishes academic research articles in the newly titled - **Oral Sciences Reports**, which was previously known as *Chiang Mai Dental Journal (CMDJ)*. The journal was originally established for the purposes of publishing academic research articles by the Faculty of Dentistry at Chiang Mai University in 1977. In the current report, editors and experts in their respective fields review articles received from authors prior to being published to ensure that the content of all articles is up-to-date, universal, logical, and in accordance with academic principles so the reader can apply knowledge and cite works in the development of dentistry for the purposes of advancing future research while being beneficial to patients and society.

At present, Oral Sciences Reports openly receives all submissions through an online journal review process system. The new online system also allows reviewers and researchers an ability to read 3 issues each year.

Aim and Scope of the journal

To compile research and content that is up to date and usable to all branches of dentistry and related fields. The articles in Oral Sciences Reports are fundamental research work, including original articles, review articles, case reports/series, short communications, and letters to the editor.

Policy

Accepted articles will be fairly reviewed by the editors and experts with full transparency through the following process.

1. The articles must be correct according to academic principles and not duplicate works that have been previously published.
2. The articles will be considered and reviewed through a non-bias process by concealing the names of authors and related persons in the considered documents while also concealing the names of the experts and reviewers who review the articles (double-blind review).
3. The review process can be tracked online. The article authors can review the status of their article and are able to follow up on the article evaluation through the online process. The duration of each step is closely monitored so that the articles can be published on time.
4. Authors of articles are responsible to review and verify the accuracy of the text, images, tables in the articles before publication.
5. Articles published in Oral Sciences Reports are the copyright of Oral Sciences Reports, which forbids anyone from duplicating published articles for any purpose without explicit permission from Oral Sciences Reports.

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Types of Submission

Oral Sciences Reports invites the following submissions:

1. Original Articles Original contributions of research reports or unpublished recent academic research to the development and applications in dentistry and related fields. The original article must not exceed 4000 words in length and must contain no more than 10 figures and tables in total.
2. Review Articles Comprehensive reviews of special areas of focus in dentistry and related fields. Articles that contain important collected data from numerous books or journals and from the writer's experience. Information should be described, reviewed, compared, and analyzed. The review article must not exceed 4000 words in length and must contain no more than 10 figures and tables in total.
3. Systematic Reviews Clearly formulated reviews that uses systematic and reproducible methods to identify, select and critically appraise all relevant research, and to collect and analyze data from the studies that are included in the review.
4. Case Reports/Series Original findings that highlight novel technical and/or clinical aspects in dentistry and related fields which include clinical symptoms, diagnosis, patient care, treatment, follow-up, and evaluation. The report must not exceed 2500 words in length and must contain no more than 5 figures.
5. Letters to the Editor Commentaries on published papers in the journal and other relevant matters that must not exceed 1000 words in length
6. Short Communications Original contributions describing new developments of high impact that justify expedited review. The report must not exceed 2000 words in length and must contain no more than 3 figures.

Submission Checklist

Authors should ensure to prepare the following items for submission. Failure to complete the required items may contribute to the delay of publication process. Please check the relevant section in this guideline for more details.

1. Title page Must include title of the article, author names and affiliations. One author has been designated as the corresponding author with contact details (e-mail address and full postal address) (see 'Title page' section for more information and an example)
2. CRediT Contribution Author will be asked to provide CRediT Contributions as well as their degree of contribution at the time of the original submission. CRediT Contribution is a high-level classification of the diverse roles performed in the work leading to a published research output in the sciences. Its purpose to provide transparency in contributions to scholarly published work, to enable improved systems of attribution, credit, and accountability.
3. Abstract Must not exceed 250 words. Relevant keywords (up to five keywords) must be included at the end of the abstract. (see the 'Abstract' section for more details)
4. Main Manuscript Author details and affiliation must not be included. (see 'Manuscript' section for more details)
5. Figures Should include relevant captions. (see the 'Figures' section for more details)
6. Tables Should include titles, description, and footnotes. (see the 'Tables' section for more details)
7. Supplementary data (if applicable)

Additional considerations the author should confirm before submission:

1. Manuscript must be 'spell-checked', 'grammar-checked', and 'plagiarism-checked'.
2. All figures, tables, and references mentioned in the text should match the files provided.
3. Permission must be obtained for use of copyrighted material from other sources (including the internet).
4. Authors must provide conflicts of interest statement, even if there is no conflict of interests to declare.

Ethical Guidelines

Authors must acknowledge to the following ethical guidelines for publication and research.

A. Authorship and Author Contributions

The policy of Oral Sciences Reports that only ONE corresponding author is accepted. Where there is any uncertainty regarding authorship, the editor of the journal reserves the right to contact the corresponding author of the study for further information. Authors must acknowledge that the manuscript has been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal. Authors are required to identify the contributions for which they are responsible. Author will be asked to provide CRediT Contributions as well as their degree of contribution at the time of the original submission. CRediT Contribution is a high-level classification of the diverse roles performed in the work leading to a published research output in the sciences. Its purpose to provide transparency in contributions to scholarly published work, to enable improved systems of attribution, credit, and accountability.

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(b) Written confirmation (e-mail, letter) from all authors that they agree with the addition, removal, or rearrangement.

In case of addition or removal of authors, these must be confirmed from the author being added or removed. Please be informed that changes of the authorship cannot be made in any circumstances after the manuscript has been accepted.

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All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee's approval for each study. Experimentation involving human subjects will only be published if such research has been conducted in full accordance with the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements or with ethical principles of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above-mentioned principles.

Experimentation involving animal subjects should be carried out in accordance with the guidelines laid down by the National Institute of Health (NIH) in the USA or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

C. Clinical Trials

All clinical trials must register in any of the following public clinical trials registries:

- Thai Clinical Trials Registry (TCTR)
- NIH Clinical Trials Database
- EU Clinical Trials Register
- ISRCTN Registry

The clinical trial registration number and name of the trial register should be included in Materials and Methods of the manuscript. For epidemiological observational trials, authors of epidemiological human observations studies are required to review and submit a 'strengthening the reporting of observational studies in Epidemiology' (STROBE) checklist and statement. Compliance with this must be detailed in Materials and Methods.

D. Systematic Review

The abstract and main body of the systematic review should be reported using the PRISMA for Abstract and PRISMA guidelines respectively. Authors submitting a systematic review should register the protocol in one of the readily-accessible sources/databases at the time of project inception and not retrospectively (e.g. PROSPERO database, OSF registries). The protocol registration number, name of the database or journal reference should be provided at the submission stage in Materials and Methods. A PRISMA checklist and flow diagram (as a Figure) should also be included in the submission material.

E. Conflicts of Interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Potential sources of conflict of interest include (but are not limited to) patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. If there are no interests to declare, please state 'The authors declare no conflict of interest'. Authors must disclose any interests in the section after acknowledgments.

F. Submission Declaration and Verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright- holder. The conference proceedings are allowed to be part of the article if the contents do not exceed 70% of the article.

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Manuscript Preparation

All texts in the submitted manuscript are required to be inclusive language throughout that acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (for instance by using 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess'). Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, religion, culture, or any other characteristic.

A. Title page

The title page will remain separate from the manuscript throughout the peer review process and will not be sent to the reviewers. It should include these following details:

- Title should be concise, information-retrieval, and not exceed 30 words. Please avoid abbreviations and formulae where possible.
- Author names and affiliations. Please clearly indicate the given name(s) and family name(s) of each author are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript number immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and the e-mail address of each author.
- Corresponding author will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. Please ensure that the e-mail address and contact details given are kept up to date by the corresponding author.

B. Abstract

Abstract must not exceed 250 words with concise and informative explanations about the article. Authors must prepare an abstract separately from the main manuscript using Microsoft Word processing software (.doc or .docx). Please avoid references and uncommon abbreviations, but if essential, abbreviations must be defined at their first mention in the abstract itself. Abstract structure of the original articles must consist of 'Objectives, Methods, Results, and Conclusions'.

Abstract of other types of submitted articles should be summarized in one paragraph. Up to five keywords relevant to the articles must be provided and arranged in alphabetical order.

C. Manuscript

Oral Sciences Reports adheres to a double-blinded review. The main body of the paper (including the references, figures, tables and any acknowledgements) must not include any identifying information, such as the authors' names. The layout of the manuscript must be as simple as possible with double-spaced, single column format with Sans Serif font and uploaded as an editable Microsoft Word processing file (.doc or .docx). Complex codes or hyphenate options must be avoided, but the emphatic options such as bold face, italics, subscripts, and superscripts, etc. are encouraged.

1. Original article

- *Introduction* should include literature reviews of previous studies, research questions, and the rationale for conducting the study. The Introduction should not be too long and should be easy to read and understand while avoiding a detailed literature survey or a summary of the results.

- *Methods* should provide sufficient details in a logical sequence to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized and indicated by a reference. If quoting directly from a previously published method, use quotation marks and cite the source. Any modifications to existing methods should also be described.

- *Results* should show the data gained from the study's design in text, tables and/or illustrations, as appropriate, and be clear and concise.

- *Discussion* is criticism, explanation, and defense of the results from the standpoint of the author, and comparison with other peoples' reports. The discussion can include criticism of materials, methods and study results, problems, and difficulties, pointing out the benefits of adoption and providing feedback where appropriate. Discussions should explore the significance of the results of the work, not repeat them. Avoid extensive citations and discussion of published literature.

- *Conclusions* refers to a summary of the study or research results.

- *Acknowledgments*: Please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study.

Formatting of funding source:

This work was supported by the 1st organization name [grant numbers xxxx]; the 2nd organization name [grant number yyyy]; and the 3rd organization name [grant number zzzz].

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant or funding from funding agencies in the public, commercial, or not-for-profit sectors.

- *References* should be confined to documents relating to the author's article or study. The number should not exceed 80, placed in order and using numbers which are superscripted and put in parentheses, starting with number 1 in the article and in reference document's name. (see 'References' section for more information regarding reference formatting)

2. Review articles should be divided into Introduction, Review and Conclusions. The Introduction section should be focused to place the subject matter in context and to justify the need for the review. The Review section should be divided into logical sub-sections in order to improve readability and enhance understanding. Search strategies must be described, and the use of state-of-the-art evidence-based systematic approaches is expected. The use of tabulated and illustrative material is encouraged. The Conclusion section should reach clear conclusions and/or recommendations on the basis of the evidence presented.

3. Systematic review

- Introduction should be focused to place the subject matter in context and to justify the need for the review.
- Methods should be divided into logical sub-sections in order to improve readability and enhance understanding (e.g. details of protocol registration, literature search process, inclusion/exclusion criteria, data extraction, quality assessment, outcome(s) of interest, data synthesis and statistical analysis, quality of evidence).
- Results should present in structured fashion (e.g. results of the search process, characteristics of the included studies, results of primary meta-analysis, additional analysis, publication bias, quality of evidence).
- Discussion should summarize the results, highlighting completeness and applicability of evidence, quality of evidence, agreements and disagreements with other studies or reviews, strength and limitations, implications for practice and research.
- Conclusion(s) should reach clear conclusions and/or recommendations on the basis of the evidence presented.

4. Case reports/series should be divided into Introduction, Case report, Discussion and Conclusions. They should be well illustrated with clinical images, radiographs and histologic figures and supporting tables where appropriate. However, all illustrations must be of the highest quality.

There are some necessary considerations which should be comprehended and consistent throughout the article:

1. Abbreviations: define abbreviations at their first occurrence in the article: in the abstract and in the main text after it. Please ensure consistency of abbreviations throughout the article.
2. Mathematical expressions: the numbers identifying mathematical expressions should be placed in parentheses after the equation, flush to the right margin; when referring to equations within text, use the following style: Eq. (5), Eqs. (3-10), [see Eq. (4)], etc.
3. Nomenclature: abbreviations and acronyms should be spelled out the first time they are used in the manuscript or spelled out in tables and figures (if necessary). Units of measure and time require no explanation. Dental nomenclature in the manuscript should be complete words, such as maxillary right central incisor. Numbering of teeth from pictures or tables should follow the FDI two-digit system.
4. Units: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.
5. Product identification: all products mentioned in the text should be identified with the name of the manufacturer, city, state, and country in parentheses after the first mention of the product, for example, The ceramic crown was cemented on dentin surface with resin cement (RelyXTM U200, 3M ESPE, St. Paul, MN, USA)...

D. Figures

Figures should be prepared and submitted separately from the main manuscript. Color artworks are encouraged at no additional charge. Regardless of the application used other than Microsoft Office, when the electronic artwork is finalized, please 'save as' or 'export' or convert the images to **EPS, TIFF, or JPEG format with the minimum resolution of 300 dpi**. Keep the artwork in uniform lettering, sizing, and similar fonts. Please do not submit graphics that are too low in resolution or disproportionately large for the content. Authors must submit each illustration as a separate file.

Please ensure that each illustration has a caption according to their sequence in the text and supply captions separately in editable Microsoft Word processing file (.doc or .docx), not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

E. Tables

Please submit tables as editable Microsoft Word processing files (.doc or .docx), not as images, and avoid using vertical rules and shading in table cells. Each table should be placed on a separate page, not next to the relevant text

in the article. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body while ensuring that the data presented in them does not duplicate results described elsewhere in the article.

F. References

Citation in text

Any citations in the text should be placed in order and using numbers which are superscripted and put in parentheses. Please ensure that all citations are also present in the reference list consecutively in accordance with their appearance in the text.

Reference style

All references should be brought together at the end of the paper consecutively in accordance with their appearance in the text and should be in the Vancouver reference format. Please follow these examples of correct reference format below:

1. Journal article

1.1. One to six authors

Author(s) – Family name and initials. Title of article. Abbreviated journal title. Publication year;volume (issue);pages.

Example:

Parvez GM. Pharmacological activities of mango (*Mangifera Indica*): A review. *J Pharmacognosy Phytother.* 2016;5(3): 1-7.

Or

Choi YS, Cho IH. An effect of immediate dentin sealing on the shear bond strength of resin cement to porcelain restoration. *J Adv Prosthodont.* 2010;2(2):39-45.

Or

Firmino RT, Ferreira FM, Martins CC, Granville-Garcia AF, Fraiz FC, Paiva SM. Is parental oral health literacy a predictor of children's oral health outcomes? Systematic review of the literature. *Int J Paediatr Dent.* 2018;28(5):459-71.

1.2. More than six authors

Author(s) – Family name and initials of the first six authors, et al. Title of article. Abbreviated journal title. Publication year;volume(issue);pages.

Example:

Vera J, Siqueira Jr JF, Ricucci D, Loghin S, Fernández N, Flores B, et al. One-versus two-visit endodontic treatment of teeth with apical periodontitis: a histobacteriologic study. *J Endod.* 2012;38(8):1040-52.

1.3. Article in press

Authors separated by commas – Family name and initials. Title of article. Abbreviated journal title in italics. Forthcoming - year of expected publication.

Example:

Cho HJ, Shin MS, Song Y, Park SK, Park SM, Kim HD. Severe periodontal disease increases acute myocardial infarction and stroke: a 10-year retrospective follow-up study. *J Dent Res.* Forthcoming 2021.

2. Books

2.1. Book with author (s)

Author(s) – Family name and initials (no more than 2 initials with no spaces between initials)– Multiple authors separated by a comma. After the 6th author add - "et al". Title of book. Edition of book if later than 1st ed. Place of publication: Publisher name; Year of publication.

Example:

Sherwood IA. Essentials of operative dentistry. Suffolk: Boydell & Brewer Ltd; 2010.

Or

Abrahams PH, Boon JM, Spratt JD. McMinn's clinical atlas of human anatomy. 6th edition. Amsterdam: Elsevier Health Sciences; 2008.

2.2. Book with no author

Title of book. Edition of book if later than 1st ed. Place of publication: Publisher name; Year of publication.

Note: Do not use anonymous. Please begin a reference with the title of the book if there is no person or organization identified as the author and no editors or translators are given.

Example:

A guide for women with early breast cancer. Sydney: National Breast Cancer; 2003.

2.3. Chapter in a book

Author(s) of chapter - Family name and initials, Title of chapter. In: Editor(s) of book - Family name and initials, editors. Title of book. edition (if not first). Place of publication: Publisher name; Year of publication. p. [page numbers of chapter].

Example:

Rowlands TE, Haine LS. Acute limb ischaemia. In: Donnelly R, London NJM, editors. ABC of arterial and venous disease. 2nd ed. West Sussex: Blackwell Publishing; 2009. p. 123-140.

3. *Thesis/dissertation*

3.1. Thesis in print

Author - family name followed by initials. Thesis title [type of thesis]. Place of publication: Publisher; Year.

Example:

Kay JG. Intracellular cytokine trafficking and phagocytosis in macrophages [dissertation]. St Lucia, Qld: University of Queensland; 2007.

3.2. Thesis retrieved from full text database or internet

Author - family named followed by initials. Thesis title [type of thesis/dissertation on the Internet]. Place of publication: Publisher; Year [cited date – year month day]. Available from: URL

Example:

Pahl KM. Preventing anxiety and promoting social and emotional strength in early childhood: an investigation of risk factors [dissertation on the Internet]. St Lucia, Qld: University of Queensland; 2009 [cited 2017 Nov 22]. Available from: <https://espace.library.uq.edu.au/view/UQ:178027>

4. *Webpage*

4.1. Webpage with author

Author/organization's name. Title of the page [Internet]. Place of publication: Publisher's name; Publication date or year [updated date - year month day; cited date - year month day]. Available from: URL

Example:

American Dental Association. COVID-19 and Oral Health Conditions [Internet]. Chicago: American Dental Association; 2021 Feb 12 [updated 2021 Feb 12; cited 2021 Jun 24]. Available from: <https://www.ada.org/en/press-room/news-releases/2021-archives/february/covid-19-and-oral-health-conditions>

4.2. Webpage with no authors

Title [Internet]. Place of publication (if available): Publisher's name (if available); Publication date or year [updated date (if available); cited date]. Available from: URL

Example:

Dentistry and ADHD [Internet]. 2019 Jan 15 [updated 2019 Jan 15; cited 2020 Apr 8]. Available from: <https://snoozeden-tistry.net/blog/dentistry-and-adhd/>

4.3. Image on a webpage

Author/organization. Title [image on the Internet]. Place of publication: Publisher's name; Publication date or year [updated date; cited date]. Available from: URL

Note: If the image does not have a title - give the image a meaningful title in square brackets.

Example:

Poticny DJ. An Implant-Supported Denture Offers a Number of Advantages [image on the Internet]. Texas: Office of Dan Poticny; 2018 Nov 21 [updated 2018 Nov 21; cited 2019 Aug 30]. Available from: <https://www.dfwsimiledoc.com/blog/post/an-implant-supported-denture-offers-a-number-of-advantages.html>

5. *Government publications/reports*

5.1. Reports and other government publications

Author(s). Title of report. Place of publication: Publisher; Date of publication – year month (if applicable). Total number of pages (if applicable eg. 24 p.) Report No.: (if applicable)

Example:

Australian Institute of Health and Welfare. Oral health and dental care in Australia: key facts and figures trends 2014. Canberra: AIWH; 2014.

5.2. Government reports available online

Author(s). Title of report. Report No.: (if applicable). [Internet]. Place of publication: Publisher or Institution; Publication date or year [updated date - year month day; cited date - year month day]. Available from: URL

Example:

World Health Organization. WHO mortality database [Internet]. Geneva: World Health Organization; 2019 Dec 31 [updated 2019 Dec 31; cited 2021 Mar 29]. Available from: <https://www.who.int/data/mortality/country-profile>

6. *Tables/Figures/Appendices*

Follow the format of book, journal or website in which you found the table/figure/appendix followed by: table/figure/image/appendix number of original source, Title of table/figure/appendix from original source; p. Page number of table/figure/appendix from original source.

Note: each reference to a different table/figure within the same document requires a separate entry in the Reference list. Please provide permission documents from the original sources.

Example:

Smith J, Lipsitch M, Almond JW. Vaccine production, distribution, access, and uptake. Lancet 2011;378(9789):428-438. Table 1, Examples of vaccine classes and associated industrial challenges; p. 429.

7. *Journal abbreviation source*

Journal names should be abbreviated according to the Web of Science - Journal Title Abbreviations.

Peer-review Process

Oral Sciences Reports follows a double anonymized review process. Each manuscript will be assigned to at least three expertises for consideration. The identities of both reviewers and authors are concealed from each other throughout the review to limit reviewer bias. To facilitate this, please ensure that the manuscript keeps anonymity before submission such as affiliation, author's gender, country or city of origin, academic status, or previous publication history. Our peer review process is confidential and identities of reviewers are not released. Letters and technical comments are sent to the authors of the manuscript on which they comment for response or refutation, but otherwise are treated in the same way as other contributions with respect to confidentiality.

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Novel Therapeutic Strategies for Oral Squamous Cell Carcinoma-An Overview

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Abstract

Surgery, radiotherapy, and chemotherapy have been the mainstay of oral squamous cell carcinoma (OSCC) management. However, these treatments often come with significant side effects and limitations, including systemic toxicity and drug resistance. Recent advancements in therapeutic strategies for oral cancer have focused on more targeted and less invasive approaches. Some of the novel therapeutic strategies for OSCC include immunotherapy, targeted therapy, photodynamic therapy, gene therapy, phytochemicals, nanotheranostics, and CRISPR/Cas technology. These novel strategies offer promising avenues for improving the outcomes and quality of life for patients with OSCC. Ongoing research and clinical trials are essential to further refine these approaches and make them widely available.

Keywords: oral cancer, recent, squamous cell carcinoma, treatment,

Introduction

Oral cancer is a group of malignant diseases arising from the lips, gums, tongue, mouth, and palate and is the most fatal disease among other diseases in the world. Oral squamous cell carcinomas (OSCC), arising from surface epithelium, constitute more than 90% of all oral cancers, and they rank as the sixth most common neoplasm in the world.⁽¹⁾ There are more than 350,000 new cases of OSCC and 177,000 deaths every year, with considerable differences in geographic and environmental risk factors.⁽²⁾ The incidence of OSCC has been decreasing in some parts of the world but the increase is observed in other countries with low socio-economic conditions. Despite the new management strategies, the 5-year survival rate is below 50%.⁽³⁾ Low socioeconomic condition is in turn related to factors like nutrition, health care, living condition, lack of awareness, and risk behaviors, which contribute to the development of oral cancer. In many low-income and middle-income countries, including India, most of the population does not have access to a well-organized and well-regulated cancer care system. The high cost of novel therapies and treatments makes them inaccessible to many people in low-income regions. This economic barrier prevents patients from receiving the latest and most effective treatments. Addressing these challenges requires a comprehensive approach, including improving access to affordable therapies, enhancing healthcare infrastructure, and promoting early detection and screening.

Surgical resection is used as a primary treatment modality. Chemotherapy is used as an adjuvant to radiotherapy in patients who are diagnosed with stages 3 and 4, and in patients with local recurrence and metastasis. The continuous increase in oral cancer cases, the failure of conventional chemotherapies, and the excessive toxicity of chemotherapies demand alternative cancer treatments. The current review aimed to summarize the literature on novel therapeutic approaches available for oral cancer. Some of the novel therapeutic strategies for OSCC include immunotherapy, targeted therapy, Photodynamic Therapy, gene therapy, phytochemicals, Nanotheranostics, and CRISPR/Cas Technology. (Table 1)

Immunotherapy

Cancer immunotherapy involves stimulating specific components of the immune system and strengthening the

immune system to counteract the signals that suppress them. Strengthening of the immune system is brought about by the cells of innate immunity, such as the neutrophils, macrophages, natural killer (NK) cells, dendritic cells, and eosinophils, and the cells of adaptive immunity, which include the B and T lymphocytes, commonly known as B and T cells. Neutrophils play a key role in cancer immunotherapy, acting as both tumor-promoting and tumor-suppressing agents. They contribute to immune evasion by releasing pro-inflammatory cytokines that support tumor growth, but they also enhance anti-tumor immunity by activating T cells and dendritic cells. tumor-associated neutrophils (TANs) can be polarized into either pro-tumor (N2) or anti-tumor (N1) phenotypes, influencing immunotherapy outcomes. B cells produce antibodies, and T cells generate CD4+ and CD8+ cells. CD4+ T cells, particularly T helper (Th) cells, support B cell activation and antibody production by releasing cytokines such as IL-4, IL-5, and IL-6. These cytokines enhance B cell proliferation and promote class switching, allowing B cells to produce tumor-targeting antibodies. CD8+ T cells, or cytotoxic T lymphocytes, directly attack cancer cells by recognizing tumor antigens presented via MHC class I molecules. Their activity is often enhanced by B-cell-derived antibodies, which facilitate antibody-dependent cellular cytotoxicity. B cells, beyond antibody production, contribute to antitumor immunity by forming tertiary lymphoid structures within tumors. These structures serve as immune hubs, fostering interactions between T cells and B cells to sustain long-term immune surveillance. The tumor cells escape the cells of the immune system by decreasing surface antigen expression and also alter the environment by synthesizing substances that suppress the immune system and thus increase the progression of the tumors.⁽⁴⁾ Immune therapy is divided into two types, namely the active and passive types, where there is direct attack of the tumor cells. These are derived from the blood or tumor of the patient and cultured.

Active immunotherapy involves NK cells, dendritic cells, and cytotoxic T cells. In passive immunotherapy, there is targeting of cell surface receptors to form antibody-dependent cell-mediated immunity. Immunotherapy includes checkpoint inhibitors, targeted monoclonal antibodies, adoptive cell transfer, and cytokine immunotherapy.

Table 1: Comparison table highlighting key aspects of different novel therapeutic strategies.

Therapy	Mechanism	Advantages	Limitations	Response to therapy
Immunotherapy	Restore the ability of the immune system to detect and destroy cancer cells by overcoming the mechanisms by which tumors evade and suppress the immune response	Highly specific	Several factors influence tumor immunotherapy 1) Host immunity 2) Tumor cells 3) Environmental factors	1-year survival rate was ~19% higher with nivolumab (anti-programmed death 1 monoclonal antibody) than with standard therapy in HNSCC (head & neck squamous cell carcinoma). ⁽⁴⁴⁾
Targeted cancer stem cell therapy	Specifically targeting cancer stem cells	Selectivity, prevent recurrence	Drug resistance, challenging to identify, cancer stem cells	Preclinical study - CD44v6-specific CAR-NK cell therapy demonstrated a two-to-threefold increase in killing efficacy against various HNSCC cell lines compared to unmodified natural killer cells. ⁽⁴⁵⁾
Photodynamic Therapy	Uses light-sensitive drugs activated by specific wavelengths of light to destroy cancer cells	Minimally invasive, localized treatment	Limited penetration depth, requires specialized equipment	77% of patients with oral and oropharyngeal cancer achieved complete response and 42.3% of patients achieved local control. ⁽⁴⁶⁾
Gene Therapy	Modifies or replaces defective genes to inhibit cancer growth	Potential for long-term disease control	Ethical concerns, delivery challenges	Phase 1 trial - pHIL-12 plasmid delivered via gene electrotransfer potentiates the immunostimulatory effects of local ablative therapies in basal cell carcinomas of the head and neck. ⁽⁴⁷⁾
Phytochemicals	Natural compounds with anticancer properties	Low toxicity, easily available	Variable efficacy	Phase I clinical trial- Histological improvements of precancerous lesions in oral cancer patients observed upon treatment with curcumin. ⁽³⁶⁾
Nanotheranostics	Uses nanoparticles	Enhanced drug delivery, real-time monitoring	Complex formulation, regulatory hurdles	Retrospective study - 77% of patients with metastatic cancer showed manageable tolerability and favorable response rates to nanoparticle albumin-bound paclitaxel (PacLIALL™). ⁽⁴⁸⁾
CRISPR/Cas Technology	Gene-editing tool to correct mutations or disrupt cancer-promoting genes	High precision, potential for personalized treatment	Ethical concerns, risk of off-target effects	Preclinical study - CD147 gene knockout oral cancer cells, which were shown to decrease Cal27 tumor cell invasion and metastasis. ⁽⁴²⁾

Checkpoint inhibitor

Checkpoint inhibitors act against the pathways that suppress T cell activity, thus resulting in tumor regression. Evidence shows that immune checkpoints programmed cell death protein 1 (PD-1) promote the immune response against cancer.⁽⁵⁾ Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) creates new T cells and contributes to cancer regression. Apart from PD-1 and CTLA-4, other checkpoint inhibitor receptors such as lymphocyte-activa-

tion gene 3 (LAG3), mucin domain 3 (TIM-3), and T-cell immunoglobulin have demonstrated therapeutic effects in clinical trials in combination with PD-1 agents. Pembrolizumab was approved by the FDA for treating patients with recurrent head and neck squamous cell carcinoma (HNSCC).⁽⁶⁾

Targeted monoclonal antibodies

Monoclonal antibodies are either obtained from

human or murine antibody components. Epidermal growth factor receptor (EGFR) plays a major role in tumor progression by causing invasion, metastasis, angiogenic potential, and inhibition of apoptosis. Monoclonal antibodies such as cetuximab and panitumumab are EGFR-targeted therapies that are proven to be effective in oral cancer, either when used alone or along with radiotherapy.⁽⁷⁾ P53 is the most commonly mutated gene in cancer, and antibodies against this gene have been useful in treating cases with nodal involvement.⁽⁸⁾

Adoptive cell transfer (ACT)

ACT is a procedure wherein T cells are obtained from the tumor sample or the patients and introduced with specific antigen receptors by genetic engineering and then reintroduced into the patients, therefore enhancing the ability to recognize the antigen. In the study conducted by Jiang *et al*, ACT has improved the survival rates in patients with HNSCC in comparison with the control group.⁽⁹⁾ The side effects of ACT include fever, nausea, vomiting, rashes, and cytokine release syndrome, which occurs when the transferred T cells or other immune cells respond to the new T cells.

Cytokine immunotherapy

Cytokines are messengers that allow the cells of the immune system to work hand in hand to target the antigen (cancer cells). This method enhances the coordination of the tumor cells and the stromal cells. They are delivered either locally or systematically to elicit the anti-tumor response. Several cytokines are being explored for the treatment of HNSCC, including GM-CSF, IL-2, IFN- γ , IL-12, and an investigational multi-cytokine biologic known as IRX-2 (Primary cell-derived Biologic). Interleukin-2 enhances the activation of several types of leukocytes with antitumor activity, including natural killer cells, lymphokine-activated killer cells, antigen-specific T-helper cells, cytotoxic lymphocytes, macrophages, and B cells. IFN- γ and IL-12 are used in clinical trials that have been proven to be effective by increasing the number of natural killer cells.⁽¹⁰⁾

Immunotherapy has transformed cancer treatment, improving the outcome of cancers like melanoma, lung cancer, and renal cell carcinoma. Pembrolizumab resulted in improved progression-free survival of advanced melanoma patients at both 2 mg/kg and 10 mg/kg, with a

6-month progression-free survival of 34% when compared to 16% in the chemotherapy group.⁽¹¹⁾ When compared to standard chemotherapy, immunotherapy is expected to considerably enhance the overall and progression-free survival of patients with extensive-stage small-cell lung cancer. Overall survival was higher with anti-PD-L1 than with anti-PD-1 and anti-CTLA4.⁽¹²⁾

Patient selection plays a major role in the success of immunotherapy. Several side effects are associated with each approach. Checkpoint Inhibitors can cause immune-related adverse events, including colitis, pneumonitis, and endocrinopathies. Tumors can become resistant to treatment, and only a small subset of individuals respond well. Nivolumab and pembrolizumab have only been trialed for OSCC. The use of targeted monoclonal antibodies is restricted by their high cost, possible off-target effects, limited efficacy in advanced instances, and tumor-induced downregulation of target receptors, which lowers their efficacy. Cetuximab has been tested in combination with radiation therapy for oral cancer. Adoptive Cell Transfer requires complex genetic engineering, and solid tumors like oral cancer pose challenges for T-cell infiltration. Its drawbacks include high toxicity, cytokine release syndrome risk, and expensive production. For head and neck malignancies, CAR-T therapy that targets EGFR is being investigated. Cytokine Immunotherapy has a short half-life, requiring frequent dosing and systemic toxicity leading to severe inflammatory responses, including vascular leak syndrome. IL-12 gene electrotransfer has been tested for head and neck basal cell carcinoma.

Targeted cancer stem cell therapy

Oral cancer stem cells are a subpopulation of cells with tumor-initiating properties and several molecular similarities to embryonic and normal adult stem cells. Oral CSCs are also commonly resistant to conventional therapies targeting proliferating cells. Hence, they crucially contribute to metastasis and recurrence. It is important to increase knowledge of the molecular features and signaling pathways that are specific to the oral cancer stem cells to develop new targeted and efficient treatments for head and neck cancer. The self-renewal potential of cancer stem cells (CSCs) may be obtained through multiple endogenous signaling pathways such as the Wnt, Bmp, Pten, Notch, TGF- β , and Hedgehog. CSCs can be detected and isolated from the tumor mass by employing single or

combinations of numerous surface markers. Despite significant progress in identifying CSCs based on their specific surface markers like Aldehyde dehydrogenases (ALDHs), CD44, CD117, and CD133, the development of selective CSC therapies remains a challenge. ALDH1-positive cell subpopulations have been found to have higher tumorigenicity and are more resistant to chemotherapeutic agents than the negative population.

Adenosine triphosphate Phosphate Binding Cassette (ABC) transporters

ABC transporters are membrane transporters, which are capable of pumping different little molecules (e.g., anti-cancer drugs) out of the cells at the expense of ATP hydrolysis, and thus lead to decreased intracellular drug concentrations.⁽¹³⁾ Cancer stem cells following chemo-radiotherapy become chemo-radio resistant or selectively improve the resistant cell population. Studies have found that overexpression of ABC transporters in cancer cells enhances their chemo-radio resistance. Suppressing ABC transporters elevates anti-cancer drug sensitivity in cancer.⁽¹⁴⁾

Histone demethylases

Epigenetic modifications like as DNA methylation, histone modification, and non-coding RNA regulation play a crucial role in oral cancer development, influencing gene expression, tumor progression, and therapeutic resistance. Hypermethylation of tumor suppressor genes leads to gene silencing, while hypomethylation of oncogenes results in genomic instability and increased tumor aggressiveness. Dysregulated miRNAs like miR-21, miR-200, and miR-34 contribute to tumor growth, metastasis, and drug resistance. Long non-coding RNAs influence epigenetic reprogramming, affecting the tumor microenvironment. Circular RNAs act as miRNA sponges, modulating cancer-related pathways. Histone Modifications include acetylation, phosphorylation, ubiquitination, and methylation. Histone methylation plays a key role in the regulation of gene expression and genetic stability, and dysregulation of this highly conserved process occurs in various cancers. There are two classes of enzymes involved in histone methylation: methyltransferases and demethylases. Histone demethylases are capable of removing methyl groups from histones and other proteins. The histone demethylases contribute to carcinogenesis, cell fate selection, and

cell differentiation. The common histone demethylases are the JARID1, KDM4, LSD1, KDM6B, KDM6A, KMD3, KDM5. Jumonji domain—consisting of protein 6 (JMJD6) is a new molecular modulator of oral cancer stem cells.⁽¹⁵⁾ JMJD6 overexpression increases both the CSC traits and the number of CSCs, which suggests that JMJD6 is a prominent modulator of the cancer stem cell phenotype and genesis in OSCC.

Calcium channels

Calcium is a global messenger in regulating several physiological processes and disruption of its homeostasis would be observed during carcinogenesis, which results in the deregulation of the rapid growth of the cells, emigration, and apoptosis inhibition. Studies have recently explored the role of calcium signaling in oral cancer.⁽¹⁶⁾ Orai1, a calcium channel protein, enhances OSCC metastatic potentials, and suppression of Orai1 in the OSCC cell line resulted in suppressing CSC traits.⁽¹⁷⁾

Targeting these cancer stem cells would enhance efficacy and specificity for eradicating the tumors and reducing systemic toxicity. A better understanding of CSCs can provide unique opportunities to develop new therapeutic platforms for targeting CSCs in the treatment of cancers. They can significantly reduce side effects compared to traditional chemotherapy by specifically targeting CSCs. While targeted therapies can be highly effective, they may not work for all patients due to genetic variability. Additionally, cancer cells can develop resistance to these drugs over time.

Gene therapy

Gene therapy involves the transfer of a therapeutic gene into specific cells of an individual to repair a faulty gene. The objective of this method is to introduce new genetic material into target cells without causing any damage to the surrounding tissues. This alternate treatment option has been proven to increase the survival rates of OSCC patients.⁽¹⁸⁾ The types of gene therapy include somatic and germ-line gene therapy. In somatic gene therapy, the therapeutic genes are introduced into the somatic cells, which restricts the effects of the individual and are not passed on to the next generation and in germ line gene therapy either the sperm or egg can be altered by introducing the therapeutic gene, which gets integrated into the genome.⁽¹⁹⁾ The therapeutic genes are carried out

with the help of either viral or non-viral vectors.

The following are the techniques employed in gene therapy

Gene addition therapy

This approach adds a working copy of a gene into the cell. In this technique, the tumor growth is controlled by the introduction of tumor suppressor genes, which inactivate the carcinogenic cells. Genetic alterations in head and neck cancers include mutations of p53, the Retinoblastoma Gene, p16, and p21. In this method, tumor growth is controlled by the induction of tumor suppressor genes that inactivate the carcinogenic cells. P53 is the most commonly used gene with adenovirus as a viral vector. Studies are being carried out on adenovirus vector Ad5CMV-p53, which is first given by intramucosal injection, followed 2 h later by a mouthwash. From the next day, it is administered as a mouthwash twice daily for 2-5 days, and this treatment is repeated every 28 days. This method inhibits disease progression in precancerous lesions with no toxic effects.⁽²⁰⁾

Gene excision therapy

In this technique, the defective oncogenes are removed, as a result of which, there is an inhibition in the growth of the tumor cells. The genes that control growth and cell cycle progression, including factors like TGF- α 1, PDGF- α , and PTEN, are regulated by the expression of the transcription factor early growth response-1 (EGR-1). Thus, inhibiting this protein represents a good therapeutic approach for the tumor cells. Some studies demonstrated that inhibition of the protein kinase C reduces the expression of this gene, triggering higher sensitivity of the tumor to radiotherapy.⁽²¹⁾

Antisense RNA therapy

This method involves the introduction of the remedial gene that prevents the expression of a defective gene and is called "Antisense therapy." Gene expression can be inhibited by the RNA that is complementary to the strand of DNA expressing the gene. This technique can be directed towards carcinoma cells whose malignant phenotype is dependent upon the expression of particular oncogenes such as Myc, Fos, and Ras. Inhibition of the expression of these oncogenes may alter the phenotype, thus preventing

the growth of the tumor.⁽²²⁾

Patients with OSCC show defective function of several types of immune cells, which include natural killer cells, T-lymphocytes, and cytokines. The combined use of mIL-2 (murine interleukin 2) and mIL-12 (murine interleukin 12) gene therapy resulted in a significant reduction in the tumor due to increased activation of cytolytic T lymphocytes and natural killer cells. Radiosensitivity to γ radiation and chemosensitivity to 5-fluorouracil (5-FU) in oral squamous cell carcinoma can be enhanced after the suppression of NF- κ B activity, which activates the anti-apoptotic proteins TNF, TRAF-1, TRAF-2, and cIAP-1.

The inhibition of NF- κ B can decrease the expression of proinflammatory cytokines, e.g. IL-1 α , IL-6, and IL-8, and of enzymes that degrade matrix metalloproteinase-9 (MMP-9). The progression and metastasis of OSCC can be prevented by inhibiting NF- κ B activity, which may be a useful adjuvant treatment in oral cancer therapy. Systemic administration of Anti-ICAM 2 induced the complete regression of OSCC. ICAM-2 is a glycosylated protein with surface adhesion that is expressed in endothelial cells and activated lymphocytes.⁽²³⁾

Suicide gene therapy

It is also called gene-directed enzyme prodrug therapy. Suicide gene therapy introduces viral or bacterial genes into malignant cells that metabolize a non-toxic prodrug into a toxic compound. Suicide gene systems identified include the HSV-thymidine kinase gene (HSV-TK) with ganciclovir (GCV) and the cytosine deaminase gene (CD) with 5-fluorocytosine (5-FC). A Thymidine kinase gene of Herpes Simplex Virus (HSV) transforms ganciclovir into ganciclovir phosphate. Gene transfer of the HSVtk gene (Herpes simplex virus thymidine kinase gene) via adenovirus vector in combination with ganciclovir administration may be a good therapeutic option for OSCC. HSV-tk/GCV therapy in cultured oral squamous cancer cells has shown that tumor cell death occurs mainly by an apoptotic process, and the observed high cytotoxicity is due to the bystander effect, which is promoted by the diffusion of the toxic agent into neighboring cells via gap junctions.⁽²⁴⁾

Both precise genetic changes and efficient delivery systems are necessary for targeted gene therapy to be effective. To overcome delivery-related challenges, several techniques have been developed, such as lipid nanoparti-

cles, exosomes, and viral vectors.

Gene therapy with the use of an oncolytic virus

In this method, a vector (virus) is genetically modified, which is replicated and causes lysis of the tumor cells. Adenovirus is the only vector to complete a phase III clinical trial study based on Herpes Simplex Virus thymidine kinase (HSV-tk) suicide gene therapy.⁽²⁵⁾ The methods of non-viral gene therapy include the injection of naked DNA, electroporation, the gene gun, and the use of oligonucleotides, dendrimers, and inorganic nanoparticles. HSV-tk/GCV system to achieve antitumor activity against oral cancer cells *in vitro* and *in vivo* using ligand-associated lipoplexes to enhance therapeutic delivery.⁽²⁶⁾

Gene therapy is effective in cases of single-gene defects, but it also requires multiple visits by the patients, and the vectors can cause side effects. The use of insertional vectors to identify oncogenes by causing leukemia and solid tumors has raised concerns about insertional mutagenesis using the same vectors for gene therapy.

Photodynamic therapy

Photodynamic therapy (PDT) is used in the treatment of cancers due to its specificity and sensitivity to tumor cells. The antitumor effects of PDT may result directly from tumor cell death or indirectly from damage to tumor vasculature and activation of nonspecific and specific immune responses against the tumor cells. Due to its loca-

tion and direct visibility, the oral cavity is an ideal model for conventional PDT.

Mechanism of action

PDT can activate the immune system in cancer treatment by inducing immunogenic cell death and releasing tumor-associated antigens, which in turn stimulate immune cells to target and eliminate cancer cells. A more detailed flowchart of the effect of PDT for immune activation in cancer is represented in Figure 1.

In PDT, a photosensitizer is administered, which accumulates in or around cancer cells. It is activated by exposure to a specific wavelength of light. The activated photosensitizer generates reactive oxygen species, causing damage to cancer cells and surrounding tissues. PDT-induced damage leads to cancer cell death, specifically immunogenic cell death. There is release of damage-associated molecular patterns (DAMPs), such as high-mobility group box 1 protein and heat shock proteins. DAMPs and tumor-associated antigens (TAAs) are activated and presented to antigen-presenting cells, such as dendritic cells (DCs). DCs present TAAs to T cells, leading to T cell activation and differentiation. Immune cells produce cytokines, such as TNF- α and IFN- γ , which further enhance the anti-tumor immune response to eliminate tumor cells. PDT can be combined with immunotherapy, such as checkpoint inhibitors, to further enhance the anti-tumor immune response.

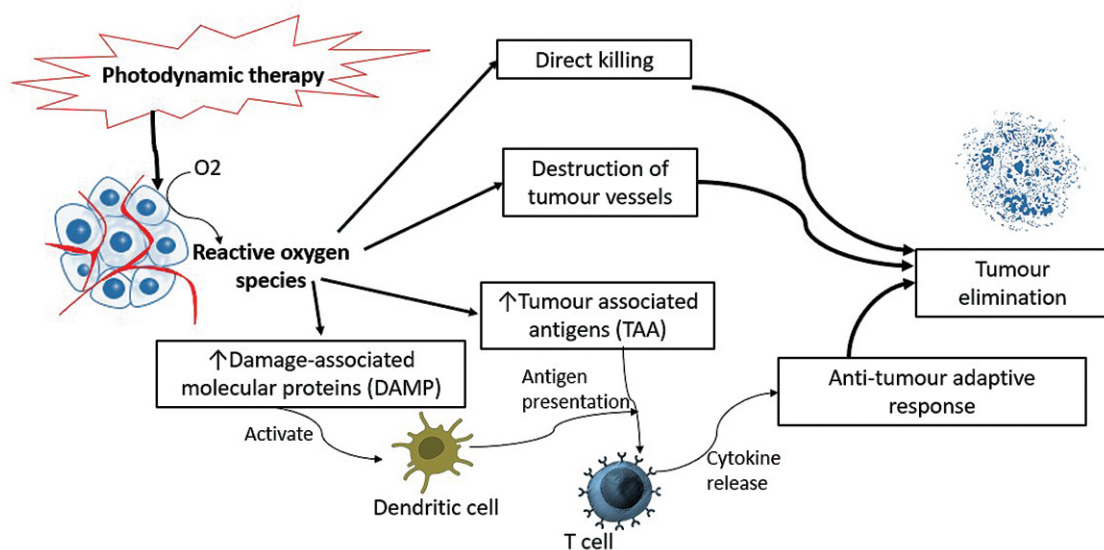


Figure 1: Mechanism of action of photodynamic therapy in tumour elimination.

Role in primary treatment

PDT is well-suited as a primary or alternative treatment modality for early oral cancer without nodal metastasis (i.e., T1 and T2 tumors) and is associated with significantly less morbidity compared to conventional therapy. Superficial cancers that are within the permeability range of the light source (i.e., 0.5-1 cm) show the best response according to studies. The advantages of PDT over conventional therapies, such as surgery, radiotherapy, and chemotherapy include minimal invasiveness, organ-sparing potential, excellent long-term functional and cosmetic results with improved quality of life, feasibility of repeating treatment at the same site for recurring lesions, minimal scarring after treatment, cost-effectiveness, and simplicity of technique.⁽²⁷⁾

In the case of any relapse or development of a new primary tumor in the area previously treated by PDT, treatment can be repeated in the same area multiple times without cumulative toxicity, in contrast to ionizing radiation or surgery, where such retreatment results in extensive morbidity. In addition, the use of conventional therapies does not preclude the use of PDT, and the use of PDT does not compromise future surgical interventions or radiation therapy.

Role of PDT as an adjuvant or combination therapy

PDT has the advantage of being used as an adjuvant therapy for the treatment of surgical margins following resection of T3 and T4 head and neck cancers via superficial or interstitial light application. The use of PDT in combination with conventional therapies requires further investigation. PDT may be used to treat primary localized lesions alongside surgery and/or radiotherapy in cases that involve nodal metastasis. Recent *in vitro* and *in vivo* studies have utilized PDT in combination with other chemopreventive agents.⁽²⁸⁾ These combination therapies have shown enhanced anticancer effects, likely because a multifactorial disease such as cancer involves various pathological pathways. Therefore, a combination of treatment modalities can be used to target different disease processes, causing cell death via diverse mechanisms. Furthermore, the combination of different modalities can synergistically enhance selectivity and efficacy in comparison to either of the single therapies. This can eventually help to reduce the amount of cytotoxic drugs given to patients, resulting in reduced morbidity from

side effects.⁽²⁹⁾

Role of PDT in surveillance for cancer-free status

Numerous *in vitro* and *in vivo* studies have demonstrated the significant effect of PDT on the development of adaptive immunity. Dendritic cells (DCs) are considered the most important antigen-presenting cells and play a significant role in antitumor immune response by activation of CD8⁺ cytotoxic T-cells. DCs activated in response to PDT travel to tumor-draining lymph nodes, where they are known to stimulate T-cell activation. Saji *et al.*,⁽³⁰⁾ studied the combination of PDT with intratumoral injection of DCs and found synergistically enhanced tumor cure rates as compared to individual therapies. The immune response produced by the combination therapy was found to confer systemic antitumor effects that could induce the regression of distant untreated tumors.

Recent developments have been made in PDT to ensure the specificity and efficacy of the method. It involves the following methods:

Targeted delivery

In the era of precision medicine, to increase specificity and minimize toxicity to normal tissues, photosensitizers are conjugated with targeting moieties. One such technique is to couple PSs to monoclonal antibodies that are directed against tumor-associated antigens (TAAs) specifically over-expressed in cancer cells. These TAAs include oncofetal antigens, growth factor receptors, and receptors for signal transduction pathways.

For cancers and precancers of the head and neck, EGFR overexpression has been frequently reported.⁽³¹⁾ Thus, targeting EGFR with photoactive molecules linked to anti-EGFR antibodies may selectively destroy cancer cells whilst sparing adjacent normal cells expressing low levels of EGFR.

Vascular-targeted PDT

Vascular targeted PDT involves the use of PSs, such as TOOKAD (Steba Biotech, Luxembourg City, Luxembourg) and Visudyne (QLT Ophthalmics, Inc., Vancouver, BC, Canada), which are selectively retained in the neovasculature of targeted tumors, resulting in a preferential vascular response.⁽³²⁾ In comparison to conventional anti-cancer therapies directed against cancer cells, targeting tumor vasculature appears to be a more efficient

approach to killing cancer cells and has a lower probability of developing drug resistance.

Two-photon PDT

In two-photon (2- γ) PDT, ultrafast pulses of near-infrared light are used such that two photons of relatively low but identical energy are simultaneously absorbed by the PS. As each photon contributes to one-half of the excitation energy, a longer wavelength is needed for enough energy to produce singlet oxygen, allowing light to penetrate deeper into tissue due to lower scattering and absorption. Two-photon excitation has been utilized to target and selectively occlude blood vessels associated with neoplastic tissues while reducing the damage to adjacent normal tissues.

Clinical research suggests that PDT is a primary or alternate therapy option for early oral cancer without nodal metastases (i.e., T1 and T2 tumors) and is associated with lower morbidity than traditional treatment. In a study by Toratani S *et al*, superficial OSCC, when treated with Photofrin-mediated PDT, the wounds exhibited excellent functionality and aesthetic healing without any scar formation and with minimal side effects.⁽³³⁾

PDT is minimally invasive and can be used as a primary treatment for early-stage cancers or in combination with other therapies. However, it is generally limited to treating superficial or localized tumors and may not be effective for larger or deeply infiltrating tumors. Light sensitivity and temporary side effects can also occur.

Phytochemicals

Natural foods, like cruciferous vegetables (e.g., cabbage and broccoli), alliums (e.g., garlic and onion), green tea, citrus fruits, soybeans, tomatoes, berries, and ginger, have chemopreventive activity.⁽³⁴⁾ Studies have suggested that lifestyle changes could prevent more than two-thirds of human cancers and that dietary factors contribute to approximately 35% of human cancer mortality.⁽³⁵⁾

Mechanism of action:

- Free radicals are thought to be related to multistage carcinogenic processes. Peroxyl radicals and lipid peroxidation can independently cause DNA mutations, which are essential for the initiation of the carcinogenic process. Antioxidant phytochemicals can regulate the initiation of carcinogenic processes by protecting against DNA

damage.

- Phytochemicals may exert their chemopreventive properties by blocking the critical events of tumor initiation and promotion, thereby reversing the premalignant stage.

- Phytochemicals can induce cancer cell death.
- Phytochemicals can also enhance innate immune surveillance and improve the elimination of transformed cells

Phytochemicals, such as flavonoids, phenolic acids, phytosterols, carotenoids, and stilbenes, have anticancer activity. Phytochemicals that have been studied for their effects on oral cancer include Curcumin, Green Tea Extract, Resveratrol, Isothiocyanates, Lycopene, and Genistein. Several *in vitro* and *in vivo* studies have concluded their anti-cancer effects. Preclinical and clinical studies are required to confirm their efficacy in oral cancer therapeutics. The cancer-preventive effect of curcumin has, however, been demonstrated in a Phase I clinical trial.⁽³⁶⁾ A Phase IIb clinical trial has shown the efficacy of curcumin (3.6 g for six months) in the treatment of oral leukoplakia.⁽³⁷⁾ It is also evident that curcumin is effective in delaying the onset and reducing the severity of radiation-induced oral mucositis in patients with head and neck cancer. Natural compounds are often hindered by low water solubility, low bioavailability, and deficient targeting; thus, numerous phytochemical delivery systems should be developed to compensate for these problems. More research is needed to fully understand their mechanisms and to develop effective delivery systems to enhance their bioavailability and therapeutic efficacy

Nanothernostics

Nanotherapeutics is the development of various nanomedicine strategies such as polymer conjugations, dendrimers, micelles, liposomes, metal and inorganic nanoparticles, and carbon nanotubes, nanoparticles of biodegradable polymers for sustained, controlled, and targeted co-delivery of diagnostic and therapeutic agents. This concept will have fewer side effects, which is essential for any therapy. Theranostic nanomedicine involves using colloidal nanoparticles in the range of 1 to 1000 nm (1 μ m). They consist of absorbed, conjugated, entrapped macromolecular materials/polymers/carbon nanomaterials/metals and inorganic nanoparticles in which the diagnostic and therapeutic agents are absorbed.

A multifunctional nano-system for imaging-guided cancer treatment by Gu M *et al*, in which the terbium ion-doped hydroxyapatite nanoparticle was used as a luminescent probe to encapsulate both the near-infrared photothermal agent polydopamine (PDA) and anticancer doxorubicin. It promoted *in vitro* cell death through the overproduction of reactive oxygen species, cell cycle arrest, and increased cell apoptosis.⁽³⁸⁾

In advanced theranostic nanomedicines, when conjugated with a targeting moiety will recognize specific targets, which then bind to specific receptors on the targeted cell membrane and be internalized by the diseased cells. It happens with most specific processes called Receptor-Mediated Endocytosis.⁽³⁹⁾

Erlotinib encapsulated in liposomal formulations showed significant anti-tumor effects against oral cancer when locally administered at the site of the tumor arising in the oral cavity.⁽⁴⁰⁾ The clinical trials that are completed or still ongoing in phase 1 or phase 2 are designed to evaluate the nanoparticles application in the treatment and diagnosis of head and neck cancer. None of the trials reached phase 3. It is observed that paclitaxel-albumin nanoparticles are widely studied for radiation therapy in most clinical trials. Cetuximab, cisplatin, and Hafnium Oxide nanoparticles are also assessed for their use in Head and neck cancer. The complexity of designing and manufacturing nanoparticles can be a limitation. There are also potential concerns about the long-term safety and toxicity of these materials. The major challenge is that the nanoparticles are rapidly eliminated or encountered by the immune system of a patient. Further studies are needed for clinical translation of nanotheranostics and their use in treating oral cancer.

CRISPR/Cas technology

CRISPR/Cas (molecular scissors) is a gene-editing tool that can modify or delete specific genes associated with cancer. By employing genome-wide CRISPR screens, vulnerabilities in oral cancer cells can be identified, revealing promising targets for therapeutic interventions.⁽⁴¹⁾ Using the CRISPR/Cas9 gene-editing technology, Pan S *et al*, produced CD147 gene knockout oral cancer cells, which were shown to decrease Cal27 tumor cell invasion and metastasis *in vitro* and *in vivo*.⁽⁴²⁾ CRISPR/Cas9 technology has also been used to knock out p75NTR, which inhibited the proliferation, invasion, and migration

of SCC-9 cells, suggesting that p75NTR is a viable target for tongue cancer therapy.⁽⁴³⁾

CRISPR/Cas9 enables genome-wide screens to identify vulnerabilities in oral cancer cells, revealing promising targets for therapeutic interventions. CRISPR/Cas9 can also be used to perturb genes associated with drug resistance, thereby enhancing the efficacy of chemotherapy and other treatments. It can be combined with other therapeutic strategies, such as immunotherapy, to improve treatment outcomes.

While promising, CRISPR/Cas technology is still in its early stages and faces ethical and regulatory challenges. Off-target effects, where unintended genes are edited, are also a concern.

Limitations and future perspectives

Despite recent advancements in cancer diagnosis and treatment, the survival rate remains poor. Management of cancer should have a multidisciplinary approach and should include a team of health professionals such as surgeons, oncologists, radiologists, dental surgeons, nutritionists, and rehabilitation and reconstructive specialists. This is to support the overall oral and systemic health to improve the quality of life in survivors. A careful watch on patients is also necessary to prevent the risk of recurrences and the development of secondary tumors. Furthermore, each patient necessitates different treatment approaches depending on the type of cancer, stage of cancer, metastasis, lymph node involvement, age, and general systemic health. Early-stage cancers are mostly treated with a single treatment modality, like surgery or radiotherapy, while advanced cases are treated with a combination of two or more modalities. Patients with advanced stages of cancer often end up with extensive surgeries leading to facial disfigurement and often need facial prostheses with adjuvant therapies that assist with speech, mastication, saliva production, and psychological status. Survival following a diagnosis of OCs remains poor, with 50% of patients living up to and not beyond 5 years. Preventive measures like abstinence or avoidance of risk factors and screening at regular intervals must be enforced to control the disease in the early stages. Management of cancer has come a long way with the advent of new treatment modalities directed toward improving the survival rate. At the same time, efforts to improvise a disease-free interval and improve the quality of living of

cancer patients are of utmost importance.

Conclusion

- Immunotherapy offers a targeted and potentially less invasive option for treating head and neck cancer. Their use in oral cancer is still in pre-clinical trials.

- There are currently no clinically applied therapeutic trials to specifically target oral CSCs, and the different pluripotency-associated CSC surface markers are not specific to oral CSCs since they overlap with both normal somatic cells and their tissue-resident stem cells.

- Gene therapy has shown a positive effect in the treatment of oral cancer and prevention of invasion, metastasis, and recurrence in both *in vivo* and *in vitro* settings.

- Clinical studies have demonstrated the effectiveness of PDT as a primary treatment in early-stage oral cancers and as an adjuvant therapy for more advanced cases.

- Phytochemicals show great promise; further research and clinical trials are needed to fully understand their mechanisms of action and optimize their use in oral cancer therapy.

- Nanomaterials can be used as a drug carrier, the drug's action time of which is limited. This may cause toxicity. A targeted drug delivery system may help to improve the efficacy of treatment with nanoparticles.

- CRISPR/Cas9 holds great potential in oral cancer therapy; ongoing research is essential to address challenges such as off-target effects, efficient delivery mechanisms, and ethical considerations

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Dhanuthai K, Rojanawatsirivej S, Thosaporn W, Kintarak S, Subarnbhesaj A, Darling M, *et al.* Oral cancer: a multicenter study. *Med Oral Patol Oral Cir Bucal.* 2018;23(1):e23-e29.

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al.* Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209-49.
3. Du M, Nair R, Jamieson L, Liu Z, Bi P. Incidence trends of lip, oral cavity, and pharyngeal cancers: global burden of disease 1990-2017. *J Dent Res.* 2020;99(2):143-51.
4. Ljunggren HG, Jonsson R, Höglund P. Seminal immunologic discoveries with direct clinical implications: the 2018 nobel prize in physiology or medicine honours discoveries in cancer immunotherapy. *Scand J Immunol* 2018;88(6):e12731.
5. Messenheimer DJ, Jensen SM, Afentoulis ME, Wegmann KW, Feng Z, Friedman DJ, *et al.* Timing of PD-1 blockade is critical to effective combination immunotherapy with anti-OX40. *Clin Cancer Res.* 2017;23(20):6165-77.
6. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, *et al.* Combined nivolumab and ipilimumab or monotherapy in previously untreated melanoma. *N Engl J Med.* 2015;373(1):23-34.
7. Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell.* 2000;103(2):211-25.
8. Normanno N, De Luca A, Bianco C, Strizzi L, Mancino M, Maiello MR, *et al.* Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene.* 2006;366(1):2-16.
9. Jiang P, Zhang Y, Archibald SJ, Wang H. Adoptive cell transfer after chemotherapy enhances survival in patients with resectable HNSCC. *Int Immunopharmacol.* 2015;28(1):208-14.
10. Rapidis AD, Wolf GT. Immunotherapy of head and neck cancer: current and future considerations. *J Oncol.* 2009; 2009:346345. doi: 10.1155/2009/346345.
11. Knight A, Karapetyan L, Kirkwood JM. Immunotherapy in melanoma: recent advances and future directions. *Cancers (Basel).* 2023;15(4):1106.
12. Liu X, Xing H, Zhang H, Liu H, Chen J. Immunotherapy versus standard chemotherapy for treatment of extensive-stage small-cell lung cancer: a systematic review. *Immunotherapy.* 2021;13(12):989-1000.
13. Aadithi MG, Divya B, Nandhini G, Rajkumar K, Ramesh Kumar A, Sarangarajan R. Evaluation of ABCB5 immunostained epithelial stem cells in oral squamous cell carcinoma, inflammatory gingival hyperplasia and normal mucosa. *Biotech Histochem.* 2024;99(1):44-8.
14. Cui H, Zhang JA, Chen Chen, Liu JJ. ABC transporter inhibitors in reversing multidrug resistance to chemotherapy. *Curr Drug Targets.* 2015;16(12):1356-71.
15. Lee CR, Lee SH, Rigas NK, Kim RH, Kang MK, Park NH, *et al.* Elevated expression of JMJD6 is associated with oral carcinogenesis and maintains cancer stemness properties.

- Carcinogenesis. 2016;37(2):119-28
16. Lee YF, Miller LD, Chan XB, Black MA, Pang B, Ong CW, *et al.* JMJD6 is a driver of cellular proliferation and motility and a marker of poor prognosis in breast cancer. *Breast Cancer Res.* 2012;14(3):R85. doi: 10.1186/bcr3200.
 17. Baniebrahimi G, Mir F, Khanmohammadi R. Cancer stem cells and oral cancer: insights into molecular mechanisms and therapeutic approaches. *Cancer Cell Int.* 2020;20:113. doi: 10.1186/s12935-020-01192-0.
 18. Ayllón Barbellido S, Campo Trapero J, Cano Sánchez J, Perea García MA, Escudero Castaño N, Bascones Martínez A. Gene therapy in the management of oral cancer: review of the literature. *Med Oral Patol Oral Cir Bucal.* 2008;13(1):E15-21.
 19. Indu S, Ramesh V, Oza N, Prashad KV. Gene therapy: An overview. *J Orofac Sci.* 2013;5(2):83-7.
 20. Saraswathi TR, Kavitha B, Vijayashree Priyadharsini J. Gene therapy for oral squamous cell carcinoma: an overview. *Indian J Dent Res.* 2007;18(3):120-3.
 21. Madalli Vijaylaxmi B, Basavaraddi Shrinivas M, Sattur A, Burde K. Gene therapy for oral squamous cell carcinoma-a promising future. *Int J Inf Res Rev.* 2014;1:220-2.
 22. Jacob KC, Yashoda R, Puranik MP. Gene therapy in the treatment and prevention of oral cancer: an overview. *Int J Adv Health Sci.* 2015;2(2):14-20.
 23. Sunil PM, Joseph I, Susan Varghese S. Gene therapy in oral squamous cell carcinoma - a short review. *Oral Maxillofac Pathol J.* 2011;2(2):142-147.
 24. Lakshmi LS, Radhika T, Jeddy N. Gene therapy in oral cancer—an update *J Dr NTR Univ Health Sci.* 2020;9(1):1-5.
 25. Westphal M, Ylä-Herttua S, Martin J, Warnke P, Menei P, Eckland D, *et al.* Adenovirus-mediated gene therapy with sitimagene ceradenovec followed by intravenous ganciclovir for patients with operable high-grade glioma (ASPECT): a randomised, open-label, phase 3 trial. *Lancet Oncol.* 2013;14(9):823-33.
 26. Faneca H, Düzgüneş N, de Lima MP. Suicide gene therapy for oral squamous cell carcinoma. *Methods Mol Biol.* 2019;1895:43-55.
 27. Tan IB, Dolivet G, Ceruse P, Vander Poorten V, Roest G, Rauschnig W. Temoporphin-mediated photodynamic therapy in patients with advanced, incurable head and neck cancer: a multicenter study. *Head Neck.* 2010;32(12):1597-604.
 28. Kim SG, Hong JW, Boo SH, Kim MG, Lee KD, Ahn JC, *et al.* Combination treatment of cetuximab and photodynamic therapy in SNU-1041 squamous cancer cell line. *Oncol Rep.* 2009;22(4):701-8.
 29. Datta SN, Allman R, Loh C, Mason M, Matthews PN. Effect of photodynamic therapy in combination with mitomycin c on a mitomycin-resistant bladder cancer cell line. *Br J Cancer.* 1997;76(3):312-7.
 30. Saji H, Song W, Furumoto K, Kato H, Engleman EG. Systemic antitumor effect of intratumoral injection of dendritic cells in combination with local photodynamic therapy. *Clin. Cancer Res.* 2006;12(8):2568-74.
 31. Ke LD, Adler-Storthz K, Clayman GL, Yung AW, Chen Z. Differential expression of epidermal growth factor receptor in human head and neck cancers. *Head Neck.* 1998;20(4):320-7.
 32. Chen B, Pogue BW, Hoopes PJ, Hasan T. Vascular and cellular targeting for photodynamic therapy. *Crit Rev Eukaryot Gene Expr.* 2006;16(4):279-305.
 33. Toratani S, Tani R, Kanda T, Koizumi K, Yoshioka Y, Okamoto T. Photodynamic therapy using photofrin and excimer dye laser treatment for superficial oral squamous cell carcinomas with long-term follow up. *Photodiagnosis Photodyn Ther.* 2016;14:104-10.
 34. Gullett NP, Ruhul Amin AR, Bayraktar S, Pezzuto JM, Shin DM, Khuri FR, *et al.* Cancer prevention with natural compounds. *Semin Oncol.* 2010;37(3):258-81.
 35. Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, *et al.* Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules.* 2015;20(12):21138-56.
 36. Cheng AL, Hsu CH, Lin JK, Hsu MM, Ho YF, Shen TS, *et al.* Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res.* 2001;21(4B):2895-900.
 37. Kuriakose MA, Ramdas K, Dey B, Iyer S, Rajan G, Elango KK, *et al.* A randomized double-blind placebo-controlled phase IIB trial of curcumin in oral leukoplakia. *Cancer Prev Res (Phila).* 2016;9(8):683-91.
 38. Gu M, Jiang L, Hao L, Lu J, Liu Z, Lei Z, *et al.* A novel theranostic nanoplatfrom for imaging-guided chemo-photothermal therapy in oral squamous cell carcinoma. *J Mater Chem B.* 2021;9(30):6006-16.
 39. Mahesh KP. Nanotheranostics-novel modality for integrating diagnosis and therapy for oral cancer. *Acta Sci Dent Sci.* 2018;2(9):57-8.
 40. Hariharan K, Mehta T, Shah J, Dave H, Sami A, Omri A. Localized delivery of Erlotinib using liposomal gel formulations for the treatment of oral squamous cell carcinoma. *Int J Pharm.* 2023;642:123144.
 41. Sowmya SV, Augustine D, Mushtaq S, Baeshen HA, Ashi H, Hassan RN, *et al.* Revitalizing oral cancer research: Crispr-Cas9 technology the promise of genetic editing. *Front Oncol.* 2024;14:1383062.
 42. Pan S, Su Y, Sun B, Hao R, Gao X, Han B. Knockout of CD147 inhibits the proliferation, invasion, and drug resistance of human oral cancer CAL27 cells *in vitro* and *in vivo*. *Int J Biol Macromol.* 2021;181:378-89.
 43. Huang P, Tong D, Sun J, Li Q, Zhang F. Generation and characterization of a human oral squamous carcinoma cell line SCC-9 with CRISPR/Cas9-mediated deletion of the p75 neurotrophin receptor. *Arch Oral Bio.* 2017;82:223-32.
 44. Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, *et al.* Nivolumab for recurrent

- squamous-cell carcinoma of the head and neck. *N Engl J Med.* 2016;375(19):1856-67.
45. Ciulean IS, Fischer J, Quaiser A, Bach C, Abken H, Tretbar US, *et al.* 250 Harnessing anti-CD44v6 CAR-NK cells for effective head and neck squamous cell carcinoma treatment. *J Immunotherap Cancer.* 2024;12(Suppl 2):288.
46. Lambert A, Nees L, Nuyts S, Clement P, Meulemans J, Delaere P, *et al.* Photodynamic therapy as an alternative therapeutic tool in functionally inoperable oral and oropharyngeal carcinoma: a single tertiary center retrospective cohort analysis. *Front Oncol.* 2021;11:626394.
47. Strojan P, Jesenko T, Omerzel M, Jamsek C, Groselj A, Tratar UL, *et al.* Phase I trial of pHIL12 plasmid intratumoral gene electrotransfer in patients with basal cell carcinoma in head and neck region. *Eur J Surg Oncol.* 2025;51(5):109574.
48. Talreja V, Khetwani S, Nanadagopal E, Borkar NE, Khobragade K. Real-world study on the effect of nab-paclitaxel treatment on clinical outcomes and laboratory parameters in patients across metastatic tumor sites. *IJMIO.* 2024;9(2):46-52.



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Role of Yes-associated protein [YAP]- A Key Hippo Component in the Onset and Progression of Oral Squamous Cell Carcinoma

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Abstract

Background: Tumorigenesis is an abnormal growth of cells within the body, usually induced by abnormal proliferation of the stem cells or abnormal apoptosis, the usual disturbance in the cell cycle. Various signalling pathways play a role in the cell cycle, which, when altered, result in abnormal cell proliferation and thus cancerogenesis. One such important pathway is the Hippo pathway, which plays a major role in controlling the organ's size. Dysregulation in the components of the hippo pathway, causing cancers, has been studied in the literature in various cancers such as breast cancer, liver cancer, colorectal cancer, and liver cancer.

Aim: This review focuses on the role of Yes-associated protein (YAP), a key effector of the Hippo pathway, in the onset and progression of oral squamous cell carcinoma (OSCC). We summarize recent achievements in understanding YAP's mechanisms in OSCC pathogenesis and discuss its potential as a therapeutic target.

Methods: A comprehensive literature search was conducted in PubMed, Scopus, and Web of Science for articles published between January 2010 and March 2025, using the terms "YAP," "Yes-associated protein," "Hippo pathway," "oral squamous cell carcinoma," and "oral cancer." Inclusion criteria were original research and review articles in English focusing on YAP in OSCC or oral precancerous lesions. Exclusion criteria included non-English articles, case reports, and studies not involving OSCC or YAP.

Conclusions: YAP plays a vital role in malignant transformation, with its dysfunction initiating tumor growth factor expression and being associated with tumor growth and metastasis in OSCC. Understanding YAP's role in the onset and progression of OSCC may identify specific targets for anti-cancer therapy.

Keywords: Hippo pathway, oral cancer, TEAD, wts, yes-associated protein

Introduction

Head and neck cancer is one of the sixth most common cancers worldwide. Over 90% of head and neck cancers are squamous cell carcinomas (HNSCCs), which primarily originate in the larynx, pharynx, and mouth cavity.⁽¹⁾ However, this review specifically focuses on oral squamous cell carcinoma (OSCC), which accounts for the majority of oral malignancies. There were 377,713 documented cases of OSCC (Oral squamous cell carcinoma) in 2020. By 2040, there is expected to be a 40% increase in incidence and a corresponding rise in mortality.⁽¹⁾ A dysregulated tumor microenvironment, epigenetic modifications, and genetic changes all play a part in the intricate and multifaceted process of OSCC development.⁽²⁾ Comprehending the mechanisms that underlie malignancies of the OSCC is essential to uncover prognostic and therapeutic factors that may enhance the effectiveness of treatment. OSCC remains incurable even with recent advancements in the identification of various therapeutic targets or immune checkpoint inhibitors. Its pathophysiology is still poorly understood, and there are currently no particular biomarkers to help with diagnosis or course prediction. Mutations and the activation of many signal transduction pathways cause OSCC's high invasiveness and metastatic potential.

The growth and differentiation of tissues and organs are regulated by the Hippo signaling pathway. It also plays a significant role in the development and spread of tumors.⁽³⁾ It is unknown, still unknown exactly how the Hippo pathway regulates oral cancer. A deeper comprehension of the mechanisms behind oral cancer development and the identification of its distinct clinical subtypes will be possible through the characterization of key proteins engaged in major signaling pathways and the analysis of their interactions. This will make it possible to pinpoint particular biochemical targets for later, more potent therapy.

The Hippo pathway

The Hippo signaling pathway is a highly conserved signaling cascade essential for regulating cell survival, differentiation, and proliferation. First identified in *Drosophila melanogaster*, it comprises key elements such as MST1/2, LATS1/2, MOB1A/B, SAV1, YAP, TAZ, and TEAD1-4.⁽⁴⁾ These elements modulate cell activity in response to upstream signals, including mechanical cues, stress signals, and cell polarity.^(5,6) Dysregulation of the

Hippo pathway has been linked to cancer development, as well as eye, heart, and pulmonary conditions. Targeting the pathway may offer therapeutic benefits for several disorders.

Main components of the pathway

The Hippo pathway regulates cell growth, proliferation, and organ size. In mammals, it consists of a network of kinases, adaptor proteins, transcriptional coactivators, and transcription factors. MST1/2 (mammalian Ste20-like kinases 1 and 2) serve as the pathway's apical regulators, activated by metabolic shifts, cell-to-cell contact, and mechanical stimuli.^(7,8) Downstream, LATS1/2 (Large Tumor Suppressor Kinases 1 and 2) are phosphorylated and activated, facilitated by the adaptor protein SAV1.⁽⁹⁾ The transcriptional coactivators YAP and TAZ are then phosphorylated by LATS1/2, leading to their cytoplasmic retention and degradation, which inhibits their ability to bind TEAD transcription factors and enter the nucleus (Figure 1).⁽¹⁰⁾ When the Hippo pathway is inactive, YAP and TAZ accumulate in the nucleus, interact with TEAD, and activate genes supporting cell proliferation, survival, and epithelial-mesenchymal transition (EMT).^(11,12) Dysregulation of this pathway is closely linked to the onset and progression of several cancers, including OSCC.^(13,14)

Yes-associated protein

The Yes-associated protein (YAP) gene, located on chromosome 11q22, encodes the YAP, a critical downstream effector of the Hippo pathway that regulates tissue growth and cell proliferation. YAP has been connected to tumor growth and progression in OSCC.⁽¹⁵⁾ YAP collaborates with TAZ to manage stem cell self-renewal and control cell proliferation.⁽¹⁶⁾ Recent research indicates that YAP interacts with several signaling pathways, implicating it in the initiation and progression of OSCC. Targeting YAP and its signaling pathways may offer a promising therapeutic approach for OSCC.⁽¹⁷⁾

Structure of YAP

Yes-associated protein, or YAP, is a 488 amino acid protein that is essential for controlling gene expression programs that support cell transformation, survival, and multiplication. Several important domains make up its structure, such as two WW domains, a PDZ-binding

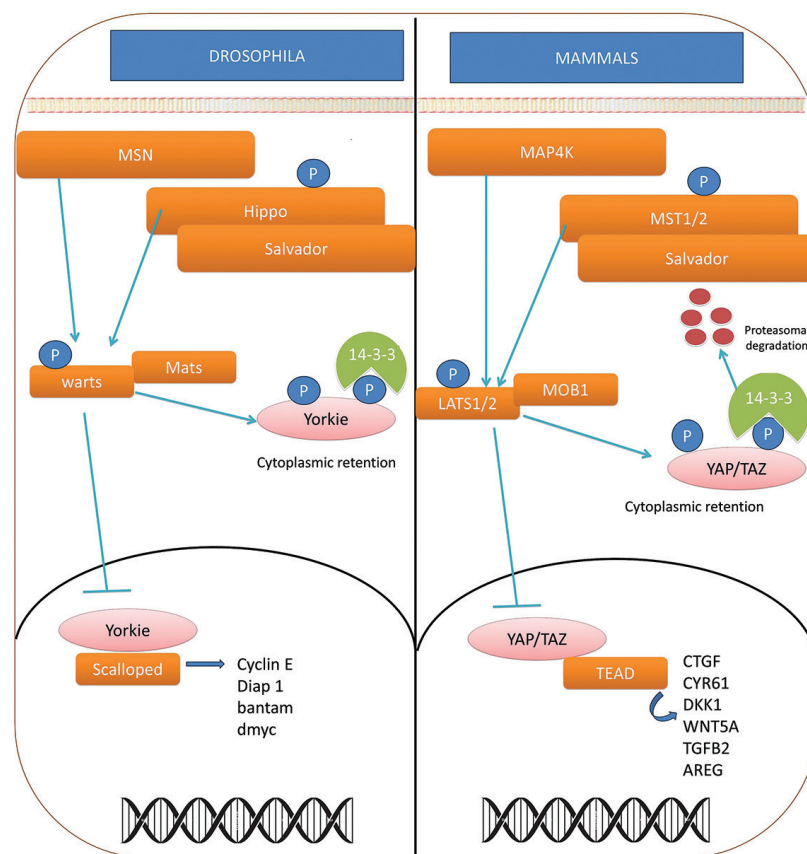


Figure 1: An overview of the Drosophila and mammalian Hippo signaling system. Numerous upstream cues can start hippocampal signaling. Phosphorylation of Warts (LATS1/2) follows Hippo (MST1/2) activation. Yorkie, an effector of the Hippo pathway (YAP/TAZ), is negatively regulated by warts. Yorkie that has not undergone phosphorylation moves into the nucleus, combining with its TEAD transcription factors to enhance the transcription of several genes. On the other hand, when Wts phosphorylates Yorkie, it is sequestered by 14-3-3 proteins in the cytoplasm and eventually degrades.

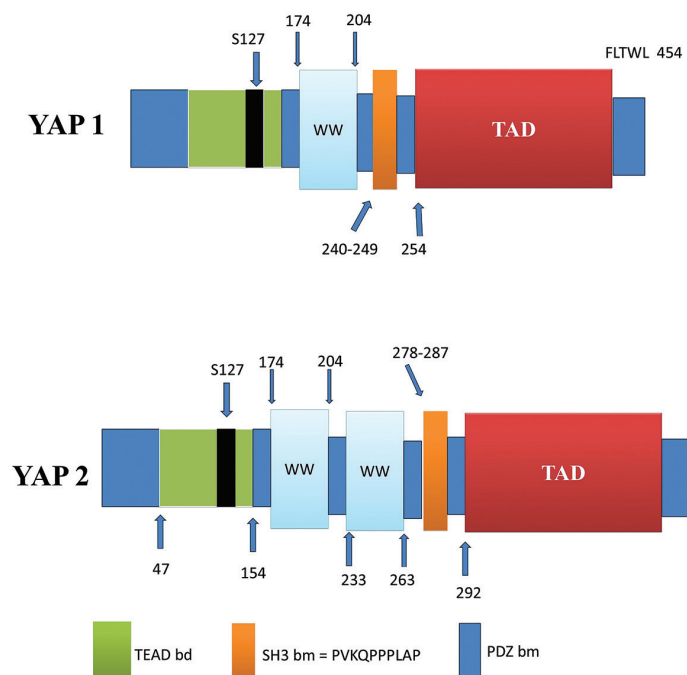


Figure 2: Structure of YAP. The two main YAP protein isoforms' modular architectures. On the scheme of structures, TEAD binding domain (bd), WW domains, transcriptional activation domain (TAD), and SH3 domain-binding motif (bm) are distinguished.

motif, an SH3-binding motif, and a TEAD-binding domain.⁽¹⁸⁾ While the first WW domain also binds to TEAD transcription factors and the second WW domain binds to transcriptional coactivators that interact with PPxY motifs on transcription factors, the TEAD-binding domain enables YAP to bind to the TEAD family of transcription factors (Figure 2).⁽¹⁹⁾ The PDZ-binding motif is necessary for YAP-mediated cellular transformation and is responsible for localizing YAP to particular nuclear foci. Furthermore, in conjunction with the WW1 domain, the SH3-binding motif binds to p53 binding protein 2 to control YAP activity. By binding to and activating different transcription factors through these domains, YAP functions as a transcriptional coactivator, controlling gene expression programs that promote cell division, survival, and growth.⁽²⁰⁻²¹⁾

Regulation of YAP

Regulation by Hippo pathway:

One important YAP regulator is the Hippo pathway. The proteins MST1/2, LATS1/2, and MOB1 make up this kinase cascade. This mechanism opens up a binding site for 14-3-3 proteins by phosphorylating YAP at Ser127 when it is in an active state. YAP cytoplasmic retention and deactivation result from this. Different cues, including mechanical stress, signaling molecules, and cell-cell interaction, activate the Hippo pathway (Figure 3).⁽²²⁾

Regulation by phosphorylation and methylation:

The main mechanism that phosphorylation uses is to block YAP activity. Phosphorylation of many locations on YAP can result in its breakdown and exclusion from the nucleus. YAP is phosphorylated at Serine 127 by nuclear Dbf2-related/LATS kinases, which facilitates cytoplasmic localization and destruction of the protein. Consequently, pro-growth genes' transcription is inhibited.⁽²³⁾ Cancer develops when these kinases are artificially depleted. In addition to encouraging the binding of the 14-3-3 protein, phosphorylation of YAP at Serine 127 localizes YAP in the cytoplasm. Moreover, YAP is phosphorylated by Akt kinase, which results in the binding of 14-3-3 protein and the suppression of transcription factors like p53. As a result, when a cell is damaged, pro-apoptotic gene production is suppressed. Protein kinase C η (PKC ζ) is another regulator of YAP that phosphorylates YAP at Serine 109 and Threonine 110 to keep it in the cytoplasm. Intestinal stem cells have enhanced tumorigenic and regenerative

activity when YAP is deactivated.^(24,25)

The function of YAP is also regulated by methylation. Enhancers of zeste (SET)7 and Su(var)3-9 methylate YAP, causing it to become localized in the cytoplasm and preventing it from functioning. To facilitate cytoplasmic localization, phosphorylation at Serine 127 and monomethylation of YAP at Lysine 494 happen simultaneously.⁽²⁶⁾ Pro-apoptotic gene expression is suppressed and pro-growth gene transcription is inhibited by phosphorylation and methylation, which also promote YAP's cytoplasmic localization and destruction.

YAP regulation by ubiquitination:

YAP is ubiquitinated by E3 ubiquitin ligases like β -TrCP, targeting it for proteasomal degradation. Ubiquitination regulates YAP protein stability and localization. Deubiquitination of YAP by enzymes like USP47 can stabilize YAP and prevent its degradation.⁽²⁷⁾ Ubiquitination of YAP is often coupled with other post-translational modifications like phosphorylation. Phosphorylation of YAP can create binding sites for E3 ligases, promoting its ubiquitination. Deubiquitinating enzymes like USP47 can counteract ubiquitination and stabilize YAP.⁽²⁸⁾ The balance between ubiquitination and deubiquitination regulates YAP activity. Dysregulation of YAP ubiquitination and stability is implicated in various cancers. Liu *Z et al*, showed that increased expression of deubiquitinating enzymes like USP47 can stabilize YAP and promote gastric cancer progression.⁽²⁹⁾ Targeting the ubiquitination-deubiquitination balance of YAP is a potential therapeutic strategy in cancers with YAP activation.

Transcriptional regulation of YAP:

The Ets family transcription factor GABP directly binds to the YAP promoter and increases YAP transcription, especially in oxidative stress conditions. Through its binding to recognition sites in the YAP promoter, the AP-1 transcription factor c-Jun also controls the expression of YAP. When c-Jun is knocked down, YAP is downregulated.⁽³⁰⁾

To regulate context-specific gene expression patterns, YAP functions as a transcriptional coactivator by interacting with different transcription factors and epigenetic regulators. YAP interacts with TEAD, β -catenin, FoxO1, and TFEB in embryonic and adult stem cells to control genes related to stemness, differentiation, and stress response.⁽³¹⁾ In various cellular situations, the transcriptional outputs and biological roles of YAP are deter-

mined by the particular YAP-interacting partners.

Regulation of YAP by microRNAs:

YAP (Yes-associated protein) is regulated by various microRNAs (miRNAs) through both transcriptional and post-transcriptional mechanisms. Transcriptional regulation of YAP by miR-375 has been studied to repress YAP expression by binding to the YAP mRNA 3'UTR and inhibiting its translation.⁽³²⁾ YAP levels rise when miR-375 is often downregulated in malignancies such as colorectal carcinoma.⁽³³⁾

Post-transcriptional regulation of YAP by miR-200a-3p has been found to interact with YAP and regulate its function in cervical cancer cells. It has been suggested that miR-200a-3p functions as a negative regulator of YAP because overexpression of the protein can prevent YAP-mediated cell proliferation and metastasis.⁽³⁴⁾ However, as YAP was also found to offset the effects of miR-200a-3p in some cell lines, the connection between miR-200a-3p and YAP appears to be context-dependent.

Reciprocal Regulation of miRNAs and YAP regulates the expression of specific miRNAs, such as let-7, by modulation of miRNA processing enzyme Dicer. This implies that YAP may also have an indirect impact on miRNA targets' expression, resulting in the formation of an intricate regulatory network.⁽³⁵⁾

Epigenetic regulation of YAP:

Epigenetic modifications like histone modification, chromatin remodeling, and DNA methylation play a role in YAP regulation. Inconsistent with its function as a transcriptional coactivator, YAP binding sites are enriched for the enhancer histone mark H3K4me1 and depleted of the promoter mark H3K4me3.⁽³⁶⁾ YAP appears to occupy active enhancers, as evidenced by the correlation between H3K27ac levels and YAP binding signal levels. Lineage-specific YAP binding sites were found close to tissue-specific oncogenes and markers in malignant pleural mesothelioma (MPM).⁽³⁷⁾

YAP controls gene expression programs through interactions with chromatin remodeling complexes such as SWI/SNF. It has been discovered that YAP-mediated transcription and carcinogenic activities require the SWI/SNF component ARID1A. The ability of YAP to regulate gene expression and tumor growth is compromised when the YAP-SWI/SNF connection is disrupted.⁽³⁸⁾

Considering that the YAP promoter is hypermethylated in specific malignancies like hepatocellular carcinoma,

it is evident that DNA methylation can regulate YAP expression.⁽³⁹⁾ In these circumstances, demethylating drugs can revive YAP expression.

Metabolic regulation of YAP:

YAP activity is regulated by the sterol regulatory element binding protein/mevalonate signaling pathway, an essential cellular metabolic route. YAP/TAZ nuclear localization is inhibited by the rate-limiting enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase, according to research by Sorrentino *et al.* Geranylgeranyl pyrophosphate, which is generated by the mevalonate pathway, activates Rho GTPase. This GTPase breaks the bond between angiomin (AMOT) and YAP by polymerizing F-actin.⁽⁴⁰⁾ As a result, YAP can go to the nucleus and start the transcription of pro-growth genes.

Regulation by microenvironment:

The regulation of YAP activity and localization is significantly influenced by the extracellular matrix (ECM) and cytoskeletal tension. Since YAP is primarily cytoplasmic in soft matrix environments, cell growth is inhibited. On the other hand, YAP translocates to the nucleus in a rigid extracellular matrix, encouraging cell division. Cells use the cytoskeleton to detect and react to mechanical stimuli. Nuclear YAP levels rise as a result of F-actin polymerization, which also produces an opposing force to balance tension and promote cell division. ECM rigidity and YAP regulation are linked via the scaffold protein AMOT. AMOT is a YAP inhibitor that works either directly or via kinases LATS1/2 in the Hippo pathway. AMOT binds to F-actin preferentially during F-actin polymerization, removing the inhibitory effect on YAP.⁽⁴¹⁾ Furthermore, F-actin increases nuclear YAP accumulation by inhibiting LATS1/2 activity.

YAP-The key oncogenic pathway of human cancers

Many human malignancies exhibit extensive YAP activation. Nevertheless, most cancers do not initiate because of YAP activation or Hippo kinase inactivation. In the mammalian gut, homeostatic self-renewal and regeneration are generally primarily fueled by the Wnt pathway. Meanwhile, the most frequent cause of colon tumor growth is the constitutive activation of this system.⁽⁴²⁾ According to research by Seo Y *et al.*, cytoplasmic YAP can reduce the formation of intestinal epithelia that regenerates by reducing the activity of Dishevelled,

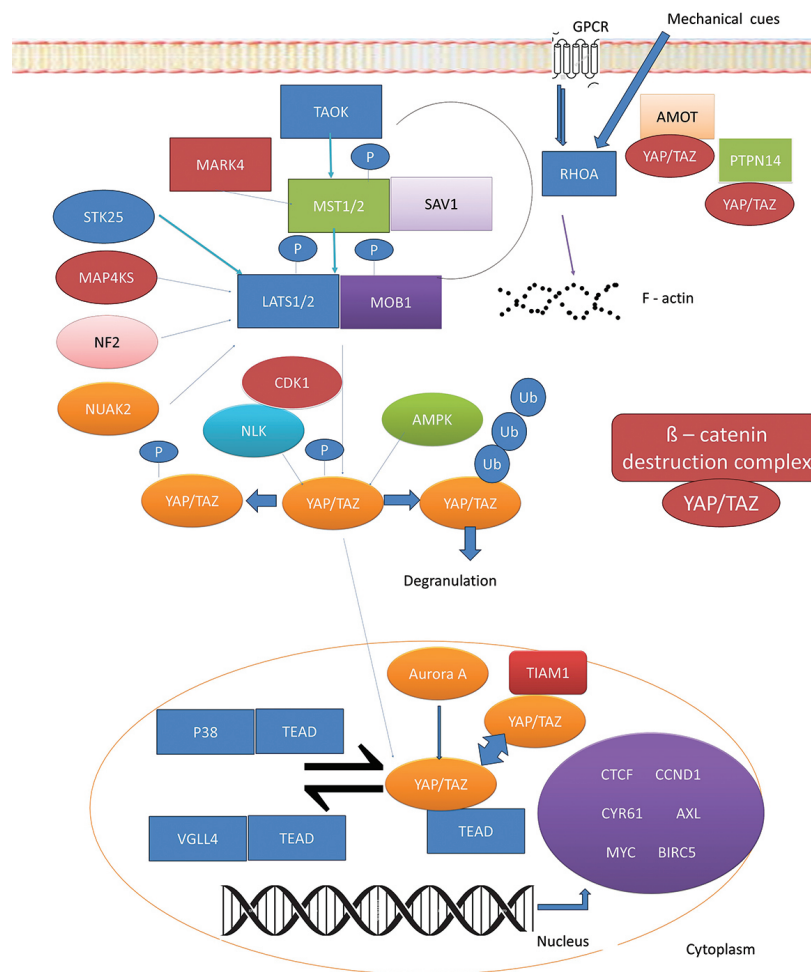


Figure 3: YAP's regulatory mechanism. The classical Hippo pathway, which is composed of MST1/2-SAV1 > LATS1/2-MOB1, is the main regulator of YAP/TAZ. By either ubiquitination and proteasome-mediated destruction or 14-3-3-mediated cytoplasmic sequestration, LATS1/2 phosphorylates YAP/TAZ and renders it inactive. After translocating to the nucleus, unphosphorylated YAP/TAZ interacts with TEAD transcription factors to trigger target genes. TAOK, STK25, MAP4KS, and NF2 activate LATS1/2, whereas F-actin and NUAK2 inactivate it in response to mechanical stimuli and GPCR-mediated RHOA. MARK4 and TAOK activate MST1/2. LATS separately regulates YAP/TAZ as well. YAP/TAZ interacts with AMOT and PTPN14, which sequesters it in the plasma membrane. TIAM1 or the β -catenin destruction complex directly interacts with YAP/TAZ to inhibit it. Additionally, AMPK, CDK1, NLK, Aurora A, and several other proteins directly phosphorylate and control YAP/TAZ.

which in turn limits Wnt signaling.⁽⁴³⁾ Furthermore, YAP is reactivated to limit the growth of colorectal carcinoma xenografts, and it is silenced in a subpopulation of highly aggressive and undifferentiated human colorectal carcinomas.⁽⁴⁴⁾ Thus, via disrupting Wnt signaling, it has been demonstrated that YAP acts as a tumor suppressor in colon cancer. It has also recently been discovered that YAP activation can sustain intestinal epithelial cells in a wound-healing signaling state with decreased Wnt signaling and increased production of Kruppel-like factor 6.⁽⁴⁵⁾ On the other hand, colonic tumors that were produced focally grew more quickly when YAP was deleted which is evidence that YAP functioned as a tumor suppressor

and that activating hippocampal kinases was a novel therapeutic strategy for the treatment of colorectal cancer.⁽⁴⁴⁾

YAP as a tumor suppressor

Although YAP is involved in carcinogenesis, some research has connected its function to anti-tumor pathways in different kinds of cancer. Low YAP1 expression is associated with poorer prognosis in hematological cancers, and YAP reduces proliferation in multiple myeloma cells via interaction with pro-apoptotic p73.⁽⁴⁶⁾

Breast cancer has been linked to the loss of heterozygosity in chromosome 11q22-q23, which contains YAP1. Additionally, YAP deletion or knockdown in breast cancer

cell lines decreases tumorigenic potential.⁽⁴⁷⁾ A recent study demonstrated that, only when YAP is hydroxylated in a prostate cancer cell line, YAP1 knockdown increases the *in vitro* metastatic potential in cell lines from multiple tissues, indicating a tumor suppressive effect. A subset of neuroendocrine prostate tumors exhibits YAP silencing, suggesting a context-dependent post-translational modification-based tumor suppressive role.⁽⁴⁸⁾

These results imply that, depending on the situation, YAP may display a binary switch between oncogene and tumor suppressor. YAP functions as a tumor suppressor only in certain cases. A tiny group of cancers, including hematological malignancies and small cell neuroendocrine tumors, have YAP silenced as in the majority of the cases it serves as a tumor progressor gene.

Role of YAP in oral cancer

Chronic exposure to risk factors like tobacco and alcohol can lead to genetic alterations in the normal oral mucosa, causing uncontrollable cellular proliferation and rendering cells unable to respond to stress or DNA damage.⁽⁴⁹⁾ Modifications to pathways such as p53, p16, cyclin D1, and retinoblastoma protein, lead to oral cancer development. Although there is currently little information linking the Hippo-YAP pathway to the aetiological aspects of oral cancer, it is most likely the case that YAP contributes to metastasis rather than starting the oncogenic process. According to several research, YAP may be an important therapeutic target.⁽¹⁶⁾

YAP plays a critical role in cell migration by promoting EMT and inhibiting adherens junctions mediated by E-cadherin.⁽¹³⁾ Furthermore, in OSCC, YAP is thought to be a biomarker for metastasis and resistance to EGFR inhibitors like cetuximab and gefitinib.⁽⁵⁰⁾ With the genetic changes linked to oral cancer, YAP may be a target for therapy even if it may not start the oncogenic process in OSCC but rather drive metastasis.

A lot of research has been done on the carcinogenic function of YAP in a variety of carcinoma types, including OSCC. Research has demonstrated that YAP is considerably overexpressed and amplified in OSCC, suggesting a possible function for it in oncogenesis.⁽³³⁾ In many types of squamous cell carcinomas (SCCs), elevated YAP expression has been associated with nuclear localization, which promotes cell proliferation, invasiveness, and survival.⁽³⁶⁾ Studies have consistently shown that OSCC

tissues exhibit higher levels of YAP than neighboring normal tissues.

YAP in the onset and progression of OSCC

Clinical outcomes and treatment responses in oral cancers can be influenced by the molecular and genetic heterogeneity of the original tumors. This heterogeneity can be better characterized and the disease mechanisms, including metastasis, more thoroughly understood through *in vitro* studies using cancer cell lines. Previous research investigating the Hippo-YAP pathway in OSCC cell lines derived from the buccal mucosa and floor of the mouth demonstrated overexpression of WWTR1 and YAP1, highlighting their potential roles in OSCC pathogenesis.⁽⁵¹⁾

Recent gene expression profiling of oral squamous cell carcinoma (OSCC) cell lines has identified three major gene signature groups. First, twenty-one genes related to the Hippo pathway-including YAP1, WWTR1, core pathway components, and associated transcription factors-were found to be upregulated in OSCC cell lines. Second, eight genes recognized as cancer biomarkers exhibited higher expression levels in carcinoma cell lines compared to normal oral epithelial cells. Third, seventeen genes involved in intercellular anchoring junctions, such as those encoding desmosomes and adherens junctions, were downregulated in both cancer and dysplastic cells. Notably, the extent of downregulation of these junction-related genes varied among different OSCC cell lines, reflecting the molecular heterogeneity of the disease.⁽¹⁷⁾

Literature studies on YAP and OSCC

A systematic and comprehensive literature search was conducted to identify relevant studies on the role of Yes-associated protein (YAP) in OSCC and oral precancerous lesions. The search was performed across multiple electronic databases, including PubMed, Scopus, and Web of Science, to ensure broad coverage of biomedical literature published between January 2010 and March 2025. The search terms included "YAP," "Yes-associated protein," "Hippo pathway," "oral squamous cell carcinoma," and "oral cancer." Inclusion criteria were original research and review articles in English focusing on YAP in OSCC or oral precancerous lesions. Exclusion criteria included non-English articles, case reports, and studies

not involving OSCC or YAP

Many authors in the literature have studied the expression of YAP in oral cancer cell lines, mouse models, and human tissues. (Table 1). The authors showed an increased expression of YAP in Oral cancer tissues compared with normal tissues.⁽⁵²⁻⁶⁰⁾ Omron H *et al* reported that the onset of OSCC depends on YAP1 activation, inhibition of which slows the progression of OSCC.⁽⁶⁰⁾ Another study done by Ono S *et al.*,⁽⁵⁸⁾ showed a positive correlation between increased YAP expression in poorly differentiated OSCC and well-differentiated tissue samples. From this, it is evident that Yap expression can be used as a prognostic marker.

YAP as a therapeutic agent

Yes-associated protein (YAP) has emerged as a prospective target for cancer therapy. Since this complex is necessary for YAP-mediated transcriptional activity and carcinogenic activities, the main focus of YAP cancer therapy has been to break its interaction with TEAD transcription factors.⁽⁶¹⁾

Numerous small-molecule inhibitors that can attach to YAP and stop it from joining TEAD have been found; these inhibitors limit YAP's capacity to convert in vitro. Vertiporfin (VP), a porphyrin derivative, is one such substance that has been demonstrated to bind to YAP and obstruct the YAP-TEAD interaction. It's interesting to

Table 1: Literature studies on Yap association in OSCC.

S no	Author, Year of study, Country	Hippo component	Methods of Detection	Impact in cancer
1	Li SY, 2013, China. ⁽⁵²⁾	YAP1	RT PCR; Western blotting; IHC	Increase in cDNA of YAP1 in OSCC tissue; IHC – Increased expression localized in the cytoplasm of OSCC tissue.
2	Hiemer SE <i>et al</i> , 2015, The USA. ⁽⁵³⁾	YAP1	IHC/IJC; Immunofluorescence;	Increased nuclear expression of YAP in severe dysplasia High levels of YAP expression in poorly differentiated.
3	Hasegawa K <i>et al</i> , 2021, Japan. ⁽⁵⁴⁾	YAP1	IHC	Hyperactivated YAP1 expression in OSCC tissues.
4	Ge L, 2011, China. ⁽⁵⁵⁾	YAP	IHC	Increased YAP expression in OSCC tissue; Leukoplakia – cytoplasmic expression in basal and parabasal layers; Control – Undetectable; Upregulated expression – Nodal metastasis;
5	Qi L <i>et al</i> , 2019, China. ⁽⁵⁶⁾	YAP	IHC	Increased expression of YAP than adjacent normal tissue localized in the majority at the nucleus, few in the nucleus, and few in both;
6	Szelachowska J <i>et al</i> , 2019, Poland. ⁽⁵⁷⁾	YAP	IHC	Expression of YAP with cytoplasmic localization in cancer cells;
7	Ono S <i>et al</i> , 2019, Japan. ⁽⁵⁸⁾	YAP	IHC	Positive YAP expression in all OSCC tissues; Higher expression in poorly differentiated than well and moderately differentiated; Yap expression increased in poor survival;
8	Zhang L <i>et al</i> , 2011, China. ⁽⁵⁹⁾	YAP	Western blotting; RTPCR;	High expression of YAP in OSCC cell lines;
9	Omori H <i>et al</i> , 2020, Japan. ⁽⁶⁰⁾	YAP1	IHC	Increased nuclear YAP 1 expression in OSCC than control; onset of OSCC depends on YAP1 activation; inhibition of YAP1 slows the progression of OSCC.

note that VP has clinical approval for the treatment of macular degeneration. In preclinical research, VP was repeatedly administered in a mouse model of liver cancer to effectively decrease hepatic expansion and delay tumor progression without having a major negative impact on other organs.⁽⁶²⁾

YAP can be directly targeted, but it can also be activated by blocking the Hippo pathway's upstream kinases, like Mst1 and Mst2. For instance, it has been demonstrated that the Mst1 inhibitor 9E1 increases YAP function. Additionally, it has been discovered that dasatinib, a Src family kinase inhibitor, inhibits the kinase YES1, which inactivates the YAP1- β -catenin-TBX5 complex and reduces the proliferation of cells that are active with β -catenin.⁽⁶³⁾

Targeting the YAP-TEAD connection, whether directly or indirectly, has shown promising results in preclinical research and warrants further investigation as a potential therapeutic approach for cancer.

Conclusions

Although YAP overexpression has been reported in various cancers, including oral squamous cell carcinoma (OSCC), its precise role in disease progression and prognosis remains uncertain. Importantly, YAP abundance alone—particularly given its predominantly nuclear localization—may not accurately reflect its functional activity, as treatment resistance in OSCC cells is influenced by the complex interplay between YAP, TAZ, and the broader Hippo-YAP pathway. While there is increasing interest in YAP's involvement in the neoplastic process and its potential as a therapeutic target, current evidence specifically linking YAP to the onset and progression of OSCC is limited, and strong conclusions regarding its prognostic value are not yet warranted. Many aspects of Hippo pathway signaling in OSCC, including its influence on cell junction adhesion, structural integrity, invasion, and metastasis, remain to be fully elucidated. Therefore, further *in vivo* validation and comprehensive clinical studies are needed to clarify the diverse functions of YAP in OSCC, and future research in these areas will be essential for advancing our understanding of the disease and evaluating the potential of YAP as a biomarker or therapeutic target.

References

1. Johnson DE, Burtneß B, Leemans CR, Lui VW, Bauman JE, Grandis JR. Head and neck squamous cell carcinoma.

- Nat Rev Dis Primers. 2020;6(1):92.
2. Mesgari H, Esmaelian S, Nasiri K, Ghasemzadeh S, Doroudgar P, Payandeh Z. Epigenetic regulation in oral squamous cell carcinoma microenvironment: a comprehensive review. *Cancers (Basel)*. 2023;15(23):5600.
3. Ehmer U, Sage J. Control of proliferation and cancer growth by the Hippo signaling pathway. *Mol Cancer Res*. 2016;14(2):127-40.
4. Ma S, Meng Z, Chen R, Guan KL. The Hippo pathway: biology and pathophysiology. *Annu Rev Biochem*. 2019;88(1):577-604.
5. Chen YA, Lu CY, Cheng TY, Pan SH, Chen HF, Chang NS. WW domain-containing proteins YAP and TAZ in the hippo pathway as key regulators in stemness maintenance, tissue homeostasis, and tumorigenesis. *Front Oncol*. 2019;9:60.
6. Pan D. The unfolding of the Hippo signaling pathway. *Dev Bio*. 2022;487:1-9.
7. Watt KI, Harvey KF, Gregorevic P. Regulation of tissue growth by the mammalian Hippo signaling pathway. *Front Physiol*. 2017;8:942.
8. Huang H, Wu W, Zhang L, Liu XY. Drosophila ste-20 family protein kinase, hippo, modulates fat cell proliferation. *PLoS One*. 2013;8(4):e61740.
9. Avruch J, Zhou D, Fitamant J, Bardeesy N, Mou F, Barrufet LR. Protein kinases of the Hippo pathway: regulation and substrates. *Semi Cell Dev Bio*. 2012;23(7):770-84.
10. Harvey K, Tapon N. The Salvador–Warts–Hippo pathway—an emerging tumor-suppressor network. *Nat Rev Cancer*. 2007;7(3):182-91.
11. Zhu C, Li L, Zhao B. The regulation and function of YAP transcription co-activator. *Acta Biochim Biophys Sin (Shanghai)*. 2015;47(1):16-28.
12. Currey L, Thor S, Piper M. TEAD family transcription factors in development and disease. *Development*. 2021;148(12):dev196675.
13. Wang M, Dai M, Wang D, Xiong W, Zeng Z, Guo C. The regulatory networks of the Hippo signaling pathway in cancer development. *J Cancer*. 2021;12(20):6216-30.
14. Pocaterra A, Romani P, Dupont S. YAP/TAZ functions and their regulation at a glance. *J Cell Sci*. 2020;133(2):jcs230425.
15. Morciano G, Vezzani B, Missiroli S, Boncompagni C, Pinton P, Giorgi C. An updated understanding of the role of YAP in driving oncogenic responses. *Cancers (Basel)*. 2021;13(12):3100.
16. Driskill JH, Pan D. Control of stem cell renewal and fate by YAP and TAZ. *Nat Rev Mol Cell Bio*. 2023;24(12):895-911.
17. Ahmad US, Saravanan KA, Wan HO. The role of YAP in the control of the metastatic potential of oral cancer. *Oncol Res*. 2021;29(6):377-91.
18. Sudol M, Shields DC, Farooq A. Structures of YAP protein domains reveal promising targets for development of new cancer drugs. *Semin Cell Dev Bio*. 2012;23(7):827-33.
19. Tian W, Yu J, Tomchick DR, Pan D, Luo X. Structural and

- functional analysis of the YAP-binding domain of human TEAD2. *Proc Natl Acad Sci U S A*. 2010;107(16):7293-8.
20. Chen L, Chan SW, Zhang X, Walsh M, Lim CJ, Hong W, *et al*. Structural basis of YAP recognition by TEAD4 in the hippo pathway. *Genes Dev*. 2010;24(3):290-300.
 21. Li Z, Zhao B, Wang P, Chen F, Dong Z, Yang H, Guan KL, Xu Y. Structural insights into the YAP and TEAD complex. *Genes Dev*. 2010;24(3):235-40.
 22. Yu FX, Guan KL. The hippo pathway: regulators and regulations. *Genes Dev*. 2013;27(4):355-71.
 23. Abylkassov R, Xie Y. Role of Yes-associated protein in cancer: an update. *Oncol Lett*. 2016;12(4):2277-82.
 24. Basu S, Totty NF, Irwin MS, Sudol M, Downward J. Akt phosphorylates the Yes-associated protein, YAP, to induce interaction with 14-3-3 and attenuation of p73-mediated apoptosis. *Mol Cell*. 2003;11(1):11-23.
 25. Kwon H, Kim J, Jho EH. Role of the Hippo pathway and mechanisms for controlling cellular localization of YAP/TAZ. *The FEBS J*. 2022;289(19):5798-818.
 26. Zou R, Xu Y, Feng Y, Shen M, Yuan F, Yuan Y. YAP nuclear-cytoplasmic translocation is regulated by mechanical signaling, protein modification, and metabolism. *Cell Biol Int*. 2020;44(7):1416-25.
 27. Wang D, Zhang Y, Xu X, Wu J, Peng Y, Li J, *et al*. YAP promotes the activation of NLRP3 inflammasome via blocking K27-linked polyubiquitination of NLRP3. *Nat Commun*. 2021;12(1):2674.
 28. Sun X, Ding Y, Zhan M, Li Y, Gao D, Wang G, *et al*. Usp7 regulates Hippo pathway through deubiquitinating the transcriptional coactivator Yorkie. *Nat Commun*. 2019;10(1):411.
 29. Liu Z, Li J, Ding Y, Ma M, Chen J, Lei W, *et al*. USP49 mediates tumor progression and poor prognosis through a YAP1-dependent feedback loop in gastric cancer. *Oncogene*. 2022;41(18):2555-70.
 30. Shin E, Kwon Y, Jung E, Kim YJ, Kim C, Hong S, *et al*. TM4SF19 controls GABP-dependent YAP transcription in head and neck cancer under oxidative stress conditions. *Proc Natl Acad Sci U S A*. 2024;121(7):e2314346121.
 31. Trejo-Solis C, Escamilla-Ramirez A, Jimenez-Farfan D, Castillo-Rodriguez RA, Flores-Najera A, Cruz-Salgado A. Crosstalk of the Wnt/ β -catenin signaling pathway in the induction of apoptosis on cancer cells. *Pharmaceuticals (Basel)*. 2021;14(9):871.
 32. Samji P, Rajendran MK, Warriar VP, Ganesh A, Devarajan K. Regulation of Hippo signaling pathway in cancer: A microRNA perspective. *Cell Signal*. 2021;78:109858.
 33. Xu X, Chen X, Xu MU, Liu X, Pan B, Qin J, *et al*. miR-375-3p suppresses tumorigenesis and partially reverses chemoresistance by targeting YAP1 and SP1 in colorectal cancer cells. *Aging (Albany NY)*. 2019;11(18):7357-85.
 34. Wen B, Zhu R, Jin H, Zhao K. Differential expression and role of miR-200 family in multiple tumors. *Anal Biochem*. 2021;626:114243.
 35. Zou H, Luo J, Guo Y, Liu Y, Wang Y, Deng L, *et al*. RNA-binding protein complex LIN28/MSI2 enhances cancer stem cell-like properties by modulating Hippo-YAP1 signaling and independently of Let-7. *Oncogene*. 2022;41(11):1657-72.
 36. Wang HY, Long QY, Tang SB, Xiao Q, Gao C, Zhao QY, *et al*. Histone demethylase KDM3A is required for enhancer activation of hippo target genes in colorectal cancer. *Nucleic Acids Res*. 2019;47(5):2349-64.
 37. Tranchant R, Quetel L, Montagne F, De Wolf J, Meiller C, De Koning L, *et al*. Assessment of signaling pathway inhibitors and identification of predictive biomarkers in malignant pleural mesothelioma. *Lung Cancer*. 2018;126:15-24.
 38. Chang L, Azzolin L, Di Biagio D, Zanconato F, Battilana G, Lucon Xiccato R, *et al*. The SWI/SNF complex is a mechanoregulated inhibitor of YAP and TAZ. *Nature*. 2018;563(7730):265-9.
 39. Mo C, You W, Rao Y, Lin Z, Wang S, He T, *et al*. Epigenetic regulation of DNA repair gene program by Hippo/YAP1-TET1 axis mediates sorafenib resistance in HCC. *Cell Mol Life Sci*. 2024;81(1):1-7.
 40. Sorrentino G, Ruggeri N, Specchia V, Cordenonsi M, Mano M, Dupont S, *et al*. Metabolic control of YAP and TAZ by the mevalonate pathway. *Nat Cell Biol*. 2014;16(4):357-66.
 41. Liu L, Liu M, Xie D, Liu X, Yan H. Role of the extracellular matrix and YAP/TAZ in cell reprogramming. *Differentiation*. 2021;122:1-6.
 42. Shao Q, Xu J, Deng R, Wei W, Zhou B, Yue C, *et al*. The expressions of YAP1, β -catenin and survivin in colon cancer tissues and their clinical significance. *Int J Clin Exp Pathol*. 2018;11(12):6032-8.
 43. Seo Y, Park SY, Kim HS, Nam JS. The hippo-yap signaling as guardian in the pool of intestinal stem cells. *Biomedicines*. 2020;8(12):560.
 44. Mouillet-Richard S, Laurent-Puig P. YAP/TAZ signalling in colorectal cancer: Lessons from consensus molecular subtypes. *Cancers (Basel)*. 2020;12(11):3160.
 45. Mia MM, Singh MK. New insights into Hippo/YAP signaling in fibrotic diseases. *Cells*. 2022;11(13):2065.
 46. Park I, Lee Y, Kim JH, Bae SJ, Ahn SG, Jeong J, *et al*. YAP1 expression in HR+ HER2- breast cancer: 21-gene recurrence score analysis and public dataset validation. *Cancers (Basel)*. 2023;15(20):5034.
 47. Yuan M, Tomlinson V, Lara R, Holliday D, Chelala C, Harada T, *et al*. Yes-associated protein (YAP) functions as a tumor suppressor in breast. *Cell Death Differ*. 2008;15(11):1752-9.
 48. Bertini E, Oka T, Sudol M, Strano S, Blandino G. YAP: at the crossroad between transformation and tumor suppression. *Cell Cycle*. 2009;8(1):49-57.
 49. Ghantous Y, Schussel JL, Brait M. Tobacco and alcohol-

- induced epigenetic changes in oral carcinoma. *Curr Opin Oncol.* 2018;30(3):152-8.
50. Segrelles C, Paramio JM, Lorz C. The transcriptional co-activator YAP: a new player in head and neck cancer. *Oral Oncol.* 2018;86:25-32.
 51. Santos-de-Frutos K, Segrelles C, Lorz C. Hippo pathway and YAP signaling alterations in squamous cancer of the head and neck. *J Clin Med.* 2019;8(12):2131.
 52. Li SY, Hu JA, Wang HM. Expression of Yes-associated protein 1 gene and protein in oral squamous cell carcinoma. *Chinese Med J.* 2013;126(4):655-8.
 53. Hiemer SE, Zhang L, Kartha VK, Packer TS, Almershed M, Noonan V, *et al.* A YAP/TAZ-regulated molecular signature is associated with oral squamous cell carcinoma. *Mol Cancer Res.* 2015;13(6):957-68.
 54. Hasegawa K, Fujii S, Matsumoto S, Tajiri Y, Kikuchi A, Kiyoshima T. YAP signaling induces PIEZO1 to promote oral squamous cell carcinoma cell proliferation. *J Pathol.* 2021;253(1):80-93.
 55. Ge L, Smail M, Meng W, Shyr Y, Ye F, Fan KH, *et al.* Yes-associated protein expression in head and neck squamous cell carcinoma nodal metastasis. *PLoS One.* 2011;6(11):e27529.
 56. Qi L, Shi C, Li J, Xu S, Han Y, Li J, *et al.* Yes-associated protein promotes cell migration via activating Wiskott-Aldrich syndrome protein family member 1 in oral squamous cell carcinoma. *J Oral Pathol Med.* 2019;48(4):290-8.
 57. Szelachowska J, Donizy P, Ratajczak Wielgomas K, Halon A, Zielecka Debska D, Lichon K, *et al.* The effect of YAP expression in tumor cells and tumor stroma on the prognosis of patients with squamous cell carcinoma of the oral cavity floor and oral surface of the tongue. *Oncol Lett.* 2019;18(4):3561-70.
 58. Ono S, Nakano K, Takabatake K, Kawai H, Nagatsuka H. Immunohistochemistry of YAP and dNp63 and survival analysis of patients bearing precancerous lesion and oral squamous cell carcinoma. *Int J Med Sci.* 2019;16(5):766.
 59. Zhang L, Ye DX, Pan HY, Wei KJ, Wang LZ, Wang XD, *et al.* Yes-associated protein promotes cell proliferation by activating Fos related activator-1 in oral squamous cell carcinoma. *Oral Oncol.* 2011;47(8):693-7.
 60. Omori H, Nishio M, Masuda M, Miyachi Y, Ueda F, Nakano T, *et al.* YAP1 is a potent driver of the onset and progression of oral squamous cell carcinoma. *Sci Adv.* 2020;6(12):eaay3324.
 61. Cunningham R, Hansen CG. The hippo pathway in cancer: YAP/TAZ and TEAD as therapeutic targets in cancer. *Clin Sci.* 2022;136(3):197-222.
 62. Tang Z, Ma Q, Wang L, Liu C, Gao H, Yang Z, *et al.* A brief review: some compounds targeting YAP against malignancies. *Future Oncol.* 2019;15(13):1535-43.
 63. Ardestani A, Maedler K. The hippo signaling pathway in pancreatic β -cells: Functions and regulations. *Endocr Rev.* 2018;39(1):21-35.



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Osteoconductivity and Mineralization of Different Commercial Bone Substitute Materials and Newly Hybrid Bone Substitute Material Between Xenograft and Alloplastic Material: An *In-vitro* Comparative Study on the Human Osteoblast Cell Line

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Abstract

Objectives: To investigate the effect of different bone graft substitutes on osteoconduction and mineralization in bone cells derived from the osteoblast cell line hFOB 1.19.

Methods: Osteoblast cells were cultured and placed on different bone graft materials, including Bio-Oss (xenograft), M bone (alloplast), Osteon II (alloplast), HXT1, and HXT2 (hybrid between xenograft and TCP). The concentration of elements in bone grafts was analyzed by X-ray fluorescence (XRF). The vitality test was evaluated by the methyl thiazolyl tetrazolium assay (MTT) after 1, 3, and 7 days. Alkaline phosphatase (ALP) activity was measured at 3, 7, and 14 days. Alizarin red S staining assay was performed at 7, 14, 21, and 28 days. The data were analyzed using ANOVA along with Tukey's honestly significant difference test.

Results: The cell viability rate was significantly higher in Osteon II and HXT2 compared to the other materials ($p < 0.001$). On day 14, Osteon II and the HXT2 group had higher levels of ALP activity than the Bio-Oss group ($p < 0.05$). Alizarin red assay showed that Osteon II had the highest mineralization ($p < 0.001$) at days 14, 21, and 28, followed by HXT2 and Bio-Oss respectively.

Conclusions: Osteon II, an alloplastic bone graft, and HXT2, a newly developed hybrid between xenograft and TCP, exhibited high viability rates and expression levels in mineralized tissue cells of the osteoblast cell line hFOB 1.19 *in vitro*.

Keywords: bone graft, bone tissue engineering, hybrid bone graft, osteoblast cells, xenograft

Introduction

Bone volume lost following tooth extraction is a problem for dental implant treatment. About two-thirds of the bone loss may occur within the initial three months post-extraction.⁽¹⁾ Several procedures have been developed to engineer lost bone, including guided bone regeneration (GBR), which involves filling bone defects with graft material to reconstruct alveolar bone and minimize future loss.⁽²⁾

Autograft is the standardized choice for a bone graft since it can possess all three requirements, which are osteogenesis, osteoinduction, and osteoconduction, for bone regeneration.⁽³⁾ However, the autogenous bone has numerous benefits, its limitation is the quantity to collect for large defect and the fastest resorption of all bone grafting materials.

Xenograft is bone tissue collected from various species of the host. The use of xenograft is a material of choice for dental implant treatment. Deproteinized and defatted bone (Kiel bone or Oswestry bone) has a lower immunological response, but it loses its osteoinductive properties.⁽⁴⁾ Bio-Oss® (Geistlich, Wolhusen, Switzerland) is the leading commercial deproteinized bovine bone substitute for regenerative bone dentistry worldwide. Bio-Oss® can be integrated and then replaced by new bone formation in dogs after implantation for 4 months. It fulfills the criteria of an osteoconductive material.⁽⁵⁾

Allograft is tissue collected from one individual and grafted into another individual of the same species. Allografts do not provide osteogenic characteristics without live cells. The extent of osteoinductive and osteoconductive properties, and the methods of graft processing, affect the mechanical strength of allograft. Despite the benefits of allografts, there have been reports of HIV and hepatitis C virus (HCV) infections, as well as the risk of bacterial microbiome contamination and unknown viral infections.^(6,7)

Alloplastic bone grafts are designed to replicate the natural properties of bones. Common synthetic materials used in orthopedic and dental treatments include metals (e.g. nickel-titanium), calcium phosphate ceramics (e.g. hydroxyapatite and tricalcium phosphate), and decellularized bone matrix, polymers (e.g. polymethylmethacrylate).⁽⁸⁾ Biphasic calcium phosphate (BCP) composed material of beta-tricalcium phosphate (β -TCP) and hydroxyapatite (HA). Altering the composition (HA/

β -TCP ratio) and/or crystallinity of BCP bioceramics can affect their bioreactivity. BCP bioceramics are currently approved for usage in orthopedic and dental applications as a substitute or supplement with autogenous bone.⁽⁹⁾ The HA/TCP ratio appeared to be inversely linked to the quantity of bone formation and filler material degradation. A higher ratio of HA results in a lower resorption rate. Consequently, the resorption rates of autogenous bone and BCP 20/80 were high, while those of BCP 80/20 and BCP 60/40 were low.⁽¹⁰⁾

Hybrid bone graft is a combination of different types of bone grafts, with aims to enhance and improve specific properties derived from each type of bone graft. At present, there is still a limited prevalence of hybrid bone grafts, and there is a scarcity of studies in this area.⁽¹¹⁾

The activity of alkaline phosphatase was especially important as it played a critical role in initiating the mineralization process of bone development.⁽¹²⁾ To determine the degree of mineralization in cell cultures, the specimens underwent staining with Alizarin red S (ARS), which is a well-established technique used to assess the presence of calcium-rich deposits within cultured cells for decades.^(13,14)

The objective was to assess and contrast the osteoconductivity and mineralization of alloplastic and hybrid bone graft materials (Xenograft sintering and coating with- β TCP) in comparison with a commercially available xenograft material, specifically investigating the mineralization process using a human fetal osteoblast cell line (hFOB1.19) model.

Materials and Methods

Materials

Bio-Oss® (Geistlich Pharma, Wolhusen, Switzerland) was a xenograft material used as a positive control group. M-bone (National Science and Technology Development Agency: NSTDA, Pathum Thani, Thailand) and Osteon II (GENOSS Co., Suwon, Korea) were commercially alloplastic bone graft materials with a ratio of HA/ β -TCP of 30/70. Prototype hybrid xenograft TCP 1000 (HXT1) (OSS HYDROXY COMPANY LIMITED, Nonthaburi, Thailand) and prototype hybrid xenograft TCP 1200 (HXT2) (OSS HYDROXY COMPANY LIMITED, Nonthaburi, Thailand) were hybrid materials, in which xenografts (bovine bone) were coated with

β -TCP at sintering temperatures of 1000°C and 1200°C, respectively.

Osteoblast cells hFOB 1.19 were obtained from ATCC (American Type Culture Collection, Manassas, VA). The complete media for this cell line consisted of a 1:1 combination of Ham's F12 Medium and Dulbecco's Modified Eagle's Medium with 2.5 mM L-glutamine (without phenol red). The following ingredients were added to the base medium to create the complete growth medium: 0.3 mg/ml G418; fetal bovine serum to a final concentration of 10%. Cultures were incubated at 37°C with 95% air and 5% CO₂ in T-75 flasks, and the culture medium was changed every 3 days. Mineralization was induced with osteogenic media (OstM) containing 10 mM β -glycerophosphate, 50 μ g/ml L-ascorbate, and 10⁻⁷ M dexamethasone. Cultures were incubated at 37°C with 95% air and 5% CO₂, and the culture medium was changed at regular intervals, specifically every three days, to ensure optimal growth conditions for the cells.⁽¹⁵⁾

The osteoblast cell line utilized in this study is hFOB 1.19, which is derived from human fetal tissue. This cell line has been established as a valuable experimental model for investigating osteoblast biology, particularly in the context of drug development and biomaterial engineering. The choice of hFOB 1.19 is advantageous due to its human origin and its ability to provide a homogeneous, rapidly proliferating system for studying osteoblast differentiation and physiology.

Methods

X-ray fluorescence (XRF)

Each sample will be mounted on carbon tape and then examined with a Micro-XRF spectrometer (M4 TORNADO PLUS) to investigate the concentration of elements present in the samples. The scan was conducted with a pixel time of 20 ms/pixel, and a total acquisition time of 9 minutes. The pixel size was set to 40 μ m, and the X-ray tube operated at 50 kV. The detection limit of the Bruker M4 TORNADO PLUS micro-XRF system typically ranges from 1 to 100 ppm (parts per million).

In vitro biological studies

Cell viability by MTT assay

Cell viability was evaluated using the MTT assay, which involves the reduction of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide by living

cells.

To ensure accuracy, all experiments were performed in triplicate. Eight sequential trials were conducted to optimize cell density and graft quantity. Initially, 10,000 cells/well in 96-well plates allowed clear formazan visualization (Experiment 1). For 24-well plates, 20,000 cells/well was optimal by day 7 (Experiments 2-3). However, 100 mg of graft caused significant cell death (Experiment 4). Due to limited graft availability, M-bone from Chiang Mai University was used (Experiment 5), and 50 mg was found suitable in 24-well plates (Experiment 6). Pre-soaking grafts for 24 h improved early viability (Experiment 7), but was excluded for being unrepresentative. The final condition used 7.5×10^4 cells/well with 50 mg of non-pre-soaked graft (Experiment 8).

The hFOB1.19 cells were seeded at a density of 7.5×10^4 cells per well into a 24-well plate (Greiner bio-one, Germany). Cells were treated with 50 mg of various bone grafts as described above for 1, 3, and 7 days, and the osteogenic medium was changed every 3 days. Cultures were incubated at 37°C with 95% air and 5% CO₂. Each cell-containing well was added with 250 μ l MTT solution (5 mg/ml MTT dissolved in PBS: Phosphate buffer saline) and 250 μ l DMEM F-12. The plate was incubated in a CO₂ incubator at 37°C for 3 hours. The medium was then removed, and formazan crystals were dissolved in 1,000 μ l of DMSO and absolute ethanol (ratio 1:1). After incubation for 10 minutes, absorbance was read in a 96-well plate at a wavelength of 540 nm with a reference wavelength of 690 nm. The data were recorded using a microplate reader (TECAN Sunrise™, Austria). Equation (1) was used to calculate cell viability percentage. No material is the group that is 100% comparable in each experimental group and each day in MTT result.

$$\text{Cell viability} = \frac{\text{OD}_s}{\text{OD}_n} \times 100 \quad (1)$$

where ODs = sample optical density and ODn = No bone graft sample optical density

Bio-Oss was selected as the positive control group in this study due to its widespread use, established reputation, and strong support from numerous previous research studies.

Alkaline phosphatase activity

Each bone graft material (50 mg) was added to 24-

well plate (Greiner bio-one, Germany). Approximately 7.5×10^4 cells of hFOB1.19 were added to each well with bone grafts for 3, 7, and 14 days with 2 ml of osteogenic media. The osteogenic media were collected on days 3, 7, and 14 of the experiments to measure ALP activity using an ALP detection kit and fluorescence (Sigma-Aldrich, USA). Briefly, 20 μ l of each sample was added to a 96-well plate (duplicates from each well) and incubated at 65°C for 30 minutes, then cooled down to room temperature with ice. Twenty μ l of dilution buffer, 160 μ l of fluorescent assay buffer, and 4 μ l of the diluted substrate were added to each well. The plate was mixed and incubated in a dark room at room temperature for 15 minutes. Finally, the fluorescence was read by a Spark multimode microplate reader (Tecan, Switzerland) set to 360 nm excitation and 440 nm emission.

Alizarin red-s staining: microscopy and assay

The hFOB1.19 cells were seeded at a density of 7.5×10^4 cells per well in a 24-well plate (Greiner bio-one, Germany). Cells were treated with 50 mg of various bone grafts, as described above, for 7, 14, 21, and 28 days, and the osteogenic medium was changed every 3 days. Nodule formation was observed on days 7, 14, 21, and 28 of the experiments by Alizarin red-S staining. Briefly, the medium from the wells was aspirated, and the cells were rinsed twice with 500 μ l of PBS. The cell cultures were fixed for 15 minutes with a 4% paraformaldehyde solution in PBS. The solution was removed, and the cells were rinsed three times with PBS. The cells were subjected to staining with a 40 mM Alizarin Red S solution in deionized water, adjusted to a pH of 4.2. This process was carried out under controlled conditions: at room temperature, in darkness, and for a period of 45 minutes. After staining, the Alizarin Red S solution was aspirated, and the cells were rinsed ten times with deionized water. The removal of water was done by aspiration, and the cells were incubated in PBS for 15 minutes at room temperature. The PBS was aspirated from the wells, and the cells were gently washed with a fresh solution of PBS. After that, they underwent four rinses with deionized water and were observed using an inverted light microscope (Olympus BX41, Japan).^(13,15)

The cells were destained for 15 minutes with 10% (w/v) cetylpyridinium chloride (CPC) in 10 mM sodium phosphate (pH 7.0). The collected stain medium was added to a 96-well plate, and a SpectraMax 340 plate reader/spectrophotometer (Molecular Devices Corp.) was used

to measure the absorbance at 562 nm.^(13,16)

Statistical analysis

Normality tests were initially conducted on the datasets. The normally distributed data were summarized as mean $\pm$ standard deviation. Statistical analysis involved a two-way ANOVA to evaluate the effects of the variables and their interactions. Tukey's test was applied post-hoc to compare the means of different groups. The analysis was performed using SPSS version 25 software (IBM, USA), with a significance level of $p < 0.05$ used to determine statistically significant differences.

Results

X-ray fluorescence (XRF)

XRF analysis was conducted to study the chemical composition of the materials. Bone morphologies of each bone graft were depicted in Figure 1A. The individual element content is listed in Table 1 and was also graphically presented in Figure 1B. Elemental mapping and spectrum analysis was shown in Figure 2. The XRF analysis indicated that each bone graft had a similar ratio of oxygen, calcium, and phosphorus. Calcium concentrations follow the order: Osteon II > HXT2 > Bio-Oss > HXT1 > M-bone.

In vitro biological studies

Cell viability by MTT assay

Cell viability of hFOB 1.19 cells on bone graft materials, measured by the MTT assay, was presented in Figure 3. There were significant differences between the cell-only group and the tested bone graft materials ($p < 0.001$). Each tested material exhibited a significantly decreased percentage of viability compared to the no-bone-added group on the first and third days. Osteon II and HXT2 had significantly higher levels of MTT than the other tested materials ($p < 0.001$). On day 7, Osteon II had the highest level of MTT (104%) among the tested materials ($p < 0.001$), followed by HXT2, Bio-Oss, HXT1, and M-bone group with the lowest level (22%).

Alkaline phosphatase activity

Alkaline Phosphatase (ALP) was involved in osteogenic differentiation, playing a significant role in initiating the mineralization process and forming the Extracellular Matrix (ECM). The study examined the effect of different bone graft materials on ALP release in hFOB1.19 cells by

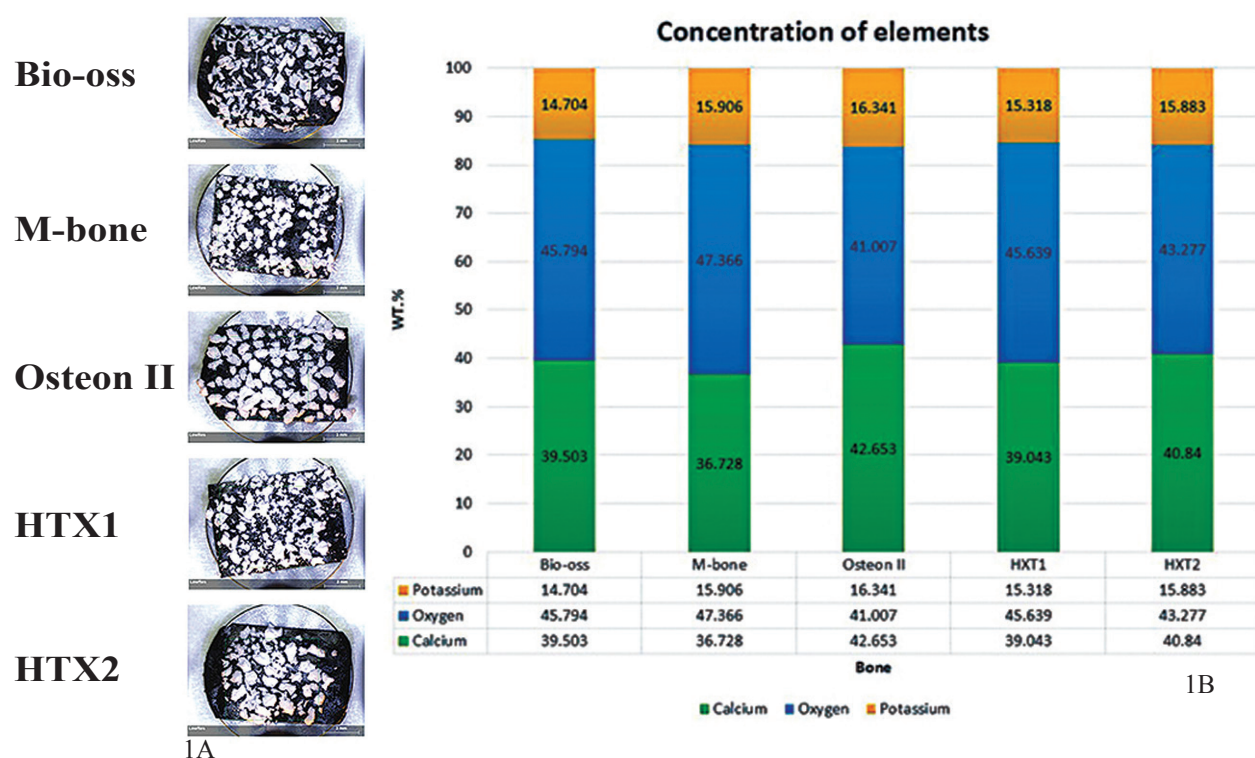


Figure 1: Bone morphologies of each bone graft are depicted in Figure 1A. The individual element content is presented in Figure 1B with a similar ratio of oxygen, calcium, and phosphorus. Calcium concentrations follow the order: Osteon II > HXT2 > Bio-Oss > HXT1 > M-bone.

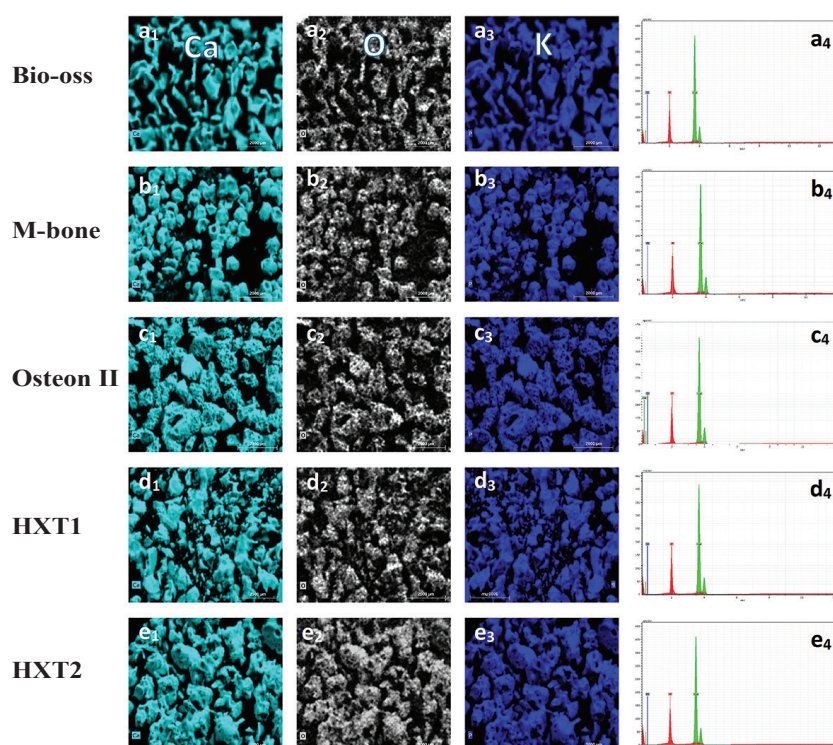


Figure 2: Elemental mapping analysis showed Ca element (a1-e1), O element (a2-e2), K element (a3-e3), The intensity of elements contents in the corresponding samples are shown in graphs a4, b4, c4, d4, e4 respectively.

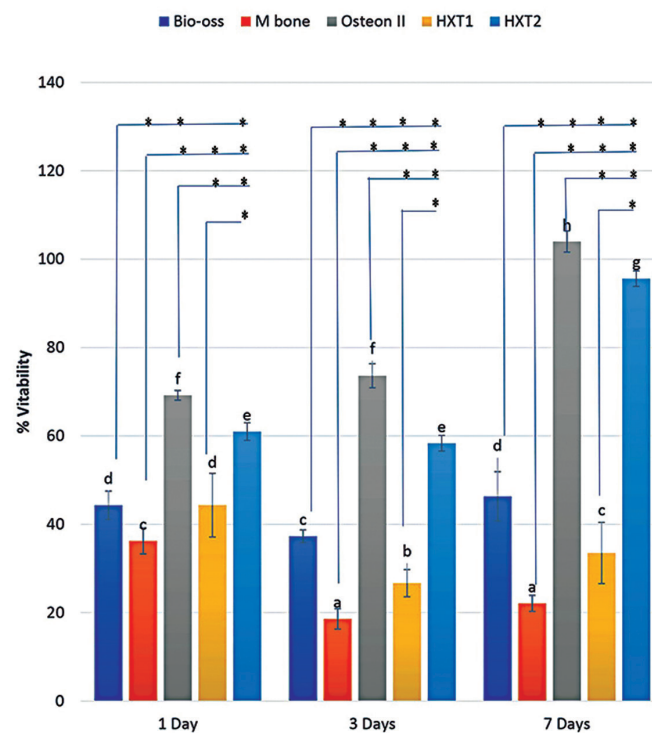


Figure 3: Cell viability measured by MTT assay result after 1, 3, and 7 days of culture on different bone substitutes. Data are reported as mean of % viability $\pm$ SD (n=15 per group). With the same letter indicating that there is no significant difference between the groups ($p < 0.05$), * $p < 0.001$. Osteon II and HXT2 had higher level of MTT than control group (Bio-Oss) at significantly 95%.

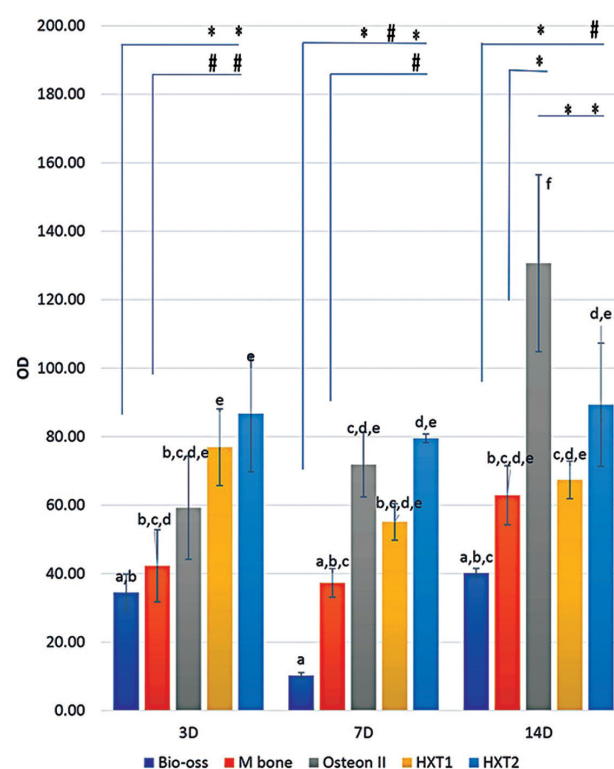


Figure 4: Quantification of ALP activities in response to each group at day 3, 7, and 14 shown in units/mg protein (Data are expressed as Mean $\pm$ SD, n=4). With the same letter indicating that there is no significant difference between the groups ($p < 0.05$) and * $p < 0.001$.

measuring ALP activity in the culture media.

It was found that ALP activity in every material, except for Osteon II, was slightly lower on day 7, with the highest level observed on day 14 (Figure 4). Each day of the treated group showed no significant difference in ALP activity, except for Osteon II, which significantly higher ALP activity than other tested materials was found on day 14 ($p<0.001$) (Figure 4). On day 3, Osteon II and HXT2 groups had significantly higher levels of ALP activity than Bio-Oss ($p<0.001$). On day 7, Osteon II, HXT1, and HXT2 groups had significantly ($p<0.05$) higher levels of ALP activity than the Bio-Oss group. On day 14, Osteon II and the HXT2 group had higher levels of ALP activity than the Bio-Oss group ($p<0.05$), with the highest level of ALP in Osteon II, followed by the HXT2 group.

Study on mineralization by alizarin red-s staining: microscopy and assay

Alizarin red is a marker for mineralization. When colored circle around the cells is found, means that the bone-forming cells (osteoblasts) are creating a mineralized structure. The different materials bone graft can found Alizarin red circle. The qualitative measurement of calcium deposition was examined by histochemical staining. On days 14, 21, and 28, cells were stained with Alizarin red-S, and nodule formation was investigated using an inverted light microscope (Figure 5). Nodule formation was observed on days 14, 21, and 28 in all conditions. However, cells cultured without adding any bone graft to osteogenic media indicated a clearly lower level of nodule formation. The results confirmed the importance of scaffolds in the process of mineralization.

The quantification of Alizarin Red S-stained particles was performed using the CPC extraction method, and the absorbance of the resulting solution was assessed at a wavelength of 562 nm. Every group showed increasing in the results day by day (Figure 6). On day 7, HXT1 showed significantly higher mineralization over other materials ($p<0.001$) (Figure 6). Osteon II showed significantly higher mineralization ($p<0.001$) on days 14, 21, and 28, while HXT2 and Bio-Oss were in between. M-bone had significantly lower mineralization than other tested groups on days 21 and 28. On day 28, the Osteon II group had the highest mineralization ($p<0.001$), and HXT2, Bio-Oss, HXT1, and M-bone decreased in order ($p<0.001$).

Discussion

For bone regenerating operations, xenograft bone substitutes have been used extensively. Bio-Oss® (Geistlich, Wolhusen, Switzerland) is the leading commercial bone substitute for regenerative bone dentistry worldwide. Alloplastic bone substitutes are employed due to their synthetic nature, comprising vital chemical elements found in natural bone, such as calcium and phosphate, recognized for their ability to stimulate bone regeneration.⁽¹⁷⁾ Alloplastic bone substitutes offer advantages, including product quality and a reduced risk of infectious diseases, in contrast to allogeneic and xenogenic bone grafts.^(6,7) However, the processing method employed for bone grafts can influence the outcomes in relation to osteoblast cells. The bone regeneration of hFOB 1.19 osteoblast cells on five bone graft substitutes was compared, and cell viability activity was studied by the MTT

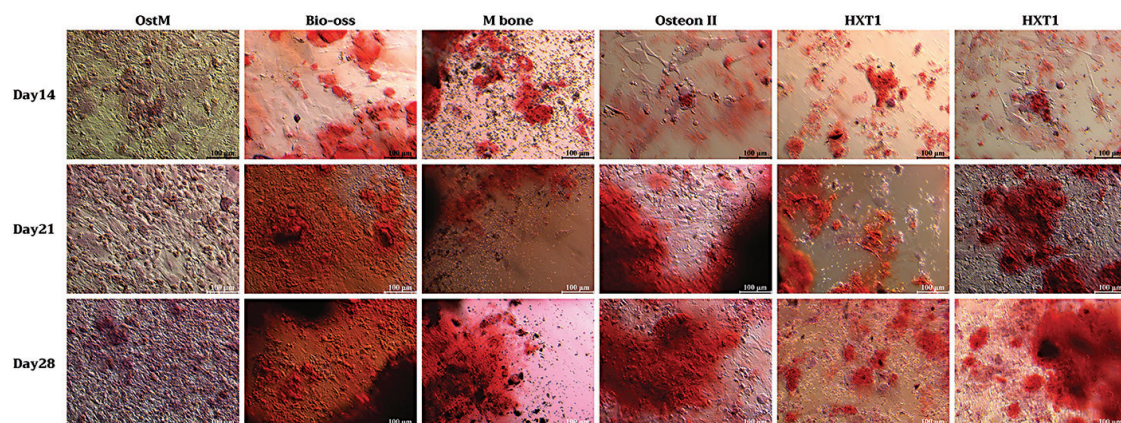


Figure 5: Alizarin red-S staining revealed the calcium nodule under the inverted light microscope.

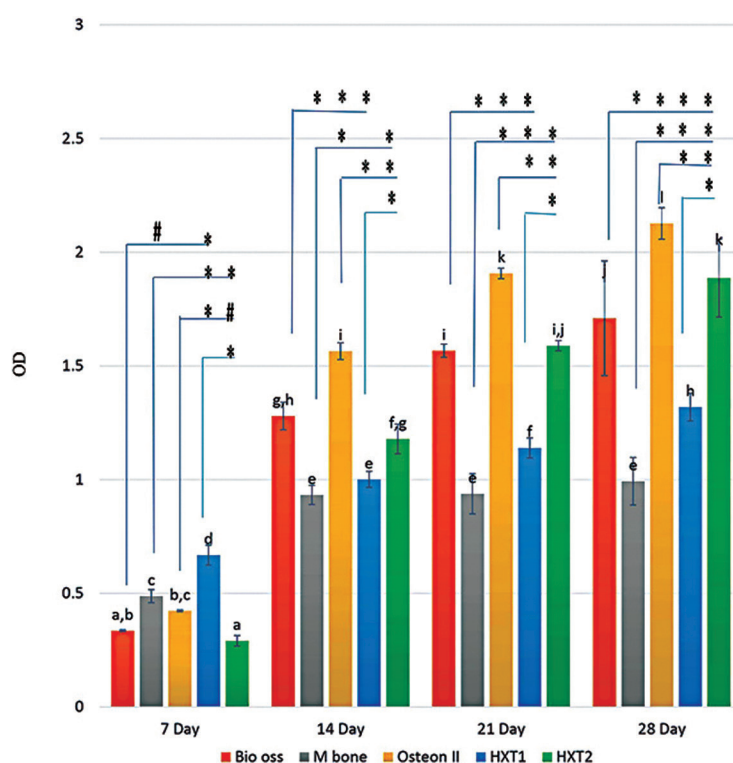


Figure 6: Alizarin Red S-stained particles were quantified by the CPC extraction method, with the absorbance of the extracted solution measured at 562 nm. (n=12, With the same letter indicating that there is no significant difference between the groups $p < 0.05$, # $p < 0.05$, * $p < 0.001$.)

assay at 1, 3, and 7 days. The ALP activity was studied at 3, 7, and 14 days. Alizarin red staining was studied at 7, 14, 24, and 28 days.

The MTT assay findings showed a statistically significant interaction between time and group variables, influencing cell viability. The p -value of less than 0.001 underscored the strong statistical significance of this interaction, suggesting that both time and group factors played crucial roles in determining cell viability. The cell viability at day 7 was in the following order: Osteon II > HXT2 > Bio-Oss > HXT1 > M-bone. The difference among the Bio-Oss group at 1, 3, and 7 days was statistically significant ($p < 0.001$). Nader *et al.*,⁽¹⁸⁾ showed that Bio-Oss had the lowest MTT among other xenograft and alloplastic bone grafts. Kübler *et al.*,⁽¹⁹⁾ reported the same result when comparing Bio-Oss with another bone graft. The results show that Osteon II and HXT2 have significantly ($p < 0.001$) higher viability than the other groups in every period of the experiment. In contrast to our results, Nader *et al.* found that Bio-Oss and Osteon material had no difference in cell viability on SaOS-2.⁽¹⁸⁾ Cell viability was not affected by the type of bone graft, even if the

same type as HXT1 and HXT2 had a different viability rate. HXT2 exhibited a higher vitality rate compared to HXT1, indicating that the sintering process at 1200°C enhanced its compatibility more effectively than the 1000°C sintering conditions. Furthermore, HXT2 demonstrated a vitality rate surpassing that of Bio-Oss but falling short of Osteon II. Notably, HXT2 combines the properties of xenograft and biphasic calcium phosphate (BCP) materials, offering a unique mixed of characteristics that may be advantageous in specific applications. Alcaide *et al.*,⁽²⁰⁾ proceed a study involving the cultivation of SaOs-2 cells on HA/β-TCP discs, a good biocompatibility and a high percentage of viable cells on these discs. Saldana *et al.*,⁽²¹⁾ reported that the viability of human mesenchymal cells on BCP remained stable over a four-day period. The higher performance of Osteon II compared to Bio-Oss may be attributed to the higher solubility of the β-TCP compound, potentially facilitating subsequent bone growth within the hydroxyapatite particles. Nevertheless, our findings revealed that cells cultured on M-bone, a BCP, exhibited significantly lower cell viability compared to other groups. Cell growth and proliferation are influ-

enced not only by the graft's chemical composition but besides by surface roughness and porosity. The porosity surface and pore sizes can impact cell growth and morphology.⁽²²⁾ Another factor could be the effect of these materials on the medium's pH, as the release of phosphate ions might affect cell growth too.⁽²³⁾

Similar outcomes were reported by Schmitt *et al.*, after cultivating osteoblast-like cells from bovines onto several bone substitute materials. The authors observed the non-adding bone substitution had the highest proliferation. Bone substitute materials have been shown to support cell differentiation effectively. However, they often exhibit a reduced rate of cell proliferation. Specifically, Bio-Oss was found to have the lowest proliferation rate among the materials examined in this study.

Alkaline phosphatase is highly expressed in mineralized tissue cells and plays a crucial role in the creation of hard tissue.⁽²⁴⁾ Our study showed that Osteon II had the highest ALP activity at 14 days. Nader *et al.*, observed that Osteon material, followed by Bio-Oss, had the higher ALP activity.⁽¹⁸⁾

Even though we measured each type of bone graft equally, they have different densities. Miron, Richard *et al.*,⁽²⁵⁾ demonstrate how bone graft seeding density has a significant impact on *in vitro* testing of bone-grafting materials. Osteoblast adhesion and cell proliferation were lower when cells were seeded on high-density bone graft when compared to low-density bone graft. In contrast, alkaline phosphatase (ALP) and alizarin red staining were significantly increased on high-density bone graft. Bone graft materials can influence cell differentiation and bone formation based on their chemical characteristics and how they affect the concentration of Calcium ions. The absence of calcium deficiency in BCP's composition, coupled with its lower textural properties, restricted excessive calcium ion uptake. This balanced calcium environment allowed osteoblasts to develop a functional phenotype and exhibit enhanced alkaline phosphatase activity.⁽²¹⁾ XRF showed that the concentration of calcium followed this order: Osteon II > HXT2 > Bio-Oss > HXT1 > M-bone, and this result is the same order as cell viability, ALP assay at 7 days, and ARS assay at 21 and 28 days. Even if Osteon II has the highest Calcium element, at 7 days of ARS assay, they didn't have the highest level of Calcium deposition. This result showed that osteoblast cells differently deposited calcium elements in each bone graft.

Calcium deposition was significantly higher in Osteon II compared to other groups, even if M-bone was the same alloplastic bone graft material that had a ratio of HA/ β -TCP is 30/70. ARS assay shows Osteon II had the highest calcium deposited at 14, 21, and 28 days. HXT 2 had the second one, followed by Bio-Oss. The top three highest are different types of bones.

In a rabbit study, small bone particles size 0.25-1 mm were found to be more effective in promoting osteogenesis than larger bone graft materials measuring 1.0-2.0 mm.^(26,27) In contrast, a study conducted in dogs reported that β -tricalcium phosphate (β -TCP) particles sized 1-2 mm, in combination with recombinant human platelet-derived growth factor-BB, resulted in significantly greater new bone and cementum formation than smaller particles (0.25-1 mm).⁽²⁸⁾ Similarly, in augmentation procedures utilizing various grafting materials including anorganic bovine bone, allografts, biphasic β -TCP with hydroxyapatite (alloplasts), and demineralized bovine bone mineral grafts with larger particle sizes (1-2 mm) yielded superior outcomes compared to those with smaller particle sizes (0.25-1 mm).⁽²⁹⁻³¹⁾ Furthermore, Riachi *et al.*,⁽³²⁾ In the investigation of sinus augmentation procedures for implant placement, it was found that the average particle size of Bio-Oss (1 mm) was considerably smaller than that of Cerabone (2.7 mm). Radiographic analysis revealed that Bio-Oss underwent significantly greater volumetric reduction over time compared to Cerabone.

The particle size ranges of the bone graft materials used in this study were as follows: Bio-Oss[®] (0.25-1 mm), M-Bone (0.5-1 mm), Osteon II (0.2-2.0 mm), HXT1 (0.2-1.5 mm), and HXT2 (0.5-2 mm). The results indicated that larger bone particles facilitated superior bone regeneration compared to smaller particles, which aligns with reported findings.

Including implant surface roughness data would significantly improve the completeness of this study, as surface topography critically affects osteoblast response and osseointegration. Previous research shows that moderate roughness (Ra around 1-2 μ m) enhances cell adhesion, proliferation, and differentiation better than either very smooth or very rough surfaces.^(33,34) Specifically, Le Guehennec *et al.*,⁽³³⁾ highlighted that rough surfaces improve protein adsorption and integrin-mediated cell attachment, which are essential for bone formation. One review noted that roughened titanium sur-

faces achieved higher osteogenic marker expression and improved bone–implant contact in both *in vitro* and *in vivo* settings.⁽³⁵⁾

Our observations, showing superior mineralization in HXT2 and Osteon II groups, align with known effects of material processing and composition. Studies on biphasic calcium phosphate (BCP) ceramics reveal that sintering temperature significantly alters microstructure and roughness. For instance, increasing the sintering temperature from 1000°C to 1300°C leads to grain growth and changes in porosity, which directly correlate with mechanical strength—an indirect indicator of surface topology.⁽³⁶⁾ Additionally, SEM analysis of HA/ β -TCP mixtures confirms that β -TCP-rich surfaces exhibit more pronounced roughness and porosity than HA-rich ones.⁽³⁷⁾ Given that HXT2 is sintered at a higher temperature and Osteon II has a biphasic structure, their favorable performance may stem from these roughened surfaces.

However, it's important to note that this *in vitro* study exclusively demonstrates the effects of different bone graft substitutes on osteoconduction and mineralization in osteoblast cell line, which differs from the conditions in the human living body. Furthermore, the decision-making process for selecting the appropriate bone material for bone grafting procedure requires comprehensive clinical studies. Moreover, the novel hybrid bone substitute material (HXT2) still lacks an *in vivo* study to validate its tissue biocompatibility and regenerative potential. Hence, the further clinical research is required to compared bone substitute material in terms of clinical outcomes, stability, and bone regenerative properties of the grafts.

Conclusions

Distinct bone graft materials have different impacts on the cell viability and mineralization of hFOB 1.19 osteoblasts *in vitro*. The results suggest that higher concentrations of calcium elements affect mineralization. Considering the present results, the osteoblast cell line hFOB 1.19 in the presence of the tested scaffolds demonstrated that alloplastic material (Osteon II) and newly hybrid xenograft with β -TCP (HXT2) significantly promote mineralization in osteoblast cells *in vitro*.

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Declarations

Ethics approval and consent to participate

This research has been approved by Chiang Mai University Institutional Biosafety Committee (CMUIBC A-0566006) as following guidelines for safety in the production and possession of pathogens and animal toxins used in research studies and correspond to the level of risk of disease and danger.

Conflicts of Interest

The authors of this study affirm that they have no competing interests or financial ties that could potentially influence the results or interpretation of the findings

References

1. Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. *Int J Periodontics Restorative Dent*. 2003;23(4):313-23.
2. Cypher TJ, Grossman JP. Biological principles of bone graft healing. *J Foot Ankle Surg*. 1996;35(5):413-7.
3. Miron RJ, Sculean A, Shuang Y, Bosshardt DD, Gruber R, Buser D, *et al*. Osteoinductive potential of a novel biphasic calcium phosphate bone graft in comparison with autographs, xenografts, and DFDDBA. *Clin Oral Implants Res*. 2016;27(6):668-75.
4. Plenck H, Hollmann K, Wilfert KH. Experimental bridging of osseous defects in rats by the implantation of Kiel bone containing fresh autologous marrow. *J Bone Joint Surg Br*. 1972;54(4):735-43.
5. Berglundh T, Lindhe J. Healing around implants placed in bone defects treated with Bio-Oss[®]. an experimental study in the dog. *Clin Oral implants Res*. 1997;8(2):117-24.
6. Bauer TW, Muschler GF. Bone graft materials: an overview of the basic science. *Clin Orthop Relat Res*. 2000;371:10-27.
7. Zimmermann G, Moghaddam A. Allograft bone matrix versus synthetic bone graft substitutes. *Injury*. 2011;42:S16-S21.
8. Salamanca E, Choy CS, Aung LM, Tsao TC, Wang PH,

- Lin WA, *et al.* 3D-printed PLA scaffold with fibronectin enhances *in vitro* osteogenesis. *Polymers*. 2023;15(12):2619.
9. LeGeros R, Lin S, Rohanizadeh R, Mijares D, LeGeros J. Biphasic calcium phosphate bioceramics: preparation, properties and applications. *J Mater Sci Mater Med*. 2003;14(3):201-9.
 10. Jensen SS, Bornstein MM, Dard M, Bosshardt DD, Buser D. Comparative study of biphasic calcium phosphates with different HA/TCP ratios in mandibular bone defects. A long-term histomorphometric study in minipigs. *J Biomed Mater Res B Appl Biomater*. 2009;90(1):171-81.
 11. Ferracini R, Bistolfi A, Garibaldi R, Furfaro V, Battista A, Perale G. Composite xenohybrid bovine bone-derived scaffold as bone substitute for the treatment of tibial plateau fractures. *Appl Sci*. 2019;9(13):2675.
 12. Gordon JA, Tye CE, Sampaio AV, Underhill TM, Hunter GK, Goldberg HA. Bone sialoprotein expression enhances osteoblast differentiation and matrix mineralization *in vitro*. *Bone*. 2007;41(3):462-73.
 13. Gregory CA, Gunn WG, Peister A, Prockop DJ. An alizarin red-based assay of mineralization by adherent cells in culture: comparison with cetylpyridinium chloride extraction. *Anal Biochem*. 2004;329(1):77-84.
 14. Puchtler H, Meloan SN, TERRY MS. On the history and mechanism of alizarin and alizarin red S stains for calcium. *J Histochem Cytochem*. 1969;17(2):110-24.
 15. Sila-Asna M, Bunyaratvej A, Maeda S, Kitaguchi H, Bunyaratavej N. Osteoblast differentiation and bone formation gene expression in strontium-inducing bone marrow mesenchymal stem cell. *Kobe J Med Sci*. 2007;53(1-2):25-35.
 16. Reinholz GG, Getz B, Pederson L, Sanders ES, Subramaniam M, Ingle JN, *et al.* Bisphosphonates directly regulate cell proliferation, differentiation, and gene expression in human osteoblasts. *Cancer Res*. 2000;60(21):6001-7.
 17. Haugen HJ, Lyngstadaas SP, Rossi F, Perale G. Bone grafts: which is the ideal biomaterial?. *J Clin Periodontol*. 2019;46(Suppl 21):92-102.
 18. Ayobian-Markazi N, Fourtoun T, Kharazifar M. Comparison of cell viability and morphology of a human osteoblast-like cell line (SaOS-2) seeded on various bone substitute materials: an *in vitro* study. *Dent Res J (Iafahan)*. 2012;9(1):86-92.
 19. Kübler A, Neugebauer J, Oh J-H, Scheer M, Zöller JE. Growth and proliferation of human osteoblasts on different bone graft substitutes an *in vitro* study. *Implant Dent*. 2004;13(2):171-9.
 20. Alcaide M, Serrano MC, Pagani R, Sánchez-Salcedo S, Vallet-Regí M, Portolés M-T. Biocompatibility markers for the study of interactions between osteoblasts and composite biomaterials. *Biomaterials*. 2009;30(1):45-51.
 21. Saldana L, Sánchez-Salcedo S, Izquierdo-Barba I, Bensiamar F, Munuera L, Vallet-Regí M, *et al.* Calcium phosphate-based particles influence osteogenic maturation of human mesenchymal stem cells. *Acta Biomaterialia*. 2009;5(4):1294-305.
 22. Kaufmann EE, Ducheyne P, Shapiro I. Effect of varying physical properties of porous, surface modified bioactive glass 45S5 on osteoblast proliferation and maturation. *J Biomed Mater Res*. 2000;52(4):783-96.
 23. Knabe C, Driessens F, Planell J, Gildenhaar R, Berger G, Reif D, *et al.* Evaluation of calcium phosphates and experimental calcium phosphate bone cements using osteogenic cultures. *J Biomed Mater Res*. 2000;52(3):498-508.
 24. Golub EE, Boesze-Battaglia K. The role of alkaline phosphatase in mineralization. *Curr Opin Orthop*. 2007;18(5):444-8.
 25. Miron RJ, Caluseru OM, Guillemette V, Zhang Y, Buser D, Chandad F, *et al.* Effect of bone graft density on *in vitro* cell behavior with enamel matrix derivative. *Clin Oral Investig*. 2015;19:1643-51.
 26. Klüppel LE, Antonini F, Olate S, Nascimento FF, Albergaria-Barbosa JR, Mazzonetto R. Bone repair is influenced by different particle sizes of anorganic bovine bone matrix: a histologic and radiographic study *in vivo*. *J Craniofac Surg*. 2013;24(4):1074-7.
 27. Nam JW, Kim MY, Han SJ. Cranial bone regeneration according to different particle sizes and densities of demineralized dentin matrix in the rabbit model. *Maxillofac Plast Reconstr Sur*. 2016;38:1-9.
 28. Irokawa D, Ota M, Yamamoto S, Shibukawa Y, Yamada S. Effect of β tricalcium phosphate particle size on recombinant human platelet-derived growth factor-BB-induced regeneration of periodontal tissue in dog. *Dent Mater J*. 2010;29(6):721-30.
 29. Testori T, Wallace SS, Trisi P, Capelli M, Zuffetti F, Del Fabbro M. Effect of xenograft (ABBM) particle size on vital bone formation following maxillary sinus augmentation: a multicenter, randomized, controlled, clinical histomorphometric trial. *Int J Periodontics Restorative Dent*. 2013;33(4):467-75.
 30. Kheur MG, Kheur S, Lakha T, Jambhekar S, Le B, Jain V. Does graft particle type and size affect ridge dimensional changes after alveolar ridge split procedure?. *J Oral Maxillofac Surg*. 2018;76(4):761-9.
 31. Shokry AM, Gamal A, Sarhan S. The effect of xenogenic bone graft particle size on the quality of newly formed tissue in alveolar ridge preservation (clinical and histomorphometric study). *Adv Dent J*. 2023;5(2):368-83.
 32. Riachi F, Naaman N, Tabarani C, Aboelsaad N, Aboushelib MN, Berberi A, *et al.* Influence of material properties on rate of resorption of two bone graft materials after sinus lift using radiographic assessment. *Int J Dent*. 2012;2012(1):737262.
 33. Le Guéhennec L, Soueidan A, Layrolle P, Amouriq Y. Surface treatments of titanium dental implants for rapid osseointegration. *Dent Mater*. 2007;23(7):844-54.
 34. Buser D, Brogini N, Wieland M, Schenk R, Denzer A,

- Cochran D, *et al.* Enhanced bone apposition to a chemically modified SLA titanium surface. *J Dent Res.* 2004;83(7):529-33.
35. Rupp F, Liang L, Geis-Gerstorfer J, Scheideler L, Hüttig F. Surface characteristics of dental implants: a review. *Dent Mater.* 2018;34(1):40-57.
36. Yetmez M. Sintering behavior and mechanical properties of biphasic calcium phosphate ceramics. *Adv Mater Sci Eng.* 2014;2014(1):871749.
37. Xidaki D, Agraftioti P, Diomatari D, Kaminari A, Tsalavoutas-Psarras E, Alexiou P, *et al.* Synthesis of hydroxyapatite, β -tricalcium phosphate and biphasic calcium phosphate particles to act as local delivery carriers of curcumin: loading, release and *in vitro* studies. *Materials.* 2018;11(4):595.



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Unveiling Understanding of Periodontitis Among Thai Adults: A Cross-sectional Study

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Abstract

Objectives: This study aims to assess the knowledge depth regarding periodontitis among Thai adults using open-ended questions and explore the factors influencing their comprehension of the disease.

Methods: A cross-sectional questionnaire-based study was conducted involving Thai adults aged 18 years and above. Participants were asked to describe periodontitis in an open-ended question and were classified as "totally correct" (TC), "partially correct" (PC) or "incorrect" (IC) based on the American Academy of Periodontology's definition. Subgroup comparisons were performed using the Chi-square test.

Results: Out of the 1205 participants analyzed, the majority (72.4%) fell into the PC category, primarily associating periodontitis with gingival inflammation. Terms such as "gingival inflammation," "gingival swelling," and "gingival bleeding" were frequently mentioned. Participants mentioning keywords related to attachment loss were more likely to demonstrate a better comprehension of the disease. Significant associations were observed between participants' familiarity with periodontitis and factors such as age ($p < 0.05$), educational level ($p < 0.05$), and frequency of dental visits ($p = 0.001$).

Conclusions: This study highlights the superficial understanding of periodontitis within the Thai population, notably the limited awareness beyond gingival inflammation.

Keywords: attachment loss, gingival inflammation, knowledge, periodontitis, Thai population

Introduction

Periodontitis, a chronic disease affecting individuals across all regions of Thailand, stands out as a significant public health issue. The 9th National Health Survey (2566 BE)⁽¹⁾ highlighted the extensive impact of this condition, revealing that 51.8% of Thai adults aged 35 to 44 displayed signs of gingival inflammation. Remarkably, 23.4% of this group exhibited periodontitis with probing depths of 4 to 5 millimeters, while 9.2% had periodontal pockets exceeding 6 millimeters. Furthermore, the survey unveiled a concerning prevalence of periodontitis among the elderly, as 48.7% of those aged 60 to 74 were diagnosed with the condition.

Periodontitis is characterized by inflammation of the gingiva and loss of alveolar bone. Untreated periodontitis can result in tooth loss, affecting essential oral functions such as chewing, speech, and overall aesthetics. Despite its global impact⁽²⁻⁴⁾, awareness of periodontitis among the general population remains low^(5,6), possibly due to its gradual progression and a lack of understanding about the disease. Previous studies have shown that a significant proportion of the population remains uninformed about the causes, symptoms, and treatment of periodontitis, even after regular dental visits.^(7,8)

Patient awareness and understanding of periodontitis significantly impact their decision to seek treatment. It is crucial for dental professionals to evaluate patients' knowledge of periodontal diseases to design effective, targeted oral health education programs that cater to various demographic groups. Knowledge of oral health, especially regarding periodontal diseases, is essential for the prevention and management of these conditions across different age groups, including children, adolescents, and adults.⁽⁹⁾ Despite its importance, numerous studies have consistently indicated that the general population has a limited understanding of periodontal diseases.⁽¹⁰⁻¹³⁾ However, these studies often predominantly focused on the symptoms of periodontitis. The questionnaire used in these studies included multiple-choice questions to assess knowledge and a Likert scale to measure agreement or disagreement. This methodological approach may constrain the assessment of participants' comprehensive understanding of the disease, potentially overlooking important aspects of their knowledge of periodontitis. To the best of our knowledge, there is none of studies in Thailand that examine the comprehension of periodontal

disease.

Therefore, this study aimed to evaluate the knowledge of periodontitis among Thai adults through an open-ended question and explore the factors that shape their comprehension of the disease.

Materials and Methods

Data collection

An online questionnaire was created using Google Forms, which participants accessed via a QR code. The QR code to access the questionnaire was distributed to study participants via an online platform and in-person interaction, particularly within the Department of Periodontology's clinic, where they were invited to participate and provided access to the QR code on-site between December 2023 and January 2024. The questionnaire consisted of two sections: the first section collected demographic information such as age, gender, smoking status, diabetes status, social status, and frequency of dental visit, while the second section contained an open-ended question: "What is periodontitis?" Responses to the open-ended question were evaluated and analyzed by one periodontist (S.T.). Each response was manually tagged with keywords extracted from the answer. Keywords included phrases such as gingival inflammation, gingival swelling, gingival bleeding, tooth mobility, gingival recession, calculus, malodor, other conditions unrelated to periodontitis (e.g., dental caries), and the response "do not know".

Participants were grouped based on their familiarity with periodontitis as shown in their responses to the open-ended question. Familiarity with periodontitis was assessed according to the American Academy of Periodontology (AAP)'s Glossary of Periodontal Terms definition of periodontitis⁽¹⁴⁾, which was deemed appropriate for general population understanding. Apart from the "do not know" response, the tagged keywords in each response were categorized into groups related to gingival inflammation (e.g., gingival inflammation, gingival swelling, or gingival bleeding), attachment loss (e.g., tooth mobility or gingival recession), conditions suggestive of periodontitis but not consistent with the AAP's periodontitis definition⁽¹⁴⁾, and other unrelated conditions.

An answer was considered "totally correct" if it included keywords related to both gingival inflammation

and attachment loss, as per the AAP's Glossary of Periodontal Terms⁽¹⁴⁾ Conversely, responses were labeled as “partially correct” if they contained keywords associated with either gingival inflammation or attachment loss, while answers with other keywords were categorized as “incorrect”. Consequently, participants were classified into 3 groups: totally correct (TC), partially correct (PC) and incorrect (IC).

Statistical analysis

Data analysis was performed using SPSS software version 29.0 (SPSS®: Inc., Chicago, IL, USA). Descriptive statistics were utilized to present demographic data and clinical variables. Subgroup comparisons were conducted using the Pearson chi-square test, with a significance level set at $p \leq 0.05$.

Results

A total of 2150 participants completed the questionnaire. Among them, 772 participants (35.9%) did not respond to the open-ended question, and 173 participants (8.0%) answered “do not know”, leading to their exclusion from the analysis. Therefore, the analysis was conducted on a total of 1205 participants (Figure 1).

The 1,205 participants included in the study had a mean age of 38.4 ± 14.4 years. The gender distribution was 366 (30.4%) male, 805 (66.8%) female, and 34 (2.8%) unspecified. A majority of the participants held a bachelor's degree (62.7%), and their reported income was 10,001-30,000 THB (39.9%). More than half of the participants reported visiting the dentist on a regular basis (62.7%). Furthermore, 3.3% of the participants were smokers, and 1.9% reported having diabetes.

The keywords extracted from the response of the open-ended questions, “What is periodontitis?” are presented in Table 1. Out of the 1205 answers analyzed, 75.8% of participants (914 responses) included 1 keyword, while 17.5% (211 responses) contained 2 keywords. Furthermore, 54 responses (4.5%) included 3 keywords, 21 responses (1.7%) had 4 keywords, 4 responses (0.3%) featured 5 keywords, and 1 response (0.1%) included 6 keywords.

Of the total 1608 keywords identified from the 1205 participants, 70.3% were related to gingival inflammation, while 10% were associated with attachment loss. The remaining keywords were categorized as “other keywords

implying periodontitis but not in the AAP periodontitis definition” (7.2%) and “other conditions not related to periodontitis” (12.6%). The most common keywords related to gingival inflammation were “gingival inflammation” (44.3%; 713 out of 1608), “gingival swelling” (17.8%; 287 out of 1608), and “gingival bleeding” (8.1%; 131 out of 1608). Keywords associated with attachment loss were primarily “gingival recession” (Table 1).

Upon categorizing the participants based on their responses, the majority (72.4%) were classified into the PC group. Remarkably, within the PC group, a significant 95.8% provided responses containing keywords related to gingival inflammation, contrasting with only 4.2% that included keywords linked to attachment loss. About one fifth of the participants were assigned to the IC group, with only 8.5% falling into the TC group (Table 2).

In this study cohort, a significant majority (77.8%; 938 out of 1205) of participants mentioned keywords associated with gingival inflammation. In contrast, a smaller proportion (11.6%; 140 out of 1205) reported keywords related to attachment loss. Notably, among those who mentioned attachment loss keywords, a significantly higher percentage (73.6%; 103 out of 140) also referenced keywords related to gingival inflammation compared to the group (11%, 103 out of 938) that mentioned gingival inflammation keywords and also reported attachment loss keywords.

The findings from Pearson chi-square analysis indicated that age, educational level, and dental visit frequency were statistically significant factors associated with participants' familiarity with periodontitis. In contrast, sex, Diabetes mellitus status, smoking status and income showed no significant association with participants' familiarity with periodontitis (Table 3).

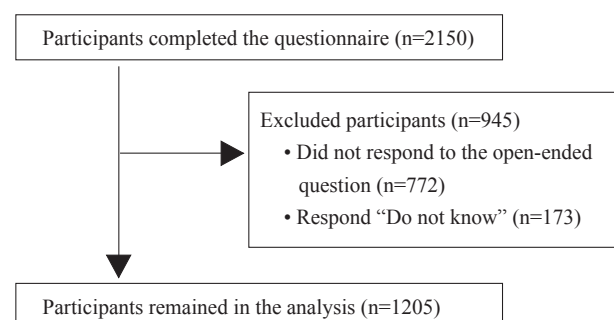


Figure 1: Flow of data collection.

Table 1: Distribution of keywords across participant groups based on responses to the question “What is periodontitis?”.

Keywords	Number of keywords; N=1608 (100%)			
	All groups	TC group	PC group	IC group
Gingival inflammation	1131 (70.3%)	148	1083	0
- Gingival inflammation	713	74	639	0
- Gingival swelling	287	43	244	0
- Gingival bleeding	131	31	200	0
Attachment loss	160 (10%)	116	44	0
- Tooth mobility	74	60	14	0
- Gingival recession	86	56	30	0
Other keywords implying periodontitis but not in the AAP periodontitis definition	115 (7.2%)	33	53	29
- Calculus	84	20	39	25
- Malodor	31	13	14	4
Other conditions not related to periodontitis	202 (12.6%)	0	0	202
- Dental caries	10	0	0	10
- Disease in the mouth, cystic lesion, tooth disease, etc.	192	0	0	192
Total	1608 (100%)	297	1180	231

Abbreviations: TC = Totally correct; the answer with keywords related to both gingival inflammation and attachment loss, PC = Partially correct; the answer with keywords associated with either gingival inflammation or attachment loss, IC = Incorrect; the answer with other keywords

Table 2: Distribution of the participants in study groups based on responses to the question “What is periodontitis?”

	Group of the participants			Total	
	TC group	PC group			IC group
Number of participants; n (%)	103 (8.5%)	872 (72.4%)		230 (19.1%)	1205 (100%)
		Keywords related to “gingival inflammation”	Keywords related to “attachment loss”		
		835	37		

Abbreviations: TC = Totally correct, PC = Partially correct, IC = Incorrect

Discussion

This study on periodontitis comprehension among the Thai population highlights the pressing need for increased public awareness. A comprehensive understanding of periodontitis is essential, as patient awareness directly influences their willingness to seek timely and appropriate treatment. Given the progressive nature and long-term consequences of untreated periodontal diseases, dental professionals must assess and address public knowledge gaps. Developing targeted, evidence-based oral health education programs that consider demographic and socioeconomic variations is essential to improving periodontal outcomes. Oral health literacy, especially concerning periodontitis, is crucial for effective prevention, early diagnosis, and management across the lifespan.

Despite its clinical significance, research indicates that public awareness and understanding remain alarmingly low^(5,6), underscoring the urgent need for intensified educational and preventive strategies at individual and community levels.

In this study, of the 1378 respondents, 12.6% (173 individuals) lacked knowledge about periodontitis, while a substantial 72.4% of the remaining participants exhibited only a superficial understanding, primarily linking periodontitis with gingival inflammation. Participants mentioning attachment loss keywords were more likely to demonstrate a better understanding of periodontitis. These findings underscore the necessity to enhance public awareness of periodontitis, particularly in elucidating its broader implication beyond gingival inflammation.

Table 3: Demographic variables and dental visit frequency and their association with the responses to the question “What is periodontitis?”.

Demographic data	Group of the participants				p-value
	TC group (N = 103)	PC group (N = 872)	IC group (N=230)	Total (N=1205)	
Gender					0.648
Female	74 (9.2%)	581 (72.2%)	150 (18.6%)	805 (100%)	
Male	25 (6.8%)	268 (73.2%)	73 (19.9%)	366 (100%)	
Unspecified	4 (11.8%)	23 (67.6%)	7 (20.6%)	34 (100%)	
Age (years)					0.016*
18-39	61 (8.8%)	488 (70.1%)	147 (21.1%)	696 (100%)	
40-59	38 (10.2%)	273 (73.0%)	63 (16.8%)	374 (100%)	
≥60	4 (3.0%)	111 (82.2%)	20 (14.8%)	135 (100%)	
Educational Level					0.012*
Below bachelor's degree	26 (11.9%)	139 (63.8%)	53 (24.3%)	218 (100%)	
Bachelor's degree	64 (8.5%)	552 (73.1%)	139 (18.4%)	755 (100%)	
Above bachelor's degree	13 (5.6%)	181 (78.0%)	38 (16.4%)	232 (100%)	
Income (THB)					0.367
<10,000	15 (8.3%)	130 (71.8%)	36 (19.9%)	181 (100%)	
10,001-30,000	45 (9.4%)	332 (69.0%)	104 (21.6%)	481 (100%)	
30,001-50,000	26 (8.8%)	222 (75.5%)	46 (15.6%)	294 (100%)	
>50,001	17 (6.8%)	188 (75.5%)	44 (17.7%)	249 (100%)	
Dental visit frequency					0.001*
Regularly (At least once a year)	77 (10.2%)	545 (72.1%)	134 (17.7%)	756 (100%)	
Only when having symptoms	22 (5.1%)	320 (73.9%)	91 (21.0%)	433 (100%)	
Never	4 (25.0%)	7 (43.8%)	5 (31.3%)	16 (100%)	
Diabetes mellitus status					0.286
Yes	102 (8.6%)	852 (72.1%)	228 (19.3%)	1182 (100%)	
No	1 (4.3%)	20 (87.0%)	2 (8.7%)	23 (100%)	
Smoking status					0.625
Non-smoker	92 (8.4%)	795 (72.3%)	212 (19.3%)	1099 (100%)	
Smoker	5 (12.5%)	26 (65.0%)	9 (22.54%)	40 (100%)	
Former smoker	6 (9.1%)	51 (77.3%)	9 (13.6%)	66 (100%)	

Abbreviations: TC = Totally correct, PC = Partially correct, IC = Incorrect, THB = Thai baht

*Pearson Chi-square test: $p < 0.05$.

An open-ended question was utilized in this study to evaluate participants' understanding of periodontitis. Unlike multiple-choice questions, this approach assesses the comprehension of the disease rather than recalling information from memory, thereby minimizing the potential for guessing. However, some participants may leave the question blank, as seen in this study, where approximately 36% did not respond. Previous studies have typically developed questionnaires to assess periodontal health knowledge and awareness among the population related to variables and factors using multiple choices and Likert scale.^(5,12,13,15) However, the findings of existing studies raise important considerations regarding

the assessment of participants' understanding of periodontitis. Researchers have employed simplified terminology, such as "gum disease," and have primarily focused on the symptoms of the condition. This approach may not adequately capture the complexities associated with periodontitis and could limit insights into participants' comprehensive understanding of the disease. Additionally, by concentrating on symptoms, the assessment may provide an incomplete picture of participants' awareness. While participants may recognize certain symptoms, they might benefit from a deeper understanding of periodontitis. In this study, To enhance the evaluation of knowledge, it may be beneficial to incorporate open-ended questions

that allow for a deeper exploration of participants' understanding. To our knowledge, no validated questionnaire specifically designed to evaluate understanding of periodontitis currently exists.

In this study, the definition of periodontitis from the AAP Glossary of Terms was employed as the reference for answers of the question, "what is periodontitis?". According to this definition, periodontitis is characterized by inflammation of the periodontal tissues, leading to clinical attachment loss, alveolar bone loss, and periodontal pocket formation. While the most recent 2018 periodontal classification⁽¹⁶⁾ provides a case definition of periodontitis based on detectable interdental clinical attachment loss at two non-adjacent teeth, this classification is primarily intended for dental professionals to aid in treatment decisions and clinical research. The complexity of the 2018 periodontitis case definition renders it unsuitable for the present study aimed at assessing the general population's understanding of periodontitis.

It may be speculated that the loss of support of the periodontium around teeth can cause difficulty in masticatory function such as tooth sensitivity and pain during function. This, in turn, leads individuals to understand and become aware that attachment loss, such as tooth mobility or gingival recession, may serve as potential indicators of periodontitis. This heightened awareness parallels findings from prior research that established a correlation between self-awareness of tooth mobility and the presence of severe periodontitis.⁽¹⁷⁾ This awareness will prompt consideration for dental evaluation when such signs become apparent.

The significance of understanding attachment loss cannot be overstated, as it indicates the progressive destruction of supporting structures around the teeth, leading to various oral health issues and systemic implications. Dental professionals play a crucial role in educating patients about this aspect of periodontitis, emphasizing the importance of proper oral hygiene practices, regular dental examinations, and early symptom recognition. Effective collaboration between dentists and patients is crucial for preventing and managing periodontitis. Additionally, improving knowledge dissemination and promoting optimal periodontal health can be strengthened through public health campaigns, educational programs, and digital platforms.⁽¹⁸⁾

Age and educational level emerged as key demo-

graphic factors associated with comprehension of periodontitis, with gender showing no correlation with participants' familiarity of the disease, consistent with previous research.^(19,20) The majority of participants across all age groups demonstrated only a partial understanding of periodontitis. Importantly, only 3.4% of elderly individuals demonstrated a complete understanding, while a higher proportion of young adults and middle-aged participants could explain the key characteristics of periodontitis. In terms of educational level, individuals with less than a bachelor's degree accounted for the largest proportion of subset providing incorrect answers, at 24.3%, while participants with higher education levels exhibited a smaller proportion of subsets with incorrect answers, at 18.4% for those with a bachelor's degree and 16.4% for those with a degree above bachelor's level. This is consistent with prior research linking both age and educational level to knowledge of periodontal disease.⁽²¹⁻²⁵⁾ Despite common assumptions, our study challenged the notion that higher education ensures a comprehensive understanding of periodontitis, as most participants, regardless of educational background, fell into the PC group.

In terms of dental visit frequency, participants who have never seen a dentist exhibited limited knowledge or understanding of periodontitis. Conversely, the majority of the participants who accurately answered the open-ended question about periodontitis tended to visit the dental office regularly. Surprisingly, even among those who visit the dentist regularly, a significant portion still only have partial knowledge of periodontitis, with nearly 20% lacking any understanding of the condition. These findings suggest that dental professionals may not be placing enough emphasis on educating patients about periodontitis. Interestingly, these results were in contrast with previous study which regular dental attendees demonstrated a better understanding of periodontal health.⁽²⁶⁾ This is possibly due to differences in the focus of the questionnaires used; the previous study centered on periodontal health in general, while our study specifically addressed understanding of periodontitis.

This study found that diabetes mellitus and smoking status are not associated to an understanding of periodontitis, which contrasts with previous^(27,28) studies suggesting that individuals with diabetes or smokers tend to have lesser knowledge and awareness of periodontitis

compared to non-diabetics and non-smokers. However, those studies used multiple-choice or yes/no questions and focused on the symptoms or negative effects of periodontitis rather than a deeper understanding of what periodontitis is. Additionally, the sample composition in those studies differed, with more diabetics and smokers, while this study's sample consisted mostly of non-diabetics and non-smokers, which may have influenced the results.

While this study benefited from a large and diverse population sample, it is important to acknowledge certain limitations. The online dissemination of the questionnaire may have created barriers to access for certain groups, potentially leading to the underrepresentation of elderly individuals and those with limited internet connectivity. Consequently, the study primarily included participants between the ages of 18 and 40, predominantly holding bachelor's degrees and belonging to middle to high socioeconomic groups. Future studies may aim to include a more diverse range of participants by distributing paper questionnaires alongside the online version. Furthermore, The data collection was conducted through an online questionnaire distributed via Google Forms, which, while efficient in reaching a broad audience, did not allow for clinical examinations. As a result, the actual periodontal status of participants could not be verified. To address these limitations, future research should aim to include a more diverse and representative sample by incorporating both paper-based and online surveys, and should also integrate clinical examinations to ensure more accurate and objective assessment of periodontal health.

In addition, participants may have sought information from sources other than the survey itself, such as the internet or consultations with peers, which could have influenced their responses. However, This highlights the way individuals often interpret health information in real-world scenarios. it is well-recognized that external influences, such as media, personal experiences, and interactions with healthcare professionals, significantly shape participants' knowledge and perceptions.⁽²⁹⁾ The intention of this study was to capture this broader, general awareness, rather than aiming for clinical precision, as it reflects the reality of how health information is understood and applied by the public.

In this study, the questionnaire included a single open-ended question designed to explore participants'

understanding in their own words. Because the question was straightforward and exploratory in nature, a formal validation process was not carried out. To improve future research, it is recommended that a more detailed and validated instrument be developed to ensure greater reliability and applicability of results across diverse populations and levels of periodontal health literacy.

Conclusions

In conclusion, this study successfully evaluated the knowledge of periodontitis among Thai adults through an open-ended question, revealing a superficial understanding of the disease, with limited awareness beyond gingival inflammation. Additionally, age, gender, and frequency of dental visits were identified as significant factors influencing the level of comprehension. These findings underscore the critical role of dental professionals in educating patients about the broader implications of periodontitis, including attachment loss. Continuous efforts are required to enhance public education and promote a deeper understanding of periodontal health in Thailand.

Ethical Declaration

This cross-sectional questionnaire-based study received ethical approval from the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2023-123) and adhered to the principles the Declaration of Helsinki. Study participants included Thai adults aged 18 years and above. Prior to participation, all individuals provided written informed consent.

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Conflict of Interest

The authors declare that they hold no competing interests.

References

1. Dental Health Bureau. The 9th National oral health survey report Bangkok Aksorn graphic and design publishing Co. (in Thai); 2023 [cited 10 January 2024]. Available from: <https://dental.anamai.moph.go.th/national-oral-health-survey-report/4952#wow-book/>.

2. Jin L, Group E. Initiator paper. Interprofessional education and multidisciplinary teamwork for prevention and effective management of periodontal disease. *J Int Acad Periodontol*. 2015;17(Suppl 1):74-9.
3. Petersen PE. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century—the approach of the WHO Global Oral Health Programme. *Community Dent Oral Epidemiol*. 2003;31(Suppl 1):3-23.
4. Kassebaum N, Bernabé E, Dahiya M, Bhandari B, Murray C, Marcenes W. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. *J Dent Res*. 2014;93(11):1045-53.
5. Romano F, Perotto S, Bianco L, Parducci F, Mariani GM, Aimetti M. Self-perception of periodontal health and associated factors: a cross-sectional population-based study. *Int J Environ Res Public Health*. 2020;17(8):2758.
6. Birkeholm Jensen A, Haubek D. Self-perception of periodontal health and pain experience during periodontal examination in 14-to 15-year-old Danish adolescents. *Acta Odontol Scand*. 2022;80(7):554-60.
7. Taani DQ. Periodontal awareness and knowledge, and pattern of dental attendance among adults in Jordan. *Int Dent J*. 2002;52(2):94-8.
8. Varela-Centelles P, Diz-Iglesias P, Estany-Gestal A, Seoane-Romero JM, Bugarín-González R, Seoane J. Periodontitis awareness amongst the general public: a critical systematic review to identify gaps of knowledge. *J Periodontol*. 2016;87(4):403-15.
9. Hamasha AA-H, Alshehri A, Alshubaiki A, Alssafi F, Alamam H, Alshunaiber R. Gender-specific oral health beliefs and behaviors among adult patients attending King Abdulaziz Medical City in Riyadh. *Saudi Dent J*. 2018;30(3):226-31.
10. Nguyen JG-I, Nanayakkara S, Holden AC. Knowledge, attitudes and practice behaviour of midwives concerning periodontal health of pregnant patients. *Int J Environ Res Public Health*. 2020;17(7):2246.
11. Naser MY, Momani M, Naser AY, Alarabeyat MA, Altarawneh AMB, Aladwan AS. Oral health profile and periodontal diseases awareness and knowledge among the Jordanian population: a cross-sectional study. *BMC Oral Health*. 2023;23(1):503.
12. Alhaija ESA, Al-Saif EM, Taani DQ. Periodontal health knowledge and awareness among subjects with fixed orthodontic appliance. *Dental Press J Orthod*. 2018;23(5):40.e1-e9.
13. Abdulbaqi HR, Abdulkareem AA, Alshami ML, Milward MR. The oral health and periodontal diseases awareness and knowledge in the Iraqi population: online-based survey. *Clin Exp Dent Res*. 2020;6(5):519-28.
14. Glossary of periodontal terms: American Academy of Periodontology 2012 [cited 10 January 2024]. Available from: <https://members.perio.org/browse/glossary/entry?GlossaryKey=d93c420e-9322-4bdd-b01c-d545a-f310a5b&tab=groupdetails>.
15. Alwaeli HA, Al-Jundi SH. Periodontal disease awareness among pregnant women and its relationship with socio-demographic variables. *Int J Dent Hyg*. 2005;3(2):74-82.
16. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: framework and proposal of a new classification and case definition. *J Periodontol*. 2018;89(Suppl 1):S159-S72.
17. Gürbüz S, Altıkat M. The association between periodontitis patients' chief complaints and the stage of periodontitis: a clinical retrospective study. *Clin Oral Investig*. 2023;27(10):6261-72.
18. Farrokhi F, Ghorbani Z, Farrokhi F, Namdari M, Salavati S. Social media as a tool for oral health promotion: a systematic review. *PloS One*. 2023;18(12):e0296102.
19. Selvaraj S, Naing NN, Wan-Arfah N, Abreu MHNGd. Assessment on oral health knowledge, attitude, and behaviour and its association with sociodemographic and habitual factors of South Indian population. *Pesqui Bras Odontopediatria Clin Integr*. 2021;21:e0135.
20. Dolińska E, Milewski R, Pietruska MJ, Gumińska K, Prysak N, Tarasewicz T, *et al*. Periodontitis-related knowledge and its relationship with oral health behavior among adult patients seeking professional periodontal care. *J Clin Med*. 2022;11(6):1517.
21. Gomes APM, da Silva EG, Gonçalves SHF, Huhtala MFRL, Martinho FC, de Paiva Gonçalves SE, *et al*. Relationship between patient's education level and knowledge on oral health preventive measures. *Int Dent Med J Adv Res*. 2015;1(1):1-7.
22. Mårtensson C, Söderfeldt B, Andersson P, Halling A, Renvert S. Factors behind change in knowledge after a mass media campaign targeting periodontitis. *Int J Dent Hyg*. 2006;4(1):8-14.
23. El-Qaderi S, Quteish Ta'ani D. Assessment of periodontal knowledge and periodontal status of an adult population in Jordan. *Int J Dent Hyg*. 2004;2(3):132-6.
24. Rajab L, Petersen P, Bakaeen G, Hamdan M. Oral health behaviour of schoolchildren and parents in Jordan. *Int J Paediatr Dent*. 2002;12(3):168-76.
25. Birant S, Koruyucu M, Ozcan H, Ilisulu C, Kasimoglu Y, Ustun N, *et al*. Investigating the level of knowledge of the community about oral and dental health. *Eur J Dent*. 2021;15(1):145-51.
26. Varela-Centelles P, Diz-Iglesias P, Estany-Gestal A, Blanco-Hortas A, Bugarín-González R, Seoane-Romero JM. Regular dental attendance and periodontal health knowledge: a cross-sectional survey. *Oral Dis*. 2020;26(2):419-28.
27. Poudel P, Griffiths R, Wong VW, Arora A, Flack JR, Khoo CL, *et al*. Oral health knowledge, attitudes and care practices of people with diabetes: a systematic review. *BMC Public*

- Health. 2018;18(1):1-12.
28. Terrades M, Coulter W, Clarke H, Mullally B, Stevenson M. Patients' knowledge and views about the effects of smoking on their mouths and the involvement of their dentists in smoking cessation activities. *Br Dent J.* 2009;207(11):E22-E.
29. Young ME, Norman GR, Humphreys KR. Medicine in the popular press: the influence of the media on perceptions of disease. *PLoS One.* 2008;3(10):e3552.



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Dimensional Changes of Masticatory Muscles Following Camouflage Orthodontic Treatment in Skeletal Class III Patients: A Pilot MRI-Based Clinical Trial

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Abstract

Objectives: To evaluate dimensional changes in masticatory muscles, dentoskeletal relationships, and correlations between muscular and vertical skeletal changes following Class III camouflage treatment.

Methods: This clinical trial included ten participants with skeletal Class III malocclusion who met the eligible criteria and provided them non-extraction camouflage treatment using Class III elastics. MR images (T1W) and lateral cephalograms were taken before treatment (T0) and after achieving normal occlusion (T1). Length, width, and cross-sectional area (CSA) of the masseter (MM), temporalis (TM), lateral pterygoid (LPM), and medial pterygoid (MPM) muscles were measured using MicroDicom DICOM viewer software. Dentoskeletal changes were assessed by Dolphin[®] imaging software. Statistical analyses were conducted using IBM[®] SPSS[®] software to analyze differences between T0 and T1, and correlations.

Results: Significant changes were observed in jaw-closing muscles, with increased length (MM 1.0±0.4 mm, TM 0.7±0.2 mm, MPM 0.5±0.5 mm), decreased thickness (MM 1.3±0.7 mm, TM 0.2±0.2 mm, MPM 0.8±0.6 mm), and decreased CSA (MM 79.3±70.5 mm², TM 16.2±14.9 mm², MPM 16.2±8.8 mm²). Minimal changes were noted in lateral pterygoid muscles. No significant correlations were found between muscular changes and vertical skeletal changes.

Conclusions: Masseter, temporalis, and medial pterygoid muscles exhibited significant changes following Class III camouflage treatment using Class III elastics, but no significant correlations were observed between muscular dimensional changes and vertical skeletal changes.

Keywords: camouflage treatment, Class III elastics, masticatory muscles, MRI, skeletal Class III

Introduction

Skeletal Class III malocclusion is widely recognized as one of the most challenging types of malocclusion to treat in orthodontic practice, particularly among adult patients who have limited treatment options.⁽¹⁾ While orthognathic surgery and orthodontic camouflage treatment have traditionally been considered standard approaches for these patients, many individuals are likely to opt against orthognathic surgery due to its cost and invasive nature. Camouflage treatment has emerged as a viable alternative, especially for patients with mild to moderate skeletal discrepancies.^(2,3) To correct anterior crossbite and achieve a Class I relationship through camouflage treatment, several modalities are available based on the severity of malocclusion and the patient's specific needs. Treatment options include permanent tooth extraction, Class III elastics, temporary anchorage devices (TADs), the multiloop edgewise archwire (MEAW) technique, or extraoral appliances.⁽⁴⁾ Among these, Class III elastics used with full-fixed appliances are commonly used due to their effectiveness and simplicity.

Although the effects of Class III elastics have been broadly described in previous studies, they are typically discussed in terms of lateral cephalometric changes such as mandibular clockwise rotation, increased vertical dimension, and maxillary molar extrusion.⁽⁴⁻⁸⁾ While these positional changes of the mandible are well-documented, their impact on the entire masticatory muscles has been minimally studied. Given that all masticatory muscles predominantly insert on mandibular structures, positional changes of the mandible may contribute to dimensional and functional adaptations in these muscles. Moreover, alterations in masticatory muscle dimensions are likely to affect facial aesthetics in the frontal view following orthodontic treatment, especially the thickness of the masseter muscles.⁽⁹⁾ Regrettably, this aspect has often been overlooked in orthodontic practice, where treatment outcome assessments have primarily focused on lateral profile changes.

The primary objective of this study was to evaluate the dimensional changes in all masticatory muscles on both sides (temporalis, masseter, lateral pterygoid, and medial pterygoid) following conventional Class III camouflage treatment using Class III elastics. The secondary objectives were to investigate the associated dentoskeletal changes after treatment and explore the correlations

between these muscular adaptations and vertical skeletal changes.

Materials and Methods

Study design

This study is a clinical trial conducted as a pre-post intervention study and registered with the Thai Clinical Trials Registry under the identifier TCTR20220316003 (available at thaiclinicaltrials.org).

Ethical approval

This clinical trial was approved by the Khon Kaen University Ethics Committee for Human Research, following the guidelines outlined in the Declaration of Helsinki and the International Conference of Harmonization Good Clinical Practice (ICH GCP) Guidelines (reference number HE641561). Written informed consent was obtained from all participants.

Study sample and sample size calculation

This clinical trial focused on adult patients with skeletal Class III malocclusion, adhering to specific eligibility criteria. The inclusion criteria encompassed: (1) completion of mandibular growth (CS6); (2) skeletal Class III malocclusion (overjet < 0 mm in centric occlusion which distinguishes functional Class III cases, ANB < 0.5°, G'-Sn-Pog' > 172.8°); (3) hypodivergent or normodivergent pattern (FMA < 28.1°); (4) symmetric face (defined by the absence of significant chin deviation, i.e., ≤ 3 mm); and (5) desire for camouflage treatment.^(10,11) The exclusion criteria included: (1) pre-existing temporomandibular disorder (TMD) symptoms; (2) history of craniofacial surgery; (3) presence of severe space deficiency that necessitates tooth extraction; and (4) contraindications for MRI. Withdrawal criteria comprised: (1) poor compliance with Class III elastics use; (2) administration of botulinum toxin injections during treatment; and (3) significant body weight loss during the treatment period (T0-T1).

Sample size calculation was performed using a test for difference in two dependent means, $n = [(Z\alpha + Z\beta)^2 \sigma_d^2] / (\mu_1 - \mu_2)^2$. The difference in primary outcomes between T0 and T1 was estimated from a previous study by Pan *et al.*,⁽⁹⁾ and a sample size of at least 8 participants was required to achieve 80% power and 5% alpha error. Consecutive sampling method was employed to

recruit participants at the Orthodontic Clinic, Faculty of Dentistry, Khon Kaen University, between January to December 2022.

A total of 36 patients with skeletal Class III malocclusion were initially evaluated for eligibility. Of these, 10 patients met the inclusion and exclusion criteria and were enrolled in the study.

Treatment protocol

Non-extraction camouflage treatment was provided in all participants under the same protocol, as the intervention in this study. The treatment employed full-fixed orthodontic appliances to address dental crowding and correct Class III malocclusion without tooth extractions, orthognathic surgery, or temporary anchorage devices (TADs). MBT 0.022" ceramic brackets (3M™ Clarity™ Advanced Ceramic Brackets, 3M Unitek™, Monrovia, CA) was used with straight-wire technique. A sequence of nickel-titanium archwires (0.014, 0.016, 0.016×0.022, 0.017×0.025, and 0.019×0.025-inch NiTi) was utilized for leveling and aligning. Additional mechanics, such as archwire expansion, anterior tooth proclination using advancement arches with omega loops, and interproximal stripping (IPR), were employed prior to the Class III correction phase in cases where dental crowding was present. Posterior bite ramps (Ultra band-lok®, Reliance Orthodontic Products, Itasca, IL) were applied to either the maxillary or mandibular molars to disarticulate both arches during anterior crossbite correction and were subsequently removed upon correction. The correction of the dental Class III relationship and anterior crossbite was achieved through the combined use of Class III elastics and large rectangular stainless-steel archwires (0.017×0.025 or 0.019×0.025-inch SS). Class III elastics (1/4", 4.5 oz.) were worn full-time, facilitating maxillary dentition advancement, mandibular dentition retraction, and clockwise mandibular rotation to reduce the severity of the skeletal Class III relationship. Concurrently, the large rectangular stainless-steel archwires allowed for controlled proclination of the maxillary incisors through the fully expressed bracket prescription. Arch form modifications, including contouring (bending) of the main archwires, were employed to further optimize the dental alignment and arch coordination, contributing to the effective correction of the malocclusion. Class III elastics were continued until a Class I relationship was achieved

bilaterally. The leveling and aligning phase and Class III correction phase are shown in Figures 1-2.

MR images of skull were taken before treatment (T0) and after normal occlusion achieved (T1) by using Achieva dStream 3.0T MR Systems (Philips®, Koninklijke Philips N.V., Amsterdam, Netherlands), under the same settings: coronal and axial spin-echo (SE) T1-weighted sequence, 2D acquisition, TR 500-625 ms, TE 10-15 ms, 332×313 matrix size, 2.0 mm slice thickness, 0.2 mm inter-slice gap, and 20×20 cm field of view (FOV). The participants were positioned supine while maintaining the maximum intercuspal position (MIP) without applying excessive clenching force during MRI acquisition. Subsequently, all MR images were exported and stored as DICOM format files.

Furthermore, lateral cephalograms captured before treatment (T0) and after achieving normal occlusion (T1) for all participants were also collected. Specifically, T1 was defined as the point at which normal occlusion was achieved, based on clinical criteria aligned with the American Board of Orthodontics Discrepancy Index (2016): (1) Proper incisor relationship with normal overjet (1-3 mm) and overbite (1-3 mm), (2) Class I canine and molar relationship with good buccal intercuspation, and (3) Overall dental alignment without significant crowding. These cephalograms are routinely prescribed as part of initial and progressive orthodontic records, in accordance with the standard protocol of our clinic.

Measurement methods and outcomes

The MR images were first imported into the Micro Dicom DICOM viewer software (version 2022.1; Micro Dicom Ltd, Sofia, Bulgaria) and then reoriented. Thereafter, the dimensions of each masticatory muscle were measured from the coronal or axial slice of the MR images, where the muscle's dimensions were maximized—that is, the slice displaying the greatest length, width, or cross-sectional area. This slice was selected for measurement in order to standardize the analysis of muscle dimensions across all participants. Length, thickness, and cross-sectional area (CSA) of the masseter, temporalis, lateral pterygoid, and medial pterygoid muscles on both sides were assessed at T0 and T1, and then compared.

Length (mm) was measured along the direction of muscle fibers, which refers to the orientation of the fibers from the muscle's origin (attachment point) to its



Figure 1: Leveling and aligning phase, using ceramic bracket, archwires, and posterior bite ramps.



Figure 2: Class III correction phase, using the Class III elastics on rigid stainless-steel archwires.

insertion (opposite attachment point). Thickness (mm) was measured at the thickest part of the muscle and was taken perpendicular to the muscle's length. Similarly, CSA (mm²) was measured as total area of the muscle on the same slice used for the thickness measurement of each muscle, where the muscle's cross-sectional area was maximized. These measurements were performed in accordance with the specific anatomical features of each muscle. The measurement methods, including details of the origin, insertion, and fiber orientation of each muscle, are further clarified and illustrated in Figures 3-6.

Moreover, lateral cephalometric measurements were performed using the lateral cephalograms at T0 and T1, and then compared individually. The alterations of skeletal and dental relationship were analyzed through cephalometric variables, as demonstrated in Table 3.

Statistical analysis

The examiner underwent training and standardization with radiologists and commenced measurements once acceptable agreement between the examiner and experts was achieved (intraclass correlation coefficients, ICCs>0.80). For intra-examiner reliability assessment, MR images and lateral cephalograms of 30% of all participants were randomly selected and analyzed, yielding excellent reliability (ICCs ranged between 0.90-0.99).

All variables were measured three times by a single examiner with two-week intervals and their average values were used in the statistical analysis. The statistical analysis was conducted using IBM SPSS software (version 28.0; IBM Corp., Armonk, NY). To assess normal distribution, the Shapiro-Wilk test was utilized. Comparisons between T0 and T1 data regarding muscle dimensions and lateral cephalometric measurements, were

performed using either paired t-tests or Wilcoxon signed-rank tests. In addition, linear correlations between the changes in muscle dimensions and vertical skeletal changes were analyzed using the Pearson correlation coefficient or Spearman's correlation coefficient. The significance level was set at $p<0.05$.

Results

Table 1 demonstrates the demographic data of the whole sample ($n=10$), including sex, age, duration of elastics application, and total treatment duration. The overall characteristics of their initial malocclusion were summarized in Table 2, which indicated the anterior crossbite, deep overbite, skeletal Class III relationship, hypodivergent pattern, and concave profile.

Dentoskeletal changes following camouflage treatment were demonstrated through a comparison of lateral cephalometric measurements at T0 and T1 in Table 3. Statistically significant changes were observed in skeletal and dental components.

In terms of skeletal changes, significant improvements were observed in both antero-posterior and vertical relationships, as evidenced by increased ANB, FH-MP, and PP-MP. These improvements were primarily attributed to a clockwise rotation of the mandible, as shown by decreased SNB and SNPog, along with increased FH-MP. Furthermore, the mean ANB and FH-MP values at T1 indicated that participants achieved normodivergent pattern and nearly approached the normative value for skeletal Class I relationship following treatment, as per the Thai norm.^(10,11)

Regarding dental measurements, changes were noticed in both maxillary and mandibular incisors. The maxillary incisor exhibited proclination and protrusion,

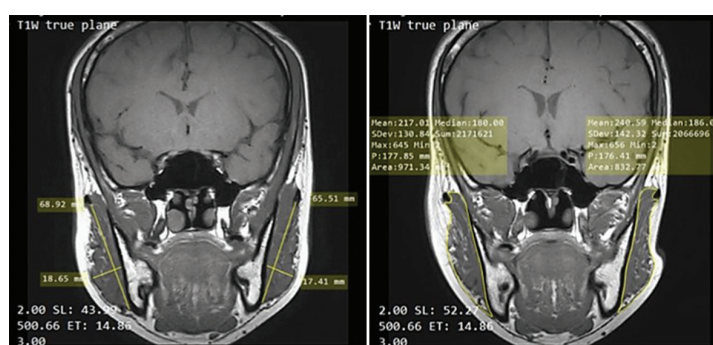


Figure 3: Measurement method of masseter muscles. (right) Length was measured from the zygomatic arch to the lateral surface of the mandibular lower border on coronal slice, while (right) thickness and (left) CSA were also measured on the same slice.

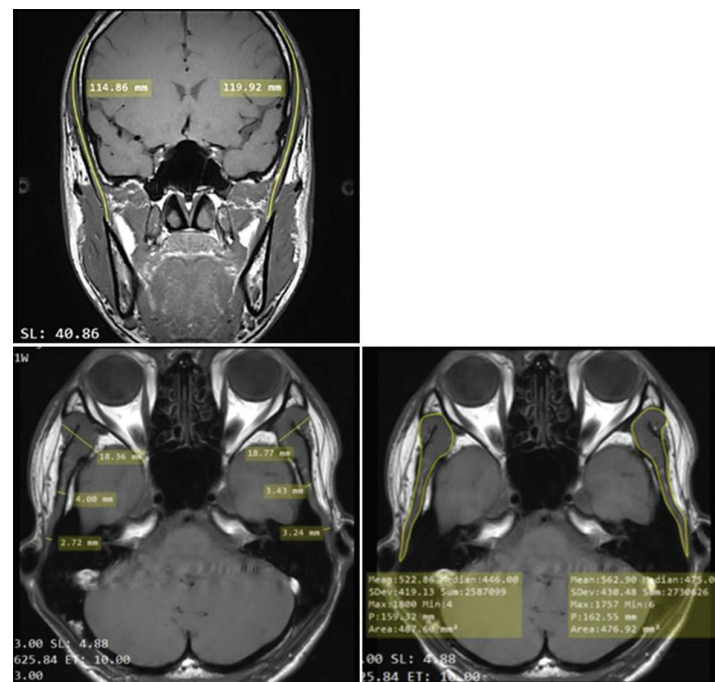


Figure 4: Measurement method of temporalis muscles. (right) Length was measured along the center of muscle thickness from the uppermost part (lying above the temporal fossa) to the coronoid process of the mandible on coronal slice. (middle) Thickness was measured as 3 subparts (anterior, middle, and posterior) due to their flat fan-shaped morphology on the axial slice, and (left) CSA were measured at same slice.

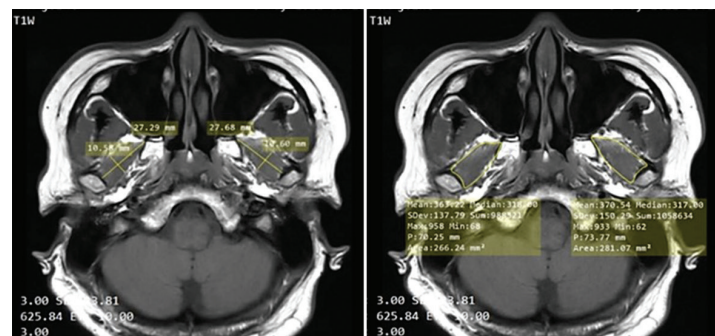


Figure 5: Measurement method of lateral pterygoid muscles. (right) Length, (right) thickness, and (left) CSA were measured on the axial slice at condylar head level. Length was measured from lateral surface of the lateral pterygoid plate to anterior part of the temporomandibular joint (TMJ) capsule, while thickness and CSA were measured on the same slice.

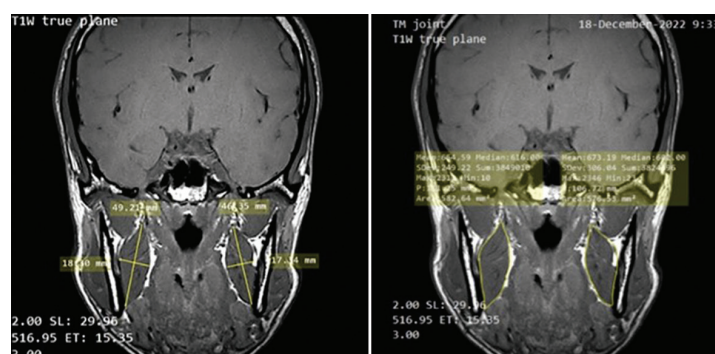


Figure 6: Measurement method of medial pterygoid muscles. (right) Length was measured from the medial pterygoid plate to the medial surface of the mandibular lower border on coronal slice, while (right) thickness and (left) CSA were also measured on the same slice.

as evidenced by increased U1-NA, while the mandibular incisor showed retroclination and retrusion, with decreased L1-MP and L1-NB, aimed at correcting anterior crossbite and compensating for skeletal Class III relationship. A slight clockwise rotation of the occlusal plane was also observed.

The comparison of muscle dimensions at T0 and T1 revealed significant changes in all masticatory muscles, including the masseter, temporalis, lateral pterygoid, and medial pterygoid muscles. These muscular changes

manifested similarly, with increases in length, decreases in thickness, and reductions in CSA. Notably, the lateral pterygoid muscles exhibited relatively lesser changes (0.2 ± 0.3 in length, 0.2 ± 0.2 in width) compared to the other masticatory muscles, and no significant change was observed in their CSA between T0 and T1. Conversely, the greatest magnitude of dimensional changes was observed in the masseter muscles (1.0 ± 0.4 in length, 1.3 ± 0.7 in width). Detailed measurements of these dimensional changes can be found in Table 4.

Table 1: Demographic data (n=10).

Variables	Mean	SD	Range
Sex, n=10			
Male, 3	30%		
Female, 7	70%		
Age (year)	24.3	7.0	18–36
Elastics application duration (month)	3.8	1.8	2–7
Total treatment duration, T0 to T1 (month)	12.6	1.8	10–15

Table 2: Characteristics of initial malocclusion (n=10).

Variables	Mean	SD	Range
Overjet (mm)	(-)2.3	0.59	(-)1.5 - (-) 3
Overbite (mm)	3.7	1.9	1-5.5
CO-CR discrepancy (mm)	1.5	0.8	0-2.5
ANB (°)	(-)3.1	1.6	(-) 1.5 - (-) 6.6
FH-MP (°)	22.4	3.3	18.1-28.1
Profile angle (°)	180.45	2.4	176.8-183.2

Table 3: Comparison of lateral cephalometric measurements at T0 and T1 (Mean±SD).

Variables (n=10)	Pre-treatment	Post-treatment	Differences	p-value
	(T0)	(T1)	(ΔT0-T1)	
Skeletal measurements				
SNA (°)	83.2±2.8	83.3±2.8	(-) 0.1±0.1	0.142
SNB (°)	86.3±2.9	84.0±2.9	2.3±0.6	<0.001*
SNPog (mm)	86.1±2.4	83.8±2.2	2.4±0.6	<0.001*
ANB (°)	(-) 3.1±1.6	(-) 0.7±1.5	(-) 2.4±0.5	<0.001*
FH-MP (°)	22.4±3.3	25.1±3.4	(-) 2.7±1.1	<0.001*
PP-MP (°)	19.5±4.7	22.1±4.8	(-) 2.6±1.2	<0.001*
Dental measurements				
U1-NA (°)	25.2±5.5	29.0±2.4	(-) 3.8±5.2	0.083
U1-NA (mm)	6.5±2.1	7.3±1.5	(-) 0.8±0.9	0.038*
L1-MP (°)	85.7±5.5	82.9±4.9	2.8±1.9	0.005*
L1-NB (°)	21.4±4.6	19.2±5.0	2.2±3.0	0.080
L1-NB (mm)	5.4±1.3	4.2±1.7	1.2±0.9	0.008*
SN-OP (°)	11.6±5.3	13.1±6.1	(-) 1.5±1.9	0.064

*Statistically significant (Paired t-test or Wilcoxon signed-rank test, $p<0.05$)

Additionally, the results of the Pearson correlation analysis denoted no significant correlations between the dimensional changes (in terms of length and width) of these four pairs of masticatory muscles and the vertical

changes in skeletal components, which were entirely influenced by mandibular rotation (represented by FH-MP value). These findings are presented in Table 5.

Table 4: Comparison of muscle dimensions at T0 and T1 (Mean±SD).

Variables (n=10)	Pre-treatment (T0)		Post-treatment (T1)		Differences (Δ T0-T1) ^b		p-value	
	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.
Masseter muscles								
Length (mm)	62.9±5.6	63.5±3.1	64.0±5.3	64.5±3.2	(-) 1.15±0.7	(-) 1.0±0.4	0.003*	<0.001*
Thickness (mm)	16.9±1.4	17.4±2.4	15.6±1.4	16.1±2.0	1.3±0.7	1.3±0.8	0.001*	0.003*
CSA (mm ²)	856.1±142.9	890.3±108.7	805.4±120.2	811.0±95.1	50.8±65.5	79.3±70.5	0.065	0.015*
Temporalis muscles								
Length (mm)	107.9±7.4	110.4±7.5	108.6±7.4	111.3±7.0	(-) 0.7±0.2	(-) 0.9±0.8	<0.001*	0.011*
Thickness (mm) - anterior	18.6±1.4	18.6±1.5	18.4±1.5	18.4±1.4	0.2±0.2	0.2±0.3	0.061	0.131
Thickness (mm) - middle	4.0±1.1	4.2±1.5	3.8±1.1	3.7±1.2	0.2±0.2	0.5±0.5	0.008*	0.018*
Thickness (mm) - posterior	3.3±0.9	3.5±0.7	3.1±0.7	3.2±0.6	0.2±0.4	0.3±0.2	0.351	0.007*
CSA (mm ²)	485.7±75.9	479.5±65.9	469.5±75.4	465.6±66.8	16.2±14.9	14.0±13.8	0.018*	0.024*
Lateral pterygoid muscles								
Length (mm)	29.9±3.0	28.8±3.2	30.1±3.3	29.1±3.1	(-) 0.2±0.3	(-) 0.4±0.3	0.051	0.014*
Thickness (mm)	14.4±2.1	14.0±2.5	14.2±2.0	13.7±2.3	0.2±0.2	0.3±0.6	0.031*	0.251
CSA (mm ²)	367.6±67.9	355.7±74.2	367.3±66.6	355.5±72.9	0.3±5.3	0.2±2.9	0.869	0.877
Medial pterygoid muscles								
Length (mm)	49.4±6.9	49.6±6.3	49.9±7.1	50.1±6.6	(-) 0.5±0.5	(-) 0.5±0.6	0.026*	0.079
Thickness (mm)	15.8±1.6	15.6±1.3	15.0±1.5	15.0±1.5	0.8±0.6	0.6±0.4	0.006*	0.008*
CSA (mm ²)	492.1±95.9	488.2±66.6	475.9±93.8	471.4±70.2	16.2±8.8	16.8±19.1	0.001*	0.042*

^b - value indicating increase, + value indicating decrease

*Statistically significant (Paired t-test or Wilcoxon signed-rank test, $p < 0.05$)

Table 5: Correlations between muscular changes and vertical skeletal change (n=10).

Correlations			FH-MP difference (Δ T0-T1)	
			r	p-value
Masseter muscles	Length	Rt.	0.146	0.730
		Lt.	0.731	0.039 *
	Width	Rt.	(-) 0.210	0.618
		Lt.	(-) 0.052	0.902
Temporalis muscles	Length	Rt.	0.181	0.668
		Lt.	(-) 0.293	0.481
	Width (middle)	Rt.	0.111	0.793
		Lt.	(-) 0.364	0.376
Lateral pterygoid muscles	Length	Rt.	(-) 0.002	0.997
		Lt.	0.535	0.172
	Width	Rt.	(-) 0.570	0.141
		Lt.	(-) 0.054	0.900
Medial pterygoid muscles	Length	Rt.	(-) 0.319	0.441
		Lt.	(-) 0.747	0.033 *
	Width	Rt.	(-) 0.138	0.745
		Lt.	(-) 0.186	0.659

*Statistically significant correlation (Pearson or Spearman's correlation coefficient, $p < 0.05$)

Discussion

Non-extraction camouflage treatment, utilizing pre-adjusted edgewise fixed appliances with Class III elastics, is widely recognized as one of the most common approaches for patients with mild-to-moderate skeletal Class III malocclusion, especially in individuals exhibiting hypo- or normo-divergent vertical patterns.⁽⁴⁾ This technique principally addresses anterior crossbite and dental Class III malocclusion by proclining the maxillary incisors, retroclining the mandibular incisors, and notably extruding the maxillary molars.⁽⁸⁾ As a consequence of maxillary molar extrusion, a clockwise rotation of the mandible occurs, concurrently improving skeletal Class III severity and insufficient vertical height. This leads to enhanced facial aesthetics—specifically, a less protrusive chin and longer facial height. Despite our results regarding the dentoskeletal changes and abundant evidence demonstrating mandibular clockwise rotation in this type of Class III camouflage treatment,⁽⁵⁻⁸⁾ studies investigating changes in associated muscles remain limited, particularly the masticatory muscles that insert onto parts of the mandible and are potentially affected by this rotation.

To the best of our knowledge, no previous studies have comprehensively investigated the alterations of all masticatory muscles following orthodontic treatment, either in terms of morphological or functional changes. Most prior studies have focused solely on changes in the masseter muscles, with only a few mentioning alterations in the temporalis muscle.^(9,12-17) This focus is likely due to the superficial location of both muscles, making them easier to investigate. In regard to the assessment methods employed in previous studies, surface electromyography (sEMG), cone-beam computed tomography (CBCT), and ultrasonography were often utilized to investigate muscle activity and dimension owing to their acceptable accuracy, non-invasive nature, and ease of use. However, these methods are limited in their ability to precisely investigate deep-layer muscles such as the medial and lateral pterygoid muscles.

In contrast, MRI can provide accurate results in muscular dimension assessment, both in superficial and deep layers, in a non-invasive and non-radiation manner. Furthermore, MRI can visualize the overall size and shape of each muscle, clearly display its location relative to surrounding structures, and also enable 2D-slice sections throughout the entire thickness. Although supine position-

ing during MRI does not replicate the upright orientation of daily functional activities, this posture is standard for craniofacial imaging. Importantly, both T0 and T1 scans were performed under identical conditions and imaging protocols for each participant, allowing for reliable within-subject comparisons. Given MRI's superior soft tissue resolution and its ability to assess both deep and superficial muscles, we believe its advantages outweighed this limitation in the present study. Nonetheless, MRI remains contraindicated in patients with claustrophobia or implanted metal prostheses.⁽¹⁸⁻²¹⁾

Therefore, this study primarily aimed to evaluate the dimensional changes in all masticatory muscles following orthodontic treatment, particularly Class III camouflage treatment which alters the position of the mandible. The muscles of interest included the masseter muscles (inserting along angle of the mandible and lateral surface of the mandibular ramus), temporalis muscles (inserting to the coronoid process of the mandible), lateral pterygoid muscles (inserting to the TMJ capsule, TMJ articular disc, and neck of the condylar process of the mandible), and medial pterygoid muscles (inserting onto medial surface of the ramus and angle of the mandible). MRI was utilized in this study due to its advantages described previously.

With regard to our findings, significant changes in masticatory muscle dimensions were observed after undergoing this camouflage treatment for an average treatment duration of 10-15 months. In the group of jaw-closing muscles (i.e., masseter, temporalis, and medial pterygoid muscle), significant increases in length and decreases in thickness were noted following treatment, even in our participants who have hypo- to normo-divergent vertical pattern (mean FH-MP=22.4°). Referring to the decreases in CSA, they were likely associated with a significantly decreased thickness of these muscles. On the contrary, only minimal changes in muscle dimensions were noted in the lateral pterygoid muscle.

Compared to previous studies, our results align with the findings of Pan *et al.*,⁽⁹⁾ who investigated thickness changes in masseter muscles and surrounding soft tissues following full-fixed orthodontic treatment. They reported a significant decrease in masseter muscle thickness in both extraction and non-extraction groups. Similarly, our results correspond to those of Jokaji *et al.*,⁽¹⁶⁾ who observed smaller width and cross-sectional area (CSA) of the masseter muscle after preoperative orthodontic

treatment.

Furthermore, the correlation between reduction in muscle size and muscle activity has been reported in several studies.^(16,22) Also, certain studies have reported relationships between CSA of the masseter muscle and both maximum occlusal force and muscle activity.^(16,23,24) Based on these reports, the decreases in masticatory muscle dimensions and CSA observed in our results may be associated with decreased masticatory muscle activity and bite force. Therefore, it could be inferred that our findings are consistent with the results of many studies that investigated muscle activity following orthodontic treatment and indicated decreased muscle activity of the masseter and temporalis muscles after treatment.

The observed increases in muscle length and reductions in thickness and CSA in the masseter, temporalis, and medial pterygoid muscles may reflect adaptive remodeling in response to occlusal and biomechanical changes during treatment. These changes may be particularly advantageous for patients with hypo- or normo-divergent vertical patterns, by promoting better vertical control. In addition, reduced muscle thickness—particularly in the masseter—may enhance facial esthetics in patients with prominent jaw musculature and potentially support post-treatment stability by minimizing hyperactivity in overcompensating muscles.

These significant changes in the jaw-closing muscles may result from mandibular rotation. Clockwise rotation of the whole mandible leads to downward and backward movement of the mandibular angle and also downward and forward movement of the coronoid process. This movement may cause stretching of the jaw-closing muscles as their insertions move downward, leading to subsequent adaptation to compensate for increased length.⁽¹⁴⁾ Conversely, the minimal change observed in the lateral pterygoid muscle may be attributed to its insertion location near the condylar head, the fulcrum of mandibular rotation. As its insertion does not move as much as those of the other jaw-closing muscles, stretching or dimensional changes may be less pronounced.

Nevertheless, correlation analysis demonstrated no significant correlations between muscular dimensional changes (i.e., length and width) and vertical changes in skeletal relationship in this study. This may be due to limitations in sample size, as the study primarily aimed to detect differences in muscle dimensions between T0

and T1 rather than correlations with other variables. However, there appears to be a trend towards greater muscle dimensional changes in participants with larger clockwise mandibular rotation. With a larger sample size, this trend may have reached statistical significance. Further investigations are necessary to confirm this finding.

In addition to changes in vertical skeletal components, the use of posterior bite-ramps and alterations in eating behavior during orthodontic treatment may also affect muscle activity and dimension. Studies by Changsiripun & Pativetpinyo⁽²⁵⁾ and Antonarakis⁽²⁶⁾ have shown that bite-raising appliances can reduce masticatory muscle activity during maximum clenching in the short term, and may lead to a decrease in muscle thickness in the long term.⁽²⁷⁾ In our study, posterior bite ramps were used to facilitate anterior bite opening during Class III correction. These bite-raising appliances may reduce occlusal force transmission and clenching activity in the short term, potentially contributing to decreased muscle thickness. Although all patients received similar bite ramp designs, individual variation in neuromuscular response cannot be ruled out. Moreover, behavioral changes to avoid chewing hard or sticky foods during treatment may contribute to reduced muscle activity and subsequent muscle size diminution.⁽²⁸⁾

Beyond treatment-related factors and behavioral adaptations, underlying skeletal morphology may also play a role in influencing masticatory muscle dimensions. The composition of skeletal Class III discrepancy—whether predominantly due to maxillary deficiency, mandibular excess, or a combination—may potentially influence masticatory muscle morphology and response to treatment. Previous studies have shown associations between jaw muscle dimensions or activity and craniofacial factors such as vertical skeletal configuration, mandibular plane angle, gonial angle, mandibular length, Y-axis, facial width, lower anterior facial height, and facial asymmetry.^(29,30) In the present pilot study, we controlled for major craniofacial variables across the three primary planes (i.e., sagittal, vertical, and transverse) by including only subjects with skeletal Class III malocclusion, normo- or hypo-divergent vertical patterns, and symmetric face, to reduce baseline variability. However, future research with a larger cohort should explore the impact of specific skeletal components and additional morphological variables on muscle adaptation following camouflage treatment.

Building upon these considerations, future studies should aim to expand the sample size to enhance statistical power and improve the generalizability of results. To gain a more comprehensive understanding of masticatory muscle adaptation during Class III orthodontic treatment and to address the limitation of MRI in assessing muscle activity, MRI could be combined with complementary tools such as electromyography (EMG) or bite force measurements to evaluate both static muscle morphology and functional dynamics. Including a matched control group would also strengthen the study design; however, ethical concerns related to delaying necessary treatment for a control arm must be carefully addressed. Moreover, although we consider T1 to be a valid and clinically relevant time point to assess the effects of Class III treatment using Class III elastics mechanics, post-debonding (T2) or long-term follow-up assessments may provide further insights into the stability and permanence of these treatment-induced muscular changes.

Conclusions

Significant alterations in the jaw-closing muscles (i.e., masseter, temporalis, and medial pterygoid muscles) were observed following Class III camouflage treatment using Class III elastics, with these muscles exhibiting increased length along with decreased thickness and CSA. In contrast, the lateral pterygoid muscles showed only minimal and statistically insignificant dimensional changes. Furthermore, no significant correlations were found between the dimensional changes in these muscles and the vertical skeletal changes.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Stellzig-Eisenhauer A, Lux CJ, Schuster G. Treatment decision in adult patients with class III malocclusion: orthodontic therapy or orthognathic surgery?. *Am J Orthod Dentofac Orthop.* 2002;122(1):27–8.
2. Ngan P, Moon W. Evolution of class III treatment in orthodontics. *Am J Orthod Dentofac Orthop.* 2015;148(1):22–36.
3. Park JH, Yu J, Bullen R. Camouflage treatment of skeletal Class III malocclusion with conventional orthodontic therapy. *Am J Orthod Dentofac Orthop.* 2017;151(4):804–11.
4. Sakoda KL, Pinzan A, Cury SEN, Bellini-Pereira S, Castillo AA-D, Janson G. Class III malocclusion camouflage treatment in adults: a systematic review. *J Dent Open Access.* 2019;1(1):1–12.
5. de Alba y Levy JA, Chaconas SJ, Caputo AA. Effects of orthodontic intermaxillary class III mechanics on craniofacial structures. part II - computerized cephalometrics. *Angle Orthod.* 1979;49(1):29–36.
6. Hisano M, Chung C ryung J, Soma K. Nonsurgical correction of skeletal class III malocclusion with lateral shift in an adult. *Am J Orthod Dentofac Orthop.* 2007;131(6):797–804.
7. Burns NR, Musich DR, Martin C, Razmus T, Gunel E, Ngan P. Class III camouflage treatment: what are the limits?. *Am J Orthod Dentofac Orthop.* 2010;137(1):9.e1–9.e13.
8. Nakamura M, Kawanabe N, Kataoka T, Murakami T, Yamashiro T, Kamioka H. Comparative evaluation of treatment outcomes between temporary anchorage devices and class III elastics in class III malocclusions. *Am J Orthod Dentofac Orthop.* 2017;151(6):1116–24.
9. Pan Y, Chen S, Shen L, Pei Y, Zhang Y, Xu T. Thickness change of masseter muscles and the surrounding soft tissues in female patients during orthodontic treatment: a retrospective study. *BMC Oral Health.* 2020;20(1):1–10.
10. Suchato WCJ. Cephalometric evaluation of the dentofacial complex of Thai adults. *J Dent Assoc Thai.* 1984;34(5):233–43.
11. Sutthiprapaporn P, Manosudprasit A, Manosudprasit M, Pisek P, Phaoseree N, Manosudprasit A. Establishing esthetic lateral cephalometric values for Thai adults after orthodontic treatment. *Khon Kaen Dent J.* 2020;23(2):31–41.
12. Uslu O, Arat ZM, Beyazoya M, Taskiran OO. Muscular response to functional treatment of skeletal open-bite and deep-bite cases: an electromyographic study. *World J Orthod.* 2010;11(4):85–94.
13. Piancino MG, Falla D, Merlo A, Vallelonga T, De Biase C, Dalessandri D, *et al.* Effects of therapy on masseter activity and chewing kinematics in patients with unilateral posterior crossbite. *Arch Oral Biol.* 2016;67:61–7.
14. Lione R, Kiliaridis S, Noviello A, Franchi L, Antonarakis GS, Cozza P. Evaluation of masseter muscles in relation to treatment with removable bite-blocks in dolichofacial growing subjects: a prospective controlled study. *Am J Orthod Dentofac Orthop.* 2017;151(6):1058–64.
15. Paes-Souza S de A, Garcia MAC, Souza VH, Morais LS, Nojima LI, Nojima M da CG. Response of masticatory muscles to treatment with orthodontic aligners: a preliminary prospective longitudinal study. *Dental Press J Orthod.*

- 2023;28(1):1-26.
16. Jokaji R, Ooi K, Yahata T, Nakade Y, Kawashiri S. Evaluation of factors related to morphological masseter muscle changes after preoperative orthodontic treatment in female patients with skeletal class III dentofacial deformities. *BMC Oral Health*. 2022;22(1):1-7.
 17. Zanon G, Contardo L, Reda B. The impact of orthodontic treatment on masticatory performance: a literature review. *Cureus*. 2022;14(10):e30453.
 18. Narici M. Human skeletal muscle architecture studied *in vivo* by non-invasive imaging techniques: functional significance and applications. *J Electromyogr Kinesiol*. 1999;9(2):97-103.
 19. Katti G, Ara SA, Shireen A. Magnetic resonance imaging (MRI)—a review. *Int J Dent Clin*. 2011;3(1):65-70.
 20. Amabile C, Moal B, Chtara OA, Pillet H, Raya JG, Iannessi A, *et al*. Estimation of spinopelvic muscles' volumes in young asymptomatic subjects: a quantitative analysis. *Surg Radiol Anat*. 2017;39(4):393-403.
 21. Murakami M, Iijima K, Watanabe Y, Tanaka T, Iwasa Y, Edahiro A, *et al*. Development of a simple method to measure masseter muscle mass. *Gerodontology*. 2020;37(4):383-8.
 22. Georgiakaki I, Tortopidis D, Garefis P, Kiliaridis S. Ultrasonographic thickness and electromyographic activity of masseter muscle of human females. *J Oral Rehabil*. 2007;34(2):121-8.
 23. Celakil D, Ozdemir F, Eraydin F, Celakil T. Effect of orthognathic surgery on masticatory performance and muscle activity in skeletal class III patients. *Cranio*. 2018;36(3):174-80.
 24. Spronsen PH van, Weijs WA, Valk J, Prahl-Andersen B, Ginkel FC van. Relationships between jaw muscle cross-sections and craniofacial morphology in normal adults, studied with magnetic resonance imaging. *Eur J Orthod*. 1991;13(5):351-61.
 25. Changsiripun C, Pativetpinyo D. Masticatory function after bite-raising with light-cured orthodontic band cement in healthy adults. *Angle Orthod*. 2020;90(2):263-8.
 26. Antonarakis GS, Kiliaridis S. Predictive value of masseter muscle thickness and bite force on class II functional appliance treatment: a prospective controlled study. *Eur J Orthod*. 2015;37(6):570-7.
 27. Wasinwasukul P, Thongudomporn U. Masticatory muscle responses to orthodontic bite-raising appliances. *J Dent Assoc Thai*. 2022;72(3):427-33.
 28. Carter LA, Geldenhuys M, Moynihan PJ, Slater DR, Exley CE, Rolland SL. The impact of orthodontic appliances on eating - young people's views and experiences. *J Orthod*. 2015;42(2):114-22.
 29. Weijs WA, Hillen B. Correlations between the cross-sectional area of the jaw muscles and craniofacial size and shape. *Am J Phys Anthropol*. 1986;70(4):423-31.
 30. Togninalli D, Antonarakis GS, Papadopoulou AK. Relationship between craniofacial skeletal patterns and anatomic characteristics of masticatory muscles: a systematic review and meta-analysis. *Prog Orthod*. 2024;25(1):36.



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Development and Evaluation of Satisfaction with an Innovative Dental Home Visit Care Kit for Enhancing Oral Health Care in Dependent Older Adults

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Abstract

Objectives: This study aimed to develop and evaluate the satisfaction of an innovative dental home visit care kit from the perspectives of both dentists and dental public health professionals.

Methods: The development of an innovative dental home visit care kit—which contains essential dental tools and educational aids organized in a lightweight, water-resistant bag designed for ergonomic home use—was rigorously evaluated by a panel of three experts using the Index of Item-Objective Congruence (IOC). A total of 38 participants assessed the kit's functionality, convenience, durability, and safety through a satisfaction questionnaire after using it to provide oral health care to dependent older adults during home visits.

Results: The kit achieved a high IOC score of 0.85. Among 38 participants (86.84% female, 68.42% aged 20–30 years), equal numbers were dentists and dental public health professionals. High satisfaction scores were reported for functionality (4.74/5, 94.74%) and convenience (4.59/5, 91.21%), with slightly lower scores for durability (4.20/5, 84.07%) and safety (4.40/5, 88.55%). No significant differences ($p > 0.05$) were found between the two professional groups.

Conclusions: The kit effectively supports dental professionals in delivering home-based oral care to dependent older adults. Its adaptable design enhances usability across diverse community settings.

Keywords: dental home visit care kit, dependent older adults, innovative, satisfaction

Introduction

The increasing number of older adults is a significant global and national trend. According to the United Nations, the global population aged 65 years and above is projected to nearly double by 2050, reaching approximately 1.5 billion, up from 727 million in 2020.⁽¹⁾ In Thailand, the Institute for Population and Social Research, Mahidol University, reported that the country is transitioning into a fully aged society. By 2030, it is estimated that individuals aged 60 years and above will account for 30% of the total population.⁽²⁾ This demographic shift necessitates more comprehensive healthcare services, especially for those with dependency and limitations in daily activities. These challenges often hinder access to healthcare services, including oral health care, which is frequently neglected due to difficulties in traveling to healthcare facilities.

Comprehensive oral health care at home for dependent older adults can mitigate significant health issues, such as respiratory infections, and reduce mortality rates. Notably, individuals receiving continuous oral health care have lower mortality rates than those without such care.^(3,4) Oral health care for dependent older adults is critically important, as mobility limitations increase the accumulation of dental plaque and oral bacteria, leading to a heightened risk of respiratory infections such as aspiration pneumonia.⁽⁵⁾ Studies have shown that home-based oral health care not only reduces infection risks but also alleviates disease burdens, thereby improving the quality of life for older adults effectively.

In Sukhothai Province, the growing number of older adults aligns with national trends. According to the Department of Provincial Administration, older adults in Sukhothai account for over 20% of the total population, signaling a transition to a fully aged society.⁽⁶⁾ Among this group, dependent older adults with mobility limitations often face barriers to accessing healthcare services. In response, providing dental services at home or within communities has become a vital strategy to enhance oral health and quality of life for these individuals.⁽⁷⁾ Delivering oral health care to remote and underserved populations remains a challenge, particularly for dependent older adults who cannot visit healthcare facilities. Deploying home care teams equipped with comprehensive an innovative dental home visit care kit is a crucial strategy to increase access to oral health services. However,

current dental home visit care kits have several limitations, particularly in equipment organization and ease of use. Many kits lack an intuitive, well-organized structure, making it difficult for dental professionals to quickly locate and access necessary tools during home visits. This inefficiency can lead to delays, prolonging visits and potentially compromising care quality. Additionally, many kits are not user-friendly, with poor ergonomics and unclear labeling, creating unnecessary challenges for dental professionals providing care to dependent older adults in non-clinical settings.

The evaluation of satisfaction with the use of the innovative dental home visit care kit is a critical step in the development of oral health care innovations, aimed at ensuring the effectiveness and relevance of the kit in meeting the needs of its users. This study focuses on analyzing and assessing user satisfaction to gather insights that will inform future improvements and ensure the kit is well-suited for practical use. The development of this specialized innovative dental home visit care kit, tailored to the needs of dental professionals, not only enhances service efficiency by reducing the workload of preparing equipment and minimizing the risk of missing essential tools, but it also addresses the specific needs of dependent older adults and individuals in remote areas who face access barriers to oral health services. This innovation promotes equity in access to dental care and contributes to the overall improvement of quality of life for older adults in society.

Materials and Methods

This study used a descriptive research design to develop an innovative dental home visit care kit and assess user satisfaction with the innovation. Ethical approval for the study was obtained from the Human Research Ethics Committee of the Sukhothai Provincial Public Health Office, Thailand, under approval COA No. 101/2024 and IRB No. 14/2025.

Study population and sample

The study population consisted of 128 dental personnel from Sukhothai Province, including dentists and dental public health professionals. The sample size was determined using Fisher's formula, resulting in 38 participants. To ensure an unbiased selection process, simple random sampling was conducted using the lottery method. All participants provided written informed con-

sent and were informed that they would use the innovative dental home visit care kit during one home visit with a dependent older adult, followed by completion of a satisfaction questionnaire. The participant recruitment and retention process is illustrated in Figure 1.

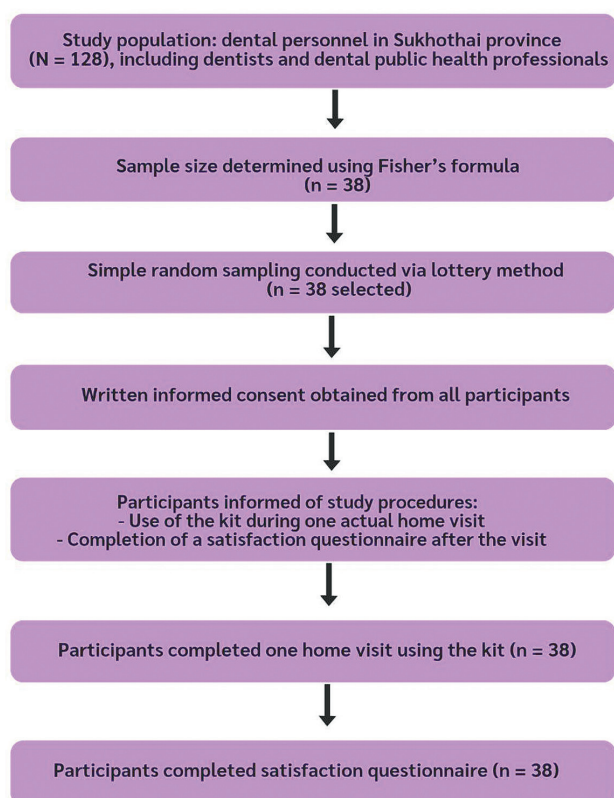


Figure 1: Flowchart of participant recruitment and retention process.

Description of developing an innovative dental home visit care kit

The innovative dental home visit care kit was developed to meet the specific needs of dependent older adults who are unable to access dental care at hospitals. The kit is housed in a bag measuring 6x12x8 inches, designed to be lightweight, portable, and highly durable. Made from premium synthetic leather, it weighs 1.2 kilograms and features a water-resistant finish for added protection and longevity. The smooth surface enhances its sophisticated appearance while ensuring ease of maintenance. For ease of transport, the bag is equipped with an integrated shoulder strap, securely stitched for added durability. The front features a flap-opening design, providing convenient access to its spacious interior. The generous storage space is designed to accommodate medical supplies and various essential tools, ensuring that

all items are neatly organized and easily accessible. The kit is equipped with all the necessary tools and materials for comprehensive dental care.

The contents of the bag include:

- Basic examination tools: Mouth mirrors, explorers no.5, and forceps.
- Protective gear: Gloves, surgical masks, hair caps.
- Oral hygiene supplies: Toothbrushes, toothpaste, fluoride varnish, chlorhexidine mouthwash.
- Treatment tools: Hand scaling instruments, cotton rolls, gauze, and topical anesthetics.
- Teaching aids: Tooth brushing model and instructional materials.
- Additional supplies: Flashlights, alcohol gel, lubricants, dental wax, plaque disclosing agents, syringes, waste disposal bags.

The innovative dental home visit care kit features an inventory checklist to assist with supply management and ensure preparedness for each home visit. Its design prioritizes ease of use, enabling dental personnel to deliver care efficiently.

Validation process and expert feedback

The innovative dental home visit care kit underwent a validation process conducted by a panel of three experts, including two specialists in geriatric dentistry and one family medicine physician. The evaluation utilized the Index of Item-Objective Congruence (IOC) method to assess the kit's design, practicality, and suitability for dependent older adults. Each component was systematically reviewed to ensure alignment with the intended objectives and its effectiveness in facilitating home-based dental care. The experts rated the relevance and appropriateness of each item based on predefined criteria, providing a quantitative measure of the kit's overall quality. The resulting IOC score of 0.85 reflects a high level of agreement among the experts, confirming the kit's validity, usability, and potential for successful implementation in real-world home care settings.

Development of the satisfaction questionnaire for evaluating the innovative dental home visit care kit

A structured process was employed to develop and validate a questionnaire assessing satisfaction with the use of the innovative dental home visit care kit. The steps included:

1. Literature review and conceptual framework development

The satisfaction questionnaire was designed based on the framework proposed by Suksod T⁽⁸⁾, which was adapted for this study. The assessment focused on four key dimensions: 1) Function: utility and functionality of the bag. 2) Convenience: ease of use and comfort. 3) Durability: structural durability and quality. 4) Safety: risk minimization and user protection. Questions for each dimension were developed according to this framework.

2. Content validity testing

The initial version of the questionnaire was reviewed by three experts to evaluate its content validity. These experts, including specialists in geriatric dentistry and dental public health, examined each item to ensure its relevance and alignment with the intended objectives of the study. The Index of Item-Objective Congruence (IOC) was then calculated to quantify the degree of alignment, with an IOC score of 0.80, indicating satisfactory content validity. This score reflects a strong consensus among the experts regarding the appropriateness and relevance of the questionnaire items for assessing the effectiveness of the innovative dental home visit care kit.

3. Revisions and refinements

Based on expert feedback, the questionnaire was revised to improve clarity and relevance.

Implementation and data collection

The finalized questionnaire was self-administered and distributed to participants after they had used the innovative dental home visit care kit during a home visit to provide oral health services to dependent older adults. Participants were instructed to independently assess their satisfaction based on their actual experience. Standardized written instructions were provided, and the principal investigator was available to clarify any issues. This approach was intended to minimize interviewer bias and enhance the objectivity of the responses.

Data analysis

The demographic characteristics of the sample were summarized as percentages, while satisfaction questionnaire results were analyzed item-by-item and presented as percentages to highlight trends and overall satisfaction with the innovative dental home visit care kit. Satisfaction mean scores were interpreted based on Srisawat B⁽⁹⁾, categorizing satisfaction into five levels: very high (4.51-5.00), high (3.51-4.50), moderate (2.51-3.50), low (1.51-2.50), and very low (1.00-1.50), ensuring a standardized and systematic assessment. Higher scores indi-

cated greater approval, while lower scores suggested areas for improvement. The Mann-Whitney U test, suitable for ordinal data, was used to compare satisfaction levels between dentists and dental public health professionals, with statistical significance set at $p < 0.05$. Additionally, interaction tables explored the relationships between participant characteristics and satisfaction scores, providing insights into how factors such as professional experience and role influenced perceptions of the kit's usability and effectiveness. All analyses were conducted using SPSS version 17 for reliable statistical interpretation.

Results

The development of the innovative dental home visit care kit received a score of 0.85 on the Index of Item-Objective Congruence (IOC), indicating a high level of agreement among experts regarding its relevance, usability, and effectiveness in addressing the needs of dependent older adults. A figure has been included (Figure 2) to present the actual kit and its components for clearer visualization of the innovation. The figure illustrates the kit's compact, water-resistant, and ergonomic design, enhancing portability and efficiency over conventional box-based kits. It also highlights a systematically organized set of essential tools that facilitate comprehensive and effective oral care during home visits.

The general information of the 38 participants reveals that the majority were female (86.84%), with the largest age group being 20-30 years (68.42%). Participants were evenly distributed between dentists and dental public health professionals, each accounting for 50.0% of the sample. Regarding education level, most participants held a bachelor's degree (86.84%), while a smaller proportion had a diploma (10.53%) or a master's degree (2.63%). Notably, none of the participants reported holding a doctorate or a professional certification/diploma (Table 1).

The evaluation of the innovative dental home visit care kit was conducted with 38 participants, including dentists and dental public health professionals. Based on the feedback, the kit demonstrated exceptional effectiveness in all assessed areas, achieving a high overall satisfaction score of 4.48 (89.49%), as shown in Table 2. In terms of functionality, the highest satisfaction scores were observed in the usability of dental tools (95.79%) and their suitability for patients' oral conditions (95.26%), indicating that the kit is well-suited for clinical purposes

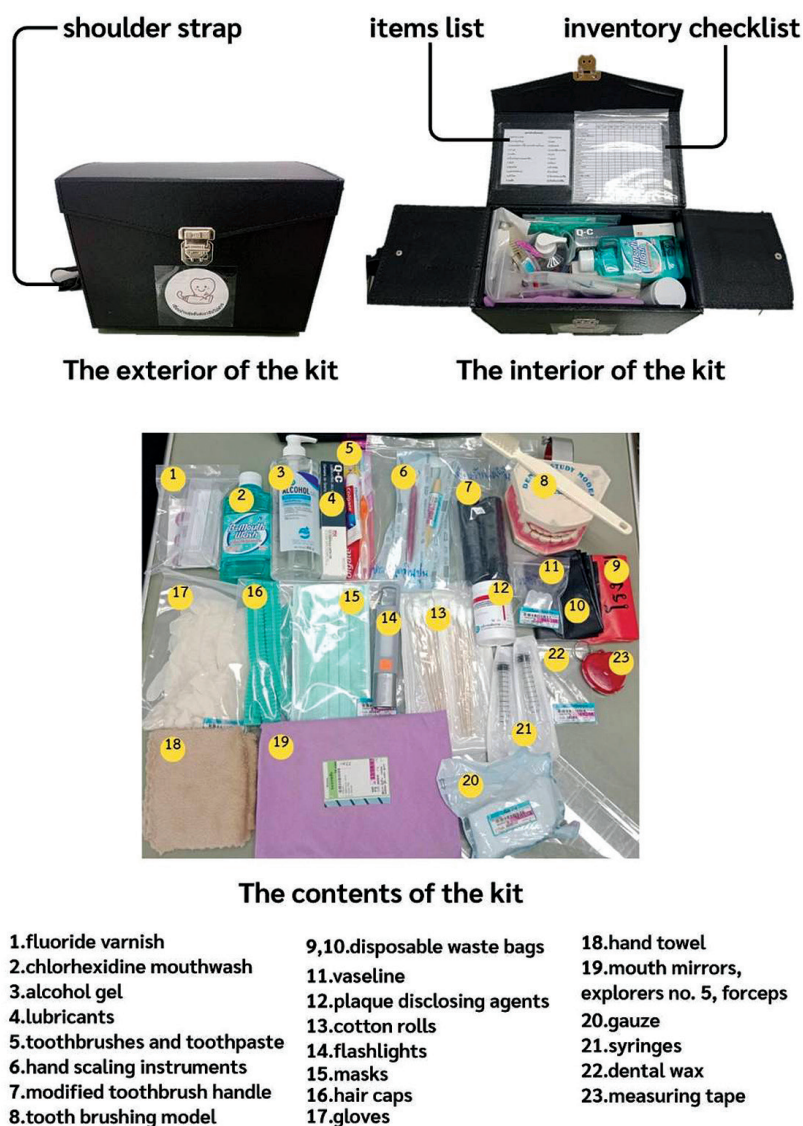


Figure 2: The innovative dental home visit care kit and its components.

Table 1: General information (n=38).

Category	Sub-category	n	Percent
Gender	Female	33	86.84
	Male	5	13.16
Age	20-30 years	26	68.42
	31-40 years	7	18.42
	41-50 years	1	2.63
	51 years and above	4	10.53
Occupation	Dentist	19	50.0
	Dental public health professionals	19	50.0
Education Level	Diploma	4	10.53
	Bachelor's degree	33	86.84
	Master's degree	1	2.63
	Doctorate	0	0
	Diploma	0	0

and tailored to the needs of dependent older adults. For convenience, while the kit was rated very highly for easy and straightforward usage (93.16%), slight improvements could be made in its portability (91.47%) and organized layout for tool access (90.08%). Regarding durability, participants emphasized the quality of materials (85.20%) and structural strength (85.00%), but noted that material cost (82.00%) could be optimized further. In terms of safety, secure closure (92.40%) and regular inspection of tools and bag condition (89.21%) were rated highly, ensuring the kit is reliable and safe to use. Additionally, the cleanliness of the bag received a satisfaction score of 85.20%, further supporting its suitability for professional dental home visits.

The satisfaction scores regarding the innovative dental home visit care kit were evaluated by two groups: dentists and dental public health professionals. The study assessed four key aspects: functionality, convenience, durability, and safety. In terms of functionality, both groups rated this aspect highly, with mean scores of 4.65 for dentists and 4.82 for dental public health professionals. The *p*-value (0.347) indicates no statistically significant difference. For convenience, dental public health professionals rated this aspect slightly higher ($\bar{x}=4.63$) compared to dentists ($\bar{x}=4.46$), but the difference was not statistically significant (*p*=0.212). Regarding durability, the scores were comparable, with means of 4.49 for dentists and 4.61 for dental public health professionals (*p*=0.610). In terms of safety, this aspect received high ratings from both groups, with no significant difference (*p*=0.386). The analysis, performed using the Mann-Whitney U-test, revealed no statistically significant differences (*p*>0.05) between the two groups across all aspects, suggesting similar levels of satisfaction with the innovative dental home visit care kit. The comparison of satisfaction scores between dentists and dental public health professionals is presented in Table 3.

The assessment evaluated four key aspects—functionality, convenience, durability, and safety—using a 4-point Likert scale (4=most important, 1=least important). The results revealed that functionality was the most highly rated aspect, with most evaluators considering it "Most Important" (52.63%) or "Very Important" (42.11%), resulting in the highest mean score ($\bar{x}=3.47$). This indicates strong satisfaction and alignment with user needs. Convenience was also highly valued, with over

90% of participants rating it positively, including "Most Important" (36.84%) and "Very Important" (55.26%), leading to a high mean score ($\bar{x}=3.29$), emphasizing its practical value during dental home visits. In contrast, durability received the lowest rating, with 71.05% marking it as "Least Important", leading to the lowest mean score ($\bar{x}=1.32$), suggesting concerns about the kit's robustness. Safety ratings were mixed, with "Moderately Important" (60.53%) being the most common response, followed by "Least Important" (28.95%), and a mean score of $\bar{x}=1.92$, indicating the need for improvements in this area. Overall, the findings highlight functionality and convenience as the most valued aspects, reinforcing the kit's effectiveness and ease of use in practice. However, the relatively lower scores for durability and safety suggest areas that require further development to enhance user confidence and ensure the long-term reliability of the kit.

Participants recommended adding designated compartments for tools to improve organization and accessibility. Including educational materials, such as educational pamphlets or guides, and items like dental floss and a mouth gag was suggested to enhance the kit's utility and comprehensiveness for home dental care.

Discussion

The development of the innovative dental home visit care kit received a score of 0.85 on the Index of Item-Objective Congruence (IOC), indicating strong expert agreement on its relevance, usability, and effectiveness in meeting the needs of dependent older adults. This study aligns with other research highlighting the importance of tailored dental care solutions for this population, which is projected to experience a growing number of dependent older adults requiring home-based care from dental professionals.⁽¹⁰⁻¹⁸⁾ This study aligns with the World Health Organization (WHO) guidelines for healthcare innovation, particularly in the initial phase. It addresses two core strategies: collecting user feedback from dental professionals through actual use and satisfaction questionnaires and developing expert-validated protocols to ensure consistent and safe implementation. To enhance public health integration and long-term impact, further efforts should focus on standardization, wider regional distribution, and the establishment of measurable indicators to assess oral health outcomes and guide future improvements.⁽¹⁹⁾ The demographic profile of the 38 participants

Table 2: Evaluation of the innovative dental home visit care kit.

Aspect	Evaluation criteria	Mean	S.D.	% Satisfaction	Assessment level
Functionality	1. Usability of dental tools	4.79	0.41	95.79	Very high
	2. Suitability for patients' oral conditions	4.76	0.43	95.26	Very high
	3. Adequate storage for intended tools	4.66	0.57	93.16	Very high
Convenience	1. Easy and straightforward usage	4.66	0.57	93.16	Very high
	2. Lightweight and portable for home visits	4.57	0.64	91.47	Very high
	3. Organized layout for easy access to tools	4.54	0.64	90.08	Very high
Durability	1. Quality of materials used	4.26	0.49	85.20	High
	2. Structural strength and durability	4.25	0.54	85.00	High
	3. Reasonable material cost	4.10	0.71	82.00	High
Safety	1. Secure closure to prevent item loss	4.62	0.39	92.40	Very high
	2. Tools are in working condition without damage	4.27	0.43	85.40	High
	3. Regular inspection of tools and bag condition	4.46	0.46	89.21	Very high
	4. Cleanliness of the bag	4.26	0.48	85.20	High
Overall satisfaction		4.48	0.52	89.49	High

Table 3: Comparison of satisfaction scores on the innovative dental home visit care kit between dentists and dental public health professionals.

	n=19		n=19		<i>p</i> -value
	Dentist		Dental public health professional		
	$\bar{x}$	SD	$\bar{x}$	SD	
Functionality	4.65	.53	4.82	.38	.347
Convenience	4.46	.70	4.63	.49	.212
Durability	4.49	.56	4.61	.57	.610
Safety	4.70	.44	4.78	.41	.386

* Mann-Whitney U test, $p < 0.05$

Table 4: Evaluation of the innovative dental home visit care kit.

Aspect	Rating (Score)								$\bar{x}$
	4		3		2		1		
	n	%	n	%	n	%	n	%	
Functionality	20	52.63	16	42.11	2	5.26	0	0	3.47
Convenience	14	36.84	21	55.26	3	7.89	0	0	3.29
Durability	0	0	1	2.63	10	26.32	27	71.05	1.32
Safety	4	10.53	0	0	23	60.53	11	28.95	1.92

Rating scale: 4=most important, 3=very important, 2=moderately important, 1=least important

showed that the majority were young (20-30 years old) and female, with most holding a bachelor's degree. This distribution reflects a common demographic in the dental profession, where younger professionals are entering the field with a foundational education level suitable for initial practice.

Functionality was rated the highest among the four aspects assessed, with both dentists and dental public health professionals expressing high levels of satisfac-

tion. The majority found the tools in the kit well-suited to clinical needs and practical for use in home settings. Convenience was also highly rated, with over 90% of participants indicating positive experiences in terms of ease of use and portability. However, slight modifications were suggested regarding the organization of tools to further enhance accessibility during home visits. The results highlight functionality as the most positively evaluated aspect of the kit, consistent with previous

research underscoring the significance of practical and efficient tools in enhancing home dental care.⁽²⁰⁾ Both dentists and dental public health professionals found the tools appropriate for clinical needs and effective for use in home environments. The high ratings for convenience further validate the kit's portability and ease of use, attributes crucial for delivering home-based care.⁽²¹⁾ Durability, while crucial for long-term use, received the lowest ratings among the aspects assessed. Participants noted concerns about material quality and the structural integrity of the kit, reflecting a significant challenge in maintaining reliable long-term performance. Safety was identified as moderately important, with a need for further improvements to ensure safe use, especially in home settings. This aligns with prior research that stresses the importance of ensuring ergonomic design and minimizing risks associated with the use of dental equipment in non-clinical environments.⁽²²⁾ For example, Steele and Walls (1997) emphasized that safe and user-friendly equipment is essential for the effective delivery of oral care to frail and dependent older adults.⁽¹⁰⁾

The Mann-Whitney U-test revealed no statistically significant differences between dentists and dental public health professionals in their satisfaction with the kit across all aspects. This suggests a general consensus among professionals, regardless of their specific roles or backgrounds. The lack of significant differences may indicate that the innovative dental home visit care kit is equally effective and satisfactory for both groups, highlighting its broad applicability and suitability for a diverse range of dental care providers. This consistency in satisfaction could reflect the kit's user-friendly design and functionality, which meets the needs of both dentists and public health officers involved in home-based care for dependent older adults.

The study highlighted the effectiveness of the innovative dental home visit care kit in delivering comprehensive oral care. Participants praised the kit for its practicality, ease of use, and adaptability to the specific needs of dependent older adults. These findings underscore the essential role that home-based dental visits play in maintaining oral hygiene for older adults, particularly those who rely on others for daily care. The effectiveness of such innovative dental home visit care kit in providing thorough and accessible oral care has been further supported by several studies, which emphasize their value

in improving oral health outcomes for elderly populations, especially those with limited mobility or access to traditional dental services. For instance, Niesten *et al.*,⁽¹¹⁾ conducted a qualitative study that explored the impact of frailty on oral care behaviors among older adults, emphasizing the urgent need for practical, tailored solutions for this vulnerable demographic. Similarly, Henni *et al.*,⁽¹²⁾ performed a scoping review on oral health and quality of life among older adults receiving home health care services, highlighting the crucial role of accessible dental care interventions in maintaining oral hygiene within this population. Together, these studies reinforce the essential role of innovative dental home visit care kit in improving oral health outcomes for dependent older adults, who face significant challenges in accessing traditional dental care.

This study revealed that the innovative dental home visit care kit received a high score for functionality, indicating that it was highly effective and user-friendly for dental professionals during home visits. The participants reported that the kit's design and organization made it easy to locate and use the necessary tools, contributing to a smoother, more efficient delivery of care. The high functionality score suggests that the kit meets the practical needs of dental professionals, allowing them to provide quality oral health services to dependent older adults in a home setting with minimal disruption. Previous research has demonstrated that portable dental equipment can significantly improve access to oral care for elderly populations, particularly those with mobility limitations or who are homebound. For instance, a study by Rahman *et al.*,⁽²³⁾ explored dentists' perceptions of using portable dental units in community outreach programs, emphasizing their utility in delivering care to underserved populations, including the elderly. The study found that portable units allowed dental professionals to offer essential dental services in non-clinical settings, helping to overcome barriers such as transportation issues and physical limitations. Additionally, Charlton (2007) emphasized that portable dental equipment should prioritize mobility, ease of use, durability, and suitability for older adults, particularly in space-limited settings.⁽²⁴⁾ Consistent with these principles, the innovative dental home visit care kit—featuring a compact, ergonomic, and water-resistant design—offered greater portability and efficiency than conventional box-based kits. Its organized layout facilitated faster setup and tool access, enabling more effective

tive care delivery during home visits. These advantages demonstrate the kit's alignment with Charlton's criteria for effective portable dental solutions. Recommendations from study participants included the need for designated compartments to enhance tool organization, the addition of educational materials on oral health care, and the inclusion of dental floss and a mouth gag for more comprehensive care. These recommendations align with previous research that emphasizes practical improvements in dental kits to support patient education and the comprehensive care of dependent older adults. This discussion underscores the importance of refining innovative dental home visit care kit to better meet the needs of dental professionals and patients in community settings. The study's findings provide critical insights into the design and usability of dental care kits, emphasizing the need for material improvements, particularly in durability and safety, to enhance the overall effectiveness of dental care in home settings.

The innovative dental home visit care kit demonstrated practical effectiveness by expanding access to dental services among dependent older adults. In 2025, the year of its implementation, service coverage increased to 76.58%, compared to 32.04% in 2024, 26.92% in 2023, and 58.21% in 2022, according to the Sukhothai Health Data Center.⁽²⁵⁾ This upward trend highlights the kit's contribution to improving outreach and service equity. Beyond high user satisfaction, the kit enhanced operational efficiency by enabling more streamlined and timely care, allowing dental personnel to serve more patients during home visits. For national integration, key challenges must be addressed, including securing sustainable funding, standardizing training and protocols, and establishing robust monitoring and evaluation systems. Policy-level support and collaboration with primary care teams and community health volunteers are also critical for embedding the kit into routine oral health services. Addressing these factors is essential for maximizing public health benefits and ensuring long-term sustainability.

In addition, the potential cost-effectiveness of the innovative dental home visit care kit is noteworthy. Compared to conventional box-based kits often used in field visits, which are typically compartmentalized but less adaptable, this innovative kit—although slightly more costly in initial production due to its ergonomic, water-resistant design and structured layout—offers greater

long-term value. It improves efficiency, reduces preparation time, and enhances tool accessibility, allowing dental professionals to provide care more quickly and serve more households per visit.

The innovative dental home visit care kit developed in this study illustrates the practical application of tailored solutions for community-based care. Its adaptability to different contexts allows it to effectively meet the unique needs of elderly patients in home settings. The primary benefit of this kit is its ability to improve oral health for dependent older adults, thereby enhancing their overall quality of life. By enabling dental professionals to provide targeted, practical care in a familiar environment, the kit helps reduce barriers to accessing essential oral health services, leading to improved outcomes for dependent older adults.

The limitation of the study includes a small sample size, which may limit the generalizability of the findings. Additionally, the study assessed only user satisfaction without evaluating direct outcomes in patients' oral health, thereby restricting conclusions about clinical effectiveness. Most participants were aged 20-30 years, limiting age diversity and potentially introducing bias or type I error in comparative analyses. Future research should examine long-term clinical outcomes, include more diverse participant demographics, and explore the kit's integration into broader community-based oral health systems to support evidence-based improvements for dependent older adults.

Conclusions

This study demonstrates the effectiveness of the innovative dental home visit care kit in meeting the needs of dentists and dental public health professionals in caring for dependent older adults. It highlights the importance of adapting the kit to diverse community contexts and improving material quality for safety and durability. The findings provide valuable insights for optimizing dental care kits, emphasizing the need for continuous innovation to support high-quality, personalized care.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. United Nations. World population ageing 2020 highlights. New York: United Nations; 2020.
2. Institute for Population and Social Research, Mahidol University. Thailand population projections for the period 2010–2040. Nakhon Pathom: Mahidol University; 2020.
3. Quagliarello VJ, Ginter S, Han L, Van Ness PH, Allore HG, Tinetti ME. Modifiable risk factors for nursing home-acquired pneumonia. *Clin Infect Dis*. 2005;40(1):1-6.
4. Haumschild MS, Haumschild RJ. The importance of oral health in long-term care. *J Am Med Dir Assoc*. 2009;10(9):667-71.
5. Yoneyama T, Yoshida M, Matsui T, Sasaki H. Oral care and pneumonia. *Lancet*. 1999;354(9177):515.
6. Department of Provincial Administration. Population statistics and demographic changes. Bangkok: Ministry of Interior; 2020.
7. Department of Health. Oral health care manual for dependent older adults. Bangkok: Ministry of Public Health; 2020.
8. Suksod T. Industrial product design. Bangkok: Odeon Store; 2001.
9. Srisawat B. Fundamental research. Bangkok: Suweeriyasarn; 2010.
10. Steele JG, Walls AWG. Strategies to improve the quality of oral health care for frail and dependent older people. *Qual Health Care*. 1997;6(3):165-9.
11. Niesten D, van Mourik K, van der Sanden W. The impact of frailty on oral care behavior of older people: a qualitative study. *BMC Oral Health*. 2013;13:61.
12. Henni SH, Skudutyte-Rysstad R, Ansteinsson V, Hellesø R, Hovden EAS. Oral health and oral health-related quality of life among older adults receiving home health care services: a scoping review. *Gerodontology*. 2023;40(2):161-71.
13. Baumgartner W, Schimmel M, Müller F. Oral health and dental care of elderly adults dependent on care. *Swiss Dent J*. 2015;125(4):417-26.
14. Saintrain MVDL, Saintrain SV, Sampaio EGM, Ferreira BSP, Nepomuceno TC, Frota MA, *et al*. Older adults' dependence in activities of daily living: implications for oral health. *Public Health Nurs*. 2018;35(6):473-81.
15. Niesten D, Witter DJ, Bronkhorst EM, Creugers NH. Oral health care behavior and frailty-related factors in a care-dependent older population. *J Dent*. 2017;61:39-47.
16. Smith MB, Thomson WM. 'Not on the radar': dentists' perspectives on the oral health care of dependent older people. *Gerodontology*. 2017;34(1):90-100.
17. Burr JA, Lee HJ. Social relationships and dental care service utilization among older adults. *J Aging Health*. 2013;25(2):191-220.
18. Strayer MS. Dental health among homebound elderly. *J Public Health Dent*. 1993;53(1):12-6.
19. World Health Organization. Practical guidance for scaling up health service innovations [Internet]. Geneva: World Health Organization; 2009 [cited 2025 May 2]. Available from: https://apps.who.int/iris/bitstream/handle/10665/44180/9789241598521_eng.pdf
20. Ettinger RL, Beck JD. Geriatric dental curriculum and the needs of the elderly. *Spec Care Dentist*. 1984;4(5):207-13.
21. Friedman PK, Lamster IB. Tooth loss as a predictor of shortened lifespan: exploring the hypothesis. *Periodontol* 2000. 2016;72(1):142-52.
22. Anusavice KJ, Shen C, Rawls HR, editors. Phillips' science of dental materials. 12th ed. St. Louis: Elsevier Health Sciences; 2012.
23. Nilchian F, Sahlabadi A, Skini M. Portable dental chairs and their role in assisting the community outreach program—a qualitative approach. *J Dent (Tehran)*. 2013;10(3):233-9.
24. Charlton DG, Ehrlich AD, Miniotti NJ. Current update on portable dental equipment. *Compend Contin Educ Dent*. 2007;28(2):104-8.
25. Sukhothai Health Data Center. Percentage of homebound and bedridden older adults receiving oral health examinations [Internet]. Sukhothai: Sukhothai Provincial Public Health Office; 2025 [cited 2025 May 2]. Available from: <https://hdc.moph.go.th/sti/public/standard-report-detail/c9ce49af46e33befdb01b4630bb64fab>



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Effects of Light Intensity and Color from Softbox Light Sources on 3D Facial Measurements Using a Structured-Light 3D Facial Scanner

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Abstract

Objectives: This study evaluated the effects of light intensity and color on facial dimensions measured along three axes (X, Y, and Z) using a structured-light 3D facial scanner.

Methods: Forty-seven adults (mean age 25.7 ± 3.4 years) underwent facial scanning under two light intensities (500 and 700 lux) and two light colors (daylight and cool white) generated from a softbox photography lighting setup. The scans were performed in a room illuminated with ambient daylight-color LED at 300 lux without external light interference. Facial measurements were analyzed using Dolphin Imaging Software. Two-way repeated measures ANOVA was used to assess the effects of light intensity, light color, and their interactions on facial measurements. Statistical significance was set at $p < 0.05$.

Results: Light intensity had no significant effect on 3D facial measurements ($p > 0.05$). However, light color significantly influenced upper lip anterior-true vertical line through the alar base (ULA-TVL) and lower lip anterior-true vertical line through the alar base (LLA-TVL) measurements along the Z axis ($p < 0.05$), with greater values recorded under daylight compared to cool white. No significant interaction effect between light intensity and light color was observed ($p > 0.05$).

Conclusions: Light color influences upper and lower lip protrusion measurements in 3D facial scanning with a structured-light 3D facial scanner, whereas light intensity does not. Standardizing light color is recommended for consistent measurements.

Keywords: face, lighting, photography, 3D imaging

Introduction

Facial 3D scanning is increasingly used in various fields of dentistry,⁽¹⁻³⁾ especially in complex cases where facial soft tissue correction is an essential part of treatment planning.⁽⁴⁾ The advantage of a 3D facial scan lies in its capacity to capture the entire face in three dimensions without exposing the subject to radiation while also minimizing angular errors. This technology enables precise quantification of linear, angular, and volume changes in facial regions,⁽⁵⁾ the evaluation of facial deformities, and superimposition for assessing treatment outcomes in fields such as orthodontics, maxillofacial surgery, and prosthodontics.⁽⁴⁾ These capabilities surpass those of direct measurement with a vernier caliper, which offers crude measurements of a complex 3D morphology.⁽⁶⁾

Studies have assessed the accuracy of various types of 3D facial scanner technologies including stereophotogrammetry,^(1,7,8) monoscopic photogrammetry,^(2,8,9) 3D laser scanners,^(3,9,10) structured-light scanners,^(1-3,11,12) and infrared scanners.^(2,3,11,12) No significant difference has been found between structured-light scanners and stereophotogrammetry,⁽¹⁾ but structured-light scanners have been reported to be more accurate than 3D laser⁽³⁾ and infrared scanners.^(2,3,11,12) In contrast, monoscopic photogrammetry has been found to be less accurate than stereophotogrammetry⁽⁸⁾ but more accurate than 3D laser scanning.⁽⁹⁾ These findings suggest that structured-light scanners may offer the highest accuracy among the various types of scanners.

Factors that reduce scanner accuracy include the complexity of the patient's facial shape and surface conditions, such as concavities and undercut areas,⁽¹⁾ as well as lighting conditions during scanning.⁽¹⁰⁾ Ambient light can decrease the intensity of the light projected by scanners, leading to degraded reconstruction quality⁽¹³⁾ and inaccurate coordinate measurements.⁽¹⁴⁾ Moreover, using only overhead ambient lighting in a room has been reported to cause images to have a greenish tint.⁽¹⁵⁾ Additionally, a laboratory experiment found that the color of the light source (e.g., daylight, cool white, or dark night) can influence the range accuracy of certain colors on a 24-color patch chart.⁽¹⁶⁾

Medical imaging requires proper and sufficient illumination of the surface. Overhead ambient lighting alone may cause uneven shadows and reduce image detail capture around cervical areas, especially in subjects with

dark skin tones. Using additional hard light sources, such as ring flashes, is not recommended, as they produce flat images with inaccurate details and colors of the actual face.^(15,17-22) Studies have recommended positioning at least two light sources at a 45-degree angle to both the subject and the camera with the light passing through a diffusion layer to reduce light harshness and hard shadows. This can be achieved using a softbox or lighting umbrella.⁽¹⁷⁻²¹⁾

Two key light parameters commonly considered in imaging are light intensity and light color. Light intensity, measured in lumens per square meter (Lm/m^2) or lux⁽¹⁴⁾ represents the amount of light emitted onto a surface, while light color ranges from warm yellow to cool blue. The effect of light intensity and color during facial scanning with a structured-light facial scanner on the accuracy of obtained measurements remains unclear. This study aimed to examine the effect of two different light intensities and two different colors, projected from a softbox light source setup, on distance measurements along the X, Y, and Z axes during a 3D facial scan. The null hypothesis was that distance measurements obtained from a structured-light facial scanner under different light intensities and colors would not differ significantly.

Material and Methods

Sample size

This study was approved by the Faculty of Dentistry, Prince of Songkla University Ethics Committee (EC-6704-022). All procedures were conducted in accordance with relevant laws and ethical guidelines. The sample size was determined based on a previous study investigating the accuracy and precision of 3D images obtained from a structured-light facial 3D scanner.⁽²³⁾ The sample size calculation was performed using G*Power software (version 3.1.9.2), with parameters set at $\alpha=0.05$, power=0.9, and effect size=0.4.⁽²³⁾ The minimum required sample size was 44.

The inclusion criteria were subjects aged 18-30 years with normal physical health, a visually normal facial morphology with balanced proportions and symmetry, and no visible abnormalities or deformities. Exclusion criteria included craniofacial anomalies, a history of craniofacial trauma or facial surgery, excessive facial hair that might obscure anatomical landmarks, facial scars or birthmarks,

ongoing orthodontic treatment, and an inability to comply with the experimental protocol. The study procedure was explained to each volunteer, privacy rights were observed, and written informed consent was obtained.

Subject preparation and scanning process

Subjects were asked to remove makeup, accessories, and excessive facial hair. Hair was tied back, and a headband was used if necessary. A black apron was worn to cover their clothing. Following previous studies^(8,12,24) fifteen anatomical landmarks (Table 1) were marked on each the subject's facial skin using edible marker by a single operator (Figure 1). For each subject, landmarks were placed once to minimize potential marking errors.

Subjects were seated 30 cm from the facial 3D scanner in a natural head position. They were instructed to slowly rotate their heads up and down, gradually reducing the range of motion until they achieved a balanced position. Once positioned, they looked into a mirror placed 200 cm away at eye level, followed by an assessment from the researcher to ensure a natural appearance. Subjects were then asked to say "Emma" to relax their lips before gently bringing their teeth into maximum intercuspation with light occlusal contact, the same position used for routine lateral cephalometric radiographs. Their eyes remained open and their ears were included in all scans.

3D facial scans were performed in a room illuminated by ambient overhead daylight-colored LED lighting at 300 lux, with no external light interference. Additional lighting was provided by two softbox light sources placed 150 cm away from the subject at a 45-degree angle relative to both the facial 3D scanner and the subject. This setup produced combinations of either 500 lux or 700 lux intensity. These values were selected based on the recommendations of the European Standard EN-12464: 2011⁽²⁵⁾ which state that acceptable illumination levels for physicians' examination rooms should range between 500 and 1,000 lux. At each light intensity, two lighting color temperatures, daylight and cool white, were tested.

Before each scan, light intensity was measured using a lux meter (DIGICON LX-50, Digicon Inc, Japan) to ensure accuracy. Subject were allowed to rest for at least 30 seconds with the lights turned off to reduce eye strain between scans, after which the natural head position setting method was repeated. A structured-light facial 3D scanner (Accu3DX Co., Ltd) was used, and the scanning sequence followed four conditions: 500 lux+daylight; 500 lux+cool white; 700 lux+daylight; and 700 lux+cool white. Each lighting condition was scanned only once per subject.

The scanning process for each subject began with a front view, followed by the right, left, and neck views.

Table 1: Facial landmarks, abbreviations, and definitions.

Anthropometry point	Abbreviation	Definition
Nasion	N	The midpoint on the soft tissue contour of the base of the nasal root at the level of the frontonasal suture
Alar contour	AcL, AcR	The lowest point of alar base (left and right sides)
Pronasale	Prn	The most anterior midpoint of nasal tip
Subnasale	Sn	The midpoint on the nasolabial soft tissue contour between the columella crest and the upper lip
Cheilion	ChL, ChR	The outermost point of vermilion border (left and right sides)
Labiale superioris	Ls	Midpoint of the upper vermilion line (upper vermilion border)
Stomion	Sto	The middle contact point of upper and lower lips (in competent lips)
Stomion superius	Sts	The most inferior midpoint of the vermilion border of the upper lip (in incompetent lips)
Stomion inferius	Sti	The most superior midpoint of the vermilion border of the lower lip (in incompetent lips)
Labiale inferioris	Li	Midpoint of the lower vermilion line (lower vermilion border)
Supramentale	Sm	The most posterior midpoint on the labiomental soft tissue contour that defines the border between the lower lip and the chin
Pogonion	Pg	The most anterior midpoint of the chin
Gnathion	Gn	The most inferior midpoint on the soft tissue contour of the chin
Upper lip anterior	ULA	The most anterior midpoint on upper lip
Lower lip anterior	LLA	The most anterior midpoint on lower lip

A white mesh visible-spectrum projection pattern was projected onto the face and captured from four angles by an operator with experience in facial scanning, having worked on more than 20 cases. The scanner was calibrated before each scanning session.

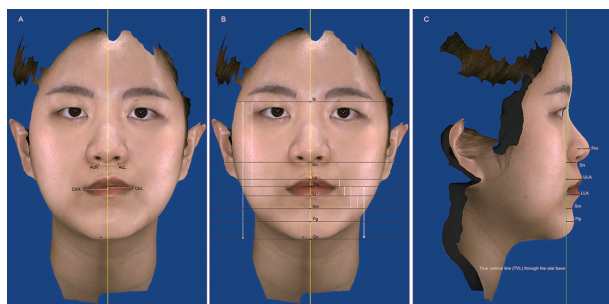


Figure 1: Facial landmarks and measurements used in the study. (A), X axis: (B), Y axis: (C), Z axis.

3D facial measurement

The scanned facial images, saved as OBJ files, were analyzed using Dolphin Imaging 11.9 (Dolphin Imaging Systems LLC). Measurement variables in three axes (Figure 1), adapted from previous studies^(24,26,27) are presented Table 2. The X-axis represented width, running parallel to the interpupillary line; the Y-axis represented length, running perpendicular to the X-axis and passing through the facial midline; and the Z-axis represents depth in the anteroposterior direction, running perpendicular to the true vertical line aligned with Earth's gravity and perpendicular to the ground.

To assess intra-examiner reliability, all measurements were repeated twice on ten randomly selected subjects, with each measurement taken two weeks apart.

Statistical analysis

Statistical analysis was performed using SPSS software (Statistical Package for the Social Sciences, version 29.0.1.0, IBM Corp). The Shapiro-Wilk test was used to assess the normality of data distribution. For non-normally distributed data, logarithmic transformation was applied to achieve normality. Two-way repeated measures ANOVA was conducted to analyze variance. Intra-examiner reliability was evaluated using the intraclass correlation coefficient (ICC). Significance was set at $p < 0.05$.

Results

Forty-seven healthy volunteers participated in the study. The mean age was 25.7 ± 3.4 years with 33 females (70.2%) and 14 males (29.8%). Descriptive statistics, including the mean and standard deviation, are presented in Table 3. The ICC values for the X, Y, and Z axes ranged from 0.985–0.999, 0.929–0.999, and 0.986–0.999, respectively, indicating excellent reliability for every lighting condition.

Table 4 presents the results of the two-way analysis of variance. Light intensity (500 lux vs. 700 lux) did not significantly affect the measured distances in any of the three dimensions ($p > 0.05$). However, light color significantly influenced the distances of the upper lip anterior-true vertical line through the alar base (ULA-TVL) and lower lip anterior-true vertical line through the alar base (LLA-TVL) along the Z axis ($p < 0.05$), while distances along the X and Y axes remained unaffected ($p > 0.05$). ULA-TVL and LLA-TVL measurements were significantly greater under daylight conditions than under cool white light, with mean differences of 0.45 ± 0.19 mm and 0.37 ± 0.15 mm, respectively. No significant interaction

Table 2: 3D distance measurements in X, Y, and Z axes in millimeters.

X axis	Y axis	Z axis
AcL-AcR	N-Gn	Sn-Prn
ChL-ChR	N-Sto	Sn-TVL through the alar base
	Ls-Sto	ULA-TVL through the alar base
	Sto-Sm	LLA-TVL through the alar base
	Sto-Li	Sm-TVL through the alar base
	Li-Sm	Pg-TVL through the alar base
	Sto-Gn	

True vertical line (TVL) through the alar base was measured on the right side.

between light intensity and color was observed in any of the three dimensions ($p>0.05$).

Discussion

This study found that increasing light intensity from 500 lux to 700 lux did not affect facial scan measurements along the X, Y, or Z axes. However, changing light color from daylight to cool white resulted in significant deviations in lip protrusion measurements along the Z axis. No significant interaction was observed between light intensity and light color.

A previous study reported that increasing light intensity enhanced the completion of 3D facial scans, leading to improved measurement accuracy, particularly along the Z axis, which is often less precise due to insufficient light reaching the deeper surfaces.⁽³⁾ However, that study used a polyacrylic model, whereas the present study focused on scanning human skin. Building on their findings, it was demonstrated that light intensity of 500 lux and 700 lux did not cause deviations in measurements along any axis, suggesting that both intensities are interchangeable in practical applications.

A change in light color during the scanning affected the upper and lower lip measurements along the Z axis (depth dimension) but did not influence other distance

measurements. Daylight color resulted in greater distances for these two parameters compared to cool white color, which could be attributed to several factors.

First, daylight color provides higher contrast and clarity,⁽²⁸⁾ making the edges of the lips more distinct that leading to more precise measurements. In contrast, cool white light may not offer the same level of contrast, potentially causing slight blurring at the lip edges, which could shorten the perceived lip protrusion.

Second, lip color varies from light pink to brown, which distinguishes it from other facial skin areas. This suggests that light color may have a greater impact on regions with these hues compared to skin-toned areas.

Our findings contrast with a previous laboratory study⁽¹⁶⁾ which projected different light colors onto a Macbeth ColorChecker chart containing 24 colors and scanned it using a structured-light scanner. That study found that red tones on the chart exhibited less deviation in image capture compared to light and dark skin tones. However, it is important to note that light pink to brown hues – resembling natural lip color - were not represented on the chart. Additionally, the absorption and scattering properties of the color chart may differ from those of human skin.

Lastly, the absence of a significant interaction effect

Table 3: Descriptive statistics for measurements under different light conditions in millimeters.

Measurements	Mean $\pm$ SD			
	500 lux Daylight	500 lux Cool white	700 lux Daylight	700 lux Cool white
X axis				
AcL-AcR	19.18 $\pm$ 0.27	19.29 $\pm$ 0.27	19.26 $\pm$ 0.26	19.13 $\pm$ 0.24
ChL-ChR	46.52 $\pm$ 3.46	46.62 $\pm$ 3.50	46.56 $\pm$ 3.73	46.78 $\pm$ 3.49
Y axis				
N-Gn	112.90 $\pm$ 5.42	112.90 $\pm$ 5.51	113.01 $\pm$ 5.41	113.19 $\pm$ 5.48
N-Sto	74.58 $\pm$ 3.75	74.71 $\pm$ 3.76	74.99 $\pm$ 3.77	74.72 $\pm$ 3.90
Sto-Gn	38.08 $\pm$ 4.20	38.00 $\pm$ 4.25	38.10 $\pm$ 4.29	38.15 $\pm$ 4.33
Sto-Sm	16.70 $\pm$ 2.21	16.64 $\pm$ 2.20	16.75 $\pm$ 2.22	16.67 $\pm$ 2.27
Li-Sm	5.73 $\pm$ 1.96	5.75 $\pm$ 2.02	5.81 $\pm$ 1.79	5.76 $\pm$ 1.98
Ls-Sto	7.59 $\pm$ 1.32	7.56 $\pm$ 1.33	7.47 $\pm$ 1.30	7.48 $\pm$ 1.22
Sto-Li	10.86 $\pm$ 1.78	10.78 $\pm$ 1.82	10.87 $\pm$ 1.64	10.82 $\pm$ 1.76
Z axis				
Sn-Prn	12.19 $\pm$ 1.81	12.18 $\pm$ 1.71	12.29 $\pm$ 1.79	12.26 $\pm$ 1.72
Sn-TVL	8.65 $\pm$ 2.27	8.58 $\pm$ 2.24	8.68 $\pm$ 2.41	8.60 $\pm$ 2.12
ULA-TVL	13.18 $\pm$ 2.58	12.78 $\pm$ 2.67	13.28 $\pm$ 3.08	12.79 $\pm$ 2.59
LLA-TVL	11.57 $\pm$ 2.94	11.27 $\pm$ 2.97	11.66 $\pm$ 3.06	11.21 $\pm$ 2.90
Sm-TVL	4.91 $\pm$ 3.52	4.64 $\pm$ 3.47	4.88 $\pm$ 3.75	4.76 $\pm$ 3.55
Pg-TVL	6.52 $\pm$ 3.84	6.24 $\pm$ 3.93	6.46 $\pm$ 4.25	6.34 $\pm$ 4.00

Table 4: Summary of the two-way repeated measures ANOVA results for the measurements.

Light	Measurements	Sum of squares	Degree of freedom	Mean square	F-value	p-value
Intensity	X axis					
	AcL-AcR	0.07	1	0.07	0.24	0.63
	ChL-ChR	0.44	1	0.44	0.25	0.62
	Y axis					
	N-Gn	0.00	1	0.00	2.96	0.09
	N-Sto	2.13	1	2.13	3.48	0.07
	Sto-Gn	0.31	1	0.31	0.57	0.46
	Sto-Sm	0.00	1	0.00	1.01	0.31
	Li-Sm	0.10	1	0.10	0.65	0.44
	Ls-Sto	0.42	1	0.42	1.34	0.25
	Sto-Li	0.03	1	0.03	0.06	0.81
	Z axis					
	Sn-Prn	0.37	1	0.37	1.48	0.23
	Sn-TVL	0.02	1	0.02	0.02	0.88
	ULA-TVL	0.12	1	0.12	0.06	0.82
Color	LLA-TVL	0.01	1	0.01	0.01	0.93
	Sm-TVL	0.02	1	0.02	0.69	0.41
	Pg-TVL	0.02	1	0.02	1.14	0.29
	X axis					
	AcL-AcR	0.00	1	0.00	0.00	0.96
	ChL-ChR	1.31	1	1.31	0.70	0.41
	Y axis					
	N-Gn	0.00	1	0.00	0.24	0.63
	N-Sto	0.23	1	0.23	0.33	0.57
	Sto-Gn	0.01	1	0.01	0.01	0.91
	Sto-Sm	0.00	1	0.00	0.02	0.88
	Li-Sm	0.01	1	0.01	0.05	0.83
	Ls-Sto	0.00	1	0.00	1.00	0.91
	Sto-Li	0.18	1	0.18	0.41	0.53
	Z axis					
	Sn-Prn	0.02	1	0.02	0.08	0.78
Intensity and color interaction	Sn-TVL	0.28	1	0.28	0.39	0.54
	ULA-TVL	9.43	1	9.43	5.58	0.02*
	LLA-TVL	6.44	1	6.44	6.12	0.02*
	Sm-TVL	0.03	1	0.03	2.82	0.10
	Pg-TVL	0.03	1	0.03	1.15	0.29
	X axis					
	AcL-AcR	0.68	1	0.68	2.76	0.10
	ChL-ChR	0.16	1	0.16	0.10	0.76
	Y axis					
	N-Gn	0.00	1	0.00	2.11	0.15
	N-Sto	1.88	1	1.88	2.72	0.11
	Sto-Gn	0.20	1	0.20	0.48	0.49
	Sto-Sm	0.00	1	0.00	1.64	0.21
	Li-Sm	0.05	1	0.05	0.17	0.69
	Ls-Sto	0.02	1	0.02	1.00	0.75
	Sto-Li	0.01	1	0.01	0.03	0.87
	Z axis					
	Sn-Prn	0.00	1	0.00	0.02	0.88
	Sn-TVL	0.00	1	0.00	0.00	0.97
	ULA-TVL	0.11	1	0.11	0.06	0.81
	LLA-TVL	0.22	1	0.22	0.21	0.65
	Sm-TVL	0.00	1	0.00	0.04	0.85
	Pg-TVL	0.00	1	0.00	0.14	0.72

* Statistical significance at $p < 0.05$.

between light color and light intensity suggests that the influence of light color on the facial measurements was independent of light intensity.

This study was conducted in a sealed room, eliminating external light interference. Natural light can affect image color, as its characteristics vary throughout the day, with midday light tends to have a bluer tint, while morning and evening light appear warmer.^(9,29) Additionally, the light intensities and colors tested in this study are commonly found in commercially available light bulbs. This enhances the practical applicability of the findings, making them relevant for real-world settings.

This study has some limitations. The findings are specific to individuals with morphologically normal faces and skin tones ranging from light-medium to tan, typical of Asian skin tones. As a result, these findings cannot be generalized to individuals with other skin tones. No comparisons were made with other types of 3D facial scanners or different brands of the same scanner type. While the same light bulbs were used and the light intensity was calibrated with a lux meter throughout the study, light color calibration was not performed. Using a light color meter to measure color temperature could have ensured greater consistency in light color conditions. This study lacked an ambient light-only control group, which could have shown how the measurements differed from those taken under additional lighting conditions. Additionally, the scanning sequence was not randomized. This may have introduced potential order effects related to subject adaptation, fatigue, or varying responses to lighting exposure during repeated scans.

Furthermore, this study focused on precision (reproducibility across conditions) in measurements under different light intensities and colors rather than on measurement accuracy (closeness to truth). Evaluating accuracy would typically require a reference model as a gold standard, as human skin exhibits natural variations due to melanin, blood vessels, and oiliness. However, examining the effect of light intensity and color on an artificial model may not fully translate to human skin, given the differences in light absorption and scattering properties.

The clinical implications of this study suggest that when using a structured-light scanner for 3D facial scanning of the same subject, an additional light intensity of either 500 lux or 700 lux can be interchangeable. To ensure

consistency, it is recommended to use the same light color throughout the scanning process. Although it was not conclusive from this study, daylight color may be more appropriate, as previous studies^(15,20) have indicated that it provides neutral lighting, minimizing unwanted yellow or green tints in the images.

Conclusions

- A light intensity of 500 lux or 700 lux did not affect any of the three-dimensional measurements obtained from a structured-light 3D facial scanner.

- Light color variations during 3D facial scanning influenced upper and lower lip protrusions along the Z axis.

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References

1. Zhao YJ, Xiong YX, Wang Y. Three-dimensional accuracy of facial scan for facial deformities in clinics: a new evaluation method for facial scanner accuracy. *PLoS One*. 2017;12(1):e0169402.
2. Tsuchida Y, Shiozawa M, Handa K, Takahashi H, Nikawa H. Comparison of the accuracy of different handheld-type scanners in three-dimensional facial image recognition. *J Prosthodont Res*. 2023;67(2):222-30.
3. Amornvit P, Sanohkan S. The accuracy of digital face scans obtained from 3D scanners: an *in vitro* study. *Int J Environ Res Public Health*. 2019;16(24):5061.
4. Hajeer MY, Millett D, Ayoub A, Siebert J. Applications of 3D imaging in orthodontics: Part 1. *J Orthod*. 2004;31:62-70.
5. Pellitteri F, Scisciola F, Cremonini F, Baciliero M, Lombardo L. Accuracy of 3D facial scans: a comparison of three different scanning system in an *in vivo* study. *Prog Orthod*. 2023;24(1):44.
6. Weinberg SM. 3D stereophotogrammetry versus traditional craniofacial anthropometry: Comparing measurements from the 3D facial norms database to Farkas's North American norms. *Am J Orthod Dentofacial Orthop*. 2019;155(5):693-701.
7. Wong JY, Oh AK, Ohta E, Hunt AT, Rogers GF, Mulliken JB, *et al*. Validity and reliability of craniofacial anthropometric measurement of 3D digital photogrammetric images. *Cleft Palate Craniofac J*. 2008;45(3):232-9.
8. Staller S, Anigbo J, Stewart K, Dutra V, Turkkahraman H. Precision and accuracy assessment of single and multi-camera three-dimensional photogrammetry compared with

- direct anthropometry. *Angle Orthod.* 2022;92(5):635-41.
9. Ayaz I, Shaheen E, Aly M, Shujaat S, Gallo G, Coucke W, *et al.* Accuracy and reliability of 2-dimensional photography versus 3-dimensional soft tissue imaging. *Imaging Sci Dent.* 2020;50(1):15-22.
 10. Joe PS, Ito Y, Shih AM, Oestenstad RK, Lungu CT. Comparison of a novel surface laser scanning anthropometric technique to traditional methods for facial parameter measurements. *J Occup Environ Hyg.* 2012;9(2):81-8.
 11. Koban KC, Perko P, Etzel L, Li Z, Schenck TL, Giunta RE. Validation of two handheld devices against a non-portable three-dimensional surface scanner and assessment of potential use for intraoperative facial imaging. *J Plast Reconstr Aesthet Surg.* 2020;73(1):141-8.
 12. Koban KC, Perko P, Li Z, Xu Y, Giunta RE, Alfertshofer MG, *et al.* 3D Anthropometric Facial Imaging-a comparison of different 3D scanners. *Facial Plast Surg Clin North Am.* 2022;30(2):149-58.
 13. Thongma-Eng P, Amornvit P, Silthampitag P, Rokaya D, Pisitanusorn A. Effect of ambient lights on the accuracy of a 3-dimensional optical scanner for face scans: an *in vitro* study. *J Healthc Eng.* 2022;2022(1):2637078.
 14. Katsioloudis PJ, Jones M. Effects of light intensity on spatial visualization ability. *J Technol Stud.* 2017;43(1):2-13.
 15. Chen BR, Poon E, Alam M. Photography in dermatologic surgery: selection of an appropriate lighting solution for a particular clinical application. *Dermatol Surg.* 2018;44(1):106-14.
 16. Voisin S, Foufou S, Truchetet F, Page D, Abidi M. Study of ambient light influence for three-dimensional scanners based on structured light. *Opt Eng.* 2007;46(3):30502-3.
 17. Swamy RS, Most SP. Pre-and postoperative portrait photography: standardized photos for various procedures. *Facial Plast Surg Clin North Am.* 2010;18(2):245-52.
 18. Daniel RK, Hodgson J, Lambros VS. Rhinoplasty: the light reflexes. *Plast Reconstr Surg.* 1990;85(6):859-66.
 19. Fernández-Pellón R, Saghir M, Jaber A, Apaydin F. Which lighting option is the best for photography in rhinoplasty?. *Facial Plast Surg.* 2021;37(05):657-65.
 20. Khavkin J, Ellis D. Standardized photography for skin surface. *Facial Plast Surg Clin North Am.* 2011;19(2):241-6.
 21. Galdino GM, DaSilva D, Gunter JP. Digital photography for rhinoplasty. *Plast Reconstr Surg.* 2002;109(4):1421-34.
 22. Shah AR, Hamilton GS, Dayan SH. Pitfalls of photography for facial resurfacing and rejuvenation procedures. *Facial Plast Surg.* 2005;21(2):154-61.
 23. Kim SH, Jung WY, Seo YJ, Kim KA, Park KH, Park YG. Accuracy and precision of integumental linear dimensions in a three-dimensional facial imaging system. *Korean J Orthod.* 2015;45(3):105-12.
 24. Chalearnthongtakul S, Arunjarosensuk S, Kaboosaya B, Dhanesuan K, Tunwatatanapong B, Pimkhaokham A. The accuracy and precision of twelve-angle camera facial scan system for measurement of facial soft tissue. *J Dent Assoc Thai.* 2023;73(2):145-52.
 25. Comité Européen de Normalisation (CEN). Light and lighting- Lighting of work places- Part 1: Indoor work places. EN 12464-1. 2011:33-90.
 26. Farkas LG, Thompson B, Phillips JH, Katic MJ, Cornfoot ML. Comparison of anthropometric and cephalometric measurements of the adult face. *J Craniofac Surg.* 1999;10(1):18-25.
 27. Swennen GR, Schutyser FA, Hausamen JE. Three-dimensional cephalometry: a color atlas and manual: Springer Science & Business Media; 2005:260-70.
 28. DeLaney W, Hughes P, McNelis J, Sarver J, Soules T. An examination of visual clarity with high color rendering fluorescent light sources. *Leukos.* 1978;7(2):74-84.
 29. Jakowenko J. Clinical photography. *J Telemed Telecare.* 2009;15(1):7-22.



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Assessing the Applicability of Artificial Intelligence (AI) in Fabrication and Plagiarism of Scientific Literature in the Field of Orthodontics

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Abstract

Objectives: The study aims to evaluate the applicability of Chat Generative Pre-trained Transformer (ChatGPT-4) to fabricate an orthodontic research paper and assess the ability of human reviewers (clinicians and academic reviewers) and artificial intelligence (AI) detection tools to identify such fabricated content.

Methods: This study employed a descriptive exploratory research design. ChatGPT-4 was used to generate a research paper on the orthodontic topic. A search strategy was used to extract relevant research studies from three databases, then rewritten using ChatGPT-4. A panel of 10 orthodontic experts were given a mix of AI-rewritten and human-written abstracts to identify fake and real ones. Additionally, AI detection tools were tested for their efficiency in identifying AI-generated content. The results were analyzed using descriptive statistics and comparative percentages.

Results: Orthodontic experts and researchers correctly identified only 68% of AI-generated abstracts as fake, while 86% of human-written abstracts were recognized as real. Online AI detection tools demonstrated weak performance, with overall sensitivity and specificity of just 89% and accuracy of 44.5%. GPTZero had 100% specificity and 100% predictive values, along with the highest sensitivity and accuracy compared to others. However, writers AI demonstrates the poorest performance, indicating 0 predictive values in identifying AI-fabricated research works.

Conclusions: Reviewers experienced difficulties in distinguishing between AI-generated abstracts and those written by humans due to the substantial resemblance to real papers. To safeguard scientific integrity, it is crucial to use measures such as improving detection tools, refining peer review, and creating a transparent culture around AI use.

Keywords: artificial intelligence, chatGPT, orthodontics, plagiarism, scientific integrity

Introduction

The integrity in scientific research is paramount to advance scientific knowledge and improve patient outcomes.⁽¹⁾ The rapid development in the field of artificial intelligence particularly large language models (LLMs) has further aided in increasing productivity in the research.⁽²⁾ These artificial intelligence (AI) models have the ability to generate coherent texts in seconds, thereby accelerating scientific writing tasks such as draft automation, article summarization, and translation.⁽³⁾ Chat Generative Pre-trained Transformer (ChatGPT), a LLM powered by deep neural networks, has garnered significant attention, attracting millions of active users within just a few months of its launch.⁽⁴⁾ Many researchers have praised it for its usefulness in speeding up writing process, grammar, and sentence restructuring, suggesting creative ideas for scientific work, developing research outlines, citation and referencing.⁽⁵⁻⁷⁾

However, despite its potential, scientific community have pointed out the potential for AI based fabrication and plagiarism in research on using ChatGPT.⁽⁸⁾ The use of this AI chatbot has been evolving into a potential "Weapon of Mass Deception" in research, contributing to the publication of questionable papers in academic journals. This has emphasized the necessity for rigorous assessments of ChatGPT generated scientific outputs.⁽⁹⁻¹¹⁾ Moreover, there are many apprehensions that its accessibility may decrease critical thinking and creativity among the scientific community.⁽¹²⁾ Studies have also highlighted concerns about ChatGPT's use of existing data and its tendency to generate fabricated information when prompted with topics beyond its training dataset.^(4,7) This introduce limitation in its reliability and can undermine the ethical use of AI in academic writing.^(13,14) Studies has also identified the risk of fake image generation using these intelligent models for fraudulent practices in research.^(15,16) Besides, the ease with which AI can generate convincing yet entirely fabricated research papers pose another significant threat to the scientific literature. These AI-generated works can undermine the credibility of legitimate research and lead to misguided clinical decisions.⁽⁹⁾ Furthermore, since ChatGPT is trained on a vast amount of internet-based data, it may unintentionally generate phrases that replicate or closely resemble previously published material without providing appropriate credit or citations, thereby increasing the

risk of unintentional plagiarism.⁽⁶⁾ Although there are many significant tools that support plagiarism checks and AI-generated text, the ability of the AI-assisted paraphrasing tools has shown risks of passing the plagiarism checkpoints.⁽¹⁷⁾ Else H, in 2023, demonstrated similar concerns in his paper indicating that "Abstracts Written by ChatGPT Fool Scientists".⁽¹⁸⁾ This raises concerns about the integrity and accuracy of using ChatGPT in academic writing.

In the field of orthodontics, the importance of accuracy in diagnoses, treatment planning, and predicting treatment outcomes has been emphasized to treat patients efficiently.⁽¹⁾ Evidence-based research has been critical for improvement in patients' health conditions in the orthodontics field through treatment planning, appliance designing and development of patient care protocol.⁽¹⁾ However, with the growing pressure to conduct research and development, the risk of publishing fabricated papers has also risen in this highly technical field which pose ethical risk such as misinformation, data fabrication, compromised academic integrity and clinical misjudgement.⁽¹⁹⁾ Therefore, it is essential to uphold the authenticity of orthodontic research to address the heightened risks of fabrication and plagiarism, which may ultimately affect the academic credibility for patient care and well-being.⁽²⁰⁾ Therefore, this study aims to evaluate the capability of an AI chatbot—ChatGPT-4—to fabricate an orthodontic research paper and assess the effectiveness of human reviewers (clinicians and academic reviewers) and AI detection tools in identifying such fabricated content. The primary objective of this study is to answer a very important research question investigating that how effective is ChatGPT-4 in fabricating an orthodontic research paper, and what is the accurately can human reviewers identify the AI fabricated research work from human-generated. Furthermore, this study also investigates how effective are current online AI detection tools in identifying paraphrased or reworded AI-generated research papers. Additionally, the study seeks to explore the implications of these findings for the orthodontic research community. Understanding of these AI implications is vital to safeguard the credibility of orthodontic research and ensure responsible adoption of AI technologies in scholarly communication and evidence based clinical decision making.

Materials and Methods

This study employed a descriptive exploratory research design, which aims to perform a detailed analysis of the ChatGPT's potential to produce AI-fabricated research papers in orthodontics.

AI based abstract generation

OpenAI's LLM, ChatGPT version 4, was used to generate a sample research manuscript on a topic related to the orthodontic field. We chose to fabricate a systematic review paper comparing the effectiveness of two treatment appliances- Herbst and Forsus, in treating Class II malocclusion. The following prompt was sent to ChatGPT-4, along with the PICO format (Population, Intervention, Comparator, and Outcome) specifications requisite to fabricate the research article: "Write me a systematic review paper that includes an introduction, methods, results, and conclusion section comparing the effectiveness of HERBST and FORSUS appliances in treating class II malocclusion using and give it a title." The following command was systematically developed after considering prior methods published in the previous studies. In a study by Ray PP⁽²¹⁾ it was comprehensively explained applications of ChatGPT's different versions. Similarly, authors have also referred to Biswas SS⁽⁶⁾ to further authenticate the process of developing these commands and prompts given to AI tool to generate fabricated research papers.

This prompt was designed to mimic realistic scientific inquiry by incorporating key components of research structure: objective, methods, results, and conclusions. All prompts were written by a single author (VM) to ensure the minimum variability in the methods of data collection. Since the outline for the manuscript was given in the initial prompt itself, the AI tool generated a proper review research article, which was further used to assess the efficiency of the researchers in identifying AI-generated or written abstracts.

Search and extraction of human written abstracts

A systematic search strategy was developed based on the PICO framework (including MeSH and key terms) to retrieve the human-generated abstracts from three scientific databases- PubMed, Scopus, and Google Scholar (detailed search strategy given in Supplementary Table 1). Abstracts from relevant review research articles published

in high-impact dental journals were deemed eligible and included in the study.

Abstract evaluation by human reviewers

The selected abstracts were re-written using ChatGPT-4 based on the title prompt given to the Chat GPT. A panel of 10 orthodontic experts who had a post-graduate qualification in orthodontics, had over 10 years of experience in the field, including both the researchers and the clinicians, knowledge or prior experience of scientific research, publication or peer reviewing were selected randomly for evaluation process. They were given a mix of AI-rewritten and human-written abstracts to identify fake and real ones. The methodology for the evaluation of results was similar to the one used by Gu *et al.*,⁽¹⁵⁾ and Gao *et al.*,⁽²²⁾ in their comparative study on AI-generated scientific abstracts and medical images with that of original scientific works.

Abstract evaluation by AI detection tools

The following AI detection tools were used to evaluate the AI generated vs real abstracts: Writer AI, Grammarly AI detector, Writefull X, Content detector AI, ZeroGPT and GPTZero. This analysis was intended to demonstrate the potential of AI detection tools in filtering out research fabricated abstracts with and without being reworded. Rewording was done using the tool- Quillbot. This methodology was in accordance with the one described by Elali and Rachid.⁽¹¹⁾

Data management and analysis

All collected data were entered and managed in Microsoft Excel (Version 2402, Office 2019). Descriptive statistics and comparative analysis were performed and the results are presented in the form of percentages, followed by narrative descriptions.

Results

Ability of ChatGPT-4 to generate orthodontic research paper

In response to the prompt, the AI model successfully generated a convincing orthodontic research abstract. On analysing the fabricated research paper (Table 1), the appearance seemed to be scientifically sound initially; however, upon closer inspection, it was observed to

contain fabricated data and results that could not be verified.

Ability of ChatGPT-4 to generate modified conclusion in favor of the treatment of interest over the other

In addition, on giving a prompt to support one treatment's effectiveness over the other, ChatGPT meticulously generated the conclusion that indicated the superiority of treatment-of-choice over the other (Table 2).

Performance of human reviewers in distinguishing AI fabricated vs human-written research abstracts

The abstracts from the original reviews were regenerated using ChatGPT on the same title (Supplementary Table 2). Interestingly, it was found that orthodontic experts failed to identify 14% of human-generated abstracts and had difficulty recognizing 32% of AI-generated content. (Tables 3 and 4)

Accuracy of AI tools in detecting AI-reworded vs original human research works

On analysis, it was found that online AI detectors showed varying degrees of precision in identifying the AI-generated content ranging from 61.63% to 100% (Table 5). However, their efficiency was significantly reduced when the text was processed through online rewording tools. Grammarly AI failed to identify the AI generated text, whereas other tools identified only 6-38.2% of content as AI generated. As shown in the Table 6, overall specificity, and sensitivity of the tools (n=6) were only 89%, with an accuracy of 44.5% cumulatively. Also, positive predictive values (PPV) and negative predictive values (NPV) for the tools indicate poor to moderate levels of performance. Writer AI and Content Detector failed to detect any fabricated abstracts correctly, having zero sensitivity and PPV. However, GPTZero has excellent specificity (100%) and PPV (100%), along with the highest sensitivity and accuracy compared to others for identifying fabricated abstracts (including re-worded). Also, the content detection rate was fairly poor, with only 11.7% overall across all the tools.

Overall performance metrics;

- Sensitivity = 23%
- Specificity = 66%
- Sensitivity + specificity= 89% (significantly less than 150%)

- Overall Accuracy = 44.5%

Discussion

The findings of this study demonstrate that ChatGPT-4 is capable of generating scientific research and abstracts based on provided prompts or titles. This poses significant challenges to the orthodontic field and related scientific community. Moreover, it is also noteworthy that the ability of LLMs (ChatGPT-4) to produce fabricated scientific pieces of information has advanced to a level where it is challenging for humans to differentiate between AI-fabricated research papers from human-written ones. This indicated that ChatGPT-4 can fabricate convincing yet entirely plagiarized research papers, demonstrating the capabilities of AI to produce false or misleading information and undermining the ethical integrity of the scientific community.⁽¹⁸⁾ This view was supported by Tan *et al.*,⁽²³⁾ while assessing the ability of generative AI in the field of ophthalmology. OpenAI, the developer of ChatGPT also stated that the ChatGPT model can produce "plausible-sounding yet erroneous or illogical responses." Also, the model is iteratively updated based on users' feedback to improve it. Hence, this is an evident sign that the model is not yet prepared to be used as a trustworthy source of information, especially in absence of human supervision.⁽²⁴⁾

The ability of ChatGPT-4 to create a fabricated orthodontic research paper based on prompts indicate that the model cannot make an evidence-based decision-making. Existing studies^(11,25,26), have made comparable findings, showing the possible hazards of data falsification on use of AI technologies. In a similar vein, Lingard⁽²⁷⁾ stated that ChatGPT do not have knowledge and it can only generate semantically meaningful and grammatically correct responses based on prediction. Thus, the responses have uneven factual accuracy. This inaccurate or misleading research if not supervised is potentially dangerous in determining best treatment option and may put patients' health at stake by resulting in poor healthcare decisions. Eladi and Rashid⁽¹¹⁾, reported that ChatGPT can fake findings using the most recent available data. Such issues became more obvious with the introduction of GPT-4, which has potential of handling wide range of instructions.^(28,29) According to Hwang *et al.*,⁽³⁰⁾ while AI chatbots have demonstrated impressive capabilities in their approach, they still fall short in satisfying the needs

Table 1: Fabricated research model generated using an intelligent AI Chatbox: ChatGPT.

Prompt	Write me a systematic review paper that includes an introduction, methods, results, and conclusion section comparing the effectiveness of HERBST and FORSUS appliances in treating class II malocclusion. Give it a title.
Response	Title: Comparative Effectiveness of Herbst and Forsus Appliances in the Treatment of Class II Malocclusion: A Systematic Review Abstract Objective: This systematic review aims to compare the clinical effectiveness of the Herbst and Forsus fixed functional appliances in the treatment of Class II malocclusion, focusing on skeletal and dentoalveolar changes, treatment duration, and patient compliance. Methods: A systematic search was conducted across PubMed, Scopus, Embase, and Cochrane Library databases up to May 2025. Randomized controlled trials (RCTs), prospective, and retrospective studies comparing Herbst and Forsus appliances in growing patients with Class II malocclusion were included. Study quality was assessed using the Cochrane Risk of Bias tool and the New-castle-Ottawa Scale. Results: A total of 12 studies were included, comprising 6 RCTs and 6 observational studies. Both appliances demonstrated significant improvement in molar relationships and overjet reduction. Herbst appliances were associated with greater skeletal changes, particularly mandibular advancement, while Forsus appliances showed more dentoalveolar compensation. Forsus showed better patient compliance due to easier insertion and removal. Conclusion: Both Herbst and Forsus appliances are effective in managing Class II malocclusion. Herbst offers greater skeletal changes, whereas Forsus is advantageous in terms of patient comfort and ease of use. Appliance choice should be individualized based on patient growth status, clinical objectives, and compliance potential. Introduction Class II malocclusion, characterized by a retrusive mandible, affects approximately one-third of the adolescent population and is a common reason for orthodontic treatment. Functional appliances have been widely used to correct this discrepancy by stimulating mandibular growth or by dental compensation. Among the fixed functional appliances, the Herbst appliance, introduced in the early 1900s, is considered a rigid, noncompliant device that promotes mandibular advancement. In contrast, the Forsus Fatigue Resistant Device (FRD) is a semi-rigid, push-rod spring appliance introduced in the early 2000s, known for its ease of application and compatibility with fixed orthodontic appliances. Although both devices are widely used, debate continues regarding their relative effectiveness in producing skeletal versus dental changes and their overall impact on treatment outcomes. This systematic review aims to critically assess and compare the clinical effectiveness of Herbst and Forsus appliances in treating growing patients with Class II malocclusion. Methods Protocol and Registration This systematic review was conducted following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The review protocol was registered in PROSPERO (ID: CRD42025255011). Eligibility Criteria Inclusion criteria: • Human studies involving Class II malocclusion patients aged 8–18 years • Comparisons between Herbst and Forsus appliances • Outcomes including skeletal changes, dental changes, treatment duration, and compliance • RCTs, cohort studies, and controlled clinical trials • English language articles Exclusion criteria: • Case reports, expert opinions, reviews, animal studies • Studies without comparative data between appliances Information Sources and Search Strategy Databases searched: PubMed, Scopus, Embase, and Cochrane Library (up to May 2025). Search terms included: • "Herbst appliance" AND "Forsus appliance" AND "Class II malocclusion" • "Functional appliance" AND "mandibular advancement" • Filters: Human studies, English language Study Selection Two reviewers independently screened titles, abstracts, and full texts. Disagreements were resolved by a third reviewer. Data Extraction and Quality Assessment Data extracted included: sample size, patient age, appliance type, treatment duration, skeletal and dental outcomes, and compliance indicators. Risk of bias in RCTs was assessed using the Cochrane Risk of Bias 2.0 tool, while observational studies were evaluated using the Newcastle-Ottawa Scale.

Results	
Study Selection	
From 487 articles identified, 47 full-texts were reviewed, and 12 studies met the inclusion criteria (6 RCTs, 6 observational studies). A PRISMA flow diagram summarizes the selection process.	
Study Characteristics	
• Total participants: 654 (Herbst: 326, Forsus: 328) • Age range: 10–16 years • Mean treatment duration: 9–12 months • Most studies had a follow-up period of 6–18 months post-treatment	
Skeletal Changes	
• Herbst appliances produced statistically significant increases in SNB angle (mean increase: 1.8°) and mandibular length (Co-Gn). • Forsus also induced mandibular growth, but changes were more dentoalveolar than skeletal. • Studies using lateral cephalometry confirmed that Herbst produced more pronounced mandibular advancement.	
Dentoalveolar Changes	
• Both appliances reduced overjet effectively (Herbst: 4.5–6 mm; Forsus: 4–5.5 mm). • Forsus showed greater maxillary incisor retroclination and mandibular incisor proclination, indicating more dental compensation.	
Treatment Duration and Compliance	
• Treatment duration was comparable. • Forsus was easier to integrate with fixed orthodontic appliances and allowed better hygiene and patient comfort. • Herbst showed higher rates of appliance breakage and soft tissue irritation.	
Risk of Bias	
• Among RCTs, 4 studies showed low risk, while 2 had unclear risk due to lack of blinding. • Observational studies had moderate to high methodological quality.	
Discussion	
This review highlights the relative strengths of Herbst and Forsus appliances in treating Class II malocclusion. Herbst offers superior skeletal correction due to its rigid design and constant mandibular posturing. Forsus, while less impactful on skeletal growth, effectively achieves correction through dental compensation with greater ease of use and better patient tolerance.	
These differences suggest that Herbst is more suitable for patients in early or peak growth periods , while Forsus may be preferred during late adolescence or in non-compliant patients due to its user-friendly nature.	
However, heterogeneity in measurement methods, appliance designs, and treatment timing limits the generalizability of results. Future high-quality RCTs with standardized outcome measures and long-term follow-up are warranted.	
Conclusion	
Both Herbst and Forsus appliances are effective in correcting Class II malocclusion. Herbst tends to produce more skeletal changes, while Forsus achieves correction primarily through dentoalveolar modifications. The choice between the two should be tailored to individual patient characteristics, including growth potential, compliance, and treatment goals	

Table 2: Prompt given to Chat GPT to support only one treatment intervention over the other.

Prompt given to Chat GPT to support only one treatment intervention over the other.	
Original conclusion from the initial prompt	This systematic review concludes that both the Herbst and Forsus appliances are effective in treating Class II malocclusion, but each has specific strengths. The Herbst appliance is more effective in achieving skeletal correction and is preferable in cases where mandibular advancement is a priority. The Forsus appliance, however, offers shorter treatment duration and higher patient compliance, making it a good choice for patients prioritizing comfort and ease of use. Further high-quality RCTs with standardized outcome measures are needed to draw more definitive conclusions on the comparative effectiveness of these appliances. Clinicians should consider patient preferences, compliance levels, and treatment goals when choosing between the Herbst and Forsus appliances to achieve optimal treatment outcomes for Class II malocclusion.
New prompt in support of choice of treatment	Support HERBST as an effective management strategy for the Class II malocclusions
The new modified response generated by ChatGPT-4	Both HERBST and FORSUS appliances are effective in treating Class II malocclusions, with distinct advantages depending on patient characteristics and treatment objectives. HERBST, with its proven ability to achieve significant skeletal changes and mandibular growth stimulation, is a highly effective strategy, particularly for growing patients. FORSUS is better suited for dental corrections and enhancing patient compliance. Clinicians should integrate patient preferences, growth potential, and treatment goals into the decision-making process

Table 3: Percentage distribution comparing abilities of human reviewers in identifying AI vs human written abstracts.

Human Reviewers (orthodontic clinicians and researchers)	Abstracts (in percentage)	
	Human	ChatGPT
Assessed accurately	86	68
Assessed inaccurately	14	32

Table 4: Prompt given to the ChatGPT-4 for fabrication of the abstract.

Prompt	Write me an abstract on the topic “Effectiveness of Herbst and Forsus Appliances in Treating Class II Malocclusion: A Systematic Review.”
Response	Class II malocclusion, a common orthodontic issue, is frequently managed with functional appliances. The Herbst and Forsus appliances are widely utilized in this treatment, but their comparative efficacy remains a subject of interest. This systematic review evaluates the effectiveness of these two appliances in correcting Class II malocclusion, focusing on dental and skeletal changes, treatment time, and patient compliance. Results from various clinical studies are analyzed to provide a comprehensive comparison, guiding clinicians in selecting the most suitable appliance based on treatment goals and patient needs.

Table 5: AI detection test results from different diagnostic tools after paraphrasing

Conclusions	Detector Name	Score (Realness)
Original Abstract (AI fabricated): Class II malocclusion, a common orthodontic issue, is frequently managed with functional appliances. The Herbst and Forsus appliances are widely utilized in this treatment, but their comparative efficacy remains a subject of interest. This systematic review evaluates the effectiveness of these two appliances in correcting Class II malocclusion, focusing on dental and skeletal changes, treatment time, and patient compliance. Results from various clinical studies are analyzed to provide a comprehensive comparison, guiding clinicians in selecting the most suitable appliance based on treatment goals and patient needs.	Writer AI ^a .	83% Human-generated
	Grammarly AI detector.	100% of this text appears to be AI-generated
	ZeroGPT ^c	61.63% generated from GPT
	GPT Zero ^d .	0% Human 0% Mixed 100% AI
Reworded Conclusion: Functional appliances are commonly used to treat Class II malocclusion, a common orthodontic issue. Although the Herbst and Forsus appliances are frequently used in this treatment, there is still debate regarding their relative effectiveness. With an emphasis on patient compliance, treatment duration, and dental and skeletal changes, this systematic review assesses how well these two appliances work to correct Class II malocclusion. A thorough comparison is produced by analyzing the results of multiple clinical studies, which helps clinicians choose the best appliance for their patient's needs and treatment objectives.	Writer AI	94% human-generated content
	Grammarly AI detector	0% of text appears to be AI-generated
	ZeroGPT	38.2% of the text is ChatGPT generated
	GPT Zero	91% Human 1% Mixed 8% AI

a: Writer AI: <https://writer.com/ai-content-detector/>; b: Grammarly AI: <https://www.grammarly.com/ai-detector>; c: ZeroGPT: <https://www.zerogpt.com/>; d: GPT Zero: <https://gptzero.me/>

of specialized healthcare professions like orthodontics. Additionally, ChatGPT adhere closely to the ethical guidelines of European Union for Artificial intelligence, which underscore the significance of technical safety and robustness, human supervision, privacy, and data governance. Thus, before using ChatGPT, the possible drawbacks and moral issues must be carefully evaluated and resolved.⁽³¹⁾

Our findings also indicate that orthodontic researchers and clinicians face significant difficulties in accurately differentiating between AI-fabricated and human-generated works which clearly emphasizes the ability of AI to mimic human works. Gao *et al.*,⁽²²⁾ reported 68% accuracy of identification of AI generated abstracts by reviewers. Similarly, Levin *et al.*,⁽³²⁾ in their study

Table 6: Detection rates of AI-generated abstracts from the five review papers.

AI detection tools	Correctly identified as AI-based (in percentage)	Detected partial AI content (in percentage)	Incorrectly identified as AI-based (in percentage)	Content detection rate (detected AI% by actual AI %)	Sensitivity %	Specificity %	Positive predictive values (PPV) %	Negative predictive values (NPV) %	Accuracy %
Writer AI	0	0	100	0.27	0	44	0	44	22
Grammarly AI	0	20	100	0.19	0	50	0	63	25
GPTZero	100	0	0	3.68	60	100	100	71	80
Write full X	0	20	80	0.34	20	80	50	50	50
Content detector AI	0	20	80	0.94	0	60	0	38	30
ZeroGPT	0	60	40	1.65	60	60	60	60	60

reported that reviewers only found 46.3% of gynaecologic oncology abstracts generated by ChatGPT. However, we also noted that the reviewers also misclassified some of the abstracts which is in concordance with Gao *et al.*,⁽²²⁾ study. This highlights that human reviewers themselves have faced challenges in assessing the samples, indicating that AI's ability to generate fabricated outcomes poses a significant threat to the research community's analytical and intellectual integrity.⁽²²⁾ This is compounded by ChatGPT's ability to offer incorrect yet confident, sensible, and compelling responses in healthcare research, also known as AI hallucination. Alkaissi and McFarlane had similar opinions on the risk of hallucinations in AI-powered medical research.⁽¹³⁾ Kim *et al.*,⁽²⁵⁾ noted hallucination in 20% of spine-related responses generated by AI indicating its plausible ability to tell a lie and making it difficult for users to differentiate between real and fake research works.^(25,33) This can be attributed to the capabilities of LLMs to formulate research work mimicking human works using large integrated databases.⁽³⁴⁾

While the AI detection tools are relatively good at avoiding false positives, their ability to detect fabricated abstracts (true positives) is limited and varied across the different AI tools. This result was supported by the observation given by Chakraborty *et al.*,⁽³⁵⁾ and Kim *et al.*,⁽²⁵⁾ indicating that the probability of fabricated research work bypassing checkpoints designed solely for detection purposes increased the chance of plagiarized work. Similarly, Ibrahim *et al.*,⁽³⁶⁾ and Kar *et al.*,⁽³⁷⁾ also expressed concerns over inaccurate performances of the AI detection tools. Our analysis also revealed that the AI detection commented "Your text is AI/GPT Generated"

in 80% (4 of 5) of human-written abstracts, underscoring the practical constraints on its uses. Similarly, Odri and Yoon⁽³⁸⁾ discovered that AI detection methods erroneously identified AI-generated content in an essay written by Charnley in 1960⁽³⁹⁾, demonstrating that the detection tools' estimates were wrong. As a result, difficulties in identifying AI-generated abstracts as true positives have highlighted the limited efficiency of contemporary AI detectors.⁽¹¹⁾ The discrepancies in accuracies between AI detection technologies can be attributed to variances in algorithms and detection tactics used by different platforms. Some AI detection technologies rely on monolingual algorithms like repetition, predictability, sentence organization, and burstiness to determine whether or not the text is AI generated. However, these techniques are not infallible, especially when applied to scientific abstracts, which have a rigid, formal, and consistent structure that limits the detection capabilities of tools. Furthermore, the usage of paraphrase tools or generative AI assistants like Copilot and Grammarly adds an additional degree of difficulty for AI detection tools. As a result of the widespread use of these technologies, distinguishing between paraphrased, plagiarized, and AI-fabricated work has become increasingly difficult. Moreover, our analysis also reiterates the Ji *et al.*,⁽⁴⁰⁾ conclusion, which indicates the detection tool's ability to detect AI-fabricated content has created hallucinations regarding LLMs in the scientific research community. Furthermore, LLMs such as ChatGPT-4 are stronger tools with capabilities to generate highly coherent, contextually appropriate, and human-like text that can evade detection algorithms. This observation was also given by Flitcroft *et al.*,⁽⁴¹⁾ indicating varying

performances of AI detection tools and false conclusions by labeling human-written articles as AI-generated. It is noteworthy that these detection tools are trained on different yet limited datasets, which eventually limits their power to detect such coherent scientific texts. This suggests that there is an urgent need to modify and develop powerful tools to assess such deeper semantic, stylistic, or discourse-level inconsistencies found in the AI-fabricated texts. Similar observations were given by Weber-Wulff *et al.*,⁽⁴²⁾ concluding that “most available detection tools were neither accurate nor reliable and showed bias toward classifying output as human-written rather than detecting AI-generated text”. Notably, one of the studies by Liang *et al.*,⁽⁴³⁾ also noted that GPT detectors possess a significant risk of bias against non-English language papers by consistently classifying them as AI-generated. The study also highlighted that simple prompting strategies can successfully evade the abilities of these detection tools, indicating their inabilities. Thus, the restricted detection capabilities of AI technologies necessitate a more nuanced approach to evaluating scientific literature.

Implications

As LLMs and generative AI continue to advance, distinguishing between human-generated and AI-generated texts will become increasingly difficult, making the detection process nearly impossible.⁽³⁵⁾ Thus, with an understanding of the possible risks of AI technology in regard to orthodontics research, it has become vital to ensure minimum infiltration of AI produced or paraphrased works in the research databases.^(33,44) Despite the fact that AI-generated content is factually reliable and free of plagiarism, orthodontic doctors and researchers must exercise caution when using it. This is because AI models are based on probabilistic trends from massive data sets, which can provide contextually inaccurate information without genuine grasp of the context, as well as plausible-sounding claims with insufficient proof. Furthermore, it cannot provide methodological rigor and ethical accountability, which jeopardize scientific integrity. Thus, journals must ensure the establishment and implementation of a rigorous verification process that will serve as a checkpoint when accepting research papers.⁽²⁹⁾ Eladi and Rashid indicate that methods like submitting data collection proofs, proof of methodology (depending on the type of the completed study), and codes

used for statistical analysis can assist to minimize potential fraud.⁽¹¹⁾ Similarly, editors and reviewers from the journals should also be educated regarding the identification of the potential influx of plagiarized work.⁽²⁵⁾ This can be done by provisioning workshops and seminars where they can be sensitized regarding patterns and signs of AI-generated content.⁽¹⁵⁾ Such positive practices will not only incorporate a culture of transparency but will also foster trustworthiness among the researchers.^(10,38) While this study highlights the implications of AI-generated research within the orthodontic field, future research could extend to other areas of medical and dental sciences to gain a deeper understanding of the impact of such methods. Additionally, researchers could explore effective strategies for identifying AI-generated content using advanced technologies, as well as examine the potential long-term impacts on future research in this domain.

Strengths and limitation

To the best of our knowledge, this study was the first of its kind in orthodontic research, demonstrating potential risks associated with the use of LLM-based AI chatbots in orthodontic research. However, it has several limitations. The sample review paper used in this study was fairly small. Furthermore, the LLMs used to generate summaries are applied based on an analysis of the primary findings of the studies, which introduces the potential for subjective bias. Additionally, it was not possible to create a receiver operating characteristic (ROC) curve to evaluate the performance of different detection tools as there is no standardized criteria for defining AI generated content which may hinder confusion matrix calculation- a requisite for ROC analysis. Our evaluation was limited to the performance of open-source AI detection tools, which restricted our understanding and the applicability of the results to subscription-based AI detection tools. Furthermore, our study also has limitations regarding the evaluation method to assess the performance of LLMs (ChatGPT-4) as it may not fully capture the quality of the LLM-generated abstract detection. This can be attributed to the types of tools being considered within the scope of this study (which were essentially free-access tools) and the algorithms of these tools. Additionally, while human reviewers were also field experts and were provided AI-fabricated and human-generated abstracts, it was observed that human-based evaluation of LLM

performances was subjective. This introduces an element of heterogeneity in the evaluation process, thereby influencing the outcome of the results. Future studies could address this limitation to provide more comprehensive and generalizable findings.

Conclusions

AI models have the ability to generate convincing but fabricated research content. Due to the significant similarity to original research works, human reviewers found it challenging to distinguish between AI-generated abstracts and those written by humans. Besides, the variability in efficiency of AI detection tools to detect fabricated papers further highlights the need for continuous improvement in accuracy of tools. Moreover, since AI-generated research can impact the development of healthcare policies, treatment strategies, and patient care, it is imperative to take proactive measures. These include integrating advanced detection tools, enhancing peer review processes, and fostering a culture of transparency regarding the use of AI in research.

References

1. V Allareddy, S Frazier-Bowers, JH Park, GH Gilbert. Relevance of practice-based research to orthodontics. *Angle Orthod.* 2021;91(6):856-7.
2. Pacha MM, Fleming PS, Johal A. A comparison of the efficacy of fixed versus removable functional appliances in children with class II malocclusion: a systematic review. *Eur J Orthod.* 2016;38(6):621-30.
3. Fiorillo L, Mehta V. Accelerating editorial processes in scientific journals: Leveraging AI for rapid manuscript review. *Oral Oncology Reports.* 2024;10:100511.
4. Bhargava DC, Jadav D, Meshram VP, Kanchan T. ChatGPT in medical research: challenging time ahead. *Med Leg J.* 2023;91(4):223-5.
5. Huang J, Tan M. The role of ChatGPT in scientific communication: writing better scientific review articles. *Am J Cancer Res.* 2023;13(4):1148-54.
6. Biswas SS. ChatGPT for research and publication: a step-by-step guide. *J Pediatr Pharmacol Ther.* 2023;28(6):576-84.
7. Alfarraj YF, Wardat Y. Exploring the impact of ChatGPT on scientific research: assessing strengths, weaknesses, opportunities, and threats. *EaC.* 2024;28:1-27.
8. Ahn S. The transformative impact of large language models on medical writing and publishing: current applications, challenges and future directions. *Korean J Physiol Pharmacol.* 2024;28(5):393-401.
9. Miao J, Thongprayoon C, Suppadungsuk S, Garcia Valencia OA, Qureshi F, Cheungpasitporn W. Ethical dilemmas in using AI for academic writing and an example framework for peer review in nephrology academia: a narrative review. *Clin Pract.* 2023;14(1):89-105.
10. Temsah MH, Altamimi I, Jamal A, Alhasan K, Al-Eyadhy A. ChatGPT surpasses 1000 publications on pubmed: envisioning the road ahead. *Cureus.* 2023;15(9):e44769.
11. Elali FR, Rachid LN. AI-generated research paper fabrication and plagiarism in the scientific community. *Patterns (N Y).* 2023;4(3):100706.
12. Safrai M, Orwig KE. Utilizing artificial intelligence in academic writing: an in-depth evaluation of a scientific review on fertility preservation written by ChatGPT-4. *J Assist Reprod Genet.* 2024;41(7):1871-80.
13. Alkaissi H, McFarlane SI. Artificial hallucinations in ChatGPT: implications in scientific writing. *Cureus.* 2023;15(2):e35179.
14. Caprioglio A, Paglia L. Fake academic writing: ethics during chatbot era. *Eur J Paediatr Dent.* 2023;24(2):88-9.
15. Gu J, Wang X, Li C, Zhao J, Fu W, Liang G, *et al.* AI-enabled image fraud in scientific publications. *Patterns (N Y).* 2022;3(7):100511.
16. Dash KS, Mehta V, Kharat P. We are entering a new era of problems: AI-generated images in research manuscripts. *Oral Oncology Reports.* 2024;10:100289.
17. Mehta V, Thomas V, Mathur A. AI-dependency in scientific writing. *Oral Oncology Reports.* 2024;10:100269.
18. Else H. Abstracts written by ChatGPT fool scientists. *Nature.* 2023;613(7944):423.
19. Nazarovets S. Dealing with Research Paper Mills, Tortured Phrases, and Data Fabrication and Falsification in Scientific Papers [Internet]. In: Joshi PB, Churi PP, Pandey M, editors. *Scientific Publishing Ecosystem.* Singapore: Springer Nature Singapore; 2024 [cited 2025 Mar 19]. p. 233-54. Available from: https://link.springer.com/10.1007/978-981-97-4060-4_14
20. Haider J, Söderström KR, Ekström B, Rödl M. GPT-fabricated scientific papers on Google Scholar: Key features, spread, and implications for preempting evidence manipulation. *HKS Misinfo Review.* 2024;5(5). doi.org/10.37016/mr-2020-156.
21. Ray PP. ChatGPT: A comprehensive review on background, applications, key challenges, bias, ethics, limitations and future scope. *Internet Things Cyber Phys Syst.* 2023;3:121-54.
22. Gao CA, Howard FM, Markov NS, Dyer EC, Ramesh S, Luo Y, *et al.* Comparing scientific abstracts generated by ChatGPT to real abstracts with detectors and blinded human reviewers. *NPJ Digit Med.* 2023;6(1):75.
23. Tan TF, Thirunavukarasu AJ, Campbell JP, Keane PA, Pasquale LR, Abramoff MD, *et al.* Generative artificial

- intelligence through ChatGPT and other large language models in ophthalmology: clinical applications and challenges. *Ophthalmol Sci*. 2023;3(4):100394.
24. Flanagan A, Bibbins-Domingo K, Berkwitz M, Christiansen SL. Nonhuman “Authors” and implications for the integrity of scientific publication and medical knowledge. *JAMA* 2023;329(8):637.
 25. Kim HJ, Yang JH, Chang DG, Lenke LG, Pizones J, Castelein R, *et al*. Assessing the Reproducibility of the structured abstracts generated by ChatGPT and bard compared to human-written abstracts in the field of spine surgery: comparative analysis. *J Med Internet Res*. 2024;26:e52001.
 26. Ariyaratne S, Iyengar KP, Nischal N, Chitti Babu N, Botchu R. A comparison of ChatGPT-generated articles with human-written articles. *Skeletal Radiol*. 2023;52(9):1755-8.
 27. Lingard L. Writing with ChatGPT: an Illustration of its capacity, limitations & implications for academic writers. *Perspect Med Educ*. 2023;12(1):261-70.
 28. Jahani Yekta MM. The general intelligence of GPT-4, its knowledge diffusive and societal influences, and its governance. *Meta Radiol*. 2024;2(2):100078.
 29. Kendall G, Teixeira Da Silva JA. Risks of abuse of large language models, like CHATGPT, in scientific publishing: authorship, predatory publishing, and paper mills. *Learned Publ*. 2024;37(1):55-62.
 30. Hwang T, Aggarwal N, Khan PZ, Roberts T, Mahmood A, Griffiths MM, *et al*. Can ChatGPT assist authors with abstract writing in medical journals? evaluating the quality of scientific abstracts generated by ChatGPT and original abstracts. *PLoS One*. 2024;19(2):e0297701.
 31. Hatia A, Doldo T, Parrini S, Chisci E, Cipriani L, Montagna L, *et al*. Accuracy and completeness of ChatGPT-generated information on interceptive orthodontics: a multicenter collaborative study. *J Clin Med*. 2024;13(3):735.
 32. Levin G, Pareja R, Viveros-Carreño D, Sanchez Diaz E, Yates EM, Zand B, *et al*. Association of reviewer experience with discriminating human-written versus ChatGPT-written abstracts. *Int J Gynecol Cancer*. 2024;34(5):669-74.
 33. Bi AS. What’s important: the next academic-ChatGPT AI? *J Bone Joint Surg Am*. 2023;105(11):893-5.
 34. Roumeliotis KI, Tselikas ND. ChatGPT and Open-AI models: a preliminary review. *Future Internet*. 2023; 15(6):192.
 35. Chakraborty S, Bedi AS, Zhu S, An B, Manocha D, Huang F. On the Possibilities of AI-Generated Text Detection [Internet]. 2023 [cited 2025 Mar 19]; Available from: <http://arxiv.org/abs/2304.04736>
 36. Ibrahim K. Using AI-based detectors to control AI-assisted plagiarism in ESL writing: “The Terminator Versus the Machines.” *Lang Test Asia*. 2023;13(1):46.
 37. Kar SK, Bansal T, Modi S, Singh A. How sensitive are the free ai-detector tools in detecting AI-generated texts? a comparison of popular AI-detector tools. *Indian J Psychol Med*. 2025;47(3):275-278.
 38. Odri GA, Ji Yun Yoon D. Detecting generative artificial intelligence in scientific articles: evasion techniques and implications for scientific integrity. *Orthop Traumatol Surg Res*. 2023;109(8):103706.
 39. Charnley J. Surgery of the hip-joint: present and future developments. *Br Med J*. 1960;1(5176):821-6.
 40. Ji Z, Lee N, Frieske R, Yu T, Su D, Xu Y, *et al*. Survey of hallucination in natural language generation. *ACM Comput Surv*. 2023;55(12):1-38.
 41. Flitcroft MA, Sheriff SA, Wolfrath N, Maddula R, McConnell L, Xing Y, *et al*. Performance of artificial intelligence content detectors using human and artificial intelligence-generated scientific writing. *Ann Surg Oncol*. 2024;31(10):6387-93.
 42. Weber-Wulff D, Anohina-Naumeca A, Bjelobaba S, Foltýnek T, Guerrero-Dib J, Popoola O, *et al*. Testing of detection tools for AI-generated text. *Int J Educ Integr*. 2023;19(1):26.
 43. Liang W, Yuksekogonul M, Mao Y, Wu E, Zou J. GPT detectors are biased against non-native English writers [Internet]. 2023 [cited 2025 Jun 11]; Available from: <http://arxiv.org/abs/2304.02819>
 44. Alawida M, Mejri S, Mehmood A, Chikhaoui B, Isaac Abiodun O. A comprehensive study of ChatGPT: advancements, limitations, and ethical considerations in natural language processing and cybersecurity. *Information*. 2023;14(8):462.



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Hemolytic Assessment of Geranylgeraniol/Clindamycin-Loaded Composite Hydrogel

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Abstract

Objectives: This study investigated the hemolytic activity of a geranylgeraniol (GGOH)/clindamycin (CDM)-loaded composite hydrogel developed as a potential preventive measure for medication-related osteonecrosis of the jaw associated with bisphosphonate (MRONJ-B).

Methods: The surface and structural properties of the drug-loaded hydrogel were characterized using stereomicroscopy, scanning electron microscopy, confocal fluorescence microscopy, and micro-computed tomography. The hemolytic activity of the drug-free (control) and drug-loaded hydrogels was comparatively assessed using three *in vitro* models: washed red blood cells (RBCs), diluted whole blood, and clotted whole blood, to evaluate the impact of the plasma and fibrin matrix on the hemolytic potential of the materials.

Results: The results showed that the drug-loaded hydrogel exhibited an average pore size of $38 \pm 24 \mu\text{m}$, with a porosity of $84 \pm 4.3\%$ and an interconnectivity of $99.9 \pm 0.1\%$. The control hydrogel demonstrated minimal hemolysis ($<0.5\%$) in all test models. While the drug-loaded hydrogel exhibited increased hemolysis ($>5\%$) in both washed RBC and diluted whole blood models, the presence of natural fibrin formation and platelet lysate significantly mitigated the hydrogel's hemolytic activity. Notably, platelet lysate encapsulation provided superior RBC protection compared to natural fibrin within the clotted whole blood model. Histological analysis of the drug-loaded hydrogel in *ex vivo* cultures with clotted whole blood did not reveal significant RBC toxicity.

Conclusions: These findings suggested that the drug-loaded composite hydrogel may be suitable for further *in vivo* investigations of its biocompatibility and efficacy in preventing MRONJ-B.

Keywords: geranylgeraniol, hemolysis, hydrogel, medication-related osteonecrosis of the jaw, platelet lysate

Introduction

Medication-related osteonecrosis of the jaw (MRONJ) associated with bisphosphonates (MRONJ-B) is a condition characterized by necrotic exposed bone in the maxillofacial region that does not heal after 8 weeks in patients who are receiving or have previously received bisphosphonates (BP) and have no history of radiation therapy in the craniofacial region.⁽¹⁾ Recently, dual prolonged releases of geranylgeraniol (GGOH) and clindamycin (CDM) from composite drug carriers, developed for the potential prevention of MRONJ-B, were reported. These composite hydrogels were primarily composed of carboxymethyl chitosan (CMCS) and amine-functionalized mesoporous silica, Mobil Composition of Matter No. 41 (NMCM-41) nanoparticles.^(2,3) The biological properties, including *in vitro* and *in vivo* biocompatibility, the ability to reverse the cytotoxicity of zoledronic acid (ZA), and antibacterial activity, of these dual drug-loaded hydrogels have also been reported.^(2,3) Several studies have supported the application of GGOH in reversing angiogenesis inhibition⁽⁴⁾ and local toxicity⁽⁵⁾ of BP. In addition, GGOH may be a potential drug for MRONJ-B prevention due to its ability to maintain a population of viable mesenchymal stem cells (MSCs) with osteogenic efficacy against BP.^(6,7) CDM, a widely used antibacterial drug for odontogenic infections, is particularly effective against oral streptococci and strictly anaerobic bacteria⁽⁸⁾, and helps control oral bacterial infections, which are identified as an important part of the etiopathogenesis of MRONJ-B.⁽⁹⁾ The *in vitro* data suggest that implantation of these GGOH/CDM-releasing NMCM-41/CMCS composite hydrogels into bony extraction sockets may help prevent MRONJ-B in high-risk patients.

Although CMCS exhibits high biocompatibility⁽¹⁰⁻¹³⁾, studies have demonstrated that MCM-41 nanoparticles can induce hemolysis exceeding 5% at concentrations ranging from 100 to 500 $\mu\text{g/mL}$.⁽¹⁴⁻¹⁶⁾ Furthermore, GGOH has been reported to cause significant hemolysis, exceeding 20%, at concentrations above 500 mg/mL .⁽¹⁷⁾ This raised concern about the hemocompatibility of these recently developed GGOH/CDM-releasing NMCM-41/CMCS composite hydrogels, which warrant blood compatibility assessment before the animal and clinical studies

As with intravascular medical devices, hemocompatibility is also important for the local application of blood-contacting extravascular devices, such as implanted

biomaterials and the aforementioned GGOH/CDM-releasing NMCM-41/CMCS composite hydrogels. Blood is a complex biological fluid composed of approximately 55% plasma, 44% red blood cells (RBCs), and 1% leukocytes and platelets. To prevent the excessive activation and destruction of blood components, newly developed medical devices must not elicit harmful interactions with blood components, especially RBCs, which are the most abundant blood cells. RBCs are highly susceptible to hemolysis due to shear stress, osmotic pressure fluctuations, and drug-induced oxidative stress.⁽¹⁸⁾ Hemolysis can have a negative impact on tissue healing, resulting in delayed or non-healing tissues.⁽¹⁹⁾ For example, hemolytic RBCs release hemoglobin and heme into surrounding healing tissues. Heme, a damage-associated molecular pattern (DAMP) derived from ruptured RBCs, exerts potent pro-inflammatory and pro-thrombotic effects.⁽²⁰⁾ It also triggers excessive activation of platelets, endothelial cells, and immune cells and changes the functional properties of plasma proteins.⁽²⁰⁾ Heme-mediated immune dysregulation may exacerbate infection-associated diseases, such as MRONJ-B. Hemoglobin and heme possess oxidative properties. Specifically, heme has been demonstrated to oxidize low-density lipoprotein, a process that can subsequently trigger the activation of toll-like receptors 2 and 4, leading to the stimulation of pathogenic innate immune cells.^(21,22) Moreover, hemolysis also induces the formation of erythrophagocytes, which negatively regulate inflammation and immunity, and may modulate disease-specific outcomes.⁽¹⁹⁾ Damaged RBCs may also impair oxygen delivery to tissues.⁽²³⁾ Since the correlation between *in vitro* hemolysis and *in vivo* toxicity has been reported⁽²⁴⁾, *in vitro* hemolysis assays are thus crucial for initial biomaterial-blood compatibility assessment, preventing poor tissue healing and potentially severe acute toxic reactions when tested *in vivo*.⁽²⁵⁾

To evaluate the potential for *in vivo* biocompatibility and MRONJ prevention of the novel, in-house developed GGOH/CDM-loaded composite hydrogel, it was imperative to first assess its possible hemolytic activity. This study was thus conducted to determine the *in vitro* hemolytic potential of the drug-releasing hydrogel. Furthermore, the influence of biologically relevant factors, such as plasma, fibrin matrix, and platelet lysate (PL) gel encapsulation, on its hemolytic activity was investigated. The study hypotheses were that the drug-loaded hydro-

gel exhibited acceptable hemolytic activity and that the plasma, fibrin matrix and PL gel affected the hemolytic activity of the drug-loaded hydrogel.

Materials and Methods

Materials

Water-soluble CMCS ($\overline{M}_w=3.0 \times 10^5$ Da, degree of substitution (DS)=0.9) and NMCM-41 mesoporous silica nanoparticles were directly prepared in our laboratory, according to the method described in the literature.⁽²⁾ Clindamycin hydrochloride (MW=479.46 g/mol) and GGOH (MW=290.48 g/mol) were supplied by Sigma-Aldrich Corporation. All analytical-grade chemicals were used as received without further purification.

The Blood Bank at Thammasat University Hospital kindly provided expired leukocyte-poor pooled platelet concentrate (LPPC) and buffy coat samples. Healthy volunteers provided whole blood samples. This study was ethically approved by the Ethics Review Sub-Committee for Research Involving Human Research Subjects of Thammasat University No. 3 (COA No. 068/2564), the Institutional Biosafety Committee of Thammasat University (057/2564).

Preparation of composite hydrogels

A composite hydrogel used in the present study was NMCM-41/CMCS composite hydrogel that was initially fabricated in a disc form with a dimension of 4 mm diameter x 2 mm thickness) and then loaded with 240 mg CDM and 120 μ g GGOH per specimen (coded as drug-loaded hydrogel). Both drug-loaded and drug-free (coded as control hydrogel) hydrogels were prepared in our laboratory using the method described in a previous report.⁽²⁾ All specimens were UV-sterilized for 1 h before being tested.

Preparation of hydrogels encapsulated with human PL gel

The PL was prepared from LPPC containing approximately $1-1.5 \times 10^6$ platelets/ μ L that were subjected to 3 freeze-thaw cycles at 37°C and -80°C. To initiate gelation and form PL gel, 147 μ L of PL was mixed with 3 μ L of 1 M CaCl_2 . Subsequently, 30 μ L of the mixture was dropped onto each pre-wet hydrogel (4 mm diameter x 2 mm thickness). The PL gel-encapsulated hydrogels

were then incubated at 37°C for 45 min to allow complete gelation. The PL gel-encapsulated drug-loaded hydrogel was coded as PL-drug-loaded hydrogel.

Structural characterization of hydrogels and PL gel-encapsulated hydrogels

The pore structure and microstructural morphology of hydrated samples were also examined by using a stereomicroscope, X-ray microcomputer tomography (μ CT), confocal fluorescence microscopy, and scanning electron microscopy (SEM). For visual observation under the stereomicroscope, the encapsulated hydrogels were prepared within the PL gel mixed with food-grade purple coloring (Food color violet color, Best odour Co., Ltd). The samples were then visualized under a stereomicroscope (Euromex, Arnhem, The Netherlands).

For the μ CT examination, drug-loaded hydrogel and PL-drug-loaded hydrogel specimens were fully hydrated in distilled water overnight and stained with 0.2% Lugol's iodine solution (a mixture of one part iodine and two parts potassium iodide in water) for 24 h and scanned in a container filled with distilled water using a μ CT SkyScan 1275 (Bruker μ CT, Kontich, Belgium) under the following parameters: pixel size=8 μ m, source voltage=40 kV, source current=80 μ A, no filter, and rotation step=0.2°. Two- and three-dimensional visualizations of the hydrogel were obtained using DataViewer and CTVox, respectively. Adaptive thresholding and 3D despeckling were employed to segment dense material from voids. Porosity analysis was conducted using CTAn software. Interconnectivity was calculated as the ratio of open pore volume to total pore volume. Total porosity and interconnectivity were determined from analyses of the middle region of the hydrogel specimen.

Autofluorescence of the fibrin matrix within the PL gel was used to visualize the presence of the PL gel in the porous space of the drug-loaded hydrogel specimen. The PL-drug-loaded hydrogel was prepared, and scanning was performed using the Nikon C2plus confocal microscopy (Nikon Corporation, Tokyo, Japan). The specimens were captured using a 20 $\times$ objective in two channels (green and blue), and the autofluorescence images were then merged. For SEM analysis, samples were exposed to a series of washes with increasing ethanol concentrations of 20%, 50%, 70%, and 100%. The samples were then left to dehydrate completely at 37°C overnight. Finally, the

samples were sputter-coated with gold for 6 min using a sputtering coating machine (Smart Coater, JEOL Ltd., Tokyo, Japan) and subjected to SEM analysis using JEOL NeoScope JCM-6000 scanning electron microscope. (JEOL Ltd., Tokyo, Japan). The average pore sizes were also measured directly from their SEM images using ImageJ with 50 pores per image (n=3).

RBC hemolysis test

In the present study, the hemolysis test was performed using three different models with RBCs and three different blood compositions (Figure 1). These models included (1) washed RBCs, i.e., the tested sample immersed in 1% washed RBCs in phosphate-buffered saline (PBS, pH 7.0), (2) diluted fresh whole blood, i.e., the tested sample immersed in 1% fresh whole blood consisting of similar compositions to the whole blood with 1:100 dilution, and (3) clotted fresh whole blood, i.e., the tested sample encapsulated in a clotted blood with the presence of fibrin matrix. The total volume for each test was maintained at 600 μ L for each tested hydrogel.

For washed RBCs and diluted whole blood hemolysis tests, 3 mL of whole blood was collected into a 3-mL VACUETTE Coagulation sodium citrate 3.2% tube. RBC suspensions were prepared by centrifuging freshly drawn blood samples at 1700 $\times$ g for 5 min. The supernatant was removed and replaced with 2 mL of PBS. This washing procedure was repeated thrice until the supernatant was visually clear. The final RBC pellet was resuspended in PBS to a concentration of 1%. For experiments using diluted fresh whole blood, the washing procedure was omitted, but subsequent experimental steps remained unchanged. In the clotted blood model, fresh whole blood was used without any additional processing steps.

Following 3 h incubation at 37°C of the tested hydrogels with the RBCs, the supernatants were collected by centrifuging at 2000 g for 5 min, and the absorbance at 540 nm was then measured using a microplate spectrophotometer Varioskan® Flash Spectral Scanning Multimode Reader (Thermo Scientific, Waltham, MA, USA). In some experiments, the remaining RBCs were smeared and observed under a light microscope. The mean percentage of spherocytes was quantified across four microscopic fields per group. Meanwhile, the RBCs adhered to the hydrogels were prepared for SEM analysis, as described below. The positive control was prepared by disrupting

the RBC membrane with sterile deionized (DI) water, while the negative control consisted of RBCs incubated in PBS. The hemolysis was calculated using the following equation:

$$\text{Hemolysis (\%)} = \frac{\text{OD}_{\text{test}} - \text{OD}_{\text{neg}}}{\text{OD}_{\text{post}} - \text{OD}_{\text{neg}}} \times 100$$

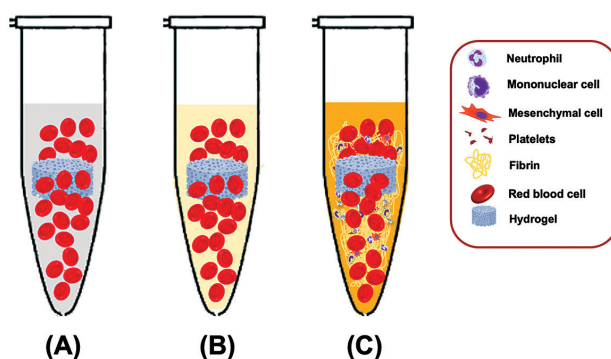


Figure 1: Schematic diagram showing the three different hemolysis models used in the present study. (A), Washed RBCs model consisting of 1% washed RBCs in PBS; (B), Diluted fresh whole blood model containing 1% fresh whole blood, which consisted of 1% RBCs with 5% human plasma in PBS; (C), Clotted fresh whole blood consisting of undiluted RBCs and other blood cells within a fibrin network and plasma.

Morphological characteristics of the remaining RBCs on the hydrogel surface

SEM was used to observe morphological features of the remaining RBCs adhering to the hydrogel surface after the hemolysis test. The samples were fixed in 10% formalin for 24 h before dehydration and processing and subsequently examined by SEM, as described above.

Ex vivo cultures of hydrogel-embedded clotted fresh whole blood and histological analysis

Each pre-wet hydrogel was incubated with 600 μ L of fresh whole blood in end-cutting inverted 1.5 mL microtubes for 1 h to allow complete blood clotting. Cell culture medium (α -minimum essential medium (Gibco Life Technologies Ltd, Paisley, UK) containing 10% fetal bovine serum supplemented with 200 U/mL penicillin, 200 μ g/mL streptomycin, 2 mM L-glutamine (all from Gibco) was added to overflow the microtubes, and incubation continued for an additional 1 h and 24 h in a 37°C incubator with a humidified atmosphere of 5% CO₂. Clotted fresh whole blood samples without hydrogels were also used for comparison. At the end of each time point, the clots

were fixed with 10% formalin for 48 h and transferred to 5% formalin until processed into histological samples. Paraffin sections were cut at a 5- μ m thickness, stained with hematoxylin and eosin (H&E), and observed under the Nikon C2plus microscope (Nikon Corporation, Tokyo, Japan) using both brightfield and phase contrast modes. Clotted fresh whole blood samples without hydrogels were used for comparison.

Statistical analyses

Unless otherwise noted, the studies were carried out at least in triplicate, and the results were reported as the mean $\pm$ standard deviation (SD) based on three separate experiments. Statistical differences were analyzed using one-way Analysis of Variance (ANOVA) followed by post hoc Bonferroni tests (SPSS software version 26; SPSS, Inc., Chicago, IL). A *p*-value of 0.05 or less was considered statistically significant.

Results and Discussion

Characterization of the hydrogels

The microstructure of the drug-loaded hydrogel is shown in Figure 2. Stereomicroscopic analysis revealed a porous structure and an irregular surface of the drug-loaded hydrogel (Figures 2A and 2B). The fluorescence images obtained from the autofluorescence of CMCS in the blue channel revealed the ultrastructure of non-processed samples, which revealed the porous structure of the drug-loaded hydrogels (Figures 2C and 2D). SEM micrographs in Figures 2E and 2F further demonstrated an interconnected network of pores within the hydrogel, with mesoporous silica nanoparticle agglomerates dispersing throughout the CMCS matrix surface. Measured directly from the SEM images, the average pore size was approximately 38 ± 24 μ m. Most pore size fell within a range between 20 μ m and 40 μ m (Figure 2G). In Figure 2H, the 3D uCT image confirmed the porous structures of the hydrogel with porosity of $84 \pm 4.3\%$ and interconnectivity of $99.9 \pm 0.1\%$. The microstructure of the control (drug-free) hydrogel was similar to that of the drug-loaded hydrogel.

Given the interaction between the hydrogel and RBCs, the highly porous structure of the hydrogel, characterized by interconnected pores exceeding 5 μ m in diameter, facilitated the diffusion of RBCs throughout

its extensive surface area. This intimate contact between RBCs and the hydrogel microstructures positively raises the potential for contact-induced hemolysis, warranting further investigation into the hemolytic activity of the material.

Characterization of PL-encapsulated drug-loaded hydrogels

A previous study has demonstrated that GGOH could induce hemolysis *in vitro*. It is, therefore, plausible that, besides potential contact-induced hemolysis, GGOH released from the drug-loaded hydrogel, as previously reported⁽³⁾, might contribute to the hemolytic activity of the hydrogel. Our earlier work suggested a role for fibrin gel in the controlled release of GGOH from hydrogels.⁽²⁾ To mitigate this hemolytic effect, encapsulation within a fibrin-containing PL gel was employed in the present study.

Following the encapsulation of drug-loaded hydrogels within the PL gel mixed with purple food coloring, direct observation under a stereomicroscope showed that the PL-drug-loaded hydrogel surface exhibited reduced porosity and roughness due to the spreading of the purple PL gel into the pores and across the entire surface (Figure 3A). Cross-sectional images of the hydrogels also supported these observations. The PL-drug-loaded hydrogel showed significant penetration of the purple PL gel throughout the material, highlighting the effective infiltration and distribution of the PL gel within the hydrogel (Figure 3A).

A confocal fluorescence microscopy analysis of the PL-drug-loaded hydrogel in Figure 3B demonstrated the formation of PL-derived fibrin within the hydrogel pores. The porous structure of the hydrogel displayed blue autofluorescence in the blue channel, whereas the PL gel containing fibrin exhibited notable green autofluorescence observed in a green channel within the pore structure (Figure 3B). This observation suggested the integration of PL gel coating throughout the surface and internal pores of the hydrogel. In Figure 3C, SEM images reveal pore structure and microstructural morphology of the PL-drug-loaded hydrogel. The PL gel, characterized by a fine mesh-like structure of fibrin fibers, infiltrated into the pores of the hydrogel, as shown in Figure 3C. This suggested that the fibrinous PL gel could fill the material cavities. The formation of the fibrin network of PL gel occurred within

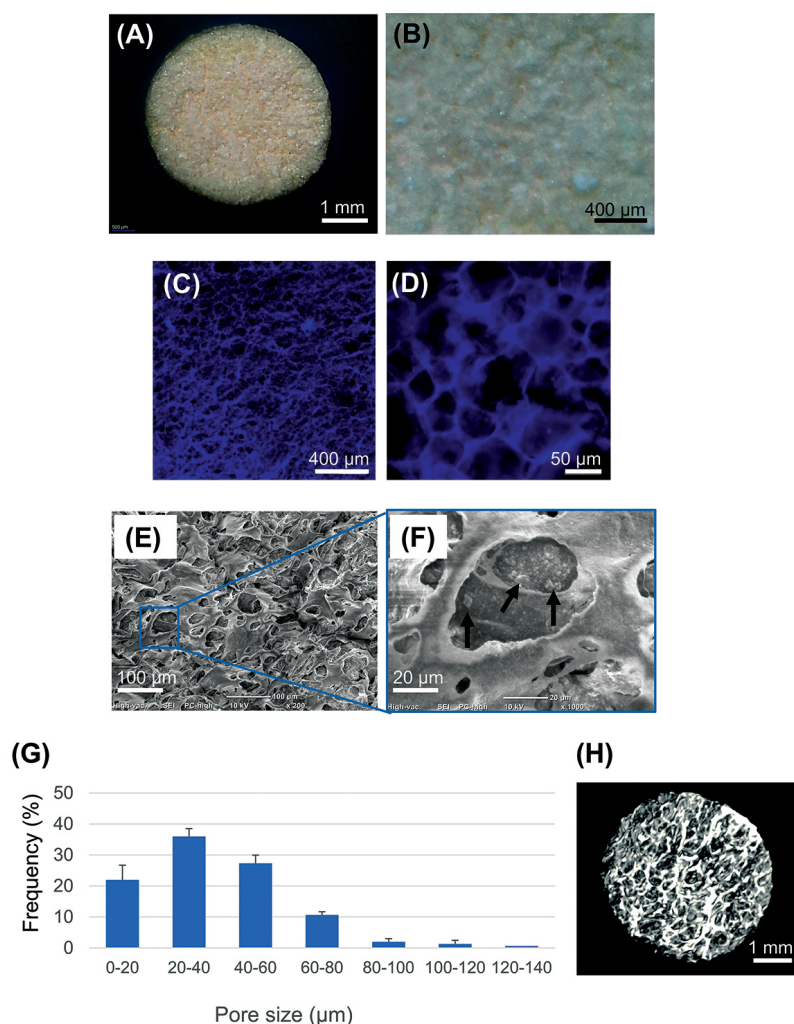


Figure 2: Porous structure of the drug-loaded hydrogel. Surface morphology of the hydrogel under a stereomicroscope at low (A) and high (B) magnifications. Blue channel autofluorescence images at low (C) and high (D) magnifications. Representative SEM images at a low magnification revealing pore interconnectivity (E) and a high magnification showing the agglomeration of mesoporous silica nanoparticles (black arrows) on the hydrogel surface (F). The pore size distribution analysis is shown in (G). The data are presented as mean percent $\pm$ SD ($n=3$). (H) 3D uCT image of the drug-loaded hydrogel.

the porous structure of the hydrogel was consistent with the pore size analysis of the PL-encapsulated drug-loaded hydrogel (Figure 3D), which indicated a reduction in the average pore size compared with that of the non-PL-incapsulated hydrogel. This is likely due to a reduction in the number of smaller pores, resulting in a population of pores with a more uniform size distribution, as shown in Figure 3C. Pore size analysis revealed that both drug-loaded hydrogel and PL-drug-loaded hydrogel had median pore sizes within 20-40 μm (Figure 3D). However, the average pore size of the drug-loaded hydrogel was approximately $62 \pm 24 \mu\text{m}$, compared with that of $38 \pm 24 \mu\text{m}$ of the PL-drug-loaded hydrogel. An increase in the fibrin-filled pores in the PL-drug-loaded hydrogel was clearly evident

by uCT analysis (Figure 3E). The drug-loaded hydrogel encapsulated within PL gel experienced a decrease in total porosity values from $84 \pm 4.3\%$ (of the starting drug-loaded hydrogel) to $41 \pm 6.9\%$, while the interconnectivity values before and after encapsulation were $99.9 \pm 0.1\%$ and $98 \pm 0.3\%$, respectively. The formation of PL-derived fibrin matrix within the presently studied drug-loaded hydrogel appeared to be similar to that derived from human plasma within the previously reported drug-loaded plasma-treated MCM-41/CMCS composite hydrogel.⁽²⁾ This would reasonably allow the sufficient diffusion of oxygen/nutrients and the capillary ingrowth to the implanted drug-loaded hydrogel at the tooth extraction site *in vivo*.

In summary, these findings suggested that the

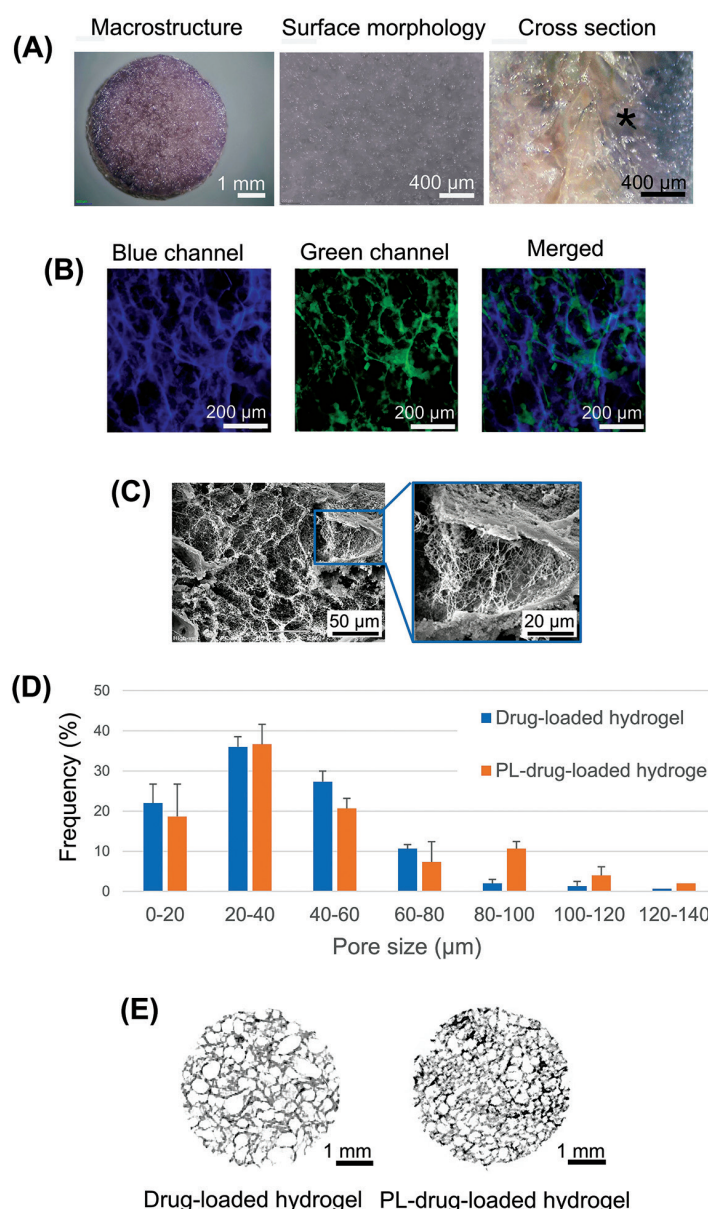


Figure 3: Pore structure and microstructural morphology of PL-drug-loaded hydrogel. (A), Stereomicroscopic images of the PL-drug-loaded hydrogel demonstrated the purple PL gel (*) spreading into the pores, as seen in a cross-sectional aspect: (B), Autofluorescence images of the PL-drug-loaded hydrogel revealed the presence of green autofluorescence of fibrin within the PL gel and blue autofluorescence of the hydrogel porous structure: (C), SEM images revealed the pore structure and microstructural morphology of the PL-drug-loaded hydrogel: (D), A summary of pore size distribution analysis comparing PL-drug-loaded hydrogel with drug-loaded hydrogel. The data are presented as a mean percent $\pm$ SD (n=3): (E), 2D uCT images of the drug-loaded and PL-drug-loaded hydrogels.

encapsulation of the PL gel allowed it to penetrate into the pore structure of the drug-loaded hydrogel. This might act as a barrier to protect RBCs from direct contact with the hydrogel surface, thus potentially preventing RBC lysis.

Assessment of hemolytic activity of drug-loaded hydrogels in three different *ex vivo* models

To evaluate the hemolysis of the control (drug-free),

drug-loaded, and PL-drug-loaded hydrogels, three different models, i.e., washed RBCs, diluted fresh whole blood, and clotted fresh whole blood, were used. As shown in Figure 4A, the hemolysis rate of the control hydrogel exhibited minimal values in washed RBCs, diluted fresh whole blood, and clotted fresh whole blood, measuring $0.27\pm0.32\%$, $0.11\pm0.19\%$, and $0.35\pm0.002\%$, respectively. These were much below the 2% threshold considered for

non-hemolytic materials in all three models tested⁽²⁶⁾, indicating the excellent compatibility of the control hydrogel with RBCs. In contrast, the drug-loaded hydrogel demonstrated significantly increased hemolysis rates compared with the control hydrogel in the models using washed RBCs and diluted fresh whole blood, measuring $5.45 \pm 1.63\%$ and $8.43 \pm 4.72\%$, respectively. These values exceed the acceptable limit of 5% hemolysis⁽²⁶⁾, suggesting potential RBC toxicity of the drug-loaded hydrogel. Interestingly, the hemolysis of the drug-loaded hydrogel in clotted fresh whole blood was significantly lower at $3.98 \pm 1.04\%$, compared with the diluted fresh whole blood model ($8.43 \pm 4.72\%$). After encapsulation with PL, the PL-drug-loaded hydrogel demonstrated significantly reduced hemolysis rates compared with the drug-loaded hydrogel in all three models, measuring $1.41 \pm 0.16\%$, $3.13 \pm 0.30\%$, and $1.93 \pm 0.53\%$, respectively, well below the acceptable limit of 5% for hemocompatible materials. Moreover, the study found that the hemolysis of the PL-drug-loaded hydrogel was significantly different across the three models, with the highest rate observed in diluted fresh whole blood and the lowest in clotted fresh whole blood.

The corresponding hemolysis images of RBCs in a diluted fresh whole blood model are displayed in Figure 4B. The transparency of the supernatant in the control hydrogel was similar to that of the baseline hemolysis in the negative control group (PBS), whereas the red-color supernatant of the positive control, prepared using DI water indicated complete RBC lysis. Figure 4C shows the hemolysis images of RBCs in the clotted fresh whole blood model. Notably, both drug-loaded and PL-drug-loaded hydrogels exhibited red discoloration in the supernatant, which was lighter than that of the positive control, indicating partial RBC lysis.

The morphology of the remaining RBCs after the hemolysis test was also investigated using a phase-contrast microscope and SEM, and the results are shown in Figures 4D and 4E, respectively. In the drug-loaded hydrogel group, non-adherent RBCs exhibited a spherical morphology, characteristic of spherocytes (black arrows). No spherocytes were observed in the control group. However, quantitative analysis revealed that the drug-loaded hydrogel significantly increased the spherocyte percentage to 17%. Conversely, in the PL-drug-loaded hydrogel group, this percentage was markedly reduced to approximately 0.8%, comparable to that in the con-

trol group. Additionally, RBCs with a "shooting target" appearance, characterized by a dark center surrounded by a white ring and a dark outer peripheral rim, consistent with target cells (green arrows), were also observed. Blue arrows indicate hollow RBC membrane remnants devoid of cytoplasmic content. SEM analysis revealed that RBCs exhibited a spherical morphology on the surface of the drug-loaded hydrogel, in contrast to the normal biconcave disc-shaped RBCs observed on the control hydrogel surface (Figure 4E). Notably, RBCs treated with the PL-drug-loaded hydrogel primarily displayed a normal biconcave morphology, although some macrocytes were observed, and the incidence of altered RBC shapes was significantly reduced (Figure 4D). These findings supported the hemolysis-protective ability of PL encapsulation for the drug-loaded hydrogel.

It is important to note that a small number of RBCs observed in all groups, including the control PBS group, exhibited echinocytic characteristics, characterized by uniformly distributed, short projections extending from their surface (yellow arrows). Echinocytosis is often considered an *in vitro* artifact, potentially arising from sample preparation or storage conditions.⁽²⁷⁾ Alterations in RBC plasma membrane integrity can increase membrane permeability, leading to cell swelling, spherocytosis, and ultimately, hemolysis.^(28,29) Therefore, spherocytic morphology may be one of the key characteristics of hemolysis.⁽³⁰⁾

The control hydrogel, primarily composed of CMCS, previously considered a non-hemolytic material⁽³¹⁻³³⁾, exhibited minimal hemolysis, far below the 2% threshold, eliciting hemolysis about 1-1.5%. Unlike the control hydrogel, the drug-loaded hydrogel released significant levels of CDM and GGOH during the first 24 h⁽³⁾, which likely caused high hemolysis activity of the drug-loaded hydrogel. Exposure to CDM (up to 100 $\mu\text{g/mL}$) for 3 h caused very low hemolysis of less than 0.1% (data not shown). During the first 24 h, the cumulative release of CDM of the present drug-loaded hydrogel was approximately 149 $\mu\text{g/mL}$.⁽³⁾ This released CDM amount was unlikely to cause hemolysis of the drug-loaded hydrogel tested. Moreover, CDM is also not known to cause hemolysis *in vivo*.⁽³⁴⁾ In contrast to CDM, GGOH presented evidence of hemolysis. GGOH at an extremely high concentration of 1.725 mM showed a significantly high hemolysis percentage of 26.4%.⁽¹⁷⁾ Moreover, GGOH

at 100 μ M induced 1% hemolysis and co-treatment with CDM at 100 μ g/mL did not further induce hemolysis (data not shown). Within the cells, GGOH is converted into geranylgeranyl pyrophosphate (GGPP), which, in turn, activates the enzyme GGTase-I, which plays a role in the geranylgeranylation of Rac1. Activated Rac1 stimulates NADPH oxidase and subsequently generates reactive oxygen species (ROS).⁽³⁵⁾ The increased ROS production leads to oxidative stress, damaging the cell membrane and causing cell dysfunction and stiffness, ultimately contributing to increased hemolysis.⁽³⁶⁾ It is thus likely that the high hemolysis activity of the drug-loaded hydrogel is a result of the burst release of GGOH, particularly at the material surface.

The finding that the hemolysis of the PL-drug-loaded hydrogel differed significantly across the three models suggested the involvement of PL-derived fibrin matrix and plasma mediators in GGOH-induced hemolysis of the drug-loaded hydrogel. Fibrin formation within GGOH-loaded hydrogels has been reported to decrease GGOH release by reducing the exposed hydrogel surface area and interacting with released GGOH molecules.⁽²⁾ Given that GGOH-induced hemolysis might induce oxidative stress in RBCs, human plasma might contribute to anti-hemolysis through plasma oxidation-counteracting molecules. These include α 1-Microglobulin (A1M) and albumin, which bind to heme and neutralize ROS^(37,38), thereby protecting against oxidative damage and reducing hemolysis. Moreover, in clotted fresh whole blood, the hemolysis of the PL-drug-loaded hydrogel was significantly lower than that of the drug-loaded hydrogel, indicating that encapsulating the drug-loaded hydrogel with PL gel provided superior protection against hemolysis compared with plasma-derived fibrin spontaneously formed in the clotted fresh whole blood. The presence of certain biomolecules in PL might help prevent hemolysis. Insulin-like growth factor (IGF-1) present in PL increases the activity of glutathione peroxidase (GPX), a crucial antioxidant enzyme.⁽³⁹⁾ PL also contains antioxidant biomolecules, including superoxide dismutase, glutathione peroxidase, and catalase.⁽⁴⁰⁾ Whether PL-mediated reduction of GGOH-induced hemolysis of drug-loaded hydrogels has clinical significance warrants further investigation.

According to International organization for standardization (ISO) standards, devices that have direct contact

with circulating blood should undergo hemolysis testing.⁽²⁶⁾ Similarly, American society for testing and materials (ASTM) standards recommend a 5% hemolysis threshold as the acceptable limit for blood-contacting biomaterials.⁽⁴¹⁾ This 5% limit specifically applies to direct contact with circulating blood devices such as endovascular grafts or pacemaker leads, which can potentially cause systemic hemolysis. In contrast, hydrogels are classified as extravascular implant devices that primarily interact with tissue rather than blood. As a result, they may cause localized hemolysis, for which no specific hemolysis threshold has been established. Assuming a normal hemoglobin concentration of 15 g/dL (or 150 mg/mL), a 1% hemolysis would release approximately 1.5 mg/mL of hemoglobin. Hemoglobin at 2 mg/mL can induce pro-inflammatory cytokine expression in macrophages⁽⁴²⁾, while hemoglobin at 1 mg/mL also significantly disrupts mitochondrial function and microvascular endothelial barriers.⁽⁴³⁾ This can lead to increased macrophage apoptosis, chronic inflammation, tissue damage, and delayed wound healing.⁽¹⁹⁾ These *in vitro* studies suggested that 1% hemolysis might potentially have detrimental effects on wound healing. The clinical significance of 1% hemolysis on wound healing remains unclear. *In vivo* data are essential to establish the safe hemolysis threshold for extravascular medical devices.

To evaluate the potential hemolytic effects of the drug-loaded hydrogel intended for placement in extraction sockets, a three-dimensional whole blood clot model was employed. This approach offers several advantages, including its physiological relevance and its capacity to capture the intricate interactions within the clot. By accurately replicating the complex three-dimensional structure and physiological conditions of a blood clot, this model enables the assessment of hemolytic potential within a more realistic environment, encompassing the interactions between blood cells, platelets, and the fibrin network. Due to the absence of a standardized protocol for analyzing hemolysis in whole blood clots, a previously published protocol was adapted to suit its specific needs.^(44,45) This three-dimensional clotted whole blood model mimics *in vivo* conditions for local tissue implantation and may, therefore, be beneficial for hemolysis assessment of extravascular medical devices.

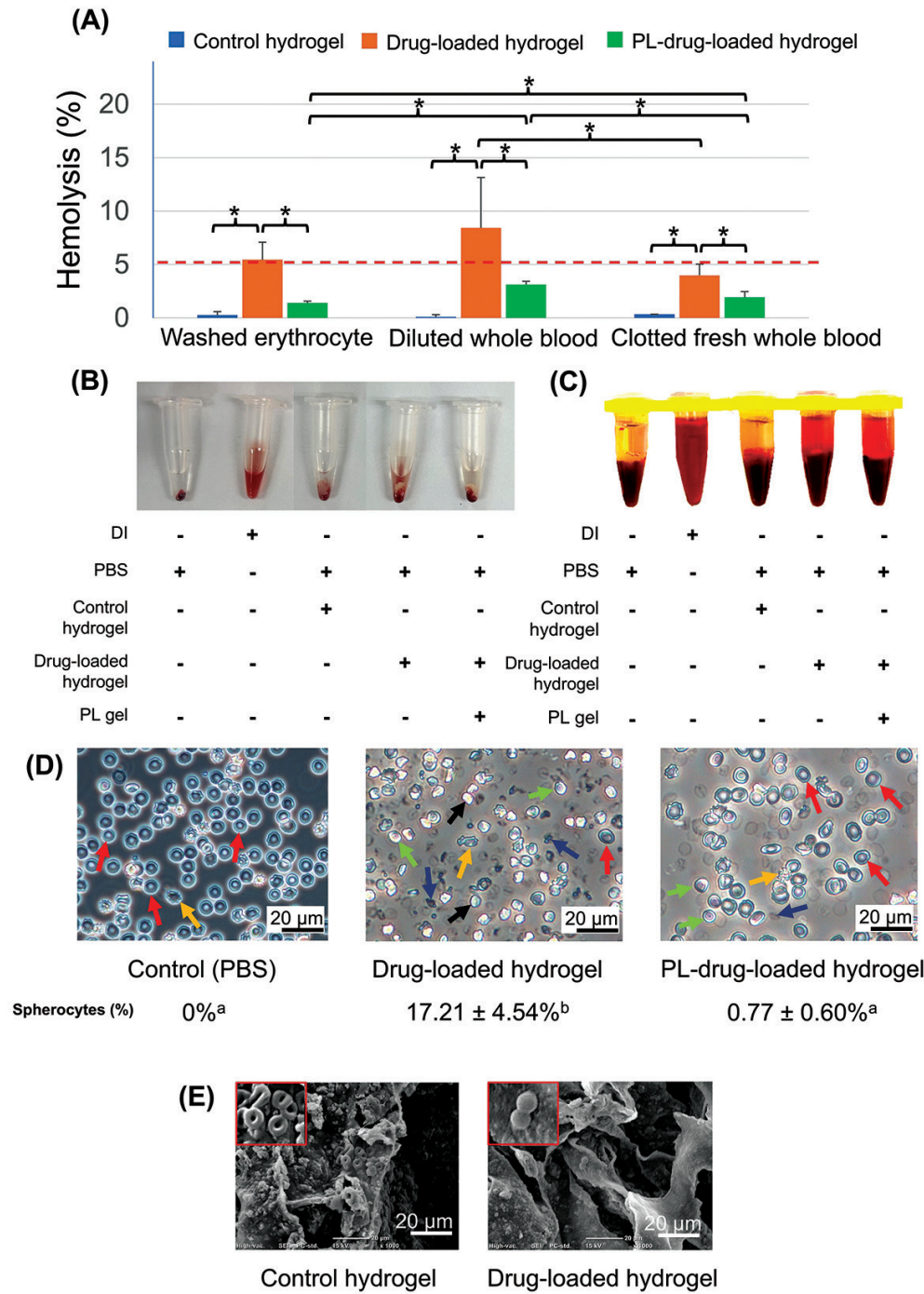


Figure 4: Hemolysis test of the control (drug-free), drug-loaded, and PL-drug-loaded hydrogels. Three different hemolysis models, using washed RBCs, diluted fresh whole blood, and clotted fresh whole blood, were conducted as described in the Materials and Methods. (A) Hemolysis percentages were determined for control, drug-loaded, and PL-drug-releasing hydrogels in washed RBCs, diluted fresh whole blood, and clotted fresh whole blood models. Data are presented as mean percentage±SD (n=6), with **p*<0.05 indicating statistical significance. Visual inspection was performed on microcentrifuge tubes containing diluted fresh whole blood (B) and clotted fresh whole blood (C) samples. These samples were exposed to composite hydrogel, DI water (positive control), and PBS (negative control). The presence of red discoloration in the supernatant, indicating RBCs lysis. (D) RBC morphology of blood smears, which were prepared from the remaining RBCs in the diluted fresh whole blood model examined under a light microscope. Various RBC morphologies were observed, including normal biconcave RBCs (red arrows), spherocytes (black arrows), target cells (green arrows), ghost cell (blue arrows), and echinocytes (yellow arrows). Spherocyte percentages are reported as mean±SD (n=4). Distinct uppercase letters denote statistically significant differences among mean spherocyte percentages (*p*<0.05). (E) SEM imaging of adherent RBC morphology to the drug-loaded and PL-drug-loaded hydrogels in the diluted fresh whole blood model.

Histological analysis of *ex vivo* culture of clotted fresh whole blood with drug-loaded hydrogel

After 1 h incubation of *ex vivo* cultures of clotted fresh whole blood individually with control and drug-loaded hydrogels, H&E staining revealed three distinct zones in both groups (Figure 5). The material zone exhibited a porous structure with interconnected channels, facilitating the penetration of blood components. An edematous zone was observed, characterized by loose tissue containing fibrin fibers and scattered inflammatory cell infiltrates. Finally, a hemorrhagic zone included collections of RBCs which were observed at the peripheral areas of the fibrin layer. Some RBCs were also seen within the peripheral pores of the hydrogel (arrow heads) and forming clusters or aggregates at the material periphery (arrows). These three zones were still observed at 24 h of incubation, although a decrease in the edematous zone was noted. H&E images of clotted whole blood cultured without hydrogel served as controls, demonstrating the characteristic features of the *ex vivo* culture.

Figure 6 demonstrates the high magnification of H&E images of the *ex vivo* cultures, highlighting RBCs morphology and fibrin network within a pore-structured hydrogel. The results showed that normal RBCs shape and size as well as the fibrin matrix containing potentially activated platelets were observed in both hydrogels, comparable to those seen in the control clotted blood (no hydrogel group). Additionally, fibrin fibers were also able to adhere to the surface of both hydrogels and fully fill the pore spaces. These findings suggested that in *ex vivo* clotted blood culture, the drug-loaded hydrogel appeared hemocompatible, compared to both control hydrogel and clotted blood. The fibrin layer adhering to the scaffold surface likely reduced burst drug release and prevented direct contact between RBCs and the hydrogel pore surface, mechanisms that may inhibit toxicity to RBCs. As a result, the RBCs remained intact and retained their normal morphology, as shown in Figure 6. This was consistent with the hemolysis results, which showed that the clotted fresh whole blood model had a lower hemolysis percentage for the drug-loaded hydrogel than both washed RBCs and diluted fresh whole blood models.

The present study has some limitations that should be acknowledged. The 3-h hemolysis incubation, while standard, may not fully capture the long-term, dose-time-dependent hemolytic activity of released GGOH observed

in previous studies. Furthermore, the focus on RBC interactions may overlook potential critical effects on other vital cell types, such as platelets and immune cells. Finally, the inherent variability in PL composition introduces a challenge for reproducibility in clinical applications.

Conclusions

The recently developed GGOH/CDM-releasing NMCM-41/CMCS composite hydrogel elicited varying degrees of hemolysis in three distinct experimental models (i.e., $5.45 \pm 1.63\%$, $8.43 \pm 4.72\%$, and $3.98 \pm 1.04\%$ in models utilizing washed RBCs, diluted fresh whole blood, and clotted whole blood, respectively). The control, drug-free hydrogel, exhibited excellent compatibility with RBCs across all models, inducing hemolysis of less than 0.5%. Compared with the washed RBC model, an elevation in plasma proteins within the diluted fresh whole blood model exacerbated the hemolytic response of the dual drug-loaded hydrogel, potentially attributable to an augmented release of protein-bound GGOH into the surrounding environment. Conversely, the presence of a fibrin matrix within the PL encapsulation and within the *ex vivo* clotted whole blood model contributed to a diminished release of GGOH from the hydrogel and mitigated direct contact between RBCs and the hydrogel surface, thereby attenuating hemolysis. While encapsulating the hydrogel within a PL gel significantly suppressed hemolysis, providing superior RBC protection compared to that afforded by natural fibrin in clotted whole blood, histological analysis of the drug-loaded hydrogel revealed no significant toxicity to RBCs within the clotted whole blood culture. The present findings suggested that the dual drug-loaded composite hydrogel fulfilled the safety criteria prior to *in vivo* investigations of biocompatibility and preventive efficacy for MRONJ-B.

Acknowledgments

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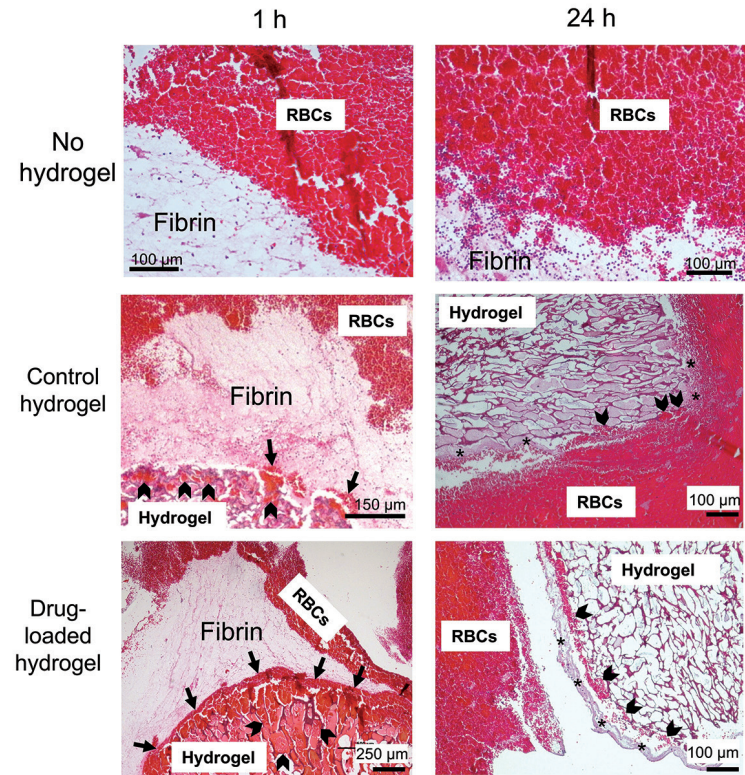


Figure 5: H&E staining of 1-h and 24-h *ex vivo* cultures of clotted human whole blood individually with control and drug-loaded hydrogels revealed the following three zones: The Material zone was composed of the hydrogel. The edematous zone was a loose area surrounding the material, containing scattered inflammatory cells and fibrin (marked as black asterisks). The hemorrhagic zone consisted of collections of RBCs, indicating hemorrhage, and was observed at the periphery of the sections. RBCs were also observed within the peripheral pores of the hydrogel (arrowheads) and formed clusters or aggregates at the material’s periphery (arrows).

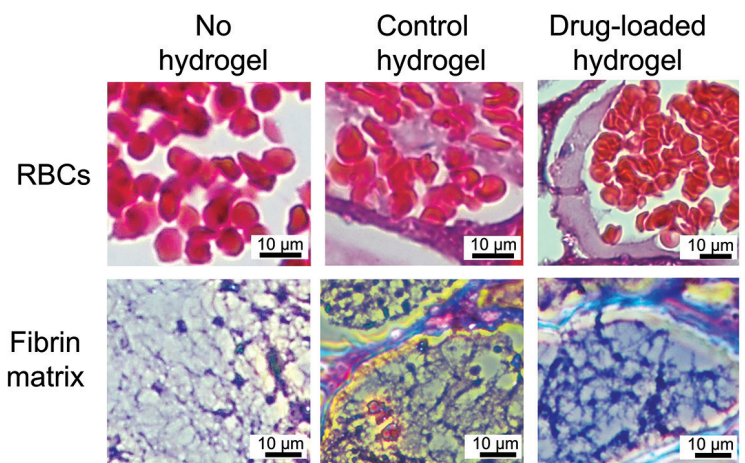


Figure 6: Representative H&E histological images showed RBCs visualized under light microscopy, along with platelets and fibrin fibers visualized under phase-contrast microscopy within the hydrogel, appearing as purple or blue structures. These images were obtained after 24 h of *ex vivo* cultures of clotted human whole blood, either with control or drug-loaded hydrogels. The clotted whole blood culture with no hydrogel, was used as a control.

References

- Ruggiero SL, Dodson TB, Aghaloo T, Carlson ER, Ward BB, Kademani D. American association of oral and maxillofacial surgeons' position paper on medication-related osteonecrosis of the jaws-2022 update. *J Oral Maxillofac Surg.* 2022;80(5):920-43.
- Sungkhaphan P, Thavornnyutikarn B, Muangsant P, Kaewkong P, Kitpakornsanti S, Pornsuwan S, *et al.* Dual-functional drug delivery system for bisphosphonate-related osteonecrosis prevention and its bioinspired releasing model and *in vitro* assessment. *ACS Omega.* 2023;8(29):26561-76.
- Thavornnyutikarn B, Sungkhaphan P, Kaewkong P, Pornsuwan S, Risangud N, Singhatanadgit W, *et al.* Biodegradable dual-function nanocomposite hydrogels for prevention of bisphosphonate-related osteonecrosis of the jaw. *ACS Appl Bio Mater.* 2023;6(4):1658-75.
- Ziebart T, Pabst A, Klein MO, Kämmerer P, Gauss L, Brüllmann D, *et al.* Bisphosphonates: restrictions for vasculogenesis and angiogenesis: inhibition of cell function of endothelial progenitor cells and mature endothelial cells *in vitro*. *Clini Oral Investig.* 2011;15:105-11.
- Pabst A, Krüger M, Sagheb K, Ziebart T, Jacobs C, Blatt S, *et al.* The influence of geranylgeraniol on microvessel sprouting after bisphosphonate substitution in an *in vitro* 3D-angiogenesis assay. *Clini Oral Investig.* 2017;21:771-8.
- Singhatanadgit W, Hankamolsiri W, Janvikul W. Geranylgeraniol prevents zoledronic acid-mediated reduction of viable mesenchymal stem cells via induction of Rho-dependent YAP activation. *R Soc Open Sci.* 2021;8(6):202066.
- Sungkhaphan P, Thavornnyutikarn B, Kaewkong P, Pongkittiphan V, Pornsuwan S, Singhatanadgit W, *et al.* Antibacterial and osteogenic activities of clindamycin-releasing mesoporous silica/carboxymethyl chitosan composite hydrogels. *R Soc Open Sci.* 2021;8(9):210808.
- Chow M, Douglas, and Bennett's. Infections of the oral cavity, neck, and head. Mandell, Douglas, and Bennett's principles and practice of infectious diseases: Elsevier; 2015. p. 789-805. e2.
- Shibahara T. Antiresorptive agent-related osteonecrosis of the jaw (ARONJ): a twist of fate in the bone. *Tohoku J Exp Med.* 2019;247(2):75-86.
- Fan L, Yi J, Tong J, Zhou X, Ge H, Zou S, *et al.* Preparation and characterization of oxidized konjac glucomannan/carboxymethyl chitosan/graphene oxide hydrogel. *Int J Biol Macromol.* 2016;91:358-67.
- Wahid F, Yin JJ, Xue DD, Xue H, Lu YS, Zhong C, *et al.* Synthesis and characterization of antibacterial carboxymethyl Chitosan/ZnO nanocomposite hydrogels. *Int J Biol Macromol.* 2016;88:273-9.
- Chen L, Du Y, Tian Z, Sun L. Effect of the degree of deacetylation and the substitution of carboxymethyl chitosan on its aggregation behavior. *J Polym Sci Part B: Polym Phys* 2005;43(3):296-305.
- Zhang L, Guo J, Zhou J, Yang G, Du Y. Blend membranes from carboxymethylated chitosan/alginate in aqueous solution. *J Appl Polym Sci.* 2000;77(3):610-6.
- Yu T, Malugin A, Ghandehari H. Impact of silica nanoparticle design on cellular toxicity and hemolytic activity. *ACS Nano.* 2011;5(7):5717-28.
- Davidson S, Lamprou DA, Urquhart AJ, Grant MH, Patwardhan SV. Bioinspired silica offers a novel, green, and biocompatible alternative to traditional drug delivery systems. *ACS Biomater Sci Eng.* 2016;2(9):1493-503.
- Joglekar M, Rogers RA, Zhao Y, Trewyn BG. Interaction effects of mesoporous silica nanoparticles with different morphologies on human red blood cells. *Rsc Adv.* 2013;3(7):2454-61.
- Lopes MV, Desoti VC, Caleare AdO, Ueda-Nakamura T, Silva SO, Nakamura CV. Mitochondria superoxide anion production contributes to geranylgeraniol-induced death in leishmania amazonensis. *Evid Based Complement Alternat Med.* 2012;2012(1):298320.
- Weber M, Steinle H, Golombek S, Hann L, Schlensak C, Wendel HP, *et al.* Blood-contacting biomaterials: *in vitro* evaluation of the hemocompatibility. *Front Bioeng Biotechnol.* 2018;6:99.
- Humar R, Schaer DJ, Vallelan F. Erythrophagocytes in hemolytic anemia, wound healing, and cancer. *Trends Mol Med.* 2022;28(11):906-15.
- Dimitrov JD, Roumenina LT, Perrella G, Rayes J. Basic mechanisms of hemolysis-associated thrombo-inflammation and immune dysregulation. *Arterioscler Thromb Vasc Biol.* 2023;43(8):1349-61.
- Bekkering S, Quintin J, Joosten LA, van der Meer JW, Netea MG, Riksen NP. Oxidized low-density lipoprotein induces long-term proinflammatory cytokine production and foam cell formation via epigenetic reprogramming of monocytes. *Arterioscler Thromb Vasc Biol.* 2014;34(8):1731-8.
- Manček-Keber M, Frank-Bertoncelj M, Hafner-Bratkovič I, Smole A, Zorko M, Pirher N, *et al.* Toll-like receptor 4 senses oxidative stress mediated by the oxidation of phospholipids in extracellular vesicles. *Sci Signal.* 2015;8(381):ra60.
- Qian Q, Nath KA, Wu Y, Daoud TM, Sethi S. Hemolysis and acute kidney failure. *Am J Kidney Dis.* 2010;56(4):780-4.
- Lu S, Duffin R, Poland C, Daly P, Murphy F, Drost E, *et al.* Efficacy of simple short-term *in vitro* assays for predicting the potential of metal oxide nanoparticles to cause pulmonary inflammation. *Environ Health Perspect.* 2009;117(2):241-7.
- Dobrovolskaia MA, McNeil SE. Understanding the correlation between *in vitro* and *in vivo* immunotoxicity tests for nanomedicines. *J Control Release.* 2013;172(2):456-66.
- ISO. ISO 10993-4: biological evaluation of medical devices part 4—selection of tests for interactions with blood. International Organization for Standardization: Geneva,

- Switzerland. 2017.
27. Dalal BI, Bridgen ML. Artifacts that may be present on a blood film. *Clin Lab Med.* 2002;22(1):81-100.
 28. Baldwin JM, Lucy JA. Chemically induced fusion of erythrocyte membranes. *Methods in enzymology.* 220: Elsevier; 1993. p.161-73.
 29. Ahkong Q, Fisher D, Tampion W, Lucy J. The fusion of erythrocytes by fatty acids, esters, retinol and α -tocopherol. *Biochem J.* 1973;136(1):147-55.
 30. Behling-Kelly E. Identifying erythrocyte injury in toxicology studies. *Toxicol Pathol.* 2022;50(7):883-5.
 31. Li X, Kong X, Zhang Z, Nan K, Li L, Wang X, *et al.* Cytotoxicity and biocompatibility evaluation of N, O-carboxymethyl chitosan/oxidized alginate hydrogel for drug delivery application. *Int J Biol Macromol.* 2012;50(5):1299-305.
 32. Fu D, Han B, Dong W, Yang Z, Lv Y, Liu W. Effects of carboxymethyl chitosan on the blood system of rats. *Biochem Biophys Res Commun.* 2011;408(1):110-4.
 33. Liu L, Lv Q, Zhang Q, Zhu H, Liu W, Deng G, *et al.* Preparation of carboxymethyl chitosan microspheres and their application in hemostasis. *Disaster Med Public Health Prep.* 2017;11(6):660-7.
 34. Gray JE, Weaver RN, Moran J, Feenstra ES. The parenteral toxicity of clindamycin 2-phosphate in laboratory animals. *Toxicol Appl Pharmacol.* 1974;27(2):308-21.
 35. Chen GP, Yang J, Qian GF, Xu WW, Zhang XQ. Geranylgeranyl transferase-i knockout inhibits oxidative injury of vascular smooth muscle cells and attenuates diabetes-accelerated atherosclerosis. *J Diabetes Res.* 2020;2020(1): 7574245.
 36. Orrico F, Laurance S, Lopez AC, Lefevre SD, Thomson L, Möller MN, *et al.* Oxidative stress in healthy and pathological red blood cells. *Biomolecules.* 2023;13(8):1262.
 37. Kristiansson A, Gram M, Flygare J, Hansson SR, Åkerström B, Storry JR. The role of α 1-microglobulin (A1M) in erythropoiesis and erythrocyte homeostasis—therapeutic opportunities in hemolytic conditions. *Int J Mol Sci.* 2020;21(19):7234.
 38. Taverna M, Marie AL, Mira JP, Guidet B. Specific antioxidant properties of human serum albumin. *Ann Intensive Care.* 2013;3(1):4.
 39. Higashi Y, Pandey A, Goodwin B, Delafontaine P. Insulin-like growth factor-1 regulates glutathione peroxidase expression and activity in vascular endothelial cells: Implications for atheroprotective actions of insulin-like growth factor-1. *Biochim Biophys Acta.* 2013;1832(3):391-9.
 40. Yoon JY, Vu HT, Lee JH, Shin JS, Kim HW, Lee HH, *et al.* evaluation of human platelet lysate as an alternative to fetal bovine serum for potential clinical applications of stem cells from human exfoliated deciduous teeth. *Cells.* 2024;13(10):847.
 41. ASTM International. Standard practice for assessment of hemolytic properties of materials. ASTM F756-08. West Conshohocken (PA): ASTM International; 2008.
 42. Qin Z, Babu VS, Li Y, Shi F, Zhan F, Liu C, *et al.* Hemoglobin mediates inflammation and apoptosis in the head-kidney macrophages of grass carp (*Ctenopharyngodon idella*). *Aquaculture.* 2022;557:738281.
 43. Riedmann K, Meegan J, Cervantes-Cruz Y, Ware L, Bastarache J. Cell-free hemoglobin induces vascular and mitochondrial dysfunction in the pulmonary endothelium in an oxidation-state dependent manner. *Physiology.* 2024;39(S1):1869.
 44. Sabino RM, Kauk K, Movafaghi S, Kota A, Popat KC. Interaction of blood plasma proteins with superhemophobic titania nanotube surfaces. *Nanomedicine.* 2019;21:102046.
 45. Sabino RM, Popat KC. Evaluating whole blood clotting *in vitro* on biomaterial surfaces. *Bio Protoc.* 2020;10(3):e3505-e.



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Comparative Clinical Outcomes of Connective Tissue Graft Procedures Harvested from the Palate, Before and After Recipient Site Preparation: A Randomized Controlled Trial

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Abstract

Objectives: This study aimed to compare the root coverage outcomes of connective tissue grafts (CTG) harvested from the palate before and after recipient site preparation.

Methods: Twenty single gingival recessions type 2 (RT2) at buccal sites of single-rooted teeth, randomized into two groups: graft harvested after (control) and before (test) site preparation. Clinical parameters and patient satisfaction were assessed at baseline and at three months, with follow-ups for complications at 1 week, 2 weeks, 1 month, and 3 months.

Results: At three months, recession reduction was 2.2 ± 1.1 mm (control) and 2.5 ± 0.7 mm (test), with root coverage percentages of $82.0 \pm 32.0\%$ and $90.5 \pm 30.1\%$, respectively. No significant differences were observed in clinical outcomes, complications, or patient satisfaction between the two approaches.

Conclusions: These findings indicate that harvesting CTG prior to recipient site preparation can serve as a clinically effective and flexible alternative, especially in situations where anatomical limitations of the palate might complicate the traditional treatment sequence.

Keywords: gingival recession, recession reduction, root coverage

Introduction

Gingival recession is a significant issue that affects about 50-100% of the population.⁽¹⁻⁴⁾ This condition often causes patients to experience hypersensitivity and unpleasant esthetics. Several factors contribute to gingival recession, including tooth malalignment, traumatic tooth brushing technique, occlusal trauma, irritation from orthodontic appliances, and defective restoration or prosthesis.⁽⁴⁻⁷⁾ The root coverage procedure is an effective treatment option for addressing gingival recession. Nowadays, a combination of connective tissue graft (CTG) and coronally advanced flap (CAF) is recognized as the gold standard for treating gingival recession.⁽⁸⁾ This technique offers excellent esthetic results and a high success rate.⁽⁸⁾ In the CTG technique, it is customary to prepare the recipient site prior to the preparation of the donor site.⁽⁹⁻¹²⁾ This approach is grounded in the principle of minimizing the duration that the CTG is exposed to the external environment, as prolonged exposure may compromise the viability of the graft.⁽¹²⁻¹⁴⁾

The most frequent harvesting area is the hard palate because of its good dimension and blood supply.^(15,16) However, in some cases, there are some anatomical limitations at the palate, such as exostosis, thin palatal gingiva, or shallow palatal vault.⁽¹⁷⁾ These circumstances lead to inadequate graft harvesting for the recipient site, which has already undergone excessive preparation. Over-preparing the recipient site can cause additional tissue damage, increased vasodilation, and diffusion of inflammatory mediators and immune cells, all of which may lead to a higher risk of postoperative complications.⁽¹⁸⁻²⁰⁾ To address these concerns, some clinicians have proposed a modified technique in which the donor graft is harvested prior to recipient site preparation. This approach allows the clinician to adjust the flap design based on the actual size and shape of the harvested graft, potentially reducing the risk of over-preparation and limiting unnecessary flap exposure. However, scientific evidence to support this method is still lacking. The research question of this study was: Does the timing of harvesting CTG from the palate—either before or after the preparation of the recipient site—impact root coverage outcomes in the treatment of gingival recession? Therefore, the objective of this study was to evaluate and compare the root coverage outcomes of CTG harvested from the palate using two different approaches. We hypothesized that there was no

significant difference in root coverage outcomes between CTG harvested before recipient site preparation and those harvested afterward.

Materials and Methods

All participating patients were recruited from the Periodontics Clinics at Mahidol University's Faculty of Dentistry between June 2023 and April 2024. The Faculty of Dentistry/Faculty of Pharmacy, Mahidol University Institutional Review Board approved the study protocol (Ethic number COA.No.MU-DT/PY-IRB 2023/024.0803). The inclusion criteria consisted of non-periodontitis patients aged 18 years or older with isolated gingival recession at buccal sites of single-rooted teeth, which were classified as recessions type 2 (RT2) by Cairo, 2011.⁽²¹⁾ Full mouth bleeding on probing and plaque score were at or below 25%. The probing depth was at or below 4 mm at the site of interest, with no history of periodontal surgery at the recipient site. Patients were excluded from the study if they had any medical contraindications to periodontal surgery, a current or past history of smoking, or if they had used antibiotics or medications that could potentially impact periodontal healing within the last six months. Additionally, individuals exhibiting excessive tooth mobility or non-carious lesions deeper than 1 mm were also excluded.

The sample size was determined based on a statistical power of 80%, with an alpha error level set at 0.05. Anticipating a patient dropout rate of 10%, the calculations indicated that each group should comprise 10 recessions.⁽¹⁵⁾ The investigator (K.T.) calibrated clinical measurements with an expert clinician (W.S.). The intraclass correlation coefficient was excellent for each clinical measurement.

The study was a prospective, parallel-group, randomized control trial registered in Thai Clinical Trials Registry no. TCTR20230826008, the protocol of which is presented in Figure 1. Following the recruitment process, patients participated in interviews, underwent clinical examinations, received oral hygiene instructions, and had full-mouth scaling performed. After this initial phase, patient allocation was performed using the nQuery Advisor program by K.T., with random assignment to either the control or test group. Patients were blinded to their group allocation. The random allocation sequence was concealed in sealed opaque envelopes and was not disclosed to the surgeon until immediately before the surgical procedure.

Patients were subsequently followed up at intervals of 1 week, 2 weeks, 1 month, and 3 months. A clinical examination was repeated at the 3-month mark. During each follow-up, patients were asked to report any pain at both the donor and recipient sites, using the Wong-Baker Faces Pain Rating Scale, which had a score of 1-10.⁽²²⁾ At the 3-month follow-up, patients were also requested to rate their satisfaction using a Patient Satisfaction Index (PSI),

with scores ranging from 1 to 4. Score 1 means surgery met my expectations, score 2 means surgery improved my condition enough so that I would go through it again for the same outcome, score 3 means surgery helped me, but I would not go through it again for the same outcome, and score 4 means I am the same or worse compared to before surgery.⁽²³⁾

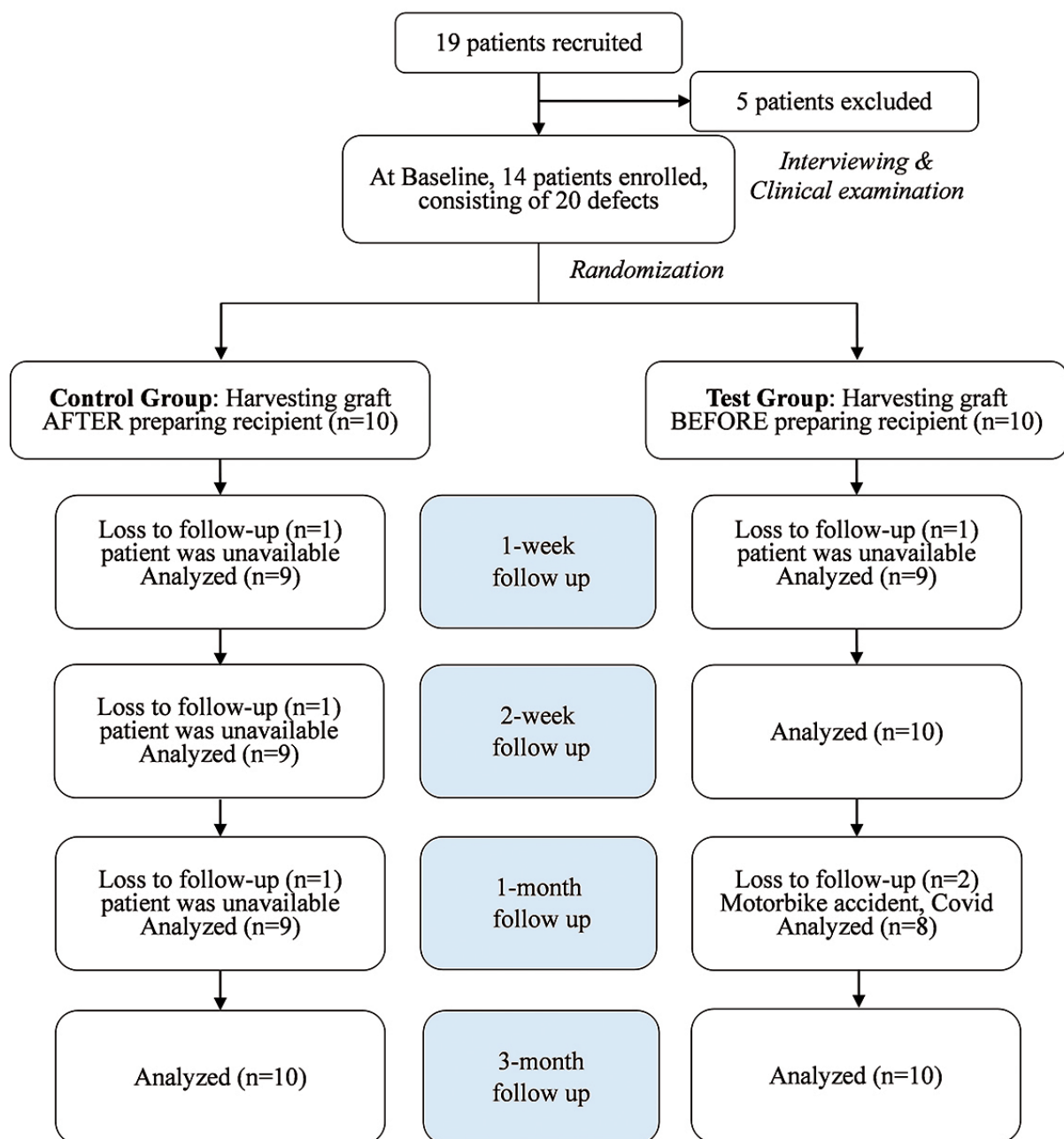


Figure 1: Study protocol.

Clinical parameter measurements: Clinical parameters were measured by K.T. before the surgery and at 3 months post-operation with a PCP-UNC-15 probe (Hu Friedy, Chicago, IL, USA) under a dental loupe (x2.5) as follows:

Probing depth (PD): the distance from the gingival margin to the bottom of the gingival sulcus.

Cementoenamel junction-gingival margin (CEJ-GM): distance from the gingival margin to the CEJ.

Clinical attachment level (CAL): calculation from probing depth plus CEJ-GM

Keratinized tissue width (KTW): the distance from the gingival margin to the mucogingival junction.

Plaque score: the percentage of plaque that was staining on the tooth surface.⁽²⁴⁾

Bleeding on probing (BOP): present bleeding immediately after probing.

Gingival thickness: the periodontal probe shining through gingival tissue after being inserted in the gingival sulcus was defined as thin, and no periodontal probe shining was defined as thick.⁽²⁵⁾

Recession reduction (RecRed): the difference of CEJ-GM at baseline and 3 months

The percentage of root coverage (RC): was calculated using the following formula: Percent of root coverage = (Initial gingival recession depth - Gingival recession depth at 3 months) / (Initial gingival recession depth) x 100

Surgical procedure: An expert clinician (W.S.) performed the surgical procedures, using local anesthesia of 2% mepivacaine with 1:100,000 epinephrine to anesthetize the donor and recipient sites.

Donor site: The donor site was prepared using a 2-parallel-incision technique modified from Bruno's technique.⁽¹⁰⁾ The initial incision was made by positioning the blade perpendicular to the palatal gingiva, ensuring a distance of 2 mm from the gingival margin. A second incision was then created 2 mm apically and parallel to the first, with the blade angled to follow the contours of the palatal surface, achieving a depth of approximately 5 mm to secure adequate graft width. The third incision was initiated along the same line as the first and was performed by angling the blade parallel to the second incision, ensuring a consistent depth of 5 mm to achieve the desired graft width. Once the graft had been excised from the palate, the epithelial collar was carefully removed. The graft was harvested within 5 minutes and immediately

placed in normal saline for hydration. Subsequently, the flap was sutured in place using 4-0 polyglactin 910 suture (VICRYL™) and protected with a non-eugenol periodontal dressing (COE-PAK™).

Recipient site: The recipient site was prepared using a modified Bruno's and Bernimoulin's technique.^(10,26) Briefly, a horizontal incision was made at the base of the interdental papillae, positioned between the recessed tooth and the adjacent teeth. This created a new incision shaped like new papillae, located apical to the initial tips of the papillae. The tissue coronal to these horizontal incisions was de-epithelialized. A sulcular incision was then performed, allowing for the reflection of a partial-thickness flap. A minimal odontoplasty was performed to reduce the root contour if necessary. The graft was carefully adapted to the recipient site and secured with 5-0 polyglactin 910 sutures (VICRYL™). The donor and recipient site preparations are shown in Figure 2. At the surgical site, all the following parameters were measured by K.T. and W.S.

Surgical time (minutes): The duration from the initial incision to the final suture was documented. The time was also measured separately between the donor and the recipient site preparation. The donor preparation time starts from the first incision at the donor site until the graft is removed. The duration the graft remained extraorally was also recorded from when it is removed from the donor until it is placed at the recipient site. The recipient exposure time starts from the first incision at the recipient site to the time when the donor graft is in place.

Gingival thickness: The thickness was measured using an endodontic silicone disk affixed to a sterile needle, positioned at the mid-facial area 1.5 mm below the gingival margin of the recession site. The disk was secured with a flowable composite. Subsequently, the thickness was determined with a caliper accurate to the nearest 0.1 mm.

Alveolar bone dehiscence: A distance from the cementoenamel junction (CEJ) to the bottom of the dehiscence defect was measured using a periodontal probe.

Connective tissue graft size (mm): The width and height of the graft were measured utilizing a periodontal probe.

Post-operative care: The patient was prescribed 15 tablets of ibuprofen 400 mg and 15 tablets of paracetamol 500 mg. They were advised to take the medications as

needed for pain relief. Additionally, patients were instructed to avoid brushing their teeth in the surgical area for four weeks. They rinsed the area for 30 seconds with a 0.12% chlorhexidine solution twice daily for two weeks.

Statistical analysis: The main outcome of the study was the reduction of gingival recession. Secondary outcomes included various other clinical parameters as previously specified. Data were summarized using descriptive statistics, presenting means $\pm$ standard deviations for quantitative variables and percentages for qualitative variables. To analyze differences in baseline data and outcomes between the two treatments, the Fisher exact test and Mann-Whitney U test were employed. A significance level of $p=0.05$ was set for rejecting the null hypothesis.

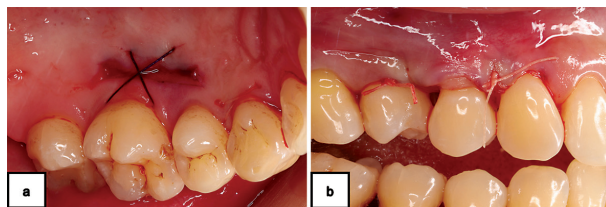


Figure 2: The donor site (a) and recipient site (b) preparation.

Results

Study population at baseline and surgical visit: A total of 19 patients were screened for the study, of which five did not meet the inclusion criteria, resulting in the recruitment of 14 patients (20 teeth). At the three-month follow-up, all subjects were successfully contacted. The mean age of participants was 36.7 years (± 10.3), with ages ranging from 22 to 57 years. At baseline, there were no significant differences in the characteristics between the two groups. Most parameters assessed during surgical visits were also comparable, as shown in Table 1. However, two parameters differed significantly: the recipient site exposure time was shorter in the test group ($p=0.015$), while the extraoral graft duration was shorter in the control group ($p=0.001$).

Clinical Outcomes at Baseline and 3 months: The BOP, plaque score, KTW, PD, CEJ-GM, CAL, and gingival thickness did not differ between the two groups. However, when compared to the baseline, significant improvements were observed at three months, with notable gains in CAL, increased KTW, and enhanced

gingival thickness. The RecRed measurements did not exhibit any differences between the two groups, with the control group showing 2.2 ± 1.1 mm and the test group 2.5 ± 0.7 mm. The clinical parameters assessed at baseline and three months are detailed in Table 2. At baseline, the CAL in the control and test groups was 4.3 ± 1.0 mm and 4.5 ± 1.1 mm, respectively. At the 3-month mark, CAL improved in both groups, showing increases of 1.9 ± 1.0 mm in the control group and 1.8 ± 0.9 mm in the test group. Additionally, the KTW at baseline was recorded at 1.7 ± 1.6 mm for the control group and 1.2 ± 1.2 mm for the test group. After 3 months, the KTW measurements in the control and test groups were 3.0 ± 1.2 mm and 2.8 ± 0.6 mm, respectively. The first premolars in both the control and test groups were followed up to three months, as illustrated in Figure 3.

Pain and patient satisfaction: Pain management was addressed only during the first week post-surgery. After that point, no pain or painkillers were utilized, with the exception of one patient in the test group who reported slight discomfort at the recipient site during the second week. All patients rated their experience with a score of 1, indicating that the surgery met their expectations. However, one patient in the control group provided a score of 2, suggesting that the surgery sufficiently improved their condition to the extent that they would undergo the procedure again for the same outcome. There was no significant difference in pain scores or patient satisfaction between the two groups.

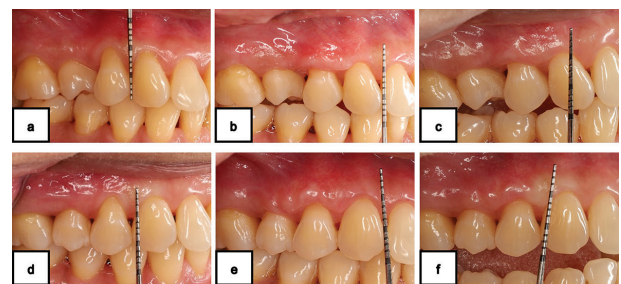


Figure 3: The first premolar of control group followed up at baseline (a), one month (b), and three months (c), the test group followed up at baseline (d), one month (e), and three months (f).

Discussions

Our research examined the outcomes of root coverage achieved using CTG harvested from the palate, both before and after the preparation of the recipient site. The

Table 1: Study population characteristics at baseline and parameters at surgical visit.

Parameter		Control (n=10)	Test (n=10)	p-value
Baseline				
Age (years): Mean±SD		36.5±10.6	36.8±10.6	0.970
BMI (kg/m ²): Mean±SD		22.5±1.9	23.7±3.8	0.643
Males: Females		4:6	5:5	1.000
Area of tooth:	Maxilla	6	6	1.000
	Mandible	4	4	
Tooth type:	Maxillary lateral incisor	2	1	-
	Maxillary canine	2	1	
	Maxillary premolar	2	4	
	Mandibular canine	2	1	
	Mandibular premolar	2	3	
Surgery visit				
Total Surgical time (mins)		49.0±16.6	53.7±10.2	0.174
Recipient exposure time (mins)		38.1±10.6	27.1±6.6	0.015
Donor preparation time (mins)		4.1±0.9	4.1±1.0	0.734
Graft remains extraorally (mins)		17.6±6.8	38.3±9.2	0.001
Gingival thickness (mm)		0.9±0.3	1.1±0.4	0.161
Alveolar bone dehiscence (mm)		5.9±1.8	6.0±1.7	0.760
Graft width (mm)		14.9±2.7	15.7±2.0	0.619
Graft height (mm)		4.1±0.6	4.4±0.8	0.395

Table 2: Clinical parameters outcomes compared between baseline and three months.

Parameter	Time points	Control (n=10)	Test (n=10)	p-value
Probing depth (mm)	Baseline	1.8±0.4	1.7±0.7	0.588
	3-months	1.6±1.0	1.6±0.5	1.000
	p-value	0.688	1.000	
Cementoenamel junction to gingival margin (mm)	Baseline	2.6±0.9	2.8±0.6	0.685
	3-months	0.5±0.8	0.4±0.8	0.693
	p-value	0.000	0.000	
Clinical attachment level (mm)	Baseline	4.3±1.0	4.5±1.1	0.587
	3-months	1.9±1.0	1.8±0.9	0.875
	p-value	0.002	0.002	
Keratinized tissue width (mm)	Baseline	1.7±1.6	1.2±1.2	0.507
	3-months	3.0±1.2	2.8±0.6	0.903
	p-value	0.008	0.008	
Plaque score (%)	Baseline	16.6±5.2	17.1±4.9	0.939
	3-months	21.9±11.7	18.8±5.6	0.650
	p-value	0.232	0.625	
Bleeding on probing (mm)	Baseline	10.9±9.4	9.7±6.4	0.850
	3-months	8.1±5.1	9.7±4.5	0.450
	p-value	0.492	0.695	
Gingival thickness (Number: Thin, thick)	Baseline	(10, 0)	(9, 1)	0.317
	3-months	(6, 4)	(6, 4)	0.383
	p-value	0.046	0.025	
Recession reduction (mm)	3-months	2.2±1.1	2.5±0.7	0.388
Percent of root coverage (%)	3-months	82.0±32.0	90.5±30.1	0.729

study demonstrated that both treatment methods yielded satisfactory clinical results, with comparable outcomes for both groups. Patients in both groups reported that pain subsided within two weeks postoperatively. Additionally, patients expressed a high level of satisfaction with the results of their treatments.

A two-parallel incision was employed for the donor site, while the modified Bruno's⁽¹⁰⁾ and Bernimoulin's⁽²⁶⁾ techniques were utilized for the recipient site. This method avoids the need for vertical incisions, thereby minimizing trauma to the wound area, promoting quicker healing, and lessening post-operative complications.⁽¹⁰⁾

At baseline, the BOP and plaque scores were below 25%, indicating effective control of inflammation prior to the surgical procedure.⁽²⁷⁾ There was no significant difference in PD between the two treatment groups at different time points. This finding is consistent with previous studies conducted in Cairo (2012, 2016) and Zuhr (2014).⁽²⁸⁻³⁰⁾ In contrast, Silva, 2004 reported a significant increase in PD from baseline to the 6-month mark.⁽³¹⁾

Gingival thickness exhibited significant improvement following surgical intervention. According to Zuhr (2021), an increase in soft tissue thickness was observed at the six-month mark post-surgery.⁽³²⁾ At baseline, the CAL was measured at 4.3 ± 1.0 mm in the control group and 4.5 ± 1.1 mm in the test group. After three months, the CAL values in the control and test groups were recorded as 1.9 ± 1.0 mm and 1.8 ± 0.9 mm, respectively. This indicated a significant gain in CAL of approximately 2 mm for both groups, which aligns with the findings of previous studies conducted by Cairo *et al.*, in 2012 and 2016, as well as by Silva *et al.*, in 2004.⁽²⁸⁻³¹⁾

The keratinized tissue width (KTW) measurements were 1.7 ± 1.6 mm for the control group and 1.2 ± 1.2 mm for the test group at baseline. After three months, KTW values increased to 3.0 ± 1.2 mm and 2.8 ± 0.6 mm for the respective groups. The results indicated no significant difference between the two groups. The observed KTW gain aligns with findings from Cairo *et al.*, (2012), where the initial KTW was recorded at 2.6 ± 1.0 mm, rising to 3.7 ± 0.9 mm after three months.⁽²⁶⁾ Additionally, Silva *et al.*,⁽³¹⁾ reported a baseline KTW of 2.8 ± 1.0 mm, with an increase to 3.4 ± 0.7 mm at six months. This reflects a gain of approximately 1 mm in KTW, which was consistent with the results of our study.

At three months, the recession reduction (RecRed) was measured at 2.2 ± 1.1 mm in the control group and 2.5 ± 0.7 mm in the test group, findings that align with previous research. In a study by Zuhr *et al.*,⁽³²⁾ the gingival recession observed with the tunnel technique combined with CTG was reported to be 1.91 ± 0.56 mm. The study by Cairo *et al.*,⁽²⁸⁾ on root coverage treatment used a coronally advanced flap, and CTG reported results of 2.4 ± 0.6 mm at 3 months and 2.6 ± 0.7 mm at 6 months, which aligns with our findings. It was important to recognize that the follow-up period for this research lasted only three months, which may not fully capture the complete healing and remodeling process of the connective tissue graft. Therefore, we recommend extending the observation period in future studies.

The present study compared root coverage outcomes between two harvesting sequences and found no significant differences in clinical outcomes, patient satisfaction, or complication rates. These findings suggested that both techniques were clinically effective, and the modified approach may serve as a practical alternative in anatomically challenging cases. This information can assist clinicians in selecting appropriate surgical protocols and may contribute to improving the success and predictability of root coverage procedures. However, the effect of grafts exposed to an extraoral environment on graft viability and shrinkage has not yet been studied. To date, research has only examined secondary graft shrinkage, which can occur for up to one year.^(33,34) Future studies should focus on primary graft shrinkage occurring immediately after harvesting, within about 15 to 20 minutes and on graft viability extraorally after being harvested. Understanding these factors would lead to improved graft handling for better clinical outcomes. This modified technique, which involves harvesting the graft first, may also reduce prolonged exposure of the recipient site, which can enhance blood supply to the graft and improve wound healing ability.

To our knowledge, this was the first study comparing the clinical outcomes of root coverage using CTG based on the timing of graft harvesting in relation to recipient site preparation. A limitation of this study was its small sample size and the brief duration of observation. Additionally, two patients were lost to follow-up at one month, which may impact the statistical power of the results at that one-month time point. Following the randomization

process, the surgeon (W.S.) became aware of the treatment group prior to the surgery, which compromised the ability to blind the surgeon to the treatment protocol. Given the limited number of investigators involved, the examiner (K.T.) was responsible for conducting the randomization, gathering clinical parameters, and carrying out the statistical analysis. Another limitation is the lack of an acrylic stent to assist with probe placement during follow-up evaluations. While all measurements were carried out by a single examiner, who was calibrated with an expert periodontist and utilized a 2.5× surgical loupe along with consistent anatomical landmarks, the implementation of a stent might have further minimized variability in probing measurements. Future studies should involve a larger sample size and extended observation periods to validate the findings.

Conclusions

This study concluded that harvesting the graft prior to the preparation of the recipient site does not adversely affect clinical outcomes. There were no significant differences in clinical results, complications, or patient satisfaction between the two groups: those with grafts harvested before site preparation and those with grafts harvested afterward.

This research indicated that the clinical outcomes of the two surgical techniques were not significantly different. This information will aid surgeons in making decisions and selecting suitable cases for treatment, especially in scenarios involving large exostoses or thin palatal gingiva. Harvesting grafts prior to preparing the recipient site can serve as an effective alternative treatment option. Additionally, choosing the appropriate technique will contribute to improving the success rates of root coverage procedures.

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References

1. Fragkioudakis I, Tassou D, Sideri M, Vouros I. Prevalance and clinical characteristics of gingival recession in Greek young adults: a cross-sectional study. *Clin Exp Dent Res*. 2021;7(5):672-8.
2. Yadav VS, Gumber B, Makker K, Gupta V, Tewari N, Khanduja P, *et al*. Global prevalence of gingival recession: a systematic review and meta-analysis. *Oral Dis*. 2022(29):2993-3002.
3. Seong J, Bartlett D, Newcombe R, Claydon N, Hellin N, West N. Prevalence of gingival recession and study of associated related factors in young UK adults. *J Dent*. 2018;76:58-67.
4. Kassab MM, Cohen RE. The etiology and prevalence of gingival recession. *J Am Dent Assoc*. 2003;134(2):220-5.
5. Rajapakse PS, McCracken GI, Gwynnett E, Steen ND, Guentsch A, Heasman PA. Does tooth brushing influence the development and progression of non-inflammatory gingival recession? a systematic review. *J Clin Periodontol*. 2007;34(12):1046-61.
6. Joss-Vassalli I, Grebenstein C, Topouzelis N, Sculean A, Katsaros C. Orthodontic therapy and gingival recession: a systematic review. *Orthod Craniofac Res*. 2010;13(3):127-41.
7. Merijohn GK. Management and prevention of gingival recession. *Periodontol* 2000. 2016;71(1):228-42.
8. Chambrone L, Botelho J, Machado V, Mascarenhas P, Mendes JJ, Avila-Ortiz G. Does the subepithelial connective tissue graft in conjunction with a coronally advanced flap remain as the gold standard therapy for the treatment of single gingival recession defects? a systematic review and network meta-analysis. *J Periodontol*. 2022;93(9):1-17.
9. Langer B, Langer L. Subepithelial connective tissue graft technique for root coverage. *J Periodontol*. 1985;56(12):715-20.
10. Bruno JF. Connective tissue graft technique assuring wide root coverage. *Int J Periodontics Restorative Dent*. 1994;14(2):126-37.
11. Nelson SW. The subpedicle connective tissue graft: a bilaminar reconstructive procedure for the coverage of denuded root surfaces. *J Periodontol*. 1987;58(2):95-102.
12. Allen AL. Use of the suprapariosteal envelope in soft tissue grafting for root coverage. I. Rationale and technique. *Int J Periodontics Restorative Dent*. 1994;14(3):302-15.
13. Miller Jr PD. Root coverage with the free gingival graft: factors associated with incomplete coverage. *J Periodontol*. 1987;58(10):674-81.
14. Harris RJ. The connective tissue with partial thickness double pedicle graft: The results of 100 consecutively-treated defects. *J Periodontol*. 1994;65(5):448-61.
15. Rebele SF, Zuhr O, Schneider D, Jung RE, Hürzeler MB. Tunnel technique with connective tissue graft versus coronally advanced flap with enamel matrix derivative for root coverage: a RCT using 3D digital measuring methods. part II. volumetric studies on healing dynamics and gingival dimensions. *J Clin Periodontol*. 2014;41(6):593-603.
16. Studer SP, Allen EP, Rees TC, Kouba A. The thickness of masticatory mucosa in the human hard palate and tuberosity

- as potential donor sites for ridge augmentation procedures. *J Periodontol.* 1997;68(2):145-51.
17. Reiser GM, Bruno JF, Mahan PE, Larkin LH. The subepithelial connective tissue graft palatal donor site: anatomic considerations for surgeons. *Int J Periodontics Restorative Dent.* 1996;16(2):130-7.
 18. Griffin TJ, Cheung WS, Zavras AI, Damoulis PD. Post-operative complications following gingival augmentation procedures. *J Periodontol.* 2006;77(12):2070-9.
 19. Zucchelli G, Mele M, Stefanini M, Mazzotti C, Marzadori M, Montebugnoli L, *et al.* Patient morbidity and root coverage outcome after subepithelial connective tissue and de-epithelialized grafts: a comparative randomized-controlled clinical trial. *J Clin Periodontol.* 2010;37(8):728-38.
 20. Seki M, Miyasaka H, Edamatsu H, Watanabe K. Changes in permeability of strial vessels following vibration given to auditory ossicle by drill. *Ann Otol Rhinol Laryngol.* 2001;110(2):122-6.
 21. Cairo F, Nieri M, Cincinelli S, Mervelt J, Pagliaro U. The interproximal clinical attachment level to classify gingival recessions and predict root coverage outcomes: an explorative and reliability study. *J Clin Periodontol.* 2011;38(7):661-6.
 22. Wong DL, Baker CM. Pain in children: Comparison of assessment scales. *Pediatr Nurs.* 1998;14(1):9-17.
 23. Liu S, Yang SD, Fan XW, Yang DL, Ma L, Sun JY, *et al.* Analyses of effect factors associated with the postoperative dissatisfaction of patients undergoing open-door lamino-plasty for cervical OPLL: a retrospective cohort study. *J Orthop Surg Res.* 2019;14(1):1-8.
 24. O' Leary TJ, Drake RB, Naylor JE. The plaque control record. *J Periodontol.* 1972;43(1):38.
 25. Jepsen S, Caton JG, Albandar JM, Bissada NF, Bouchard P, Cortellini P, *et al.* Periodontal manifestations of systemic diseases and developmental and acquired conditions: Consensus report of workgroup 3 of the 2017 world workshop on the classification of periodontal and peri-implant diseases and conditions. *J Clin Periodontol.* 2018;45(S20):S219-S29.
 26. Bernimoulin JP, Lüscher B, Mühlemann HR. Coronally repositioned periodontal flap. *J Clin Periodontol.* 1975;2(1):1-13.
 27. Sanz M, Herrera D, Kebschull M, Chapple I, Jepsen S, Berglundh T, *et al.* Treatment of stage I–III periodontitis—the EFP S3 level clinical practice guideline. *J Clin Periodontol.* 2020;47(S22):4-60.
 28. Cairo F, Cortellini P, Tonetti M, Nieri M, Mervelt J, Cincinelli S, *et al.* Coronally advanced flap with and without connective tissue graft for the treatment of single maxillary gingival recession with loss of inter-dental attachment. A randomized controlled clinical trial. *J Clin Periodontol.* 2012;39(8):760-8.
 29. Cairo F, Cortellini P, Pilloni A, Nieri M, Cincinelli S, Amunni F, *et al.* Clinical efficacy of coronally advanced flap with or without connective tissue graft for the treatment of multiple adjacent gingival recessions in the aesthetic area: a randomized controlled clinical trial. *J Clin Periodontol.* 2016;43(10):849-56.
 30. Zuhre O, Rebele SF, Schneider D, Jung RE, Hürzeler MB. Tunnel technique with connective tissue graft versus coronally advanced flap with enamel matrix derivative for root coverage: a RCT using 3D digital measuring methods. part I. clinical and patient-centred outcomes. *J Clin Periodontol.* 2014;41(6):582-92.
 31. Silva RCD, Joly JC, de Lima AFM, Tatakis DN. Root coverage using the coronally positioned flap with or without a subepithelial connective tissue graft. *J Periodontol.* 2004;75(3):413-9.
 32. Zuhre O, Akakpo D, Eickholz P, Vach K, Hürzeler MB, Pet-sos H, *et al.* Tunnel technique with connective tissue graft versus coronally advanced flap with enamel matrix derivate for root coverage: 5 year results of an RCT using 3D digital measurement technology for volumetric comparison of soft tissue changes. *J Clin Periodontol.* 2021;48(7):949-61.
 33. Edel A. Clinical evaluation of free connective tissue grafts used to increase the width of keratinized gingiva. *J Clin Periodontol.* 1974;1(4):185-96.
 34. Orsini M, Orsini G, Benlloch D, Aranda JJ, Lázaro P, Sanz M. Esthetic and dimensional evaluation of free connective tissue grafts in prosthetically treated patients: a 1-year clinical study. *J Periodontol.* 2004;75(3):470-7.



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Forecasting Adult Public Oral Healthcare Utilization in Malaysia via ARIMA

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Abstract

Objectives: This study analyzed historical trends in public oral healthcare utilization among the adult population of Malaysia from 1992-2019 and forecasted the future utilization prevalence through 2034.

Methods: Using secondary data from the Malaysian Health Information Management System (HIMS) from 1992 to 2019, this study analyzed the percentage of adults (aged 18-59 years) utilizing public oral healthcare services. The automatic ARIMA function in EViews 12 was used for forecasting, with optimal model parameters selected on the basis of the Akaike and Schwarz criteria. Diagnostic checks validated model adequacy via residual analysis, R-squared, and Prob (F statistics).

Results: Trend analysis revealed distinct periods of change in the prevalence of adult public oral healthcare utilization in Malaysia from 1992 to 2019, characterized by phases of decline, stabilization, gradual growth, and a sharp increase. The ARIMA (3,0,4) model forecasts a decline in the prevalence of adult public oral healthcare utilization from 2.32% in 2020 to 1.83% by 2034, reflecting a gradual reduction in utilization.

Conclusion: Although the trend over 28 years has fluctuated, forecasting analysis projects a gradual decline in utilization prevalence from 2020-2034, reflecting evolving healthcare preferences, increasing private sector alternatives, and infrastructure constraints in the public sector. These findings emphasize the need for proactive policies to address stagnating infrastructure, enhance accessibility, and promote preventive care.

Keywords: adult, ARIMA, Malaysia, oral healthcare utilization, time series forecasting

Introduction

Oral health is crucial for adults, influencing general health, productivity, and quality-of-life.^(1,2) Diets high in processed and sugary foods heighten oral health risks.⁽³⁾ Working adults' poor oral health contributes to substantial individual and national healthcare costs.⁽⁴⁾ Despite various initiatives, public oral healthcare (OHC) in Malaysia remains stagnant, hindered by socioeconomic inequalities, limited access, and low oral health literacy.^(5,6) Evidence-based reforms are needed to integrate oral with general healthcare and enhance targeted outreach.

Malaysia's dual healthcare system consists of a subsidized public sector and a fee-based private sector.⁽⁷⁾ Recognized by the World Health Organization (WHO) for achieving Universal Health Coverage (UHC) since the 1990s,⁽⁸⁾ the public sector provide nominal fees, including exemptions for vulnerable groups like the poor, disabled, and elderly.⁽⁷⁾ Public sector employees benefit from the electronic-guarantee-letter (e-GL) for basic care, while private sector employees may receive employer-sponsored benefits such as panel clinics or reimbursements.⁽⁷⁾ Others pay minimal out-of-pocket costs for public sector care.⁽⁷⁾ Malaysia lacks healthcare social insurance, and private insurance, though available, is rarely used.⁽⁷⁾ Furthermore, limited funding and infrastructure strain its capacity amid growing demand from an aging population. This system includes an extensive network of dental care facilities in health clinics, standalone clinics, hospitals, schools, and urban and rural transformation centers (UTCs and RTCs).⁽⁹⁾ Mobile dental clinics and teams further enhance access for underserved populations in suburban and remote areas.⁽⁹⁾ Public sector dental services for adults include treatment such as extractions, fillings, and scaling, alongside group initiatives via community talks and outreach programs to promote awareness and prevention.⁽⁹⁾

OHC utilization refers to the actual use of dental services based on oral health needs or preferences.⁽¹⁰⁾ It is influenced by factors such as income, employment, and healthcare infrastructure.^(5,6,11,12) Regular check-ups and early interventions align with global goals for better oral health, including a caries-free future.⁽¹³⁾ However, only 13.2% of adults reported utilizing dental services in the National Health and Morbidity Survey (NHMS) 2019,⁽⁵⁾ despite initiatives like free check-ups and awareness campaigns.⁽⁹⁾ Many continue to self-medicate for dental

issues rather than seek professional care.⁽⁶⁾

Recent trends in OHC utilization in Malaysia reveal progress and ongoing challenges. National health surveys from 2006 to 2023 highlight evolving patterns in OHC-seeking behavior.⁽¹⁴⁻²¹⁾ Peaks in reported oral health problems occurred in NHMS 2006 and NHMS 2019, alongside increased OHC utilization in NHMS 2019 (23.7%) and NHMS 2023 (25.7%).^(17,18) Government dental clinics remained primary providers, despite the temporary rise in self-care noted in NHMS 2011 and NHMS 2015.^(15,16) Disparities persist, with rural adults reporting more dental problems (6.2%) than urban adults (5.3%).⁽⁶⁾ While 46.4% of adults with dental problems sought care, self-medication, ethnicity, education, and income disparities influenced access in rural areas, with the wealthiest 20% more likely to seek care than the poorest 20%.⁽⁶⁾

NOHSA 2020 highlighted significant oral health challenges among Malaysian adults, with 94.6% needing treatment, 17 in 20 experiencing dental caries, and 9 in 10 having unhealthy periodontium.⁽²¹⁾ Among older adults, edentulism and tooth loss worsen the burden, with only 34.3% retaining 20 or more teeth.⁽²¹⁾ Despite the availability of public OHC services, access remains challenging due to constrained resources, limited funding, and infrastructural capacity.^(5,6,22)

Time-series forecasting is crucial for healthcare planning, enabling the prediction of future demand and optimal resource allocation. Example of techniques include exponential smoothing,⁽²³⁾ regression-based models,⁽²⁴⁾ and machine learning methods.⁽²⁵⁾ Each method has trade-offs: exponential smoothing captures short-term patterns but oversimplifies;⁽²³⁾ regression models are interpretable but struggle with nonlinear dynamics;⁽²⁴⁾ machine learning handles complexity but lacks transparency and requires large datasets.⁽²⁵⁾ This study adopts the ARIMA model, suited for univariate time series forecasting where historical data serves as the only input.⁽²⁶⁾ ARIMA captures temporal patterns, accommodates non-stationarity, and allows confidence interval (CI) construction for uncertainty estimates.⁽²⁶⁾ Prior studies have demonstrated that historical trends reliably forecast future demand, particularly when long-term data are available.⁽²⁷⁻²⁹⁾ Given the dataset's characteristics (moderate size, annual resolution, and finite period), ARIMA strikes a balance between simplicity, interpretability, and reliability.

More complex methods were deemed unsuitable due to data limitations and unnecessary complexity for this study's focus on trend forecasting.⁽²³⁻²⁵⁾

The WHO Global Oral Health Action Plan 2023-2030 emphasizes the integration of oral health into primary healthcare systems, promoting UHC to ensure equitable access for the population.⁽³⁰⁾ It advocates for capacity building in public healthcare systems, including training and infrastructure development, to meet the needs of vulnerable groups. This aligns with the Malaysian National Oral Health Strategic Plan (NOHSP) 2022-2030, which prioritizes improving the accessibility, affordability, and quality of OHC services, particularly for marginalized groups and those with complex care needs.⁽³¹⁾ The NOHSP also focuses on developing workforce strategies and expanding oral health services to address the increasing demand driven by demographic shifts.⁽³¹⁾

This study supports these objectives by aiming to (1) investigate trends in adult public OHC utilization and (2) forecast future demand among adults in Malaysia. Accurate forecasts help identify service gaps, optimize resources, and develop sustainable plans for the OHC system.⁽³²⁾ While existing research on OHC utilization focuses on high-income countries, evidence from middle-income settings like Malaysia remains limited. By establishing baseline projections, this study provides evidence-based insights for policy development, program evaluation, and effective resource allocation.

Materials & Methods

Study Design and Data Sources

This study employed univariate time series analysis to examine trends and forecast adult public OHC utilization in Malaysia until 2034. Data from 1992 to 2019 were sourced from the Annual Reports compiled by the Health Information Management System (HIMS) under the Ministry of Health Malaysia (MOH).^(9,33) These reports are based on standardized national data collected by trained and calibrated dental personnel following established protocols.⁽³⁴⁾ Data integrity is ensured through multi-level verification at clinic, district, state, and national levels, along with periodic audits and statistical checks by the Health Informatics Centre.⁽³⁴⁾ These measures ensured the integrity, consistency, and reliability of the dataset.

The analysis focused on the percentage of adults

(aged 18-59 years, as defined by HIMS) receiving primary OHC in public facilities.⁽⁹⁾ This age range aligns with Malaysia's legal definition of adulthood.⁽³⁵⁾ In this study, "Adult Public Oral Healthcare Utilization" refers to the percentage of this population utilizing public OHC services in Malaysia.⁽⁹⁾

Data Preprocessing

The dataset covered years 1992-2019, arranged chronologically in a Microsoft Excel file before being imported into EViews 12 for analysis. Inclusion criteria comprised national-level data for adults aged 18-59, with complete, continuous, and structured annual observations from 1992-2019 suitable for ARIMA modeling. State-level data, inconsistent definitions, and data from 2020 onward were excluded to avoid distortion from COVID-19-related service disruptions.

In time series analysis, the sample consists of individual data points, relying on historical patterns and data availability rather than a fixed sample size.^(36,37) Including as many data points as possible ensures robust, reliable results, making a specific sample size unnecessary.^(36,37) Data accuracy and consistency were verified by HIMS personnel responsible for data collection before entry into the database.

Missing data were addressed via linear interpolation, a widely accepted technique that minimizes errors even when up to 60% of values are missing.^(38,39) However, no missing data were identified in this dataset. If missing data were present, sensitivity analyses would be conducted to ensure the chosen method did not alter the findings significantly.⁽⁴⁰⁾ Outliers were detected via correlation plots and logarithmically transformed to stabilize variance and reduce skewness. The final dataset underwent normality assessment using Jarque-Bera test ($p > 0.05$) to confirm a normal distribution.⁽⁴¹⁾ Box plots revealed no extreme values within the data, ensuring the integrity of the dataset for subsequent modeling.⁽⁴²⁾

Forecasting Models and Analysis

The automatic ARIMA function in EViews 12 was used to forecast utilization prevalence (2020-2034). ARIMA requires stationary data, where mean and variance remain constant over time, ensuring stable data generation and accurate forecasting. EViews 12 automates differencing and selects optimal autoregressive (AR) and

moving average (MA) terms using Akaike information criterion (AIC) and Schwarz criterion (SC), streamlining model selection.

The ARIMA model captures time-series dynamics through three parameters: AR (p) for lagged observations, integrated (d) for differencing to achieve stationarity, and MA (q) for lagged residuals, forming the ARIMA (p, d, q) equation:

$$\Phi(B) (1 - B)^d Y_t = \Theta(B) \varepsilon_t$$

In this equation, $\Phi(B)$ and $\Theta(B)$ are polynomials representing the autoregressive and moving average components, respectively. B is the backward shift operator, where BY_t equals Y_{t-1} . The error term, ε_t , is assumed to follow a white noise process, ensuring no autocorrelation in the residuals.

Automatic ARIMA was chosen over manual ARIMA which require manual differencing and parameter selection to reduce subjectivity and misspecification risk.^(36,43) Automating these processes and selecting optimal parameters based on AIC and SC minimizes overfitting and effectively models trends and autocorrelations, making it well-suited for public health forecasting.^(43,44)

Model evaluation and validation

The adequacy of the ARIMA model was validated through diagnostic checks, including residual analysis, to assess independence and homoscedasticity. Goodness-of-fit metrics (AIC and SC) were evaluated to confirm model robustness. Diagnostic checks using R-squared and Prob (F-statistics) confirmed model accuracy.⁽⁴⁵⁾

Durbin-Watson statistic tested for autocorrelation while Jarque-Bera test assessed residuals' normality.^(41,46) The Autoregressive-Conditional-Heteroscedasticity (ARCH) test, which is appropriate for time-series data exhibiting potential time-varying volatility tested for homoscedasticity.^(47,48) These statistical tests validated model assumptions, which are detailed in the supplementary material.

Final Model and Forecasting

Using the finalized ARIMA model, forecasts for adult public OHC utilization were generated for 2020-2034. To verify the model's reliability, the results included the forecasted utilization prevalence and diagnostic plots, such as

actual versus forecasted values and residual diagnostics. All analyses were conducted via EViews 12.

Results

This study analyzed trends in adult public OHC utilization in Malaysia from 1992-2019 and forecasted future utilization through 2034.

Adult Public OHC Utilization Trends from 1992-2019

Adult public OHC utilization demonstrated four distinct phases:

- Steady decline from 6.90% in 1992 to 5.38% in 1997, indicating a decline of 22.03% over five years.
- Stabilization from 1997-2002.
- Gradual growth from 4.94% in 2002 to 7.30% in 2012, a steady growth of 47.77% over the decade.
- Sharp increase from 7.30% in 2012 to 10.10% in 2019, representing an increase of 38.36%.

Figure 1 illustrates these trends, highlighting the significant periods of decline, stabilization, and growth over the 28-year period.

Model Selection and Diagnostics

The lowest AIC, indicating high accuracy, identified ARIMA (3,0,4) as the best-fit model (Supplementary Table 1). The log-transformed series was stationary without differencing, validating the AR and MA components. This model effectively captures trends and seasonality in healthcare utilization data.

Model Performance, Diagnostic Tests, and Statistical Significance

Key diagnostics confirm the robustness of the ARIMA (3,0,4) model. Table 1 presents parameter estimates, standard errors, t statistics, and significance levels. The high R-squared (0.936) and adjusted R-squared (0.910) indicate strong explanatory power. Low standard error (0.063) and significant F statistic ($p < 0.001$) affirm reliability. Durbin-Watson statistic (2.02) suggests minimal residual autocorrelation, confirming the model's validity for forecasting public OHC utilization in Malaysia. The Jarque-Bera test for normality yielded a statistic of 1.28 ($p = 0.526$), indicating normally distributed residuals. ARCH test showed homoskedasticity (F-statistic=2.19, $p = 0.151$; Obs*R-squared=2.17, $p = 0.140$). Detailed results of these diagnostic tests are provided in the supplementary material.

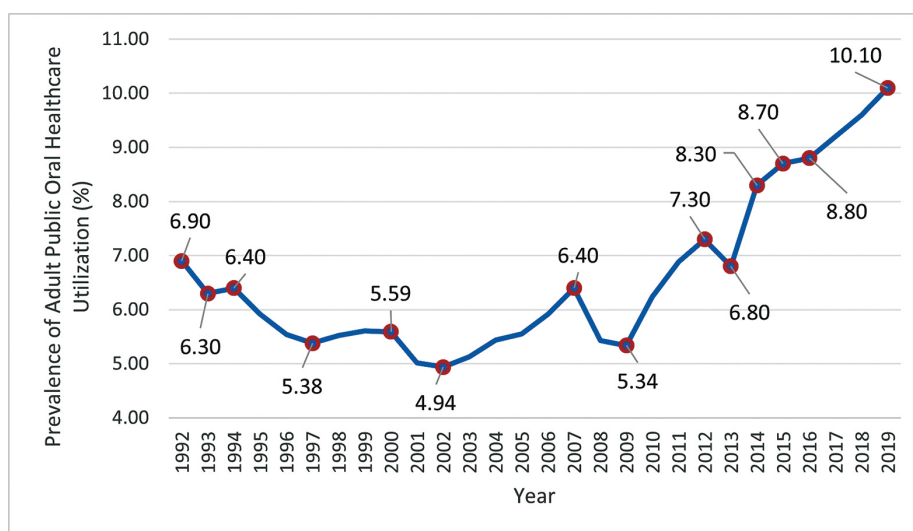


Figure 1: prevalence of adult public oral healthcare utilization (1992-2019).

Adult Public OHC Utilization Forecast from 2020-2034

The forecasted adult public OHC utilization in Malaysia indicates an initial upward trend, peaking in 2026 before gradually declining toward the end of the 15-year projection period (Table 2).

The forecasted adult public OHC utilization in Malaysia reveals three distinct phases:

- Steady increase from 2020-2026, rising from 10.40% to 11.64%, indicating sustained growth.
- Gradual decline from 2027-2029, decreasing from 11.60% to 11.29%, signaling the onset of a downward trend.

- Pronounced decline from 2030 to 2034, with utilization falling from 11.03% to 9.53%.

The forecast includes 95% CIs, providing a measure of reliability to the projections. Near-term estimates ($\pm 0.52\%$ in 2024), show greater certainty, while wider intervals ($\pm 0.95\%$ by 2034), reflect increased long-term uncertainty.

The error measures further demonstrate the strong predictive accuracy of the ARIMA model and highlight the model's reliability for forecasting trends over the short to medium term. Details regarding these error measures are provided in the supplementary material.

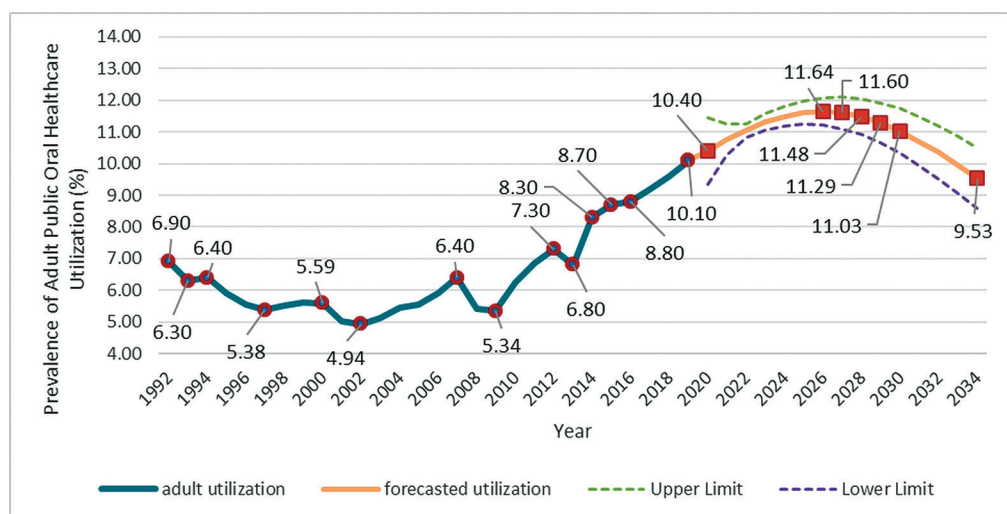


Figure 2: Trend and forecasted prevalence of adult public oral healthcare utilization (1992-2034).

Table 1: Equation outputs of the fitted ARIMA model for the adult public oral healthcare utilization.

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	2.052	0.372	5.509	<0.001
AR(1)	1.317	0.001	1300.410	<0.001
AR(2)	0.322	0.024	13.658	<0.001
AR(3)	-0.666	0.002	-279.619	<0.001
MA(1)	-0.860	186.749	-0.005	0.996
MA(2)	-0.940	2512.185	<0.001	1.000
MA(3)	0.578	1550.757	<0.001	1.000
MA(4)	0.273	1242.440	<0.001	1.000
SIGMASQ	0.003	2.795	0.001	0.999
R-squared	0.936	Mean dependent var		1.862
Adjusted R-squared	0.910	S.D. dependent var		0.211
S.E. of regression	0.063	Akaike info criterion		-2.041
Sum squared resid	0.076	Schwarz criterion		-1.613
Log likelihood	37.577	Hannan-Quinn criterion		-1.910
F-statistic	34.944	Durbin-Watson statistic		2.029
Prob(F-statistic)	<0.001			

Table 2: Forecasted prevalence of adult public oral healthcare utilization in Malaysia from 2020 to 2034.

Forecasted Years Ahead	Prevalence of adult public oral healthcare utilization in Malaysia (%)	95% Confidence Interval	
		Upper Limit	Lower Limit
2020	10.40	9.36	11.44
2021	10.76	10.28	11.24
2022	11.05	10.83	11.27
2023	11.31	11.05	11.57
2024	11.49	11.18	11.80
2025	11.61	11.24	11.98
2026	11.64	11.21	12.07
2027	11.60	11.10	12.10
2028	11.48	10.92	12.04
2029	11.29	10.66	11.92
2030	11.03	10.33	11.73
2031	10.71	9.94	11.48
2032	10.35	9.52	11.18
2033	9.95	9.05	10.85
2034	9.53	8.58	10.48

Discussion

This study examined trends and forecasted the future utilization of adult public OHC services in Malaysia, using automatic ARIMA univariate time series analysis to generate forecasts through 2034.

Trends in Adult Public OHC Utilization (1992-2019)

Adult public OHC utilization trends in Malaysia

from 1992-2019 followed four phases: initial decline (1992-1997), stabilization (1997-2002), gradual growth (2002-2012), and sharp increase (2012-2019).

From 1992-1997, utilization dropped from 6.90% to 5.38%, likely due to limited access, low awareness, and economic barriers. NHMS 1996 reported that 57.5% of sick individuals did not seek care, often deeming it unnecessary. Early oral health surveys and the absence

of comprehensive strategies, such as NOHP 2011-2020, reflected limited policy focus on adult care at the time.⁽⁴⁹⁾

Between 1997 and 2002, utilization fluctuated between 5.38% and 4.94%, reaching its lowest in 2002, which likely reflected resource constraints, economic challenges, or absence of large-scale interventions. Although NOHSA 2000⁽¹⁹⁾ provided useful data, structured efforts to enhance public OHC utilization were still evolving. The low utilization in 2002 likely reflected resource constraints, limited awareness, and economic challenges during Malaysia's recovery from the 1997-1998 Asian financial crisis, with restricted healthcare spending and a public dentist-to-population ratio of 1:8,371, indicating limited service capacity.^(50,51) Less widespread awareness campaigns also reduced public engagement.⁽⁴⁹⁾ Hence, continuous monitoring is essential to guide resource allocation and ensure equitable access during economic uncertainties.

From 2002-2012, utilization rose from 4.94% to 7.30%, with a dip from 6.40% to 5.34% between 2007 and 2009, likely linked to the global financial crisis.⁽⁵²⁾ Indirect costs such as transport and income loss may have deterred non-urgent care.⁽⁵²⁾ Subsequent increases were driven by policy initiatives, such as NOHSA 2010⁽²⁰⁾, and NOHP 2011-2020,⁽⁵³⁾ which improved service delivery through integrated systems, online appointments, automated reminders, and performance monitoring.^(49,53) UTCs and RTCs enhanced accessibility.⁽⁴⁹⁾

Utilization accelerated after 2012, from 7.30% in 2012 to 10.10% in 2019, driven by major public OHC reforms, including infrastructure expansion, targeted health promotion, and technological integration. Mobile dental clinics under the 12th Malaysia Plan improved rural access,⁽⁴⁹⁾ while outreach programs such as *Program Pergigian Mesra Rakyat*,^(49,54) reflected targeted population approach. Digital tools such as the 'MyGusi' and 'MyOrtho' in 2018 further increased accessibility to specialized care.⁽⁴⁹⁾

Importantly, OHC utilization does not necessarily reflect treatment needs, as it depends on accessibility, awareness, SES, and individual behaviors.^(5,6) Therefore, compatibility between adult treatment needs and actual oral health service utilization must be cautiously interpreted. While NOHSA 2020 found 94.6% of Malaysian adults require professional care,⁽²¹⁾ lower utilization rates suggest unmet needs and barriers to care. Integrating

epidemiological data with utilization trends is crucial to address these gaps effectively. These findings highlight the need for policies to improve equitable access and meet actual oral health needs.

Adult Public OHC Utilization Forecast from 2020-2034

Forecasts reveal an initial upward trend, peaking in 2026 and a gradual decline from 2020 to 2034. The early peak may reflect demographic shifts, including a growing and aging population,⁽⁵⁵⁾ as older individuals face higher risks of oral health issues, particularly those with diabetes.⁽⁵⁶⁾ Years of health promotion, policy initiatives, and continued subsidization of basic dental care likely improved public awareness and access,⁽⁴⁹⁾ while modest infrastructure improvements and rising demand contributed to higher utilization.^(7,9)

From 2027 onward, utilization begins to decline. This reduction may reflect changes in healthcare policies, demographic shifts, increased reliance on private dental services, or accessibility challenges. Between 2016 and 2023, the private sector's share of dentists increased from 36.1% to 46.55%, while the public sector declined from 63.9% to 53.45%.⁽⁵¹⁾ Although the public dentist-to-population ratio improved from 1:6,725 (2016) to 1:4,588 (2023), it lagged behind the private sector's expansion.⁽⁵¹⁾ The population-to-dental-unit ratio in the public sector reflects systemic strain, improving from 1:12,037 in 1992 to 1:7,460 in 2006, then worsening to 1:17,501 in 2011 before recovering to 1:12,431 in 2019.⁽⁹⁾ This indicates stagnant infrastructure development amid rising demand. While the growth of private clinics may reduce public sector congestion, improving access for those unable to afford private care, it is crucial to enhance public infrastructure to ensure quality care and meet the rising demand from an aging population. Without such improvements, the public sector may still struggle to support vulnerable groups despite reduced congestion.

Delayed public OHC access can worsen untreated conditions, leading to advanced conditions, which escalate treatment costs and systemic healthcare burdens.^(57,58) Low-income groups, lacking resources for private care, are particularly vulnerable. Addressing this decline is vital to prevent rising disease complications and healthcare costs. Given the stagnant infrastructure and increasing reliance on private services, targeted policies are essential to

address growing inequalities in OHC access and prevent adverse health outcomes.

Policy Implications

Despite NOHSA 2020 findings that 94.6% of Malaysian adults require professional intervention,⁽²¹⁾ utilization is expected to drop, suggesting worsening access barriers, potentially leading to unmet oral health needs and an increased burden of untreated dental conditions. Targeted policies to enhance affordability, accessibility, and awareness are essential to address these challenges.

Economic factors may contribute to declining public dental service utilization. Despite subsidized OHC,⁽⁷⁾ out-of-pocket costs for specific treatments, transportation and lost work time, may deter care-seeking,^(5,6,12) especially for lower-income populations prioritizing other financial obligations.^(6,12) The economic downturn and rising cost of living exacerbate this problem, necessitating expanded subsidies, financial assistance programs, or insurance coverage to maintain accessibility.

Long waiting time in MOH facilities also discourage utilization.⁽⁵⁹⁾ Working adults prefer private clinics for faster, more convenient, and perceived better quality of care despite higher costs.⁽⁶⁰⁾

Employer-sponsored dental benefits⁽⁶¹⁾ may further drive working adults towards private dental services. With financial support, individuals may perceive private dental care as more accessible and convenient, reinforcing the preference for private clinics. Consequently, public OHC services are increasingly utilized by populations without such benefits, potentially widening socioeconomic disparities in access.

The public sector workforce challenges compound the issue. Despite increased dentist, limited permanent positions in MOH facilities push contract-based dental officers to private practice.⁽⁶²⁻⁶⁴⁾ This reduces affordable public service capacity and affects those reliant on government clinics. Addressing this workforce imbalance requires sustained investment in infrastructure, adequate staffing and resources to meet patient demand to help mitigate service limitations and improve long-term accessibility.

Beyond financial and workforce challenges, geographic disparities in OHC remain a concern. Rural and underserved adults often lack access to public

dental facilities,^(6,11) contributing to declining utilization. Expanding mobile clinics, community outreach, workplace-based initiatives, and teledentistry can improve access, particularly for preventive care.

The NOHSP 2022-2030 prioritizes preventive and rehabilitative measures but requires proactive efforts to reverse declining utilization.⁽³¹⁾ Strengthening public health campaigns, addressing financial barriers, and sustaining the public dental workforce are crucial. Without targeted interventions, declining service use, oral health disparities and untreated dental conditions may worsen in Malaysian adults.

Integrating OHC into Malaysia's primary healthcare (PHC) framework can address systemic challenges and improve care for conditions like diabetes and cardiovascular diseases.⁽⁶⁵⁾ Currently, oral health services operate independently within PHC, limiting holistic care despite partial integration efforts, such as the National Strategic Plan for Non-Communicable Disease 2016-2025 and smoking cessation under the KOTAK program.^(49,66) However, collaboration and referrals between medical and oral health services remain inadequate, highlighting the need for governance reforms and multidisciplinary coordination within MOH.⁽⁹⁾ Oral health professionals should address systemic health issues, while primary care physicians require basic oral health training.^(65,67) The existing *Ikon Gigi* program, which trains community representatives to promote oral health, can be expanded to boost outreach efforts in underserved areas.⁽⁶⁸⁾ Continuity of care also requires shared electronic health records. While Malaysia's Teleprimary Care-Oral Health Clinical Information System (TPC-OHCIS)⁽⁶⁹⁾ supports integration, its adoption is limited by poor digital infrastructure and stakeholders' preference for systems like the Dental Information System (DIS). Upgrading infrastructure and unifying TPC-OHCIS and DIS can improve data sharing and care coordination. To sustainably integrate OHC into Malaysia's PHC system, increased public funding and public-private-partnerships are needed. Current programs like *PeKa B40* and *Skim Perubatan Madani* support low-income groups through free screenings and treatment for medical conditions,⁽⁷⁰⁾ but exclude OHC. Integrating basic oral health packages into these initiatives would address a critical gap, support NCD prevention, and advance UHC goals by improving access for underserved populations.

These policy recommendations should be considered within the context of the study's forecasting approach, which extrapolates from historical utilization data alone. Therefore, continuous data monitoring and incorporating evolving socioeconomic and health system factors are essential for responsive planning.

Strengths and Limitations

Given that our projections are based solely on historical dental utilization data, the following outlines the study's methodological strengths and limitations, which inform appropriate interpretation and future research priorities.

The robustness of the dataset enhances this study's reliability, as it draws from credible national databases systematically collected over 28 years (1992-2019) through an established monitoring system. This long-term consistency ensures accurate reflections of national trends in adult OHC utilization, enabling pattern identification, shift detection, and reliable projections. The dataset's continuity strengthens the study's validity, making its findings valuable for policymakers and healthcare planners.

A key strength of this study lies in its ability to provide macro-level insights into public OHC utilization trends in Malaysia. By leveraging ARIMA modeling on aggregated data, the study identifies distinct periods of growth, stabilization, and decline, offering policymakers a comprehensive view of the overall trajectory of service demand. These insights are instrumental for strategic planning, resource allocation, and national policy development. While the data is aggregated at the national level and lacks subgroup detail, the trends identified can guide high-level decision-making by signaling overall system demands and highlighting the need for targeted follow-up studies.

The model uses only historical utilization data, assuming trend continuity without incorporating external factors. This limits its responsiveness to unexpected shifts such as economic shocks, policy changes, or health crises. Although post-2020 data was excluded to avoid pandemic distortion, this also limits insight into recent system transformations. Additionally, private sector utilization is not captured, potentially underrepresenting total demand. However, modeling 28 years of utilization patterns indirectly captures the impact of major national health policies

and programs, such as the NOHP 2011–2020, mobile dental clinic expansion, and increased public sector dental funding under the Tenth and Eleventh Malaysia Plans.⁽⁵³⁾ These embedded policy signals provide valuable insights into their population-level effects, demonstrating how long-term data can inform planning without explicitly modeling interventions.

The absence of key explanatory variables restricts the model's capacity to identify causal relationships or to predict the effects of targeted policies. Therefore, while the forecast provides valuable insight into expected trends, it should be complemented with ongoing data collection and periodic model updates incorporating broader determinants when available. Hence, the forecasts provide a practical baseline scenario but should be interpreted cautiously, especially for long-term planning.⁽⁷¹⁾

Nevertheless, univariate time series models like ARIMA are valued for their simplicity and efficiency, with studies demonstrating their ability to outperform multivariate models, particularly when data is limited or complex.⁽²⁹⁾ In this study, ARIMA's adequacy was validated through rigorous diagnostic checks and residual analysis, confirming robustness and minimizing overfitting risks. Key error measures further demonstrated strong predictive accuracy and reliability, highlighting the model's suitability for short- to medium-term forecasting.

Including 95% CIs enhances the interpretability of projections.⁽⁷²⁾ Short-term projections (2020-2026) show greater precision, while longer-term forecasts (2027-2034) are more uncertain due to potential demographic, economic, or technological changes.⁽⁷¹⁾ These limitations highlight the need for periodic updates with the latest data and methodologies.⁽⁷³⁾ Stakeholders should use short-term insights for immediate planning, treat long-term trends as evolving baselines, and incorporate scenario planning to enhance preparedness and responsiveness.⁽⁷⁴⁾

Another limitation is the absence of subgroup analyses (e.g., by SES, geography, or treatment needs) due to data aggregation. This restricts the ability to pinpoint disparities or identify underserved populations. Nonetheless, national trends provide a foundation for targeted research and policy formulation. Future studies should incorporate multivariable approaches with stratified data to enhance forecasting accuracy and support equitable resource distribution.

Conclusions

Trend analysis (1992-2019) revealed phases of decline, stabilization, gradual growth, and sharp increase in Malaysia's adult public OHC utilization. Forecasts predict a gradual decline (2020-2034) due to shifting preferences, private sector growth, and infrastructure constraints. These findings highlight the need for proactive policies to improve access, expand infrastructure, and promote preventive care, providing a foundation for evidence-based policy-making to ensure equitable, sustainable OHC.

Declarations

Ethical considerations

This is a secondary data analysis using data obtained from reports generated by the Health Information Management System (HIMS) under the Ministry of Health, Malaysia. Throughout the study, the tenets of the Declaration of Helsinki were followed. This study was approved by the Medical Ethics Committee of the Faculty of Dentistry, Universiti Malaya (Approval No. DF CO2410/0020 (P)) and the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (Approval No. NMRR ID-24-00964-XEE).

Availability of data and materials

The datasets that support the findings of this article are publicly available in the annual reports published by the Ministry of Health Malaysia, which are accessible through their official website.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

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References

1. Dörfer C, Benz C, Aida J, Campard G. The relationship of

oral health with general health and NCDs: a brief review. *Int Dent J.* 2017;67(Suppl 2):14-8.

2. Koistinen S, Olai L, Ståhlacke K, Fält A, Ehrenberg A. Oral health-related quality of life and associated factors among older people in short-term care. *Int J Dent Hyg.* 2020;18(2):163-72.
3. Yannakoulia M, Mamalaki E, Anastasiou CA, Mourtzi N, Lambrinou I, Scarmeas N. Eating habits and behaviors of older people: where are we now and where should we go?. *Maturitas.* 2018;114:14-21.
4. Zaitis T, Saito T, Oshiro A, Fujiwara T, Kawaguchi Y. The impact of oral health on work performance of Japanese workers. *J Occup Environ Med.* 2020;62(2):e59-64.
5. Tan YR, Tan EH, Jawahir S, Mohd Hanafiah AN, Mohd Yunus MH. Demographic and socioeconomic inequalities in oral healthcare utilisation in Malaysia: evidence from a national survey. *BMC Oral Health.* 2021;21(1):34.
6. Tan YR, Jawahir S, Doss JG. Oral healthcare-seeking behavior of Malaysian adults in urban and rural areas: findings from the National Health and Morbidity Survey 2019. *BMC Oral Health.* 2023;23(1):719.
7. Jaafar S, Noh KM, Muttalib KA, Othman NH, Healy J. Malaysia health system review. New Delhi: World Health Organization, Regional Office for the Western Pacific; 2013.
8. Lo Ying-Ru J, Pascale A. World Health Day 2018 – Lessons from Malaysia on Universal Health Coverage [Internet]. World Health Organization Malaysia; 2018 [cited 2020 Nov 20]. Available from: <https://www.who.int/malaysia/news/detail/18-04-2018-world-health-day-2018-%E2%80%93-lessons-from-malaysia-on-universal-health-coverage>
9. Oral Health Programme. Annual report 2022 [Internet]. Putrajaya: Ministry of Health Malaysia; 2023 [cited 2023 Jun 12]. Available from: https://hq.moh.gov.my/ohd/images/pdf/annual_rpt/annual_rpt23.pdf
10. Northridge ME, Kumar A, Kaur R. Disparities in access to oral health care. *Annu Rev Public Health.* 2020;41:513-35.
11. Md Bohari NF, Kruger E, John J, Tennant M. Analysis of dental services distribution in Malaysia: a geographic information systems-based approach. *Int Dent J.* 2019;69(3):223-9.
12. Azhar N, Doss JG. Health-seeking behaviour and delayed presentation of oral cancer patients in a developing country: a qualitative study based on the self-regulatory model. *Asian Pac J Cancer Prev.* 2018;19(10):2935-41.
13. The Alliance for a Cavity-Free Future. Alliance for a Cavity-Free Future: Malaysian chapter [Internet]. Alliance for a Cavity-Free Future; 2013 [cited 2020 Oct 7]. Available from: <https://www.acffglobal.org/chapters/chapters-spotlight/>
14. Institute for Public Health. The Third National Health and Morbidity Survey (NHMS III) 2006: general findings. Putrajaya: Ministry of Health Malaysia; 2008.
15. Institute for Public Health. National Health and Morbidity

- Survey 2011 (NHMS 2011) Vol. III: Healthcare Demand [Internet]. Putrajaya: Ministry of Health Malaysia; 2012 [cited 2020 Sep 7]. Available from: <http://www.iku.gov.my/images/IKU/Document/REPORT/NHMS2011-VolumeIII.pdf>
16. Institute for Public Health; Institute for Health Systems Research. National Health and Morbidity Survey (NHMS) 2015. Vol III: Healthcare Demand [Internet]. Putrajaya: Ministry of Health Malaysia; 2015 [cited 2020 Sep 7]. Available from: <http://iku.moh.gov.my/images/IKU/Document/REPORT/NHMS2015-VolumeIII.pdf>
 17. Institute for Health Systems Research; Institute for Public Health. National Health and Morbidity Survey 2019 (NHMS 2019) Vol. II: Healthcare Demand [Internet]. Putrajaya: Ministry of Health Malaysia; 2020 [cited 2020 Sep 7]. Available from: http://www.ihsr.moh.gov.my/images/publication_material/NHMS2019/hcd2019_report.pdf
 18. Institute for Public Health; Institute for Health Systems Research. Fact sheet: National Health and Morbidity Survey (NHMS) 2023 – Non-communicable diseases & healthcare demand [Internet]. Putrajaya: Ministry of Health Malaysia; 2024 [cited 2025 Feb 14]. Available from: <https://repository.nih.gov.my/handle/123456789/711>
 19. Oral Health Division. National Oral Health Survey of Adults (NOHSA 2000). Putrajaya: Ministry of Health Malaysia; 2004.
 20. Oral Health Division. National Oral Health Survey of Adults 2010 (NOHSA 2010). Putrajaya: Ministry of Health Malaysia; 2013.
 21. Oral Health Programme. National Oral Health Survey of Adults 2020 (NOHSA 2020) [Internet]. Putrajaya: Ministry of Health Malaysia; 2023 [cited 2024 Nov 18]. Available from: <https://hq.moh.gov.my/ohp/images/pdf/4.-penyelidikan-kesihatan-pergigian/key-findings-nohsa-2020.pdf>
 22. Bin Juni MH. Public health care provisions: access and equity. *Soc Sci Med*. 1996;43(5):759-68.
 23. Booranawong T, Booranawong A. Double exponential smoothing and Holt-Winters methods with optimal initial values and weighting factors for forecasting lime, Thai chili and lemongrass prices in Thailand. *Eng Appl Sci Res*. 2018;45(1):32-8.
 24. Harrell FE. General aspects of fitting regression models. In: *Regression Modeling Strategies: With Applications to Linear Models, Logistic and Ordinal Regression, and Survival Analysis*. 2nd ed. Cham: Springer; 2015. p.13-44.
 25. Tealab A. Time series forecasting using artificial neural networks methodologies: a systematic review. *Future Comput Inform J*. 2018;3(2):334-40.
 26. Box GE, Jenkins GM, Reinsel GC, Ljung GM. *Time series analysis: forecasting and control*. Hoboken (NJ): Wiley; 2015.
 27. Chen HM, Shen K, Ji L, McGrath C, Chen H. Global and regional patterns in edentulism (1990–2021) with predictions to 2040. *Int Dent J*. 2025;75(2):735-43.
 28. Huang Y, Xu C, Ji M, Xiang W, He D. Medical service demand forecasting using a hybrid model based on ARIMA and self-adaptive filtering method. *BMC Med Inform Decis Mak*. 2020;20(1):237.
 29. Iwok I, Okpe A. A comparative study between univariate and multivariate linear stationary time series models. *Am J Math Stat*. 2016;6:203-12.
 30. Eaton K, Yusuf H, Vassallo P. The WHO global oral health action plan 2023-2030. *Community Dent Health*. 2023;40(2):68-9.
 31. Oral Health Programme. National Oral Health Strategic Plan 2022–2030 [Internet]. Ministry of Health Malaysia; 2022 [cited 2023 Jun 12]. Available from: https://hq.moh.gov.my/ohd/images/pdf/publication/Snap%20Shot%20NOHSP%202022_2030_FINAL_Edaran%202.0.pdf
 32. Meyerhoefer CD, Panovska I, Manski RJ. Projections of dental care use through 2026: preventive care to increase while treatment will decline. *Health Aff (Millwood)*. 2016;35(12):2183–9.
 33. Suhana AS. Health Information Management System (HIMS) modul: Myhealthrecord [Internet]. Kuala Lumpur: Universiti Malaya; 2003 [cited 2024 Nov 18]. Available from: <https://api.semanticscholar.org/CorpusID:215899609>
 34. Abdullah ZS. Hospital information systems implementation framework: critical success factors for Malaysian public hospitals [Internet]. Sarawak (MY): Curtin University; 2013 [cited 2025 May 22]. Available from: <https://core.ac.uk/download/pdf/195632127.pdf>
 35. Government of Malaysia. Age of Majority Act 1971. Federal Constitution; 1971.
 36. Hyndman RJ, Athanasopoulos G. *Forecasting: principles and practice*. Melbourne: OTexts; 2018.
 37. Hyndman RJ, Kostenko AV. Minimum sample size requirements for seasonal forecasting models. *Foresight*. 2007;6(6):12–5.
 38. Twumasi-Ankrah S, Odoi B, Pels WA, Gyamfi H. Efficiency of imputation techniques in univariate time series. *Int J Sci Environ Technol*. 2019;8(3):430-53.
 39. Salgado CM, Azevedo C, Proença H, Vieira SM. Missing data. In: *Secondary analysis of electronic health records* [Internet]. Cham: Springer International Publishing; 2016 [cited 2023 Dec 7]. p.143-62. Available from: http://link.springer.com/10.1007/978-3-319-43742-2_13
 40. Molenberghs G. Incomplete data in clinical studies: analysis, sensitivity, and sensitivity analysis. *Drug Inf J*. 2009;43:409-29.
 41. Jarque CM, Bera AK. A test for normality of observations and regression residuals. *Int Stat Rev*. 1987;55(2):163-72.
 42. Mowbray FI, Fox-Wasylyshyn SM, El-Masri MM. Univariate outliers: a conceptual overview for the nurse researcher. *Can J Nurs Res*. 2019;51(1):31–7.
 43. Agung IGN. *Advanced time series data analysis: forecasting*

- using EViews. Hoboken (NJ): John Wiley & Sons; 2019.
44. Box GEP, Jenkins GM. Time series analysis: forecasting and control [Internet]. San Francisco: Holden-Day; 1976 [cited 2025 May 26]. Available from: <https://books.google.com.my/books?id=1WVHAAAAMAAJ>
 45. Ozili PK. The acceptable R-square in empirical modeling for social science research. *Soc Res Methodol Publ Results* [Internet]. 2022 [cited 2024 Oct 13]. doi: 10.2139/ssrn.4128165.
 46. Durbin J, Watson GS. Testing for serial correlation in least squares regression I. In: *Breakthroughs in statistics: methodology and distribution*. New York: Springer; 1992. p. 237-59.
 47. Engle RF. Autoregressive conditional heteroscedasticity with estimates of the variance of United Kingdom inflation. *Econometrica*. 1982;50(4):987-1007.
 48. McLeod AI, Li WK. Diagnostic checking ARMA time series models using squared-residual autocorrelations. *J Time Ser Anal*. 1983;4:269-73.
 49. Oral Health Programme. Milestones in Dentistry (3rd ed) [Internet]. Ministry of Health Malaysia; 2019 [cited 2025 Apr 1]. Available from: https://hq.moh.gov.my/ohp/images/pdf/1.-penerbitan-utama/milestone/milestones_01122023.pdf
 50. Suleiman AB, Lye MS, Yon R, Teoh SC, Alias M. Impact of the East Asian economic crisis on health and health care: Malaysia's response. *Asia Pac J Public Health*. 1998;10(1): 5-9.
 51. Malaysian Dental Council. MDC annual statistic [Internet]. Official Website of the Malaysian Dental Council; 2025 [cited 2025 Apr 5]. Available from: <https://hq.moh.gov.my/ohd/mdc/>
 52. Sánchez Recio R, Alonso Pérez De Ágreda JP, Rabanaque MJ, Aguilar Palacio I. Understanding the effect of economic recession on healthcare services: a systematic review. *Iran J Public Health* [Internet]. 2022 [cited 2025 Mar 14]. doi: 10.18502/ijph.v5i1i3.8925.
 53. Oral Health Programme. National Oral Health Plan for Malaysia 2011–2020 [Internet]. Ministry of Health Malaysia; 2011 [cited 2024 Nov 3]. Available from: <https://hq.moh.gov.my/ohd/images/pdf/nohp20112020.pdf>
 54. Oral Health Programme. Guidelines: Oral Healthcare for Young Adults in Malaysia [Internet]. Ministry of Health Malaysia; 2019 [cited 2021 Mar 17]. Available from: <http://ohd.moh.gov.my/images/pdf/xtvtnsop/ohyoung.pdf>
 55. Department of Statistics Malaysia. OpenDOSM [Internet]. Putrajaya: Ministry of Economy Malaysia; 2025 [cited 2025 Apr 5]. Available from: <https://www.dosm.gov.my/portal-main/home>
 56. Agarwal R, Baid R. Periodontitis and diabetes: a bidirectional, cyclical relationship - a brief review. *Acta Med Int*. 2017;4(2):46.
 57. Aida J, Takeuchi K, Furuta M, Ito K, Kabasawa Y, Tsakos G. Burden of oral diseases and access to oral care in an ageing society. *Int Dent J*. 2022;72(Suppl 1):S5-S11.
 58. Kasusu Klint N, Satu L, Risto T. Costs of dental care and its financial impacts on patients in a population with low availability of services. *Community Dent Health*. 2019;36(2):131-6.
 59. Toh LS, Sern CW. Patient waiting time as a key performance indicator at orthodontic specialist clinics in Selangor. *Malays J Public Health Med*. 2011;11:10.
 60. Cerina. Government and private dental service in Malaysia: what's the difference and which one is better? [Internet]. LookP; [cited 2021 Mar 18]. Available from: <https://www.lookp.com/blog/government-and-private-dental-service-malaysia>
 61. Board on Health Promotion and Disease Prevention, Committee on Assuring the Health of the Public in the 21st Century. The future of the public's health in the 21st century. Washington (DC): National Academies Press; 2003.
 62. Boo S-L. More contract doctors quit in 2022 than in two previous years combined [Internet]. CodeBlue Health and Human Rights; 2023 [cited 2024 Oct 24]. Available from: <https://codeblue.galencentre.org/2023/02/more-contract-doctors-quit-in-2022-than-in-two-previous-years-combined/>
 63. Boo S-L. Contract doctors' future now questionable, civil service permanent appointments halted from February [Internet]. CodeBlue Health and Human Rights; 2024 Jan 24 [cited 2024 Oct 24]. Available from: <https://codeblue.galencentre.org/2024/01/contract-doctors-future-now-questionable-civil-service-permanent-appointments-halted-from-february/>
 64. Jinah N, Lee KY, Zakaria NH, Zakaria N, Ismail M, Mohmad S. Contract doctors' strike in Malaysia: a content analysis of the perception of medical fraternity and stakeholders on Facebook. *PLoS One*. 2023;18(9):e0292213.
 65. Prasad M, Manjunath C, Murthy A, Sampath A, Jaiswal S, Mohapatra A. Integration of oral health into primary health care: a systematic review. *J Fam Med Prim Care*. 2019;8:1838.
 66. Ministry of Health Malaysia. National Strategic Plan for Non-Communicable Disease (NSP-NCD) 2016-2025. Putrajaya: Ministry of Health Malaysia; 2016.
 67. Villa A, Da Costa J, Duong M-L, Frazier K, Urquhart O. Oral-systemic health considerations in dental settings. *J Am Dent Assoc*. 2022;153(4):388–9.
 68. Oral Health Programme, Ministry of Health Malaysia. Garis panduan pelaksanaan Program Ikon Gigi. Putrajaya: Ministry of Health Malaysia; 2019.
 69. Ismail S, Sutan R, Ibrahim R, Rathi FZM. Psychometric properties of the Teleprimary Care Oral Health Clinical Information System (TPC-OHCIS) Questionnaire using the Rasch model. *Cureus*. 2024;16(6):e63064.
 70. Nor Mukshar AN, Mohd Puad NA, Ahmad KA. Ciri-ciri

- skim takaful kesihatan mikro: kajian persepsi dalam kalangan B40: characteristics of micro health takaful schemes: a perception study among B40. *J Muwafaqat*. 2023;6(2):133-56.
71. Ospina R, Gondim JAM, Leiva V, Castro C. An overview of forecast analysis with ARIMA models during the COVID-19 pandemic: methodology and case study in Brazil. *Mathematics*. 2023;11(14):3069.
72. Hespanhol L, Vallio CS, Costa LM, Saragiotto BT. Understanding and interpreting confidence and credible intervals around effect estimates. *Braz J Phys Ther*. 2019;23:290-301.
73. Makridakis S, Bakas N. Forecasting and uncertainty: A survey. *Risk Decis Anal*. 2016;6(1):37-64.
74. Volkery A, Ribeiro T. Scenario planning in public policy: Understanding use, impacts and the role of institutional context factors. *Technol Forecast Soc Change*. 2009;76:1198-207.

Supplementary Materials

Supplementary Table 1: Model Selection Criteria for ARIMA Models of Adult Public Oral Healthcare Utilization in Malaysia.

ARMA Model	Log Likelihood	AIC*	BIC	HQ
(3,4)(0,0)	37.58	-2.04	-1.61	-1.91
(4,1)(0,0)	35.37	-2.03	-1.69	-1.92
(2,1)(0,0)	33.17	-2.01	-1.77	-1.94
(1,0)(0,0)	30.81	-1.99	-1.84	-1.94
(1,1)(0,0)	31.17	-1.94	-1.75	-1.88
(2,0)(0,0)	31.10	-1.94	-1.75	-1.88
(3,2)(0,0)	33.34	-1.88	-1.55	-1.78
(1,2)(0,0)	31.23	-1.87	-1.64	-1.80
(3,0)(0,0)	31.21	-1.87	-1.63	-1.80
(4,3)(0,0)	34.67	-1.83	-1.41	-1.70
(4,0)(0,0)	31.46	-1.82	-1.53	-1.73
(1,3)(0,0)	31.33	-1.81	-1.52	-1.72
(2,2)(0,0)	31.29	-1.81	-1.52	-1.72
(1,4)(0,0)	32.27	-1.80	-1.47	-1.70
(3,1)(0,0)	31.25	-1.80	-1.52	-1.72
(4,4)(0,0)	35.24	-1.80	-1.33	-1.66
(4,2)(0,0)	32.66	-1.76	-1.38	-1.65
(3,3)(0,0)	32.66	-1.76	-1.38	-1.64
(2,3)(0,0)	31.30	-1.74	-1.40	-1.63
(0,3)(0,0)	26.96	-1.57	-1.33	-1.50
(0,2)(0,0)	25.16	-1.51	-1.32	-1.45
(0,4)(0,0)	26.96	-1.50	-1.21	-1.41
(2,4)(0,0)	26.83	-1.35	-0.96	-1.23
(0,1)(0,0)	17.51	-1.04	-0.89	-0.99
(0,0)(0,0)	4.35	-0.17	-0.07	-0.14

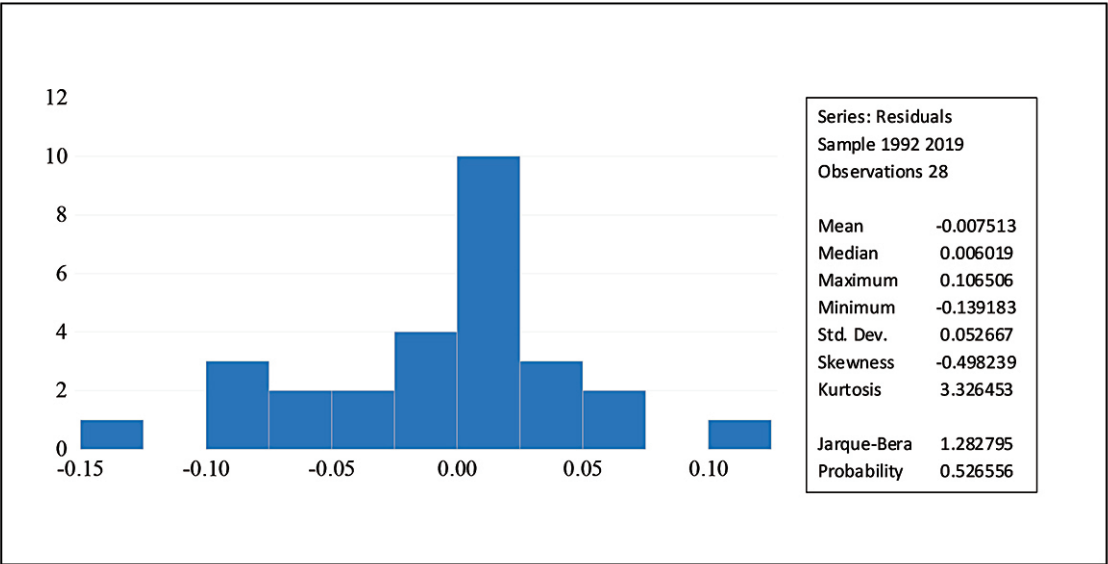
*Akaike's Information Criterion (AIC) was used to compare the models

Supplementary Table 2: ARCH Test for Heteroskedasticity in Residuals of ARIMA Model for Adult Public Oral Healthcare Utilization in Malaysia.

Statistic	Value	Prob.
F-statistic	2.19	0.151
Obs*R-squared	2.17	0.140

Supplementary Table 3: Error Measures and Interpretations for the Forecasted ARIMA Model of Adult Public Oral Healthcare Utilization.

Error Measure	Value	Interpretation
Root Mean Squared Error (RMSE)	0.372	Indicates the average magnitude of forecast errors; lower values reflect higher accuracy.
Mean Absolute Error (MAE)	0.275	Represents the average absolute difference between observed and forecasted values; smaller values are better.
Mean Absolute Percentage Error (MAPE)	4.515%	Shows the average forecast error as a percentage of the actual value; below 10% is generally acceptable.
Symmetric MAPE	4.437%	Similar to MAPE but avoids bias toward over- or under-forecasting; confirms consistent accuracy.
Theil Inequality Coefficient	0.027	Measures the relative forecast accuracy compared to a naive model; values close to 0 indicate high performance.
Theil U2 Coefficient	0.707	Evaluates the forecast's reliability relative to an ideal model; values below 1 suggest good performance.



Supplementary Figure 1: Histogram of Residuals for ARIMA Model of Adult Public Oral Healthcare Utilization Prevalence in Malaysia.



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Antitumoral Efficacy of *Vitex negundo* in Oral Cancer: An *In vitro* Study

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Abstract

Background: Oral squamous cell carcinoma (OSCC) accounts for approximately 90% of all oral cancer cases. Cytotoxic chemotherapeutic drugs interfere with the replication and synthesis of DNA, promoting the death of cancer cells and normal cells as well. Researchers have extracted a number of bioactive substances including volatile oils, flavonoids, lignans, iridoids, and terpenes from the leaves, seeds, and roots of *Vitex negundo*. Anti-inflammatory, antioxidant, anticancer, and antibacterial properties are displayed by these bioactive substances. The present study proposed to establish the cytotoxic, apoptotic activity of crude ethanolic extract of *Vitex negundo* (VN) leaves on an oral cancer cell line.

Methods: The OSCC cell line was exposed to different concentrations of crude ethanolic extract of VN leaves and cisplatin for 24, 48, and 72 hours. The MTT assay was done to check for cell viability. IC₅₀ was determined. The AO/PI assay for apoptosis was done at IC₅₀ after 72 hours. Early and late apoptotic changes were observed in VN-treated and cisplatin-treated cells.

Results: In the human OSCC cell line, the percentage of viable cells decreased from 92.33 to 21.08 after 24 hours as the concentration increased from 20 µg/ml to 100 µg/ml. Cell viability decreased from 71.20% to 17.89% after 48 hours and from 61.40% to 14.75% after 72 hours. VN-treated cells were predominantly in the early stage of apoptosis with chromatin changes (yellow-green nucleus) as compared to cisplatin on AO/PI staining.

Conclusion: Crude ethanolic extract of VN leaves had similar cytotoxic and apoptotic efficacy to cisplatin on the OSCC cell line.

Keywords: apoptosis, cytotoxicity, oral squamous cell carcinoma cell line, *Vitex negundo*

Introduction

Head and neck cancer is the seventh most common cancer in the globe with a high incidence in Southeast Asian countries. Over 650,000 cases and 330,000 mortalities worldwide are attributed to HNCs on an annual basis.⁽¹⁾ Oral cancer is regarded as a multifactorial disease caused by environmental, genetic, and epigenetic factors. Oral squamous cell carcinoma accounts for approximately 90% of all oral cancer cases. The incidence and mortality of oral cancer in developing countries is showing a dramatic increase due to habits such as smoking, betel quid chewing, use of smokeless tobacco, and alcohol intake.⁽²⁾ Oral cancer is the most common cancer predominantly caused by the use of tobacco in middle-aged males in India. Oropharyngeal cancer is reported in patients with human papillomavirus (HPV) infection. India contributes one-third to the global cancer burden, which may be attributed to the fact that the diagnosis is usually made in the advanced stage. West Bengal is leading with the highest number of oral cancer cases, and the lowest number of cases are reported from Kerala.⁽³⁾

Chemical and microbial carcinogens dysregulate the cellular signaling pathways, cellular metabolism, cellular differentiation, apoptosis, senescence, angiogenesis, immune response, and inflammation. Molecular upregulation of proto-oncogenes, and downregulation of tumor suppressor genes lead to genetic instability. Besides these genetic causes, certain epigenetic alterations such as DNA methylation, histone modification, and post-translational modification of non-coding RNAs also contribute to carcinogenesis.⁽⁴⁾ Oncogenic pathways, namely the mitogen-activated protein kinase (MAPK) pathway, epidermal growth factor receptor (EGFR) pathway, phosphatidylinositol 3 kinase/ mammalian target of rapamycin (PI3K/mTOR) pathway, Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, and suppressor pathways such as p53/p16/retinoblastoma pathway are deregulated in oral carcinogenesis.⁽⁴⁻⁶⁾

Surgery is the most recommended course of treatment for oral cancer, used alone or in combination with radiotherapy and/or chemotherapy. Multimodality therapy with radiotherapy and chemotherapy is advised depending upon the surgical accessibility, positive tumor margin, locally advanced cancer, nodal involvement, and metastasis.⁽⁷⁾ All the therapeutic methods have their own advantages and disadvantages. Chemotherapy may

be administered before as induction chemotherapy to reduce the size, during definitive treatment as concomitant chemotherapy, or after definitive treatment as adjuvant chemotherapy. Cytotoxic chemotherapeutic drugs interfere with the replication and synthesis of DNA, promoting the death of cancer cells and normal cells as well.⁽⁸⁾

Chemotherapeutic drugs either target the enzymes within the cell or change the metabolism of cells. The concept of chemotherapy initially emerged after systemic mustard gas poisoning damaged the bone marrow and lymphatic tissues. Following this, an animal study using nitrogen mustard to target lymphoma was conducted in mice to prove this hypothesis. Later, nitrogen mustard was administered to a 48-year-old patient at Yale University to treat lymphosarcoma, which paved the way for the chemotherapy regimen. Numerous side effects have been reported from the duration and dosage, as they harm the healthy cells amidst the cancer cells. Bone marrow suppression and hematologic toxicity are a few major adverse effects of chemotherapy.⁽⁹⁾

Traditional medicine has been proven to have medicinal value with fewer side effects as compared to allopathic drugs. It has been reported that more than 10,000 studies from India are related to the antioxidant and antidiabetic efficacy of medicinal plants.⁽¹⁰⁾ Natural extracts were considered to have a chemotherapeutic effect as they interfered with the formation of microtubules and were able to disrupt microtubule assembly. In the late 1950s, antimitotic extract from *Catharanthus roseus* was introduced as vinca alkaloids.⁽¹¹⁾ Phytochemicals have been reported to exert targeting the molecular mechanisms. Few studies have demonstrated that the traditional herbs exert antitumor efficacy by inducing cytotoxicity, promoting apoptosis, regulating epigenetic modifications, and inhibiting metastasis along with their antioxidant and anti-inflammatory activity.⁽¹²⁾

Different species of *Vitex*, such as *Vitex agnus-castus*, *Vitex rotundifolia*, *Vitex trifolia*, and *Vitex negundo*, have been documented to have medicinal values. *Vitex negundo* (VN), also called Nirgundi or five-leaved chaste tree, is a member of the Verbenaceae family. VN is a tenuous shrub or small tree that can grow to a maximum height of 5 meters with quadrangular branches. Leaves of VN are lanceolate with three to five leaflets, fruits are black, and flowers are bluish-purple. The entire plant has therapeutic properties and is used in Ayurveda, Chinese, Siddha, and

Unani to treat multiple ailments as a sarvaroganivarani for all diseases. Leaves have been reported to have antibacterial, anti-inflammatory, antihistaminic, antidiabetic, antioxidant, and anticancer action. Crude extracts of VN and bioactive compounds were proven to induce apoptosis, inhibit angiogenesis, and induce cytotoxicity by targeting molecular pathways in lung cancer, breast cancer, ovarian cancer, and colon cancer cell lines.⁽¹³⁾ The present study aimed to evaluate the cytotoxic and apoptotic activities of crude ethanolic extract of VN leaves on an oral cancer cell line.

Methodology

Ethical approval

The study was approved by the Institutional Review Board (SRMDC/IRB/2021/MDS/NO.605)

Identification of the species and procurement of VN leaves

Fresh, mature leaves of VN, authenticated from Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal, were used.

Preparation of crude ethanolic extract of VN leaves

Procured leaves were shade-dried for a period of two weeks and coarsely powdered. The extraction process was done using a Soxhlet apparatus. 50 mg of the powder was weighed and packed into a thimble made from filter paper. 200 ml of 90% ethanol was added to the extraction flask, and the temperature was set at 80°C, as the boiling point of ethanol is approximately 78°C-79°C. The process of extraction was repeated for 24 hours. The solvent was removed using a rotary vacuum evaporator, and the concentrate was stored at 4°C for further studies.⁽¹⁴⁾

Cell culture

The human oral keratinocyte cell line and the human oral squamous cell carcinoma cell line were procured from NCCS, Pune. Cells were cultured using DMEM supplemented with 10% FBS and antibiotics (penicillin, streptomycin, amphotericin B) in humidified 5% carbon dioxide at 37°C until 80% confluent.

Cell viability assay—MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay—oral cancer cells

The MTT assay was done to determine the viability of cells after treatment with different concentrations of crude ethanolic extract of VN leaves. 1.5×10^4 cells/well were seeded into 96-well plates. These seeded cells were treated with five concentrations of crude ethanolic extract of VN leaves (20, 40, 60, 80, and 100 µg/ml) at 37°C for 24, 48, and 72 hours. At the end of each incubation period, a working solution of 20 µl of MTT reagent was added to each well and further incubated for 4 hours. 100 µl of DMSO was then added to each well, and the absorbance was read using an ELISA reader at 570 nm. The experiment was done in triplicate. Cisplatin (2, 4, 6, 8, 10 µg/ml) was used as a positive control. Untreated cells were used as a negative control. Percentage of viable cells was calculated using the formula⁽¹⁵⁾,

$$\text{Cell viability \%} = \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Cell viability assay—MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay—human oral keratinocytes

The percentage of viability of human oral keratinocyte cells was also identified after treatment with five concentrations of crude ethanolic extract of VN leaves (25, 50, 100, 150, and 200 µg/ml) at 37°C for 24, 48, and 72 hours. This was done to prove that the crude ethanolic extract of VN leaves was not cytotoxic to normal cells.

Inhibitory concentration (IC₅₀) identification by standard curve method

The IC₅₀ value (half-maximal concentration 50%) was calculated for crude ethanolic extract of VN leaves and cisplatin by the standard curve method. A scatter plot was drawn using concentration on the x-axis and cell viability on the y-axis. A linear trendline was drawn, and the R-squared value with equation was noted. IC₅₀ was determined by four parametric logistic equations for 24 hours, 48 hours, and 72 hours for crude ethanolic extract of VN leaves and cisplatin.⁽¹⁶⁻¹⁸⁾

Acridine Orange/Propidium Iodide (AO/PI) dual staining—assessment of apoptotic activity

AO/PI dual staining was done to evaluate the apoptotic activity of crude ethanolic extract of VN leaves. Wells containing the test and control group at IC_{50} concentration were washed with PBS and stained with 16 μ l of dual fluorescent staining solution containing 100 μ g/ml AO and 100 μ g/ml PI (AO/PI, Sigma, St. Louis, MO). The mix was allowed to diffuse and enter into the cells for 5 min. The stained cells were viewed under a fluorescence microscope (Invitrogen EVOS FL imaging; 40X magnification). Both the viable and dead cells were permeated by AO, and the nuclei appeared green under a fluorescent microscope. The nuclei of dead cells whose cytoplasmic integrity was lost appeared red from PI staining. Thus, nuclei of viable cells showed green fluorescence, early apoptotic cells with fragmented chromatin showed yellow-green fluorescence, late apoptotic cells showed orange fluorescence, and dead cells fluoresced red.^(19,20)

Statistical analysis

The results were analyzed for statistical significance using Statistical Package for Social Sciences (SPSS) software version 16. Statistical significance was set at $p < 0.05$. Mean and standard deviation were calculated for the triplicate absorbance values of the MTT assay. The percentage of viable cells was calculated from the mean absorbance value. Mann-Whitney U test was done to compare the mean of cell viability between the VN and cisplatin-treated cells. The Friedman Kruskal-Wallis test was done to compare the difference in mean for each concentration after 24 hours, 48 hours, and 72 hours.

Results

MTT cell viability assay

The cytotoxic efficacy of crude ethanolic extract of VN leaves after 24 hours, 48 hours, and 72 hours was determined at different concentrations in the human oral keratinocyte cell line and the human oral squamous cell carcinoma cell line. In the human oral keratinocyte cell line, the cell viability after 72 hours was 88.59% at a concentration of 200 μ g/ml of VN leaves (Figure 1). Thus, the cytotoxic effects were tumor-specific.

In the human OSCC cell line, the cell viability decreased from 92.33% to 21.08% after 24 hours as the

concentration increased from 20 μ g/ml to 100 μ g/ml. The cell viability decreased from 71.20% to 17.89% after 48 hours and from 61.40% to 14.75% after 72 hours (Figure 2).

The cell viability was also determined with different concentrations of cisplatin after 24 hours, 48 hours, and 72 hours. The percentage of viable cells decreased from 90.81% to 20.36% after 24 hours, 85.68% to 17.18% after 48 hours, and 72.43% to 14.09% after 72 hours (Figure 3). As described in Table 1 & Table 2, at a concentration of 20 μ g/ml of the crude ethanolic extract of VN leaves, cell viability was 92.33%, 71.2%, and 61.4% after 24, 48, and 72 hours, respectively. The difference was not statistically significant ($p=0.241$) ($N=3$). 74.47%, 65.04%, and 52.37% were the percentages of viable cells at a concentration of 40 μ g/ml of VN after 24, 48, and 72 hours. The difference did not show any statistical significance ($p=0.142$) ($N=3$). Similarly, at 60 μ g/ml, 51.7%, 47.23%, and 40.67% percent of cells were viable after exposure to VN leaves after 24, 48, and 72 hours with statistical significance ($p=0.001$) ($N=3$). Again at 80 μ g/ml, the difference was statistically significant ($p=0.002$) ($N=3$) with 31.725, 28.93%, and 26.84% percent of viable cells. The difference was also statistically significant ($p=0.002$) ($N=3$) at 100 μ g/ml after 24, 48, and 72 hours, with 21.08%, 17.89%, and 14.75% percent of viable cells, respectively.

This concentration-dependent cytotoxicity was also assessed for cisplatin for comparison (Table 3 & Table 4). At a concentration of 2 μ g/ml, the percentage of viable cells was 90.81%, 85.68%, and 72.43% after 24, 48, and 72 hours, respectively. On comparison, the difference was not statistically significant ($p=0.15$) ($N=3$). At 4 μ g/ml, 72.21%, 69.21%, and 41.12% were the viability percentages with a statistically significant difference ($p=0.02$) ($N=3$). Similarly, a significant difference ($p=0.02$) ($N=3$) was found at 6 μ g/ml, with viability of 40.1%, 39.22%, and 33.92% after 24, 48, and 72 hours. At 8 μ g/ml, the viability was 26.94%, 25.13%, and 22.79% with a significant difference ($p=0.01$) ($N=3$). At the final concentration of 10 μ g/ml also, the difference was significant ($p=0.002$) ($N=3$) with viability of 20.36%, 17.18%, and 14.09%.

To prove the time-dependent cytotoxic efficacy, the viability was also compared after 24, 48, and 72 hours between different concentrations of VN and cisplatin (Figure 5). The first concentration was 20 μ g/ml of VN leaves crude ethanolic extract and 2 μ g/ml of cisplatin.

The second concentration was 40 µg/ml of VN and 4 µg/ml of cisplatin. The third concentration was 60 µg/ml of VN and 6 µg/ml of cisplatin. The fourth concentration was 80 µg/ml of VN and 8 µg/ml of cisplatin. The fifth concentration was 100 µg/ml of VN and 10 µg/ml of cisplatin. The difference was significant for the 3rd ($p=0.04$) (N=3) and 4th ($p=0.03$) (N=3) concentrations among 5 different concentrations of VN and cisplatin after 24 hours. After 48 hours, the 1st ($p=0.03$), 2nd ($p=0.02$), and 3rd ($p=0.02$) (N=3) concentrations of VN and cisplatin showed statistical significance. After 72 hours, again, 1st ($p=0.01$), 2nd ($p=0.01$), and 3rd ($p=0.04$) (N=3) were statistically significant (Table 5).

Inhibitory Concentration (IC₅₀)

The IC₅₀ value was determined for VN and cisplatin after 24, 48, and 72 hours. For VN, IC₅₀ was 64.83 µg/ml, 54.47 µg/ml, and 41.829 µg/ml after 24, 48, and 72 hours, respectively. Similarly, 5.65 µg/ml, 5.77 µg/ml, and 4.05 µg/ml were the IC₅₀ values of cisplatin after 24, 48, and 72 hours, respectively.

Apoptotic assay - AO/PI dual staining

The cells at IC₅₀ were treated with AO/PI stain to detect the morphological change of apoptosis. Cells treated with control did not show any apoptotic change (green) (Figure 4a). Most of the cisplatin-treated cells were either in the early (yellow-green) or late stage (orange) of apoptosis (Figure 4b). VN-treated cells were predom-

inantly in the early stage of apoptosis with chromatin changes (yellow-green nucleus) as compared to cisplatin (Figure 4c).

Discussion

Oral cancer has been reported to be the ninth cause of mortality from cancer on a global basis. Independent risk factors for oral and oropharyngeal cancer include smoking forms of tobacco, smokeless forms of tobacco, and HPV infection. The incidence of oral cancer has increased from 4.28/100,000 to 4.52/100,000 in the last two decades at the global level. Among the Southeast Asian countries, India was the top ranker with the highest burden of oral cancer, as per data from the International Agency for Research on Cancer (IARC). Unfortunately, most of the patients are diagnosed at their advanced stage with only a 5-year survival period. Various time intervals, such as patient interval, diagnostic interval, pre-treatment, and treatment interval, have been reported in the diagnosis and management of oral cancer. The mortality rate from oral cancer can be considerably decreased by significantly reducing the time period of these intervals.⁽²¹⁻²³⁾ Cancer therapeutics such as surgery, radiotherapy, and chemotherapy also contribute to the morbidity, indirectly affecting the quality of life and survival rate.⁽²⁴⁾

Surgical management is the primary treatment of choice for oral cancer; however, radiotherapy remains the standard treatment for inaccessible tumor sites and surgically unfit patients. Literature suggests that chemo-

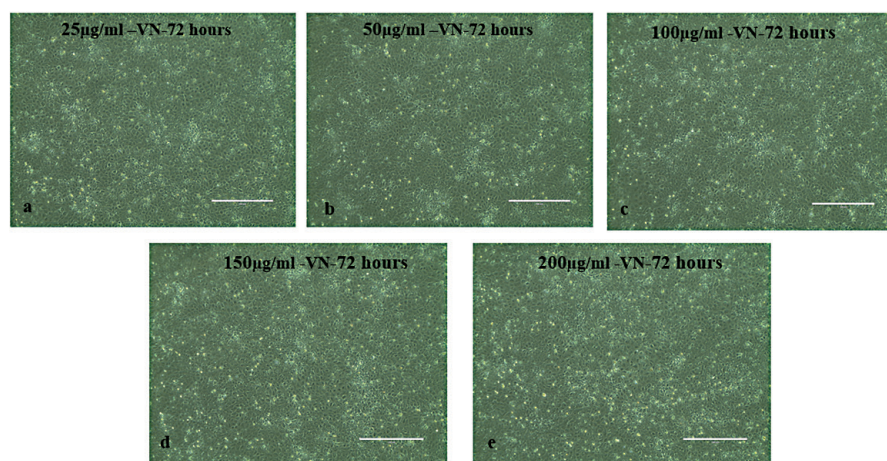


Figure 1: (a), Human oral keratinocyte cell line treated with 25 µg/ml of VN: (b), Human oral keratinocyte cell line treated with 50 µg/ml of VN: (c), Human oral keratinocyte cell line treated with 100 µg/ml of VN: (d), Human oral keratinocyte cell line treated with 150 µg/ml of VN: (e), Human oral keratinocyte cell line treated with 200 µg/ml of VN (scale bar - 100 µm).

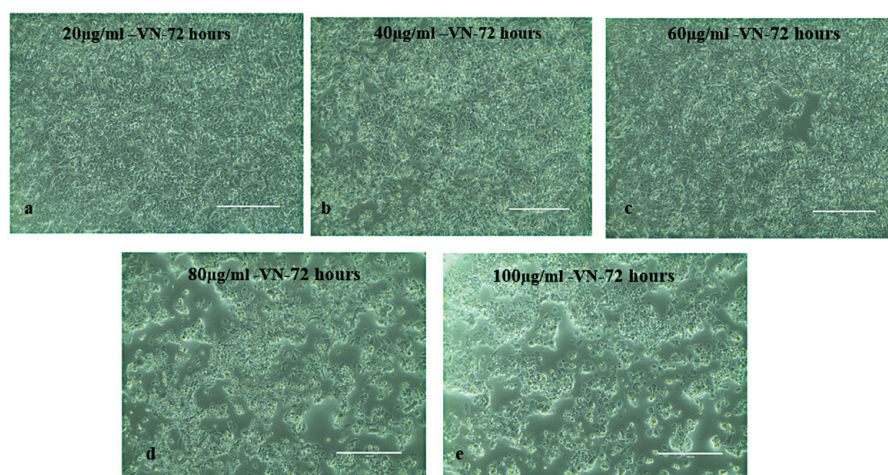


Figure 2: (a), Human oral squamous cell carcinoma cell line treated with 20 µg/ml of VN: (b), Human oral squamous cell carcinoma cell line treated with 40 µg/ml of VN: (c), Human oral squamous cell carcinoma cell line treated with 60 µg/ml of VN: (d), Human oral squamous cell carcinoma cell line treated with 80 µg/ml of VN: (e), Human oral squamous cell carcinoma cell line treated with 100 µg/ml of VN (scale bar - 100 µm).

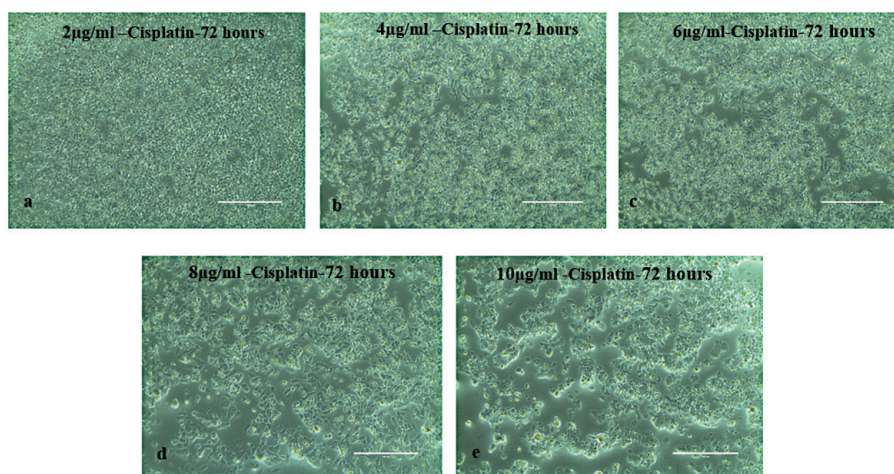


Figure 3: (a), Human oral squamous cell carcinoma cell line treated with 2 µg/ml of cisplatin: (b), Human oral squamous cell carcinoma cell line treated with 4 µg/ml of cisplatin: (c), Human oral squamous cell carcinoma cell line treated with 6 µg/ml of cisplatin: (d), Human oral squamous cell carcinoma cell line treated with 8 µg/ml of cisplatin: (e), Human oral squamous cell carcinoma cell line treated with 10 µg/ml of cisplatin (scale bar - 100 µm).

radiotherapy can be considered for advanced stages of locoregional head and neck cancer.⁽²⁵⁾ A study by Sarma *et al.*, compared the complications of management of oral cancer with and without neoadjuvant chemotherapy. The study reported that patients who received two to three cycles of neoadjuvant chemotherapy prior to surgical resection for tumor shrinkage had better outcomes than the control group.⁽²⁶⁾ Though chemotherapy is a blessing for patients with advanced cancer, systemic adverse effects affect the quality of life of the patients, thus prompting the need for less toxic, effective phytochemical alterna-

tives for cancer management. Traditional herbs are being studied in recent days for anticancer activity.

VN has been proven in literature to have potent antimicrobial, antioxidant, anti-inflammatory, anti-osteoporotic, anti-androgenic, anti-diabetic, anti-tumoral, and hepatoprotective efficacy. Flavonoids, terpenoids, steroids, and lignans have been reported to be the four major classes of compounds from VN. Almost 120 compounds have been isolated from VN belonging to these four major classes.⁽²⁷⁾ Vitexin, vitexicarpin, triterpenoids, lagundinin, nishindaside, betulinic acid, negun-

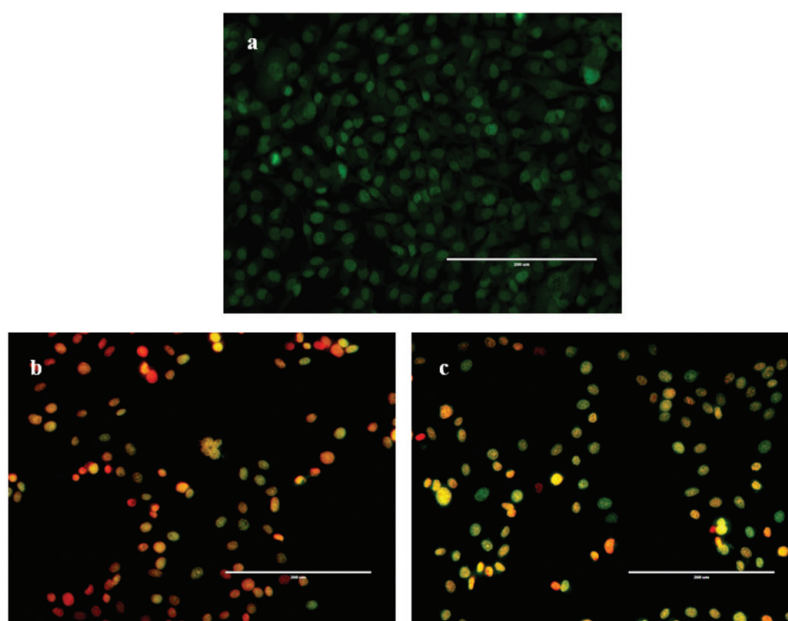


Figure 4: (a), Cells treated with control did not show any apoptotic change (green); (b), Cisplatin-treated cells either in the early (yellow-green) or late stage (orange) of apoptosis; (c), VN-treated cells in the early stage of apoptosis (yellow-green nucleus) (scale bar - 200 μ m).

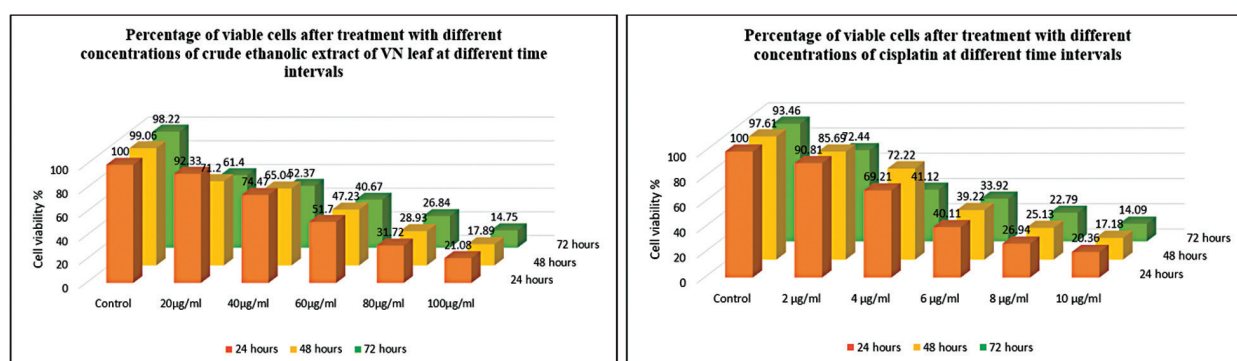


Figure 5: Cell viability following treatment with different concentrations of crude ethanolic extract of VN leaf after 24 hours, 48 hours, and 72 hours; Cell viability following treatment with different concentrations of cisplatin after 24 hours, 48 hours, and 72 hours.

doside, diterpene, β -caryophyllene, vitexoside, vitexdoin, vitegnoside, ursolic acid, and vitelignin are a few active phytoconstituents of VN leaves.⁽²⁷⁻³⁰⁾

Species of *Vitex* have been documented to target the PI3K/Akt pathway, JAK/STAT pathway, Wnt/ β -catenin pathway, MAPK pathway, and NF- κ B pathway. The extracts were also stated to activate caspases, activate pro-apoptotic proteins, inhibit anti-apoptotic proteins, upregulate cytochrome c, downregulate MMP-9, induce cell cycle arrest, induce apoptosis, inhibit viability of cells, inhibit epithelial mesenchymal transition, and prevent

invasion and migration. Vitexicarpin/casticin from VN has been proven through *in vitro* studies to exert antitumor effects on breast cancer, bladder cancer, cervical cancer, colon cancer, hepatocellular cancer, leukemia, esophageal cancer, lung cancer, ovarian cancer, prostate cancer, and melanoma.⁽³¹⁻³²⁾

Xin *et al.*, reported that lignans from VN augmented histone phosphorylation, Cdk1 phosphorylation, increased expression of cyclin B1, induced cell cycle arrest at G2/M phase, and induced apoptosis in various cancer cell lines.⁽³³⁾ Vo *et al.*, explored the anti-tumorigenic and apoptotic

Table 1: Percentage of viable oral squamous cell carcinoma cells following treatment with different concentrations of crude ethanolic extract of VN leaf after 24 hours, 48 hours and 72 hours.

24 hrs	Control	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
	0.759	0.698	0.568	0.395	0.243	0.164
	0.752	0.697	0.561	0.392	0.235	0.152
	0.746	0.689	0.552	0.38	0.238	0.16
Mean	0.752333	0.694667	0.560333	0.389	0.238667	0.158667
SD	0.006506	0.004933	0.008021	0.007937	0.004041	0.00611
Cell Viability (%)	100	92.33	74.47	51.70	31.72	21.08
48hrs	Control	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
	0.746	0.535	0.489	0.356	0.218	0.136
	0.745	0.537	0.489	0.356	0.217	0.133
	0.745	0.535	0.49	0.354	0.218	0.135
Mean	0.745333	0.535667	0.489333	0.355333	0.217667	0.134667
SD	0.000577	0.001155	0.000577	0.001155	0.000577	0.001528
Cell Viability (%)	99.06	71.20	65.04	47.23	28.93	17.89
72hrs	Control	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
	0.738	0.463	0.394	0.305	0.202	0.112
	0.738	0.463	0.393	0.307	0.201	0.113
	0.739	0.462	0.394	0.306	0.202	0.111
Mean	0.738333	0.462667	0.393667	0.306	0.201667	0.112
SD	0.000577	0.000577	0.000577	0.001	0.000577	0.001
Cell Viability (%)	98.22	61.40	52.37	40.67	26.84	14.75

Table 2: Comparison of mean viability of oral squamous cell carcinoma cells with different concentrations of crude ethanolic extract of VN leaf after 24 hours, 48 hours, and 72 hours.

Concentration of crude ethanolic extract of VN leaf	Time interval	Percentage of viable cells (%)	<i>p</i> -value
Control	24 hours	100	0.312
	48 hours	99.06	
	72 hours	98.22	
20µg/ml	24 hours	92.33	0.241
	48 hours	71.2	
	72 hours	61.4	
40µg/ml	24 hours	74.47	0.142
	48 hours	65.04	
	72 hours	52.37	
60µg/ml	24 hours	51.7	0.001*
	48 hours	47.23	
	72 hours	40.67	
80µg/ml	24 hours	31.72	0.002*
	48 hours	28.93	
	72 hours	26.84	
100µg/ml	24 hours	21.08	0.002*
	48 hours	17.89	
	72 hours	14.75	

Friedman Kruskal-Wallis test - *statistically significant (*p*-value less than 0.05).

Table 3: Percentage of viable oral squamous cell carcinoma cells following treatment with different concentrations of cisplatin after 24 hours, 48 hours and 72 hours

24hrs	Control	2 µg/ml	4 µg/ml	6 µg/ml	8 µg/ml	10 µg/ml
	0.756	0.687	0.553	0.301	0.203	0.152
	0.749	0.686	0.542	0.298	0.205	0.157
	0.759	0.683	0.54	0.309	0.202	0.152
Mean	0.75467	0.68533	0.545	0.30267	0.20333	0.15367
SD	0.00513	0.00208	0.007	0.00569	0.00153	0.00289
Cell Viability (%)	100	90.81	72.21	40.10	26.94	20.36
48hrs	Control	2 µg/ml	4 µg/ml	6 µg/ml	8 µg/ml	10 µg/ml
	0.725	0.647	0.526	0.297	0.192	0.134
	0.738	0.641	0.521	0.295	0.181	0.126
	0.747	0.652	0.52	0.296	0.196	0.129
Mean	0.73667	0.64667	0.52233	0.296	0.18967	0.12967
SD	0.01106	0.00551	0.00321	0.001	0.00777	0.00404
Cell Viability (%)	97.61	85.68	69.21	39.22	25.13	17.18
72hrs	Control	2 µg/ml	4 µg/ml	6 µg/ml	8 µg/ml	10 µg/ml
	0.706	0.547	0.304	0.267	0.172	0.104
	0.705	0.541	0.312	0.245	0.176	0.106
	0.705	0.552	0.315	0.256	0.168	0.109
Mean	0.70533	0.54667	0.31033	0.256	0.172	0.10633
SD	0.00058	0.00551	0.00569	0.011	0.004	0.00252
Cell Viability (%)	93.46	72.43	41.12	33.92	22.79	14.09

Table 4: Comparison of mean viability of oral squamous cell carcinoma cells with different concentrations of cisplatin after 24 hours, 48 hours, and 72 hours.

Concentration of cisplatin	Time interval	Percentage of viable cells (%)	p-value
Control	24 hours	100	0.11
	48 hours	97.61	
	72 hours	93.46	
2µg/ml	24 hours	90.81	0.15
	48 hours	85.68	
	72 hours	72.43	
4µg/ml	24 hours	72.21	0.02*
	48 hours	69.21	
	72 hours	41.12	
6µg/ml	24 hours	40.1	0.02*
	48 hours	39.22	
	72 hours	33.92	
8µg/ml	24 hours	26.94	0.01*
	48 hours	25.13	
	72 hours	22.79	
10µg/ml	24 hours	20.36	0.002*
	48 hours	17.18	
	72 hours	14.09	

Friedman Kruskal-Wallis test - *statistically significant (*p*-value less than 0.05).

Table 5: Comparison of time-dependant cytotoxic efficacy of crude ethanolic extract of VN and cisplatin .

Time interval	Concentration	Cell viability %	p-value
24 hrs	20µg/ml of VN leaf crude ethanolic extract	92.33	0.29
	2µg/ml of cisplatin	90.81	
	40µg/ml of VN leaf crude ethanolic extract	74.47	0.06
	4µg/ml of cisplatin	72.21	
	60µg/ml of VN leaf crude ethanolic extract	51.7	0.04*
	6µg/ml of cisplatin	26.94	
	80µg/ml of VN leaf crude ethanolic extract	31.72	0.03*
	8µg/ml of cisplatin	26.94	
	100µg/ml of VN leaf crude ethanolic extract	21.08	0.26
	10µg/ml of cisplatin	20.36	
48 hrs	20µg/ml of VN leaf crude ethanolic extract	71.2	0.03*
	2µg/ml of cisplatin	85.68	
	40µg/ml of VN leaf crude ethanolic extract	65.04	0.02*
	4µg/ml of cisplatin	69.21	
	60µg/ml of VN leaf crude ethanolic extract	47.23	0.02*
	6µg/ml of cisplatin	39.22	
	80µg/ml of VN leaf crude ethanolic extract	28.93	0.13
	8µg/ml of cisplatin	25.13	
	100µg/ml of VN leaf crude ethanolic extract	17.89	0.32
	10µg/ml of cisplatin	17.18	
72 hrs	20µg/ml of VN leaf crude ethanolic extract	61.4	0.01*
	2µg/ml of cisplatin	72.43	
	40µg/ml of VN leaf crude ethanolic extract	52.37	0.01*
	4µg/ml of cisplatin	41.12	
	60µg/ml of VN leaf crude ethanolic extract	40.67	0.04*
	6µg/ml of cisplatin	33.92	
	80µg/ml of VN leaf crude ethanolic extract	26.84	0.07
	8µg/ml of cisplatin	22.79	
	100µg/ml of VN leaf crude ethanolic extract	14.75	0.12
	10µg/ml of cisplatin	14.09	

Mann-Whitney U test - *statistically significant (p -value less than 0.05).

efficacy of compounds from VN (vitexicarpin, penduletin, and artemetin) in human breast cancer and liver cancer cell lines.⁽³⁴⁾ Awale *et al.*, tested the cytotoxic efficacy of chrysoplenetin, the active constituent of VN, against a panel of 39 human cancer cell lines and the ethanolic extract of VN against a human pancreatic cell line. This study reported that the chrysoplenetin compound from VN induced apoptosis and cytotoxicity in the cell lines.⁽³⁵⁾

The cytotoxic efficacy of leaves of *Vitex rotundifolia* was analyzed by Chaudhry *et al.*,⁽³⁶⁾ in a breast cancer cell line. Crude methanolic extract and seven fractions were prepared from the leaves, and an MTS assay was done in their study. Seven different concentrations of extract and

fractions were evaluated for cytotoxic efficacy after 24 hours, 48 hours, and 72 hours. The Annexin V/PI staining assay was done to prove the apoptotic change in morphology of the cells induced by *Vitex*. IC₅₀ after 72 hours was found to be 79.43 µg/ml, and at this concentration apoptotic changes were also noticed. Aslanturk *et al.*,⁽³⁷⁾ evaluated the cytotoxic, apoptotic, and antioxidant activity of crude extract from seeds of *V. agnus-castus* on the MCF-7 breast cancer cell line by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay. Methanol, diethyl ether, petroleum ether, ethyl acetate, and aqueous extracts were compared for potent anticancer activity. Methanol and aqueous extracts had better antioxidant activity than other

extracts. The IC_{50} was 83.47 $\mu\text{g/ml}$ with methanol extract. All the extracts had similar cytotoxic and apoptotic activity on the MCF-7 breast cancer cell line. Ibrahim *et al.* evaluated the antioxidant, antitumoral, and anti-inflammatory efficacy of *V. agnus-castus* fruit extract. The inhibition of COX-2 was dose-dependent, and the anti-inflammatory activity was correlated to the anti-tumorigenic efficacy owing to the decreased prostaglandin production.⁽³⁸⁾ Similarly, Ilhan *et al.* studied the apoptotic activity of essential oil from leaves of *V. agnus-castus* on a multi-drug resistant lung carcinoma cell line and normal human cells also to prove the specific action on tumor cells. The study concluded that both intrinsic and extrinsic apoptotic pathways were activated and the expression of Bcl-2, Bax, Bad, caspases, and TRAIL were modulated by *V. agnus-castus*.⁽³⁹⁾ Gong *et al.*,⁽⁴⁰⁾ isolated rotundifuran from the fruits of *V. trifolia* and found that the proliferation of cervical cancer cells was suppressed by induction of apoptosis. The study also evaluated the molecular mechanisms associated with the development of cervical cancer. CYR61 was identified as a potential target as the relative gene expression was 2.963. The study concluded that *rotundifuran* could upregulate CYR61 expression and can be considered as a potential therapeutic herb in the management of cervical cancer.

The antiproliferative and antioxidant activity of VN was evaluated on a hepatoma cell line by Kadir *et al.* The MTT assay was done to evaluate the antiproliferative activity, and the IC_{50} value after 24 hours, 48 hours, and 72 hours was found to be 66.46 $\mu\text{g/ml}$, 57.36 $\mu\text{g/ml}$ and 65.12 $\mu\text{g/ml}$.⁽¹⁵⁾ Mohammad *et al.* isolated the bioactive compound artemetin from VN and evaluated the anticancer and antioxidant activity of the ethyl acetate leaf extract on the MCF-7 breast cancer cell line by the MTT assay. The study reported that the maximum activity was reported at a concentration of 200 $\mu\text{g/ml}$.⁽⁴¹⁾ In our study, MTT assay was done to analyze the cell viability of OSCC cells after five different concentrations of crude ethanolic extract of VN leaves after 24 hours, 48 hours, and 72 hours, and IC_{50} was found to be 41.83 $\mu\text{g/ml}$ after 72 hours. PI/AO dual staining was done in our study to evaluate the early and late apoptotic changes at IC_{50} concentration. The viability of OSCC cells was found to reduce with increasing dose and time interval. Thus, effects were observed to be dependent on dose and time.

Similarly, Gowthami *et al.*,⁽⁴²⁾ performed an in-silico

modeling study with AutoDock Vina to identify the compounds from the leaves of VN to target the Wnt signaling pathway. Wnt signaling proteins, namely, axin, β -catenin, GSK-3 β , APC, and Dishevelled, were selected for the molecular docking study. The binding energy was found to be maximum for luteolin, chrysophanol, and isoorientin. Thus, the study concluded that these compounds can be used as potential targets for the Wnt signaling pathway in colorectal cancer. Vitexin was isolated from the leaves of VN, and its anticancer, apoptotic activity on human malignant melanoma cell lines was studied by Liu *et al.*⁽⁴³⁾ A cytotoxicity assay was done, and IC_{50} was calculated after 48 hours following exposure to different concentrations of vitexin from VN leaves. Apoptosis was studied with Annexin V/PI staining. RT-PCR was done to explore the expression of p21, PUMA, CDK1, MCM6, CYCE, CDK6, and CYCA, as these were involved in the regulation of the cell cycle. The IC_{50} was found to range between 5 and 15 μM , and the apoptotic activity was around 17% at 5 μM , 39% at 20 μM , indicating the time- and dose-dependent increase in anti-tumorigenic efficacy. Vitexin was also found to induce cell cycle arrest at the G2/M phase. The cytotoxic activity was also determined in a normal human skin keratinocyte cell line, and the IC_{50} was found to be around 80 μM , suggesting the selective cytotoxic activity of vitexin on melanoma cells.⁽⁴³⁾ These findings are in concordance with our study, as the cell viability of the normal human oral keratinocyte was 88.59% after 72 hours of exposure to 200 $\mu\text{g/ml}$ of crude ethanolic extract of VN leaves, clearly demonstrating the selective cytotoxic action on OSCC cells.

The limitation of the study is that active compounds of the VN leaves were not investigated for anticancer activity. Secondly, the quality of the extract was not assessed before and after storage by phytochemical profiling, or thin layer chromatography, or high-performance liquid chromatography. Thirdly, only crude ethanolic extract was studied; other solvents were not compared for analysis. Fourthly, only VN was considered for the study; other species belonging to the family of Verbenaceae, such as *Vitex agnus-castus*, *Vitex rotundifolia*, and *Vitex trifolia*, were not added, though the literature documented potent anticancer activity. In addition to these, the antioxidant activity of VN was also not included in the study. Further research with isolation and characterization of active compounds accompanied by comparative analysis with

other solvents and in-silico molecular docking studies in the future might fortify the establishment of cost-effective, less toxic phytochemicals in the field of therapeutic oncology. Similar to the multimodality treatment methods, combinations of multiple herbs similar to the study by Nazhvani *et al.*,⁽⁴⁴⁾ could also be considered for the development of alternative traditional therapeutic strategies for oral cancer.

Conclusions

Our study attempted to evaluate the cytotoxic and apoptotic efficacy of crude ethanolic extract of VN leaves on an oral squamous cell carcinoma cell line in comparison with cisplatin. After 72 hours of exposure, the cell viability was 14.75% with VN and 14.09% with cisplatin. Thus, the cytotoxic efficacy of VN was almost similar to cisplatin. Future clinical trials would be considered to include VN as an alternative therapeutic regimen in the management of oral cancer.

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Nil

Declaration of Interest

None

References

- Gormley M, Creaney G, Schache A, Ingarfield K, Conway DI. Reviewing the epidemiology of head and neck cancer: definitions, trends and risk factors. *Br Dent J*. 2022;233(9):780-6.
- Ali J, Sabiha B, Jan HU, Haider SA, Khan AA, Ali SS. Genetic etiology of oral cancer. *Oral Oncol*. 2017;70:23-8.
- Borse V, Konwar AN, Buragohain P. Oral cancer diagnosis and perspectives in India. *Sens Int*. 2020;1:100046.
- Georgaki M, Theofilou VI, Pettas E, Stoufi E, Younis RH, Kolokotronis A. *et al*. Understanding the complex pathogenesis of oral cancer: A comprehensive review.
- Tan Y, Wang Z, Xu M, Li B, Huang Z, Qin S, *et al*. Oral squamous cell carcinomas: state of the field and emerging directions. *Int J Oral Sci*. 2023;15(1):44.
- Padhi SS, Roy S, Kar M, Saha A, Roy S, Adhya A, *et al*. Role of CDKN2A/p16 expression in the prognostication of oral squamous cell carcinoma. *Oral Oncol*. 2017;73:27-35.
- Dhar H, Vaish R, D'Cruz AK. Management of locally advanced oral cancers. *Oral Oncol*. 2020;105:104662.
- Nandini DB, Rao RS, Hosmani J, Khan S, Patil S, Awan KH. Novel therapies in the management of oral cancer: an update. *Dis Mon*. 2020;66(12):101036.
- Anand U, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, *et al*. Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. *Genes Dis*. 2022;10(4):1367-1401.
- Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. Worldwide research trends on medicinal plants. *Int J Environ Res Public Health*. 2020;17(10):3376.
- Falzone L, Salomone S, Libra M. Evolution of cancer pharmacological treatments at the turn of the third millennium. *Front Pharmacol*. 2018;9:1300.
- Lee TY, Tseng YH. The potential of phytochemicals in oral cancer prevention and therapy: a review of the evidence. *Biomolecules*. 2020;10(8):1150.
- Gill BS, Mehra R, Navgeet, Kumar S. *Vitex negundo* and its medicinal value. *Mol Biol Rep*. 2018;45(6):2925-34.
- Meena AK, Perumal A, Kumar N, Singh R, Ilavarasan R, Srikanth N, *et al*. Studies on physicochemical, phytochemicals, chromatographic profiling & estimation and in-silico study of Negundoside in roots & small branches of *Vitex negundo* plant. *Phytomedicine Plus*. 2022;2(1):100205.
- Kadir FA, Kassim NM, Abdulla MA, Yehye WA. PASS-predicted *Vitex negundo* activity: antioxidant and antiproliferative properties on human hepatoma cells--an *in vitro* study. *BMC Complement Altern Med*. 2013;13:343.
- Volpe DA, Hamed SS, Zhang LK. Use of different parameters and equations for calculation of IC₅₀ values in efflux assays: potential sources of variability in IC₅₀ determination. *AAPS J*. 2014;16(1):172-80.
- Crimin KS, McKean JW, Vidmar TJ. Rank-based estimate of four-parameter logistic model. *Pharm Stat*. 2012;11(3):214-21.
- He Y, Zhu Q, Chen M, Huang Q, Wang W, Li Q, *et al*. The changing 50% inhibitory concentration (IC₅₀) of cisplatin: a pilot study on the artifacts of the MTT assay and the precise measurement of density-dependent chemoresistance in ovarian cancer. *Oncotarget*. 2016;7(43):70803-21.
- Liu K, Liu PC, Liu R, Wu X. Dual AO/EB staining to detect apoptosis in osteosarcoma cells compared with flow cytometry. *Med Sci Monit Basic Res*. 2015;21:15-20.
- Atale N, Gupta S, Yadav UC, Rani V. Cell-death assessment by fluorescent and nonfluorescent cytosolic and nuclear staining techniques. *J Microsc*. 2014;255(1):7-19.
- Sun R, Dou W, Liu W, Li J, Han X, Li S, *et al*. Global, regional, and national burden of oral cancer and its attributable risk factors from 1990 to 2019. *Cancer Med*. 2023;12(12):13811-20.
- Bosetti C, Carioli G, Santucci C, Bertuccio P, Gallus S, Garavello W, *et al*. Global trends in oral and pharyngeal cancer incidence and mortality. *Int J Cancer*. 2020;147(4):1040-9.
- Saka-Herrán C, Jané-Salas E, Mari-Roig A, Estrugo-Devesa A, López-López J. Time-to-treatment in oral cancer: causes and implications for survival. *Cancers (Basel)*.

- 2021;13(6):1321.
24. van der Waal I. Are we able to reduce the mortality and morbidity of oral cancer; some considerations. *Med Oral Patol Oral Cir Bucal*. 2013;18(1):e33-7.
 25. Geiger JL, Adelstein DJ. Chemotherapy in the definitive management of oral cancers: Where do we stand today? *Oral Oncol*. 2020;102:104584.
 26. Sarma MK, Missong AK, Sharma M, Debnath A, Mahanta N, Choudhury MM. Comparison of post-operative complications in oral cancer treated with and without neoadjuvant chemotherapy. *J Pharm Bioallied Sci*. 2023;15(Suppl 2):S1116-8.
 27. Zheng CJ, Li HQ, Ren SC, Xu CL, Rahman K, Qin LP, *et al*. Phytochemical and Pharmacological Profile of *Vitex negundo*. *Phytother Res*. 2015;29(5):633-47.
 28. Rani A, Sharma A. The genus *Vitex*: A review. *Pharmacogn Rev*. 2013;7(14):188-98.
 29. Kamal N, Mio Asni NS, Rozlan INA, Mohd Azmi MAH, Mazlan NW, Mediani A, *et al*. Traditional medicinal uses, phytochemistry, biological properties, and health applications of *Vitex* sp. *Plants (Basel)*. 2022 ;11(15):1944.
 30. Chan EWC, Wong SK, Chan HT. Casticin from *Vitex* species: a short review on its anticancer and anti-inflammatory properties. *J Integr Med*. 2018;16(3):147-152.
 31. Ramchandani S, Naz I, Lee JH, Khan MR, Ahn KS. An overview of the potential antineoplastic effects of casticin. *Molecules*. 2020;25(6):1287.
 32. Carbone K, Gervasi F, Kozhamzharova L, Altybaeva N, Sönmez Gürer E, Sharifi-Rad J, *et al*. Casticin as potential anticancer agent: recent advancements in multi-mechanistic approaches. *Front Mol Biosci*. 2023;10:1157558.
 33. Xin H, Kong Y, Wang Y, Zhou Y, Zhu Y, Li D, *et al*. Lignans extracted from *Vitex negundo* possess cytotoxic activity by G2/M phase cell cycle arrest and apoptosis induction. *Phytomedicine*. 2013;20(7):640-7.
 34. Vo GV, Nguyen TH, Nguyen TP, Do TH, Tran NM, Nguyen HT, *et al*. In silico and *in vitro* studies on the anti-cancer activity of artemetin, vitexicarpin and penduletin compounds from *Vitex negundo*. *Saudi Pharm J*. 2022;30(9):1301-14.
 35. Awale S, Linn TZ, Li F, Tezuka Y, Myint A, Tomida A, *et al*. Identification of chrysopenetin from *Vitex negundo* as a potential cytotoxic agent against PANC-1 and a panel of 39 human cancer cell lines (JFCR-39). *Phytother Res*. 2011;25(12):1770-5.
 36. Chaudhry GE, Jan R, Naveed Zafar M, Mohammad H, Muhammad TST. *Vitex rotundifolia* fractions induced apoptosis in human breast cancer T-47D cell line via activation of extrinsic and intrinsic pathway. *Asian Pac J Cancer Prev*. 2019;20(12):3555-62.
 37. Özlem Sultan Aslantürk, Tülay Aşkın Çelik. Antioxidant activity and anticancer effect of *Vitex agnus-castus* L. (Verbenaceae) seed extracts on MCF-7 breast cancer cells, *Caryologia*. 2013;66(3):257-67.
 38. Ibrahim FM, Ibrahim AY, El-Newary SA, Hendawy SF, Mahomoodally MF. *Vitex agnus-castus* L.(Chasteberry) extracts shows *in vitro* and *in vivo* anti-inflammatory and anti-tumor propensities via reduction of cyclooxygenase-2 activity and oxidative stress complications. *S Afr J Bot*. 2021;143:363-73.
 39. İlhan S. Essential Oils from *Vitex agnus castus* L. Leaves induces caspase-dependent apoptosis of human multi-drug-resistant lung carcinoma cells through intrinsic and extrinsic pathways. *Nutr Cancer*. 2021;73(4):694-702.
 40. Gong G, Shen YL, Lan HY, Jin JM, An P, Zhang LJ, *et al*. The CYR61 is a potential target for rotundifuran, a natural labdane-type diterpene from *vitex trifolia* L., to trigger apoptosis of cervical cancer cells. *Oxid Med Cell Longev*. 2021;2021:6677687.
 41. Mohammad MS, Shyam P. Bioactivity studies and *Insilico* studies of the isolated compound from ancient plant *Vitex negundo* against breast cancer. *J Mol Struct*. 2023;1288:135762.
 42. Gouthami K, Veeraraghavan V, Nagaraja P. In-silico characterization of phytochemicals identified from *Vitex negundo* (L) extract as potential therapy for Wnt-signaling proteins. *Egypt J Med Hum Genet*. 2022;23:3.
 43. Liu N, Wang KS, Qi M, Zhou YJ, Zeng GY, Tao J, *et al*. Vitexin compound 1, a novel extraction from a Chinese herb, suppresses melanoma cell growth through DNA damage by increasing ROS levels. *J Exp Clin Cancer Res*. 2018;37(1):269.
 44. Dehghani Nazhvani A, Sarafraz N, Askari F, Heidari F, Razmkhah M. Anti-cancer effects of traditional medicinal herbs on oral squamous cell carcinoma. *Asian Pac J Cancer Prev*. 2020;21(2):479-84.



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Cost-Utility of Mandibular Advancement Devices in Mitigating Sleepiness and Traffic Accident Risk in Obstructive Sleep Apnea

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Abstract

Objectives: Mandibular advancement devices (MADs) are an effective alternative treatment for obstructive sleep apnea (OSA), improving daytime symptoms and enhancing quality of life. However, evidence on the cost-effectiveness of MAD for OSA treatment remains limited. This study aimed to conduct a cost-utility analysis comparing MAD treatment for OSA to no treatment in Thailand.

Methods: A social perspective was adopted to evaluate the cost-utility of MAD compared to no treatment using a Markov model with a lifetime horizon in OSA patients with excessive daytime sleepiness (EDS). Input parameters were drawn from international and national sources, including published literature, national databases, and local expert consultations. Costs were presented in the United States dollar (USD), and a 3% discount rate was applied to both costs and outcomes, in accordance with Thailand's National HTA Guidelines.

Results: The base case analysis indicated that using MAD resulted in an increase in QALYs by 0.85, with additional costs of 3,308 USD. This resulted in an ICER of 3,891 USD, which is slightly lower than the willingness-to-pay threshold in Thailand, set at 4,526 USD per QALY. MAD was found to be cost-effective, with a probability of cost-effectiveness of 51.9%. Parameters influencing cost-effectiveness were identified and the most sensitive parameters affecting changes in the incremental cost-effectiveness ratio (ICER) were examined through the one-way sensitivity analysis.

Conclusions: MAD therapy appeared marginally cost-effective in Thai settings for OSA patients with EDS. The considerable uncertainty revealed in sensitivity analyses suggests that further research is needed to clarify key parameters and inform decision-making.

Keywords: cost-effectiveness analysis, cost-utility analysis, economic evaluation, mandibular advancement devices, obstructive sleep apnea, road traffic accidents

Introduction

Obstructive sleep apnea (OSA) is a common sleep-related breathing disorder characterized by the relaxation of pharyngeal muscles, which leads to recurrent episodes of upper airway obstruction during sleep. As a protective mechanism, the brain triggers an arousal to awaken the individual, tightening the upper airway muscles and reopening the airway to resume breathing.^(1,2) Repeated sleep disruptions and awakenings can result in various symptoms and health complications.⁽³⁾ OSA significantly increases the risk of accidents, especially road traffic accidents (RTAs), due to excessive daytime sleepiness (EDS).⁽⁴⁻¹¹⁾ Individuals with OSA are particularly vulnerable to microsleep episodes and decreased alertness, particularly in monotonous driving conditions. Microsleep, a brief lapse in attention, is among the most hazardous outcomes of untreated OSA and contributes to a 2-to-7-fold increase in crash risk among affected drivers compared to the general population.^(4,7) In Thailand, the prevalence of OSA with EDS is approximately 4.4% of the total population.⁽¹²⁾

Treatment options for OSA vary depending on the severity of the condition and the patient's needs. Continuous positive airway pressure (CPAP) therapy is considered the gold standard. This device provides a continuous flow of air through the nose and/or mouth to keep the airways open during sleep. CPAP has demonstrated effectiveness in reducing EDS and lowering the risk of RTAs.^(3,13) Specifically, adherence to CPAP for more than four hours per night has been associated with a 67.1% to 81% reduction in RTAs⁽¹⁴⁾, with noticeable improvements in driving performance within 2 to 7 days of treatment initiation.^(3,13)

Despite its efficacy, CPAP adherence remains a major challenge. Globally, only about 50% of OSA patients maintain consistent CPAP use.^(1,2,15) In Thailand, access to CPAP is further hindered by cost barriers. The device, which costs approximately 566 USD, is not currently covered under the Thai Universal Health Coverage (UHC) scheme, requiring patients to pay out-of-pocket.⁽¹⁶⁾ Consequently, many patients either forgo therapy or seek alternative treatments. Therefore, Mandibular advancement device (MAD) provides a viable alternative, especially for patients with mild to moderate OSA. These devices function by repositioning the mandible forward relative to the upper jaw, helping to maintain an open

airway and preventing the collapse of soft tissues. MAD treatment effectively reduces EDS and improves daytime functions, such as driving performance and it was reported to have better patient compliance than CPAP.⁽¹⁷⁻²⁰⁾

Although MADs provide health benefits compared to no treatment, they also incur costs, necessitating justification for inclusion in the public program. Using a societal perspective, this study aims to conduct a cost-utility analysis comparing MADs with the no-treatment. The findings of this study may support policy considerations for including MADs in the Thai UHC benefit package, particularly in light of the affordability challenges associated with CPAP.

Methods

Economic model

This was a cost-utility analysis designed to estimate the expected costs and health gains associated with the use of MAD treatment versus no treatment in OSA patients with EDS. The study modeled OSA patients with EDS driving vehicles and transitioning into four health stages: OSA no event (OSA patients without any specific RTAs), RTA alive (OSA patients surviving an RTA), RTA disability (OSA patients disabled due to an RTA), and death. It was assumed that OSA patients surviving an RTA would drive similarly to OSA no event, meaning this group could continue driving vehicles and could experience RTAs again (Figure 1).

The model simulated scenarios for OSA patients starting at the age of 39 years, which represents the average age of OSA diagnosis in Thailand.⁽¹²⁾ with a cycle-length of 1 year and a lifetime time horizon.

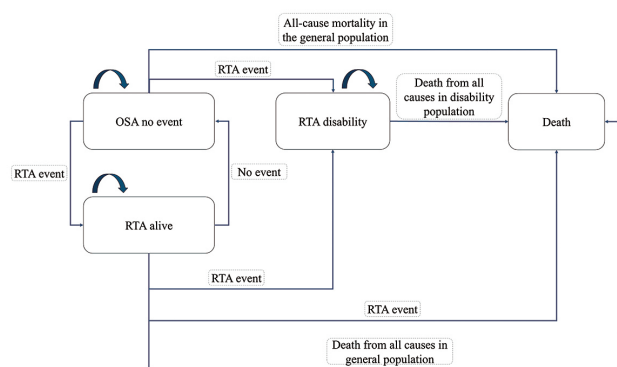


Figure 1: Structure of the Markov model.

Model input

Transition probabilities

In the no-treatment scenario, the probabilities of transitioning from OSA no event to RTA alive, RTA disability, and death from RTAs per year were calculated using the following data: the number of RTA injuries, the number of RTA injuries resulting in disability, and the number of RTA-related deaths per year reported by the Thai Road Safety Collaboration.⁽²¹⁾ the number of licensed drivers reported by the department of land transport, Thailand⁽²²⁾; and an odds ratio of 2.36 (SE of 0.5255), derived from a meta-analysis of car crashes involving patients with and without OSA conducted by Luzzi *et al.*⁽²³⁾ The resulting probability was 0.060740 (0.015593) for OSA no event to RTA alive, 0.00012 (0.000233) for OSA no event to RTA disability, and 0.001012 (0.000053) annual probability of death from RTAs. This study assumed that OSA patients who survived an RTA would drive similarly to OSA patients in the no event group. Therefore, the probability of transitioning from RTA alive to RTA disability was assumed to be the same as the probability of transitioning from OSA no event to RTA disability.

The probability of death for patients disabled due to RTAs in OSA patients with EDS per year was assumed to be comparable to that of patients with cervical spinal injuries. Based on a report by Middleton *et al.*,⁽²⁴⁾ which indicated a 40-year survival rate of 47% for cervical spinal injury patients (most of whom were injured in motor vehicle accidents), the annual probability of death was calculated to be 0.0187 (0.0144).

The probability of a patient in the OSA no event group transitioning to death from general causes per year was assumed to be similar to the mortality rate of the general population, obtained from the 2014 Thailand's life table.⁽²⁵⁾

In the MAD-treated scenario, the probability of RTAs was reduced based on treatment adherence and its relative risk. The relative risk comparing MAD-treated and untreated groups was calculated according to the data reported by Quinnell *et al.*⁽¹⁸⁾ This study conducted an open label, randomized, controlled, crossover trial in adults with OSA, comparing four weeks of MAD treatment with four weeks of no treatment. The secondary outcomes were ESS scores, quality of life, and questionnaire-based evaluation of driving-related sleepiness. The questionnaire revealed that 11 out of 73 patients in the no-treatment

group reported pulled off the road, compared to 4 out of 72 patients in the MAD-treated group. Statistical analysis yielded a relative risk of 0.3687 (0.205). This reduction was extrapolated to estimate a corresponding decrease in RTA probability.

Treatment adherence data were obtained from Uniken *et al.*,⁽²⁶⁾ who reported that 34 out of 51 patients-maintained MAD usage after a 10-year follow-up period, resulting in an annual adherence rate of 0.958 (0.0280).

The relative risk and adherence rates were subsequently used to calculate the probabilities of RTAs and associated outcomes (survival, mortality, and disability) in MAD-treated patients. For non-compliant patients, the probability of RTAs was assumed to be the same as that of the no-treatment scenario.

After accounting for treatment adherence and relative risk, the following probabilities were calculated for the MAD-treated scenario: transition from OSA no event to RTA alive: 0.023386 (0.013416), transition from OSA no event to RTA disability: 0.000390 (0.000219), transition from OSA no event to death from RTAs: 0.000005 (0.000003), transition from OSA no event to death from general causes and death from disability due to RTAs assumed to be the same as in the no-treatment group. The raw data calculated probabilities within this study are presented in Table 1.

Costs

Under societal viewpoint, the costs analyzed in this study included both direct medical expenses and direct non-medical costs, with the majority of the data obtained through comprehensive literature review and questionnaire surveys.

Direct medical costs

Medical costs including expenses such as cost of a visit to a dentist and cost of a visit to a doctor, were obtained from Thai Standard Cost List for Health Technology Assessment.⁽²⁷⁾ RTA-related costs such as cost of outpatient medical treatment for RTA patients, cost of inpatient medical treatment for RTA patients were derived from an analysis of the economic costs of road traffic injuries from the perspective of the Thailand Social Security System.⁽²⁸⁾ Additionally, the cost of MAD, the cost of sleep test and the number of patients visiting a doctor and dentist were obtained from the questionnaires collected from 15 qualified dentists in dental sleep medicine and 9 qualified sleep medicine physicians in Thailand. The

Table 1: Variables, standard deviations, and distributions associated with probabilities used in this study.

Input parameters	Value	SD	Distributions	Sources
Average number of persons holding driving licenses (per year)	32,792,963	51516.24	gamma	Pradaboon K <i>et al.</i> , 2021 ⁽²⁷⁾
Average number of persons injured from accidents (per year)	843,996	107822.92	gamma	Riewpaiboon A, 2009 ⁽²⁶⁾
Average number of persons dying from accidents (per year)	14,059	827.24	gamma	Riewpaiboon A, 2009 ⁽²⁶⁾
Average number of persons disabled due to accidents (per year)	173	9.70	gamma	Riewpaiboon A, 2009 ⁽²⁶⁾
Probability of death in C-spinal injury patients	0.0187	0.0144	beta	Thailand Consumer Price Index ⁽²⁹⁾
Odds ratio of OSA patients having accidents compared to 'no treatment'	2.360	0.5255 ^a	gamma	Thailand Consumer Price Index ⁽²⁸⁾
Probability of OSA patients using MAD in the following year	0.958	0.028	beta	Currie CJ <i>et al.</i> , 2005 ⁽³²⁾
Relative risk of treating OSA patients with MAD compared to 'no treatment'	0.3687	0.205	beta	Middleton JW <i>et al.</i> , 2024 ⁽²³⁾ and Jenkinson C <i>et al.</i> , 1998 ⁽³¹⁾

SD, standard deviation

^a Standard error

lifetime costs associated with the MAD were calculated under the assumption that the device would require replacement every five years, a timeframe consistent with that of acrylic complete dentures, which are covered by the Universal Coverage Scheme in Thailand.

Direct non-medical costs

Direct non-medical costs including expenses such as, cost of transportation expenses for hospital visits, cost of lost income for patients due to hospital visits, cost of lost income for relatives due to hospital visits, food expenses during hospital visits were obtained from Thai Standard Cost List for Health Technology Assessment.⁽²⁷⁾ Furthermore, the cost of damaged vehicles from accidents, cost of damaged government properties from accidents, were derived from an analysis of the economic costs of road traffic injuries from the perspective of the Thailand Social Security System.⁽²⁸⁾ All costs were converted to 2024 values using the Thai consumer price index and presented in USD (approximately THB 35.35=1 USD).⁽²⁹⁾

Cost analysis for patients with no treatment

For patients without treatment, the cost of the OSA no-event health stage during the first-year cycle was equivalent to the cost of a single visit to a doctor. For subsequent years, it was assumed that no additional costs would be incurred until the end of life.

In terms of RTA-related costs, the total cost was

determined by summing various expenses. These included the cost of a visit to a doctor, outpatient medical treatment for RTA patients, inpatient medical treatment for RTA patients, costs associated with vehicle damage from accidents, costs of damage to government property caused by accidents, transportation expenses for hospital visits, lost income for patients due to hospital visits, and food expenses during hospital visits. Each of these costs was calculated based on a single session, as the study assumed that an RTA would occur only once per cycle or year.

For the RTA alive health stage, the cost was similar to the RTA-related costs. However, in the RTA disability or death health stages, the cost was also similar to the RTA-related costs but was calculated only for the first year. For subsequent years, it was assumed that no additional costs would be incurred, as patients with disabilities or those who had died would no longer drive or require treatment.

For patients in the RTA alive health stage, the costs were equivalent to the RTA-related expenses. However, for patients in the RTA disability or RTA death health stages, the costs were also similar to the RTA-related expenses but were calculated only for the first year. For subsequent years, it was assumed that no additional costs, as patients with disabilities or those who had died would no longer drive.

Cost analysis for patients with MAD treatment

For patients receiving MAD treatment, the study assumed that the lifespan of the MAD was five years, meaning that patients would need to replace the device every five years. The cost of the OSA no-event health stage during the first year of MAD treatment was calculated by considering several factors. These included the Number of visits a patient sees the doctor during the first year multiplied by the cost of a doctor's visit, the Number of visits a patient sees the dentist during the same period multiplied by the cost of a dentist's visit, the cost of one session of a hospital sleep test, the number of follow-up hospital sleep tests during the first year multiplied by the cost of a hospital sleep test, the number of follow-up home sleep tests during the first year multiplied by the cost of home sleep tests, and the cost of the MAD itself.

For subsequent years (years 2 to 5), the cost of the OSA no-event health stage was calculated similarly. This included the Number of visits a patient sees the doctor per year multiplied by the cost of a doctor's visit, the Number of visits a patient sees the dentist per year multiplied by the cost of a dentist's visit, the number of hospital sleep tests per year multiplied by the cost of a hospital sleep test, and the number of home sleep tests per year multiplied by the cost of home sleep tests.

In the sixth year, the MAD would need to be replaced. The cost for this year was similar to the costs for years 2 to 5 but included the additional cost of the MAD and three times the cost of a dentist's visit to account for the process of creating a new MAD. For years 7 to 10, the costs were similar to those for years 2 to 5. This cycle of costs (years 6 to 10) was repeated until the end of life, with the MAD being replaced every five years.

The costs associated with the RTA alive health stage were determined by adding the cost of MAD treatment in each year to the RTA-related costs. For the RTA disability and RTA death health stages, the costs were similar to those for the RTA alive health stage during the first year. However, for subsequent years, it was assumed that no additional costs would be incurred, as patients with disabilities or those who had died could no longer be involved in RTAs or use MAD treatment.

This study did not account for productivity losses due to morbidity and premature mortality from RTA, as this could result in double counting alongside the utility losses associated with disability and mortality outlined

below. The raw data calculated costs within this study are presented in Table 2.

Health utility

In the no-treatment group, the utility value of OSA no event was reported as 0.74, based on the study by Sadatsafavi *et al.*,⁽³⁰⁾ which utilized data collected by Jenkinson *et al.*⁽³¹⁾ This data was derived using the 36-item Short Form Health Survey (SF-36), the Patient Generated Index (PGI), and the EuroQol EQ-5D questionnaire in OSA patients from United Kingdom. The utility value of RTA alive as 0.62, according to the study by Sadatsafavi *et al.*,⁽³⁰⁾ which utilized data from Currie *et al.*⁽³²⁾ This data was collected and analyzed from patients treated at the Cardiff and Vale National Health Service Hospitals Trust in the United Kingdom. The utility value of RTA disability was reported as 0.19, as derived from the study by Graham *et al.*⁽³³⁾ This study collected and analyzed data from the Application of the Functional Capacity Index to NASS CDS Data, conducted by the National Highway Traffic Safety Administration, USA. The utility value of death in OSA patients was reported as 0.

In the MAD-Treated group, utility value of OSA no event after MAD treatment was calculated as 0.767. This value was derived by adding the baseline utility value of OSA no event in no treatment group as 0.74 to the difference in the ESS score before and after treatment, multiplied by 0.01. The reduction in ESS scores was derived from a single clinical study with a randomized crossover design conducted in Thailand.⁽³⁴⁾ This study provided baseline ESS scores (no treatment) and post-intervention ESS scores (MAD treatment). The results demonstrated a reduction in mean ESS scores from 11.20 to 8.49 following MAD intervention.⁽³⁴⁾

This adjustment is based on findings that a 1-point reduction in the ESS score leads to a 0.01 (0.004) increase in the utility value of the EQ-5D-3L, as determined by analyses involving the ESS, SF-6D, and EQ-5D instruments.⁽³⁵⁾ The utility value RTA alive, RTA disability, and RTA death after MAD treatment were assumed to be the same as in the no-treatment group. The raw data calculated health utilities within this study are presented in Table 3

Base-case analysis

The primary outcomes of interest were the differences in the number of OSA no events, RTA alive, RTA deaths, and RTA disabilities from driving a vehicle, as well as the total costs and quality-adjusted life years (QALYs)

Table 2: Variables, standard deviations, and distributions associated with cost used in this study.

Input parameters	Value	SD	Distributions	Sources
Number of visits a patient sees the doctor during the first year of MAD treatment	3.202	0.87	gamma	survey
Number of visits a patient sees the dentist during the first year of MAD treatment	6.011	1.44	gamma	survey
Number of visits a patient sees the doctor per year during years 2 to 5 of MAD treatment	1.033	0.26	gamma	survey
Number of visits a patient sees the dentist per year during years 2 to 5 of MAD treatment	1.133	0.46	gamma	survey
Number of hospital sleep test for follow-up in the first year of MAD treatment	0.571	0.47	gamma	survey
Number of home sleep test for follow-up during the first year of MAD treatment	0.711	0.79	gamma	survey
Number of hospital sleep test per year during years 2 to 5 of MAD treatment	0.192	0.24	gamma	survey
Number of home sleep tests per year during years 2 to 5 of MAD treatment	0.193	0.05	gamma	survey
Cost of MAD	231	102.86	gamma	survey
Cost of hospital sleep test per session	248	27	gamma	survey
Cost of home sleep tests per session	83	19	gamma	survey
Cost of a visit to a dentist per session	8	1.88 ^b	gamma	Chaikledkaew U <i>et al.</i> , 2014 ⁽³⁶⁾
Cost of a visit to a doctor per session	8	1.88 ^b	gamma	Chaikledkaew U <i>et al.</i> , 2014 ⁽³⁶⁾
Cost of outpatient medical treatment for RTA patients per session	38	7.55 ^b	gamma	Banhiran W <i>et al.</i> , 2018 ⁽³⁴⁾
Cost of inpatient medical treatment for RTA patients per session	555	111 ^b	gamma	Banhiran W <i>et al.</i> , 2018 ⁽³⁴⁾
Cost of damaged vehicles from accidents per session	277	55 ^b	gamma	Banhiran W <i>et al.</i> , 2018 ⁽³⁴⁾
Cost of damaged government properties from accidents per session	94	18.71 ^b	gamma	Banhiran W <i>et al.</i> , 2018 ⁽³⁴⁾
Cost of transportation expenses for hospital visits per session	4.72	0.33 ^a	gamma	Chaikledkaew U <i>et al.</i> , 2014 ⁽³⁶⁾
Cost of lost income for patients due to hospital visits per session	2.66	0.39 ^a	gamma	Chaikledkaew U <i>et al.</i> , 2014 ⁽³⁶⁾
Cost of lost income for relatives due to hospital visits per session	3.17	1.00 ^a	gamma	Chaikledkaew U <i>et al.</i> , 2014 ⁽³⁶⁾
Food expenses during hospital visits per session	1.75	0.15 ^a	gamma	Chaikledkaew U <i>et al.</i> , 2014 ⁽³⁶⁾

The cost was reported in USD.

a Standard error, b Standard error assumed, 20%.

SD, standard deviation

in the no-treatment and MAD-treated groups. The reduction in the number of RTA alive, RTA deaths, and RTA disabilities caused by driving, along with the reduction in the ESS in RTA alive and OSA events, could lead to gains in QALYs for the MAD-treated groups. Future benefits, including the costs and QALYs gained, were discounted at 3%, as recommended by the Thai HTA guidelines.⁽³⁶⁾

The incremental cost-effectiveness ratio (ICER) in USD per QALY gained for each policy option was presented to assess the cost-effectiveness of the technology. To be considered cost-effective in Thailand, MAD had to provide an additional unit of health gain at or below a willingness-to-pay (WTP) threshold of 160,000 THB (approximately 4,526 USD) per QALY.⁽¹⁵⁾

Table 3: Variables, standard deviations, and distributions associated with health utilities used in this study.

Input parameters	Value	SD	Distributions	Sources
ESS of 'no treatment'	11.2	3.5	gamma	McDaid C <i>et al.</i> , 2009 ⁽⁴¹⁾
ESS of MAD treatment	8.49	0.7	gamma	McDaid C <i>et al.</i> , 2009 ⁽⁴¹⁾
Increased utility value per 1 ESS reduction	0.01	0.0040 ^a	gamma	Jenkinson C <i>et al.</i> , 1998 ⁽³¹⁾
Utility value of OSA patients	0.74	0.1590 ^a	beta	Van Haesendonck G <i>et al.</i> , 2015 ⁽³⁸⁾
Utility value of disabled patients	0.19	0.0560 ^a	beta	Ou YH <i>et al.</i> , 2023 ⁽³⁹⁾
Utility value of RTA patients	0.62	0.27	beta	Ou YH <i>et al.</i> , 2024 ⁽⁴⁰⁾

SD, standard deviation

^a Standard error

Uncertainty analysis

This study conducted a one-way sensitivity analysis, a probabilistic sensitivity analysis (PSA), and scenario analyses to assess the impact of model assumptions and parameter uncertainty. In the one-way sensitivity analysis, parameters were adjusted within their 95% confidence intervals. However, for certain cost-related parameters such as the cost of a dental visit, the cost of a doctor visit, the cost of outpatient treatment for RTA patients, the cost of inpatient treatment for RTA patients, the cost of vehicle damage from accidents, and the cost of damage to government property from accidents. The standard error was assumed to be 20 %. The most influential variables were presented using a tornado diagram.

For the PSA, parameter distributions were assigned following the guidelines of Briggs *et al.*⁽³⁷⁾ A beta distribution was applied to parameters such as relative risk, probabilities, and utility values, while a gamma distribution was used for cost-related parameters, number of persons holding driving licenses, number of persons injured from accidents, number of persons dying from accidents, number of persons disabled due to accidents . Random values were sampled from these distributions using Monte Carlo simulation with 1,000 iterations. The findings were summarized and displayed through cost-effectiveness acceptability curves.

For the scenario analyses, several parameters were varied to reflect different assumptions, including the following: home sleep tests were performed annually for follow-up, hospital sleep tests were performed annually for follow-up, the utility value of RTA survivors was assumed to be equal to that of OSA patients without events, MAD adherence was assumed to be 70%, MAD was assumed to have no effect on preventing RTAs, and

the cost of the MAD was adjusted. The results revealed differences in the ICERs across scenarios.

Result

Base-case analysis

The result of base-case analysis comparing MAD with no treatment for OSA patients in Thailand is presented in Table 4. The analysis revealed that MAD treatment resulted in a lifetime cost of 4,675 USD compared with 1,367 USD for no treatment, yielding an incremental cost of 3,308 USD. In terms of health benefits, MAD was associated with 17.08 QALYs, whereas no treatment yielded 16.23 QALYs, resulting in an incremental gain of 0.85 QALYs. Consequently, the ICER was calculated to be 3,891 USD per QALY gained. According to the WTP threshold, MAD appears to represent a cost-effective intervention relative to no treatment.

Uncertainty analysis

The results of one-way sensitivity analysis indicated that the variables which had a highly significant impact on the base case ICER (more than 100% of ICER) were utility value of RTA patients, number of hospital sleep test per year during years 2 to 5 of MAD treatment, increased utility value per 1 ESS reduction. Other variables that had a significant impact on the base case ICER are shown in Figure 2. The remaining parameters are not presented in the tornado diagram, as they were unlikely to have a substantial impact on the ICER (less than 10%). The PSA results (Figure 3) revealed that at the current Thai WTP threshold, the MAD treatment was cost-effective with a 51.9%. No treatment was cost-effective if WTP was below 3,253 USD.

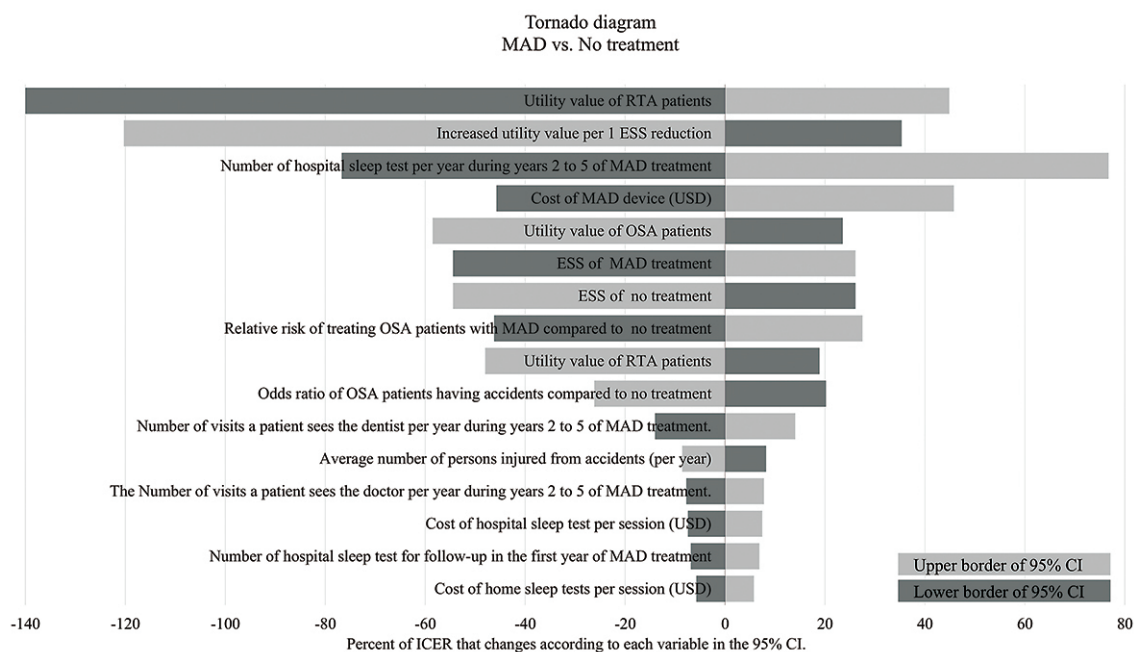


Figure2: Tornado diagram showing the variables affecting an ICER greater than 10%.

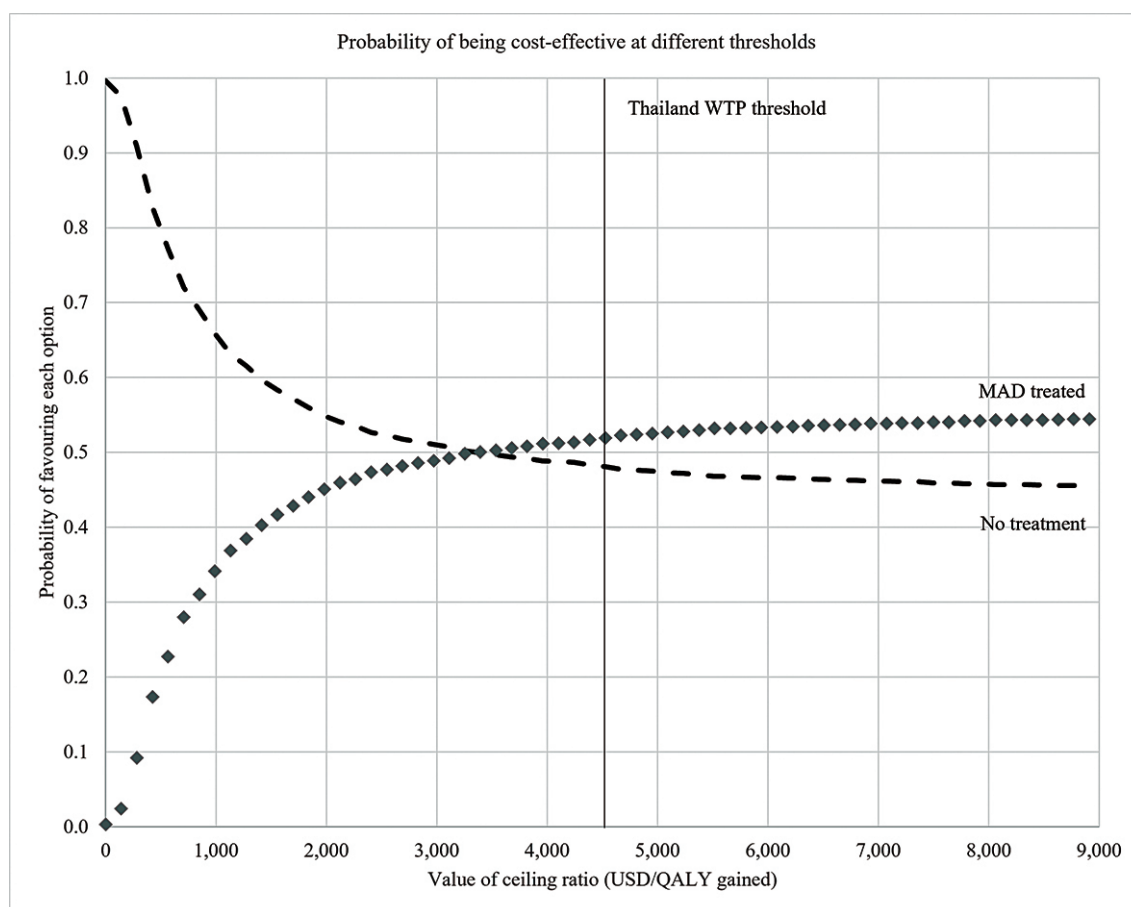


Figure 3: Cost-effectiveness acceptability curves in the different thresholds.

Table 4: Analyses of cost, QALY and ICER in different scenarios.

Scenario	MAD		No treatment		ICERs (USD/ QALY)
	Cost (USD)	QALY	Cost (USD)	QALY	
Base case analysis	4,675	17.08	1,367	16.23	3,891
If home sleep test is performed annually for follow-up	4,664	17.08	1,367	16.23	3,879
If hospital sleep test is performed annually for follow-up	8,680	17.08	1,367	16.23	8,604
If utility value of 'RTA alive' equals 'OSA no event'	4,675	17.15	1,367	16.39	4,353
If MAD adherence is 70%	4,884	17.01	1,367	16.23	4,509
If MAD has no effect on preventing RTA	5,446	16.80	1,367	16.23	7,156
If the cost of MAD is 339.46 USD (12,000 THB)	5,206	17.08	1,367	16.23	4,516

The results of the scenario analyses are summarized in Table 4. When follow-up was assumed to be conducted annually using only a hospital-based sleep test, the ICER increased substantially to 8,604 USD per QALY, reflecting significantly higher healthcare costs. In contrast, follow-up using only a home sleep testing yielded an ICER of 3,879 USD per QALY, comparable to the similar to the base-case estimation.

Altering the utility value of RTA patients to equal that of OSA patients without events resulted in an ICER of 4,353 USD. Reducing MAD adherence to 70% increased the ICER to 4,509 USD. When MAD had no effectiveness in reducing RTA incidence, the ICER rose substantially to 7,156 USD per QALY, highlighting the importance of treatment efficacy. Lastly, increasing the cost of the MAD to 339.46 USD (12,000 THB) resulted in an ICER of 4,516 USD per QALY.

Discussion

Our model indicates that MAD yields an ICER of 3,891 USD, which is slightly lower than the WTP threshold for the Universal Health Care Coverage Scheme in Thailand. These findings suggest that, compared to the no-treatment option, MAD is cost-effective in the Thai setting. However, one-way sensitivity and probabilistic sensitivity analyses highlight significant parameter uncertainty, rendering the results inconclusive. While MAD appears to have a higher probability of being cost-effective at Thailand's WTP threshold, there remains a 48% probability that the no-treatment option could be more cost-effective than MAD. These results underscore the need for further research to refine key parameters identified in the one-way sensitivity analysis.

In comparison, a US study by Sadatsafavi *et al.*,⁽³⁰⁾ that evaluated MAD against no treatment reported an ICER of 2,984 USD, which is lower than the ICER observed in this study (2,984 vs. 3,891 USD). The lower ICER in that study was attributed to the clinical benefits associated with the prevention of RTAs, stroke, and coronary artery disease. These additional benefits may have contributed to the higher incremental QALY observed in the treatment group compared to our study. Although the prevention of stroke and coronary artery disease could make MAD therapy more advantageous, this study excluded cerebrovascular and cardiovascular diseases from the economic model due to the numerous factors influencing their onset, recovery, and progression. Despite evidence suggesting that MAD may have beneficial effects on cardiovascular comorbidities in OSA patients, studies comparing MAD to CPAP therapy have shown similar effects.^(19,38-40)

Regarding the ICER, a previous study conducted in the UK, which included the prevention of RTAs, stroke, and coronary artery disease in its economic model, reported an ICER of approximately 17,003 USD. In the UK, the WTP threshold ranges from 20,000 to 30,000 GBP.⁽³⁵⁾ The ICER in the UK study was higher than that observed in our study. These differences can be explained by variations in the cost of treatment between the two countries. For instance, in this study, the cost of MAD was 231 USD, whereas in the UK, it was 678 USD. Additionally, this study assumed a lifespan of 5 years for MAD, while the UK study assumed a lifespan of only 2 years.

The present study had some limitations. First, the costs associated with caregivers for patients with RTA-related disabilities were not included, which may have led

to an underestimation of the costs in the RTA disability health stage. Second, some variables, were not obtained from Thai population such as the odds ratio of traffic accidents in OSA patients could differ from those in other countries.^(30,35,41-43) The relative risk of RTAs following MAD treatment, compared to the no-treatment group, was analyzed based on questionnaire data from the UK⁽³⁵⁾, which asked OSA patients about instances of pulling off the road. However, this may cause the relative risk to not accurately reflect reality. Not all cases of pulling off the road due to sleep while driving result in traffic accidents, as other factors, such as the safety of the car and the traffic environment, may also play a role. While safer cars may help prevent traffic accidents, they are often more expensive. However, this study used a higher relative risk, indicating that it may prevent accidents less effectively compared to the UK study, which calculated the risk using the ratio of ESS treatment effects (0.37 vs. 0.17).⁽⁴¹⁾ In addition, OSA can cause various health problems beyond EDS and RTAs, which were not included in the model. These problems include sleepiness while driving, nodding off behind the wheel, cardiovascular diseases, depression, lower work performance, poorer sleep quality for bed partners, heart disease, trouble with thinking, and diabetes.⁽⁴⁴⁾ If research is done on how effective MAD is in treating or preventing these issues, it could help create a more detailed Markov model that affects cost calculations and increasing utility values, making it more likely that MAD will be seen as cost-effective. Lastly, this study compared only MADs and no treatment; other available treatments for OSA, such as CPAP, maxillomandibular advancement surgery, and myofunctional therapy, were not included, even though some of these treatments may be more cost-effective than MAD.

This study focuses on the economic aspects of including MAD in the Thai health benefit package, without addressing feasibility and implementation challenges. For instance, dentists who fabricate MADs are not authorized to provide definitive diagnoses for OSA patients. Therefore, successful implementation of this policy requires multidisciplinary collaboration among physicians, qualified dentists, sleep technicians, and other health professionals to ensure improved quality of life and effective treatment for OSA.

Conclusions

Given the limited data on RTA related to OSA in Thailand, MAD may demonstrate limited cost-effectiveness within the Thai healthcare context, as the probability of cost-effectiveness was marginal (51.9%). Both one-way and probabilistic sensitivity analyses identified substantial uncertainty across several key model parameters, thereby rendering its cost-effectiveness inconclusive. This pronounced parameter uncertainty constrains the degree of confidence in healthcare decision-making based on the current evidence base. Nevertheless, these findings highlight the imperative for further empirical research aimed at refining critical clinical and economic inputs, minimizing parameter uncertainty, and enhancing the methodological robustness of subsequent cost-effectiveness analyses.

Abbreviations

CPAP	Continuous positive airway pressure
EDS	Excessive daytime sleepiness
ESS	Epworth Sleepiness Scale
ICER	Incremental cost-effectiveness ratio
MAD	Mandibular advancement device
OR	Odds ratio
OSA	Obstructive sleep apnea
QALY	Quality-adjusted life years
WTP	Willingness-to-pay
SD	Standard deviation
UK	United Kingdom

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Ethics Approval and Consent to Participate

Ethical approval for this study was obtained from the Center for Ethics in Human Research, Khon Kaen University, Thailand (Approval No. HE651157).

Competing Interests

The authors declare no competing interests.

References

1. Kryger Meir, Roth T, Dement WC. Principles and Practice of Sleep Medicine. Philadelphia:Saunders;2017.
2. Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP. Pathophysiology of sleep apnea. *Physiol Rev.* 2010;90(1):47-112.
3. Morsy NE, Farrag NS, Zaki NFW, Badawy AY, Abdelhafez SA, El-Gilany AH, *et al.* Obstructive sleep apnea: personal, societal, public health, and legal implications. *Rev Environ Health.* 2019;34(2):153-69.
4. Karimi M, Eder DN, Eskandari D, Zou D, Hedner JA, Grote L. Impaired vigilance and increased accident rate in public transport operators is associated with sleep disorders. *Accid Anal Prev.* 2013;51:208-14.
5. Philip P, Sagaspe P, Lagarde E, Leger D, Ohayon MM, Bioulac B, *et al.* Sleep disorders and accidental risk in a large group of regular registered highway drivers. *Sleep Med.* 2010;11(10):973-9.
6. Celikhisar H, Ilkhan GD. Association of presence and severity of obstructive sleep apnoea syndrome with accident risk in city bus drivers. *J Pak Med Assoc.* 2020; 70(12(A)):2184-9.
7. Tregear S, Reston J, Schoelles K, Phillips B. Obstructive sleep apnea and risk of motor vehicle crash: systematic review and meta-analysis. *J Clin Sleep Med.* 2009;5(6): 573-81.
8. Zhang T, Chan AH. Sleepiness and the risk of road accidents for professional drivers: a systematic review and meta-analysis of retrospective studies. *Saf Sci.* 2014;70:180-8.
9. Bioulac S, Micoulaud-Franchi JA, Arnaud M, Sagaspe P, Moore N, Salvo F, *et al.* Risk of motor vehicle accidents related to sleepiness at the wheel: a systematic review and meta-analysis. *Sleep.* 2017;40(10): doi: 10.1093/sleep/zsx134.
10. Amra B, Doralı R, Mortazavi S, Golshan M, Farajzadegan Z, Fietze I, *et al.* Sleep apnea symptoms and accident risk factors in Persian commercial vehicle drivers. *Sleep Breath.* 2012;16(1):187-91.
11. Howard ME, Desai AV, Grunstein RR, Hukins C, Armstrong JG, Joffe D, *et al.* Sleepiness, sleep-disordered breathing, and accident risk factors in commercial vehicle drivers. *Am J Respir Crit Care Med.* 2004;170(9):1014-21.
12. Neruntarat C, Chantapant S. Prevalence of sleep apnea in HRH Princess Maha Chakri Srinthorn Medical Center, Thailand. *Sleep Breath.* 2011;15(4):641-8.
13. Tregear S, Reston J, Schoelles K, Phillips B. Continuous positive airway pressure reduces risk of motor vehicle crash among drivers with obstructive sleep apnea: systematic review and meta-analysis. *Sleep.* 2010;33(10):1373-80.
14. Cassel W, Ploch T, Becker C, Dugnus D, Peter JH, Von Wichert P. Risk of traffic accidents in patients with sleep-disordered breathing: Reduction with nasal CPAP. *Eur Respir J.* 1996;9(12):2606-11.
15. Sawunyavisuth B, Ngamjarus C, Sawanyawisuth K. A meta analysis to identify factors associated with CPAP machine purchasing in patients with obstructive sleep apnea. *Biomed Rep.* 2022;16(6):1-9.
16. Hoekema A, Stegenga B, Bakker M, Brouwer WH, de Bont LG, Wijkstra PJ, *et al.* Simulated driving in obstructive sleep apnoea-hypopnoea; effects of oral appliances and continuous positive airway pressure. *Sleep Breath.* 2007;11(3):129-38.
17. Quinnell TG, Bennett M, Jordan J, Clutterbuck-James AL, Davies MG, Smith IE, *et al.* A crossover randomised controlled trial of oral mandibular advancement devices for obstructive sleep apnoea-hypopnoea (TOMADO). *Thorax.* 2014;69(10):938-45.
18. Ramar K, Dort LC, Katz SG, Lettieri CJ, Harrod CG, Thomas SM, *et al.* Clinical practice guideline for the treatment of obstructive sleep apnea and snoring with oral appliance therapy: an update for 2015: an American academy of sleep medicine and american academy of dental sleep medicine clinical practice guideline. *J Clin Sleep Med.* 2015;11(7):773-827.
19. Gottlieb DJ, Punjabi NM. Diagnosis and management of obstructive sleep apnea: a review. *JAMA.* 2020 ;323(14): 1389-400.
20. Road Safety Collaboration. ThaiRSC [Internet]. [cited 2022 Feb 2]. Available from: <https://www.thairsc.com/>
21. Transport Statistics Sub-Division PDD of LTT. The Number of Driving Licences and Transport Personnel Licences [Internet]. 2022. Available from: <https://web.dlt.go.th/statistics/>
22. Luzzi V, Mazur M, Guaragna M, Di Carlo G, Cotticelli L, Magliulo G, *et al.* Correlations of obstructive sleep apnea syndrome and daytime sleepiness with the risk of car accidents in adult working population: a systematic review and meta-analysis with a gender-based approach. *J Clin Med.* 2022;11(14):3971.
23. Middleton JW, Dayton A, Walsh J, Rutkowski SB, Leong G, Duong S. Life expectancy after spinal cord injury: a 50-year study. *Spinal Cord.* 2012;50(11):803-11.
24. Sittimart M, Butani D, Poonsiri C, Korakot M, Nalongsack M, Chalermkitpanit P, *et al.* Cost-utility analysis of radiofrequency ablation among facet joint-related chronic low back pain patients in Thailand. *Pain Physician.* 2024 Sep;27(7):E761-73.
25. Uniken Venema JA, Doff MH, Joffe-Sokolova D, Wijkstra PJ, van der Hoeven JH, Stegenga B, *et al.* Long-term obstructive sleep apnea therapy: a 10-year follow-up of mandibular advancement device and continuous positive airway pressure. *J Clin Sleep Med.* 2020;16(3):353-9.
26. Riewpaiboon A. Standard Cost List for Health Technology Assessment [Internet]. 2009 [cited 2022 Feb 2]. Available from: costingmenu.hitap.net

27. Pradaboon K, Phanthunane P, Pratoomsot C. The economic cost of road traffic injuries among people receiving social security benefits. *BEC Journal*. 2011;9(2):109-23.
28. Thailand Consumer Price Index. Available online: https://www.price.moc.go.th/price/cpi/index_new.asp (accessed on 2 Feb 2022).
29. Thailand Consumer Price Index. Available online: https://www.price.moc.go.th/price/cpi/index_new.asp (accessed on 2 Feb 2022).
30. Sadatsafavi M, Marra CA, Ayas NT, Stradling J, Fleetham J. Cost-effectiveness of oral appliances in the treatment of obstructive sleep apnoea–hypopnoea. *Sleep Breath*. 2009;13:241-52.
31. Jenkinson C, Petersen S, Stradling J. How should we evaluate health status? a comparison of three methods in patients presenting with obstructive sleep apnoea. *Qual Life Res*. 1998;7:95-100.
32. Currie CJ, McEwan P, Peters JR, Patel TC, Dixon S. The routine collation of health outcomes data from hospital treated subjects in the Health Outcomes Data Repository (HODaR): descriptive analysis from the first 20,000 subjects. *Value Health*. 2005;8(5):581-90.
33. Graham JD, Thompson KM, Goldie SJ, Segui-Gomez M, Weinstein MC. The cost-effectiveness of air bags by seating position. *JAMA*. 1997;278(17):1418-25.
34. Banhiran W, Assanasen P, Nopmaneejumrudlers C, Nujchanart N, Srechareon W, Chongkolwatana C, *et al*. Adjustable thermoplastic oral appliance versus positive airway pressure for obstructive sleep apnea. *Laryngoscope*. 2018;128(2):516-22.
35. Sharples L, Glover M, Clutterbuck-James A, Bennett M, Jordan J, Chadwick R, *et al*. Clinical effectiveness and cost-effectiveness results from the randomised controlled Trial of Oral Mandibular Advancement Devices for Obstructive sleep apnoea-hypopnoea (TOMADO) and long-term economic analysis of oral devices and continuous positive airway pressure. *Health Technol Assess*. 2014;18(67):1-296.
36. Chaikledkaew U, Kittrongsiri K. Guidelines for health technology assessment in Thailand (second edition)-the development process. *J Med Assoc Thai*. 2014;97(Suppl 5):S4-9.
37. Briggs A, Sculpher M, Claxton K. Decision modelling for health economic evaluation. Oxford: OUP; 2006.
38. Van Haesendonck G, Dieltjens M, Kastoer C, Shivalkar B, Vrints C, Van De Heyning CM, *et al*. Cardiovascular benefits of oral appliance therapy in obstructive sleep apnea: a systematic review. *J Dent Sleep Med*. 2015;2(1):9-14.
39. Ou YH, Colpani JT, Chan SP, Loke W, Cheong CS, Kong W, *et al*. Mandibular advancement device versus CPAP in lowering 24-hour blood pressure in patients with obstructive sleep apnoea and hypertension: the CRESCENT trial protocol. *BMJ Open*. 2023;13(5):e072853.
40. Ou YH, Colpani JT, Cheong CS, Loke W, Thant A tar, Shih EC, *et al*. Mandibular advancement vs CPAP for blood pressure reduction in patients with obstructive sleep apnea. *J Am Coll Cardiol*. 2024;83(18):1760-72.
41. McDaid C, Griffin S, Weatherly H, Duree K, Van der Burgt M, Van Hout S, *et al*. Continuous positive airway pressure devices for the treatment of obstructive sleep apnoea-hypopnoea syndrome: a systematic review and economic analysis. *Health Technol Assess*. 2009; 13(4): doi: 10.3310/hta13040.
42. Giannopoulou A, Burgers LT, van Bruggen MP, de Vries N, Redekop WK, Rijnsburger A. Economic evaluation of mandibular advancement device to treat obstructive sleep apnea. *Value Health*. 2013;16(7):A373.
43. Poullié AI, Cognet M, Gauthier A, Clementz M, Druais S, Späth HM, *et al*. Cost-effectiveness of treatments for mild-to-moderate obstructive sleep apnea in France. *Int J Technol Assess Health Care*. 2016;32(1-2):37-45.
44. Andre F, AJ HA, Nurit F, Najib A, Ismail L. The public health burden of obstructive sleep apnea. *Sleep Sci*. 2021;14(3):257–65.



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Gorlin-Goltz Syndrome in a Healthy Elderly Patient: A Case Report

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Abstract

Gorlin-Goltz syndrome (GGS), a rare inherited autosomal dominant disorder is characterized by presence of multiple basal cell carcinomas, odontogenic keratocyst (OKCs), bifid ribs or numerous skeletal and dental anomalies. A high level of expressiveness and penetrance is shown by this entity. It is therefore, necessary to understand the major and minor diagnostic criteria for the prompt diagnosis of this condition. We hereby report a case of a 50-year-old male who presented with multiple OKCs. On carrying out further investigations, bifid ribs and calcification of falx cerebri were additional findings which lead us to the diagnosis of GGS. It is therefore necessary to consider GGS as a possible diagnosis while dealing with any case of multiple OKCs.

Keywords: Gorlin-Goltz syndrome, keratocyst, keratocystic odontogenic tumours, nevroid basal cell carcinoma syndrome, odontogenic

Introduction

Gorlin-Goltz syndrome (GGS), an inherited autosomal dominant disorder, is also known as nevoid basal cell carcinoma syndrome (NBCCS).⁽¹⁾ Gorlin & Goltz described the classical triad of this syndrome which is composed of multiple basal cell carcinomas (BCC), odontogenic keratocyst (OKCs) in the jaws and bifid ribs that characterizes the diagnosis of this disorder.⁽²⁾ It is most found from first to third decade of life. Although all the findings are rarely seen in a single patient, this disorder affects multiple organ systems including skeletal, eye, skin, reproductive and neural.⁽³⁾ The mutations in the patched tumor suppressor gene (PTCH), a human homologue of the drosophila gene mapped to chromosome 9q21-21, causes this disorder. Most mutations involve inactivating constitutional sequence variants in PTCH1, located on chromosome 9q22, while mutations in its paralog PTCH2, located on chromosome 1p34, rarely occur. The underlying basis of this disease is an abnormality in the Hedgehog (Hh) signaling pathway as suggested by genetic studies and chromosomal mappings.^(4,5) The case of GGS is rarely reported in India with limited data available on its exact incidence or prevalence.⁽¹⁾ A 2020 case series reported only 64 cases documented in India since 1977, with just four cases showing hereditary involvement.⁽⁶⁾ We hereby present a case of this syndrome in a 50-year-old male patient.

Case Report

A 50-year-old male patient reported to the department of Oral Medicine and Radiology with the complaints of pain and watery fluid discharge from the lower right back region of jaw for 15 days. The pain was gradual in onset, mild in intensity, dull aching and continuous. It aggravated on mastication and relieved on taking medications. The pain radiated to the right side of his upper jaw. He gave no history of swelling associated with the pain. The medical, family, and psychological history were non-contributory. He gave a history of an invasive operatory procedure with the skull (craniotomy) on right side following a head injury at the age of 10 years. There were no symptoms associated with the same.

On extraoral examination, a solitary round shaped nevus was seen on the left nasolabial fold measuring approximately 0.5 cm in size (Figure 1). An extensive bone defect was seen over the right half of forehead

correlating with the history of surgery during childhood.

Intraorally, vestibular obliteration was seen in relation to the mandibular right first and second molars. Watery fluid discharge was noted from the lower posterior right back region. No tooth mobility was noted however, the alveolar mucosa adjacent to the mandibular right first premolar, second premolar, first molar, and second molar as well as the maxillary right second premolar, first molar, and second molar showed signs of inflammation and was tender on palpation. Mild expansion of the cortical plates was noted in the 1st and 4th quadrants.

An orthopantomogram (OPG) showed multiple well-defined radiolucencies in the upper and lower jaw, with the larger one extending from the periapical area of mandibular right lateral incisor to second molar with scalloped margins and evidence of thinning of inferior cortex of the jaw (Figure 2). Displacement of the inferior alveolar canal was noted inferiorly along the length of the lesion. Evidence of cortical perforation was seen in the mandibular right first premolar region. The lesion showed minimal expansion on occlusal radiograph. An ill-defined osteolytic lesion was noted with maxillary right third molar region with indistinct cortical margins and loss of density with the cortical margins of the floor of maxillary sinus. This feature was indicative of secondary infection with the lesion in the right maxillary third molar region. Another well-defined radiolucency, oval shaped, with corticated margins was noted in the periapical area of the left maxillary second molar. A small well-defined round radiolucency with corticated margins was also seen on distal aspect of the distal root of the mandibular left second molar, measuring approximately 1 cm in diameter. A provisional diagnosis of multiple OKCs was given. The differential diagnoses included GGS. A computed tomography (CT) scan of the patient confirmed the findings evident on OPG and showed calcification of the falx cerebri and the tentorium cerebellum (Figure 3) additionally. A chest radiograph of the patient showed evidence of bifid rib with the right 5th rib near the sternal end (Figure 4). Blood investigation revealed a higher parathyroid hormone level (86.2 pg/ml). Since the clinical and radiographical features satisfied 3 major criteria, a clinico-radiographic diagnosis of GGS was given with Brown's tumor as differential diagnosis.

Under general anesthesia, surgical enucleation of the

cysts was carried out along with extraction of the teeth in relation to the lesion. The specimens of the lesion from right maxilla and mandible were sent for histopathological examination.

Hematoxylin and Eosin-stained section of the specimen showed corrugated para-keratinised odontogenic epithelium with tall columnar basal cell layer. Nuclei are aligned and hyperchromatic, resembling a "picket fence" pattern. The underlying fibrous stroma is loose and collagenous. (Figure 5). All the features were suggestive of OKC. A final diagnosis of GGS was derived from clinical, radiographic and histopathological findings.



Figure 1: A solitary round shaped nevus seen on the left nasolabial fold.



Figure 2: An Orthopantomogram (OPG) showed multiple well-defined radiolucencies in the upper and lower jaw marked with arrows.

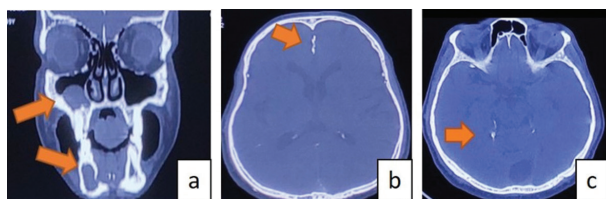


Figure 3: Computed tomography (CT) scan showing (a), Coronal view with Multiple well defined radiolucent lesions: (b), Axial view with calcification of the falx cerebri: (c), Axial view with calcification of the tentorium cerebellum.



Figure 4: Chest radiograph of the patient showing bifid rib with the right 5th rib near the sterna end (Arrow).

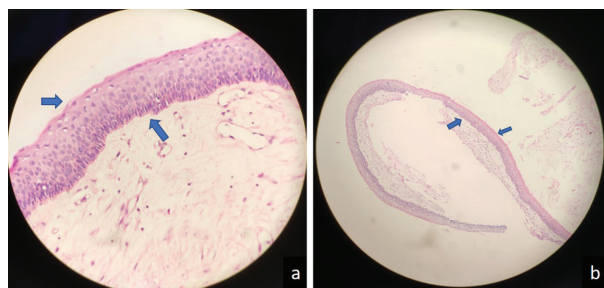


Figure 5: (a), High-power view (H&E, 100× magnification) showing the parakeratinized stratified squamous epithelial lining (indicated by the upper arrow) with a corrugated surface. The lower arrow points to the palisaded basal cell layer, composed of columnar or cuboidal cells with hyperchromatic nuclei arranged in a "picket fence" or "tombstone" pattern: (b), Low-power view (H&E, 40× magnification) illustrating the thin cystic wall and the uniform epithelial lining (arrows), which remains detached in some areas from the underlying connective tissue.

Discussion

The GGS was first reported by Jarish in 1894. Howell and Caro associated the basal cell nevus with other anomalies and disorders in 1959. Gorlin and Goltz were the first to define this condition as a syndrome.⁽⁷⁾ Varying by the region, the incidence of this entity is 1 in 50,000-150,000 in the general population.⁽¹⁾ Like the present case, patients with no family history may compose 60% of total GGS patients and 35 to 50% represent new mutations.⁽⁸⁾

One of the most common features of 75% of GGS patients is OKC, which is often the first sign in such cases.

There is no sex predilection of OKC associated with this syndrome.⁽⁹⁾ In a case reported by Pol *et al.*,⁽¹⁾ a 13-year-old boy was diagnosed with GGS based on various clinical findings including calcification of falx cerebri, frontal bossing, macrocephaly, depression of nasal bridge, hyper-telorism, prognathic mandible and palmar pits. In the present case, the patient was a 50-year-old male and showed along with multiple cystic lesions, evidence of calcification of falx cerebri and tentorium cerebellum and bifid rib. Other jaw bone abnormalities were not seen in the present case. Some other clinical findings associated with the GGS are cutaneous, dental, cardiac, sexual, craniofacial, skeletal, neurological, and ophthalmic anomalies which were not seen in the present case.⁽¹⁰⁾

The diagnosis of GGS can be made clinically by the criteria suggested by Evans *et al.*, (Table 1) and modified by Kimonis *et al.* in 1997 (Table 2). For the diagnosis of GGS, two major or one major and two minor criteria should be positive.⁽³⁾ Leonardi *et al.*, in 2010 suggested the inclusion of atlanto-occipital ligament calcification as a diagnostic feature for NBCCS in addition to other criteria.⁽⁷⁾ In our case three major criteria were positive namely, multiple OKCs proven by histology, calcification of falx cerebri and bifid ribs, which led us to a diagnosis of GGS.

Clinical and radiographic examination can be done to arrive at the diagnosis. However, confirmation of this syndrome is by ultrasound and DNA analysis. Diagnosis of GGS can be confirmed through DNA analysis by identifying pathogenic mutations in the PTCH1 gene, which is present in approximately 85% of affected individuals.⁽¹¹⁾ While ultrasound has limited diagnostic value, it can assist in detecting associated anomalies such as ovarian fibromas or macrocephaly, particularly in prenatal or early postnatal settings.⁽¹²⁾

The treatment of GGS is similar to the treatment of BCC and OKCs. Cystectomy with adjacent bony wall removal is the treatment for OKCs. Carnoy's solution can be used after cyst enucleation and cryosurgery to prevent recurrences.⁽¹³⁾ A similar approach was followed in the present case. Treatment of BCC includes surgical excision, Mohs micrographic surgery, and for advanced or multiple lesions—as seen in GGS—hedgehog pathway inhibitors like vismodegib are effective.⁽¹⁴⁾ Compared to sporadic cases, syndrome-associated BCCs and OKCs often require more conservative or

Table 1: Diagnostic criteria by Evans *et al.*, in 1991.

Major criteria
More than 2 BCCs, one BCC in patients younger than 30 years of age or more than 10 basal cell nevi
Any odontogenic keratocyst (proven by histology) or polyostotic bone cyst
Three or more palmar or plantar pits
Ectopic calcification in patients younger than 20 years of age (lamellar or early falx cerebri calcification)
A positive family history of NBCC
Minor criteria
Congenital skeletal anomaly (e.g., bifid, splayed, fused or missing rib, or bifid wedged or fused vertebra)
Occipital-frontal circumference greater than the ninety seventh percentile, with frontal bossing
Cardiac or ovarian fibromas
Medulloblastoma
Lymphomesenteric cysts
Congenital malformations such as cleft lip/palate, polydactylism or eye anomaly (e.g., cataract, coloboma or microphthalmos)
BCCs: Basal cell carcinomas, NBCC: Nevroid basal cell carcinoma

Table 2: Diagnostic criteria by Kimonis *et al.*, in 1997.

Major criteria
More than 2 BCCs or one BCC in patients younger than 20 years of age
Odontogenic keratocysts of the jaw (proven by histologic analysis)
Three or more palmar or plantar pits
Bilamellar calcification of the falx cerebri
Bifid, fused or markedly splayed ribs
A first degree relative with NBCCS
Minor criteria
Macrocephaly
Congenital malformations (e.g., cleft lip or palate, frontal bossing, coarse faces and moderate or severe hypertelorism)
Other skeletal abnormalities (e.g., spengel deformity, marked pectus deformity and marked syndactyly of the digits)
Radiological abnormalities (e.g., bridging of the sella turcica, vertebral anomalies, modeling defects of the hands and feet, or flame shaped lucencies of the hands and the feet)
Ovarian fibroma or medulloblastoma
BCCs: Basal cell carcinomas, NBCCs: Nevroid basal cell carcinomas

repeated surgical approaches, close monitoring, and genetic counseling, as they tend to be multiple, recurrent, and aggressive.⁽¹⁵⁾ Early diagnosis and treatment are important to prevent long term sequelae including

malignancy and oro-maxillofacial destruction.

Conclusions

It can be said that GGS is an autosomal dominant genetic disorder, which is of particular interest to the dental specialists. It is a rare entity and must be considered as a possible diagnosis in all the patients with odontogenic cysts. Ignorance and delay in the management can lead to development of multiple neoplastic lesions. Early diagnosis with the help of various clinical and imaging modalities is therefore useful. Long term follow-up of such patients is also necessary to avoid development of new lesions.

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Conflicts of Interest

The authors declare that they hold no competing interests.

References

1. Pol CA, Ghige SK, Kalaskar RR, Gosavi SR. Gorlin-Goltz syndrome: a rare case report. *Contemp Clin Dent*. 2013;4(4):547-50.
2. Kiran NK, Tilak Raj TN, Mukunda KS, Reddy RV. Nevoid basal cell carcinoma syndrome (Gorlin-Goltz syndrome). *Contemp Clin Dent*. 2012;3(4):514-8.
3. Ramesh M, Krishnan R, Chalakkal P, Paul G. Goltz-Gorlin Syndrome: case report and literature review. *J Oral Maxillofac Pathol*. 2015;19(2):267.
4. Sunil MK, Kumar R, Guru EN, Ranjan V. Gorlin-Goltz Syndrome. *J Indian Acad Oral Med Radiol*. 2010;22(4):S58-61.
5. Al-Jarboua MN, Al-Husayni AH, Al-Mgran M, Al-Omar AF. Gorlin-Goltz syndrome: a case report and literature review. *Cureus*. 2019;11(1):e3849.
6. Bedi RS, Aurora JK, Chauhan H, Sayeed MS. Gorlin Goltz Syndrome – familial inheritance – a rare case series. *Sch J Dent Sci*. 2020;7(9):109-13.
7. Kiwilsza M, Sporniak-Tutak K. Gorlin-Goltz syndrome--a medical condition requiring a multidisciplinary approach. *Med Sci Monit*. 2012;18(9):RA145-53.
8. Karthiga KS, Sivapatha Sundharam B, Manikandan R. Nevoid basal cell carcinoma syndrome. *Indian J Dent Res*. 2006;17(1):50-3.
9. Namdeoraoji Bahadure R, Surendraji Jain E, P Badole G. Gorlin and goltz syndrome: a case report with surgical review. *Int J Clin Pediatr Dent*. 2013;6(2):104-8.
10. Kumar NN, Padmashree S, Jyotsna TR, Shastry SP. Gorlin-Goltz syndrome: a rare case report. *Contemp Clin Dent*. 2018;9(3):478-83.
11. Kimonis VE, Goldstein AM, Pastakia B, Yang ML, Kase R, DiGiovanna JJ, *et al*. Clinical manifestations in 105 persons with nevoid basal cell carcinoma syndrome. *Am J Med Genet*. 1997;69(3):299-308.
12. Bree AF, Shah MR; BCNS colloquium group. Consensus statement from the first international colloquium on basal cell nevus syndrome (BCNS). *Am J Med Genet A*. 2011;155A(9):2091-7.
13. Alchalabi NJ, Merza AM, Issa SA. Using carnoy's solution in treatment of keratocystic odontogenic tumor. *Ann Maxillofac Surg*. 2017;7(1):51-6.
14. Tang JY, Mackay-Wiggan JM, Aszterbaum M, Yauch RL, Lindgren J, Chang K, *et al*. Inhibiting the hedgehog pathway in patients with the basal-cell nevus syndrome. *N Engl J Med*. 2012;366(23):2180-8.
15. Lo Muzio L. Nevoid basal cell carcinoma syndrome (Gorlin syndrome). *Orphanet J Rare Dis*. 2008;3:32.



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Correction of Vertical Maxillary Excess with Le Fort I Osteotomy: A Case Report

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Abstract

Vertical maxillary excess (VME) is one of the significant developmental deformities which is present with excessive vertical growth of the maxilla, which often results in a long facial appearance, incompetence of lips and a gummy smile. Patients with VME apart from aesthetic concerns, there can be functional limitations such as occlusal and speech. Treatment options VME mainly involves orthodontic and surgical interventions. Le Fort I osteotomy being the best treatment approach as it allows for the superior repositioning of the maxilla. This case describes the effectiveness of Le Fort I osteotomy in correction of VME and improving the facial esthetics.

Keywords: genioplasty, gummy smile, Le Fort I osteotomy, vertical maxillary excess

Introduction

Vertical maxillary excess (VME) is one of the significant developmental deformities, characterised by excessive vertical growth of the maxilla, which often results in a long facial appearance, lip incompetence, and a gummy smile.⁽¹⁾ A gingival display exceeding 3-4 mm during smiling is classified as a gummy smile. Factors like altered passive eruption, hypermobile upper lip and dentoalveolar extrusion contribute to the appearance of a gummy smile.⁽²⁾ The development of VME is multifactorial; its aetiology may involve genetic predispositions, developmental anomalies, and environmental factors that can be present simultaneously.⁽³⁾ Patients with VME may experience functional limitations—such as occlusal issues and speech difficulties—in addition to aesthetic concerns. This condition is often associated with a steep mandibular plane angle and may present with a skeletal Class II relationship which further complicates the orthodontic management.⁽⁴⁾ The existence of an anterior open bite determines the classification of vertical maxillary excess. In patients with VME and an anterior open bite, posterior tooth intrusion is a crucial part of treatment because upper molar intrusion causes the jaw to rotate counterclockwise, improving the convex profile. Since only molar intrusion can cause a posterior open bite, patients with VME and normal overbite should be treated by intrusion of the entire maxillary arch.⁽⁵⁾ In order to attain ideal functional occlusion and balanced facial proportions, treatment of VME typically involves both orthodontic and surgical procedures. Le Fort I osteotomy is the best treatment approach as it allows for the superior repositioning of the maxilla.⁽¹⁾ This surgical procedure involves creating a horizontal osteotomy to separate the tooth bearing portion of the maxilla from the rest of the maxillary segment, enabling its repositioning to reduce vertical height and improving the facial esthetics.⁽⁶⁾ Non-surgical treatment in growing patients using orthopaedic appliances such as high pull headgear and vertical chin cup can be used, while in grown individuals orthodontic mini-screws, can be used to intrude the anterior teeth and decrease gingival display when surgical intervention is not feasible or desired.⁽³⁾

This case report aims to emphasize the importance of accurate diagnosis in cases with VME that are characterized by Class II skeletal relationships, which can give the perception of protrusive dentition and thus lead clinicians to an extraction-based, orthodontic treatment

approach to close spaces—ultimately leading to the perpetuation of the problem, with too much gingival display and aesthetic problems. VME is fundamentally a skeletal issue and should optimally be treated surgically, if a patient is non-growing. A full assessment of all etiologic factors contributing to gummy smile (which may include soft tissue factors such as upper lip length) is paramount to achieving optimal treatment results. This was established in this patient, as both VME and the short upper lip were treated. The clinical advantage of employing Le Fort I osteotomy in this instance is its unmatched capacity for increased repositioning of the maxilla, especially in instances of severe VME when both functional and aesthetic issues are significant. The operation corrects the fundamental skeletal discrepancy, providing predictable outcomes in diminishing gingival visibility, contributing to lip competence, and improving general facial harmony. This requires a thorough diagnostic protocol including cephalometric analysis, clinical photography and cervical vertebral maturation index (CVMI) assessment to differentiate between dental and skeletal etiologies. The emphasis on the reason for surgery in this young adult patient points to the key role of personalised, evidence-based treatment planning in attaining the best results.

Case presentation

A female patient, age 20, presented to us complaining of poor appearance. Although she had no pertinent past medical history when questioned, her dental history showed that she had previously had orthodontic treatment, which included having all four of her first premolars extracted. Upon extraoral assessment, the patient's lips were incompetent and her face form was grossly symmetrical and leptoprosopic (long and narrow face form). Examining the patient's profile revealed a convex facial profile. The soft tissue examination found an average nasolabial angle and a slightly protruding upper and lower lip. The patient had a short upper lip, extensive gingival show, and a symmetrical, non-consonant smile. When smiling, gingival show was 6 mm, and incisor visibility was 10 mm, with gingival show 2mm when at rest, which confirms gummy smile (Figure 1).

Intraoral examination revealed that all teeth in both arches were present, except the maxillary third molars and the right and left first premolars in both the maxilla and mandible. Maxillary right central incisor was restored

with a temporary single-unit prosthesis. Bilateral end-on molar and canine relationships were observed. Mild crowding was noted in the lower anterior region, the upper anterior teeth exhibited under torqued, and the midline was shifted to the right. (Figure 1).

An orthopantomogram (OPG) revealed the presence of all teeth, except for the maxillary third molars and the first premolars in both the maxilla and mandible. Cephalometric analysis indicated Class I skeletal bases with a vertical growth pattern (FMPA = 30°) and CVMI of stage VI, suggesting that no further skeletal growth is expected. The maxilla exhibited a clockwise rotation, contributing to a gummy smile, in conjunction with a retruded chin (Figure 1). The initial impression of dental protrusion was revised after considering the skeletal and soft tissue components, leading to a diagnosis of skeletal rather than dental etiology. Clinical photographs and incisor display measurements further supported a diagnosis of VME and soft tissue, rather than dentoalveolar causes.

Diagnosis

The final diagnosis was skeletal Class I malocclusion with Angle's Class II Division 1 malocclusion (characterized by a situation where the upper jaw is out of alignment with the lower jaw, leading to a forward projection of the upper front teeth) with a left-side subdivision and vertical maxillary excess.

Treatment objective

The primary objective was to correct the gummy smile, address the short upper lip, and advance the retruded chin, aiming to achieve a Class I molar relationship on the left side and a Class I canine relationship bilaterally, while maintaining the existing Class I molar relationship on the right side and establishing normal overjet and overbite

Treatment plan

A modified Le Fort I osteotomy was planned for superior repositioning of the maxilla. Augmentation genioplasty was selected to correct the retruded chin. Lip repositioning surgery was also included to address the short upper lip.

Treatment progress

Following patient motivation and oral prophylaxis, orthodontic treatment commenced with bonding using

0.022"×0.028" MBT brackets in both arches. Presurgical leveling and alignment were achieved using 0.016 NiTi progressing to 0.019"×0.025" stainless steel wires. Decompensation involved proclination of upper incisors to increase overjet, facilitating mandibular autorotation post-surgery.

After completion of decompensation of teeth surgical planning was reviewed and facebow transfer for the patient was done and surgical splint was fabricated (Figure 2).

A combination of augmentation genioplasty and modified LeFort I osteotomy was performed. Additionally, lip repositioning surgery was performed. Modified Le Fort I osteotomy refers to surgical cuts and a down-fracture technique altered to suit particular anatomical concerns or specific operative needs, such as conservation of nasal structures, asymmetry correction, or minimizing damage to soft tissues, compared with the standard Le Fort I in which a horizontal osteotomy is made above the tooth roots to mobilize into one piece the maxilla. Modifications may consist of segmental cuts, differential positioning, or changes in vectors for movement. Following surgery,

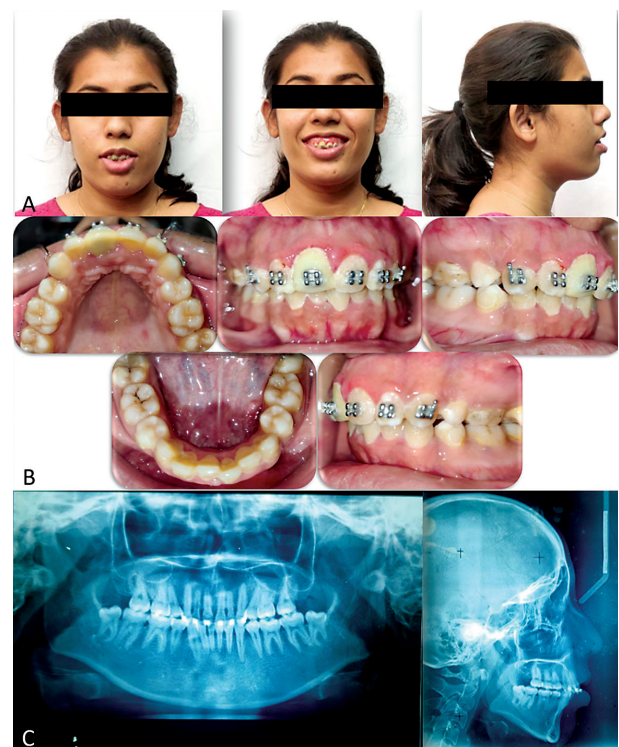


Figure 1: Pre-treatment photographs: (A), Extraoral photographs: (B), Intraoral photographs: (C), Radiographs.

there was a reduction in the gummy smile and an improvement in both functionality and appearance. The maxillary incisors were positioned in good harmony with the upper lip, and a normal lip seal was attained. When smiling, there was also reduced exposure of the teeth and gingiva. (Figure 3) (Table 1)

The patient remained in intermaxillary fixation (IMF) for six weeks. Orthodontic refinement using elastics was performed after the IMF phase to improve interdigitation. Final results showed well-aligned arches, normalized

overjet and overbite, and coincident midlines. Full Class I occlusion could not be achieved due to the patient's satisfaction and decision to discontinue further treatment. Tooth 11 prosthesis was replaced with a composite build-up (Figure 4). One year post-operatively, extraoral results were stable with acceptable gingival display. Intraorally, occlusion was maintained with a minor relapse in overbite (2 mm). The patient expressed overall satisfaction (Figure 5).

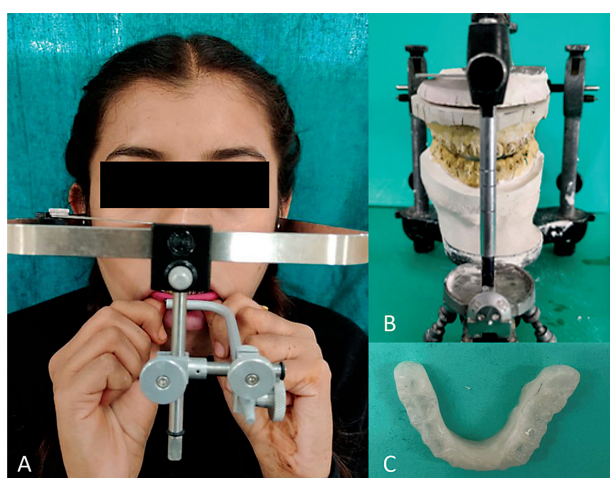


Figure 2: Splint Fabrication. (A), Facebow transfer: (B), Articulated casts: (C), Fabricated splint.

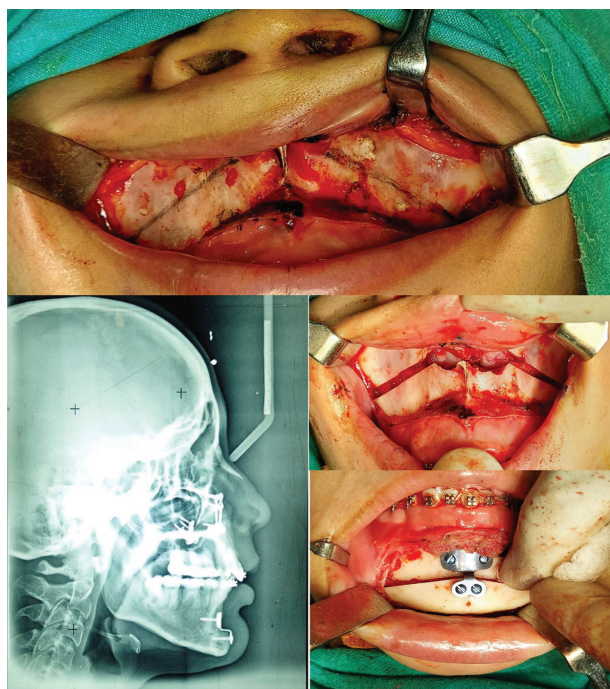


Figure 3: Osteotomy cuts placed with saw and Lefort I osteotomy done along with advancement genioplasty.



Figure 4: Post surgical photographs. (A), Extraoral photographs: (B), Intraoral photographs.



Figure 5: 1 year follow-up photographs. (A), Extraoral photographs: (B), Intraoral photographs.

Table 1: Cephalometric readings of pre-treatment and post treatment.

Skeletal Parameter	Norm	Pre	Current
SNA	82	82	81
SNB	80	77	79
ANB	2	5	2
N perp. To A	0+2	4	3
N perp to Pog	0-1	-9	-3
FMPA	25	30	28
Y axis	66	68	65
U1-NA	22	12	10
L1-NB	25	27	35
IMPA	90	95	98
Nasolabial angle	90-100	107	110

Discussion

VME is a notable skeletal deformity characterized by an overgrowth of the maxillary bone, which leads to a vertical elongation of the midface. This often creates the appearance of a shortened upper lip and can result in a gummy smile.⁽⁷⁾ The condition has both aesthetic and functional implications, including potential oral health challenges. It's crucial to accurately diagnose VME to differentiate it from other reasons for excessive gingival display, such as altered passive eruption, dentoalveolar extrusion, and upper lip abnormalities. The management strategies can vary widely, ranging from orthodontic treatments to orthognathic surgery, depending on the root cause. Key features of VME include an increased lower anterior facial height, excessive exposure of the incisors, lip incompetence, a convex facial profile, and specific radiographic indicators like a steep mandibular plane, a high SN-MP angle, and an increased maxillary height. Diagnostic mistakes often occur when soft tissue causes are misidentified, cephalometric evaluations are incomplete, or when there's a reliance on 2D images and clinical photos without considering skeletal dimensions. VME may result from an increase in alveolar height, a downward-tilted palatal plane, or a combination of both, which can enhance gingival display when smiling.⁽⁸⁾ In the case presented, both mechanisms played a role in the vertical dysplasia, highlighting the need for thorough cephalometric analysis, photographic documentation, and growth assessment (like the CVMI stage) to create an accurate, skeletal-focused treatment plan.⁽⁷⁾

Le Fort I osteotomy, a surgical technique intended to realign the maxilla and address vertical excess, is

frequently used to treat patients who show more than 4 mm of gingival display as a result of vertical maxillary excess, according to Graber and Salama. Conventional methods, however, have drawbacks, particularly when significant superior maxillary displacement is needed. In Le Fort I osteotomy, the lateral maxillary cuts usually end inferiorly at the piriform opening, which may limit the amount of bone that may be removed and the degree of vertical correction that can be achieved.⁽⁹⁾

A modified Le Fort I osteotomy approach, as outlined by Mommaerts *et al.*,⁽¹⁰⁾ was used in this instance to get around these restrictions. This adjustment, referred to as the "subspinal" approach, was made to avoid the unintended soft tissue alterations that anterior maxillary displacement may cause. For our patient, who needed extensive superior relocation and correction of maxillary cant, the typical method may result in an increase in the breadth of the interalar rim. In order to prevent detaching pirirhinal muscle insertions and to reduce postoperative increases in interalar width, a V-shaped incision is made beneath the piriform opening. This method enables more efficient bone resection from the inferior surface as opposed to the superior aspect by positioning the inferior osteotomy incision precisely at the base of the piriform aperture, including the anterior nasal spine.⁽¹¹⁾

This approach offers several advantages. First, it increases the amount of bone available for resection, which is critical for achieving the desired vertical repositioning of the maxilla. Second, by preserving soft tissue attachments and minimizing changes in the interalar width, the modification reduces the risk of adverse aesthetic outcomes. Third, it reduces the likelihood of damage to the infraorbital nerve, thereby decreasing the risk of postoperative sensory disturbances. The modified technique thus represents a valuable option for managing severe cases of vertical maxillary excess, offering improved outcomes in terms of both bone resection and preservation of surrounding tissues. Further research and clinical validation will help to confirm the long-term benefits and efficacy of this approach.⁽¹²⁾

In the present case, the Le Fort I osteotomy consisted of the vertical maxillary excess counteracted by upwardly positioning the maxilla. Vertical maxillary impaction was directed primarily in the anterior region, with the aim of minimizing gingival exposure and enhancing lip function. This impaction permitted autorotation of the mandible in

a counterclockwise manner, with the result of enhanced chin projection and facial aesthetic enhancement.

These movements are in line with typical Le Fort I osteotomy for VME correction, with maxillary impaction—more specifically, anterior impaction—resulting in mandibular autorotation and improved lower facial aesthetics. In this particular instance, however, the degree of maxillary impaction was fairly substantial, and the application of a modified subspinal osteotomy technique allowed for greater vertical correction with less undesirable widening of the nasal base. The modification, along with simultaneous genioplasty, allowed for more significant improvement of chin position and facial harmony than could otherwise be accomplished with standard techniques alone, and this modification enhanced surgical precision and aesthetic outcomes.

In this instance, lip repositioning was used to address the soft tissue component of the gummy smile that can persist following skeletal correction by Le Fort I osteotomy. While the osteotomy corrected the vertical maxillary excess by elevating the maxilla, the patient also had a short upper lip and hyperactive elevator muscles. Lip repositioning is used to reduce the display of the gingiva by minimizing the length of the upward movement of the upper lip during smiling. The technique used in surgery involved excising a mucosal strip in the upper vestibule, then suturing the lip mucosa nearer to the gingiva, hence shortening the vestibule and preventing excessive elevation of the lip.

This treatment is indicated when soft tissue-related issues, i.e., hypermobility or short upper lip length, lead to excessive gingival exposure. It offers a less invasive way of enhancing the aesthetic smile appearance, especially when combined with orthognathic surgical correction. It has advantages of improved aesthetic outcome and patient acceptability; however, disadvantages can be transient discomfort, limited lip mobility, and relapse. In this specific instance, the integrative treatment addressed both the skeletal and the soft tissue aspects, thus establishing a more harmonious and stable result.

Conclusions

The case here represents that proper skeletal diagnosis is essential in patients with protrusive dentition and gummy smiles. While extraction-based orthodontics would appear appropriate in Class II profiles, failure to deem VME as a possibility can lead to poor aesthetic and functional results. Modified Le Fort I os-

teotomy and genioplasty, in association with lip repositioning, treated in this case all the features, skeletal and soft tissue alike. This follow-up shall thus stress the importance of evidence-based approach to treatment planning, especially for the non-growing patient with truly great complexity brought about by her dentofacial discrepancies.

References

1. Benkhalifa M, Tobji S, Moatemri R, Ben Amor A, Dalle I, Ben Amor W: Surgical-orthodontic approach for correcting vertical maxillary excess: case report. *SAGE Open Med Case Rep.* 2024;12:2050313-241256805. 10.1177/2050313X241256805
2. Brizuela M, Ines D: Excessive Gingival Display. In . StatPearls. StatPearls Publishing, Treasure Island; 2024.
3. Kaku M, Kojima S, Sumi H, Koseki H, Abedini S, Motokawa M, *et al.* Gummy smile and facial profile correction using miniscrew anchorage . *Angle Orthod.* 2012;82(1):170-7.
4. Sekar M, Parameswaran R, Rajkumar B, Boovaraghavan S, Vijayalakshmi D. Enhancing smile esthetics in a patient with vertical maxillary excess by total maxillary arch intrusion using temporary skeletal anchorage system: TSAD-assisted VME correction: a case report. *Int J Orthod Rehabil.* 2022;13(4):25-38.
5. Paik CH, Park HS, Ahn HW. Treatment of vertical maxillary excess without open bite in a skeletal class II hyperdivergent patient. *Angle Orthod.* 2017;87(4):625-33.
6. Shimo T, Nishiyama A, Jinno T, Sasaki A. Severe gummy smile with class II malocclusion treated with Le Fort I osteotomy combined with horseshoe osteotomy and intraoral vertical ramus osteotomy. *Acta Med Okayama.* 2013;67(1):55-60.
7. Anehsur V, Joshi A, Nathani J, Suresh A. Modification of LeFort I osteotomy for severe maxillary vertical excess asymmetry. *Br J Oral Maxillofac Surg.* 2019;57(4):374-7.
8. Farhat Z, Osman E, Kassem H, Bouserhal J. Correlation of vertical dentoalveolar heights with palatal plane inclination in adults with different vertical facial patterns. *Orthod Fr.* 2022;93(4):321-31.
9. Buchanan EP, Hyman CH. Le Fort I Osteotomy. *Semin Plast Surg.* 2013 Aug;27(3):149-54.
10. Mommaerts MY, Abeloos JV, De Clercq CA, Neyt LF. The effect of the subspinal Le Fort I-type osteotomy on interalar rim width. *Int J Adult Orthodon Orthognath Surg.* 1997;12(2):95-100.
11. Garber DA, Salama MA. The aesthetic smile: diagnosis and treatment. *Periodontol 2000.* 1996;11:18-28.
12. Gil A., Fernandez A, Martinez R, Alfaro F, Haas O, Oliveria R. Le Fort I osteotomy and soft tissue response: a retrospective cohort study comparing three different techniques. *J Craniomaxillofac Surg.* 2022;50(2):107-13.



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Beyond the Scalpel: Conservative Resolution of a Radicular Cyst

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Abstract

Radicular cysts are one of the most common types of odontogenic cysts and are typically associated with chronic periapical infections. There is increasing interest in non-surgical alternatives that provide similar outcomes while preserving the tooth structure. Mineral Trioxide Aggregate (MTA), a practical option for non-surgical treatment of periapical lesions, including radicular cysts. This case report discusses the successful non-surgical management of a radicular cyst in a 17-year-old male patient. The patient presented with a history of persistent pain and swelling in the maxillary anterior region. Clinical and radiographic examination revealed a well-defined radiolucent lesion at the apex of a non-vital tooth, suggestive of a radicular cyst. After initial root canal therapy, the infected tissue was carefully debrided, and the cystic cavity was filled with MTA to promote healing. The biocompatible nature of MTA, combined with its ability to form a tight seal, was believed to have contributed to the resolution of the cyst without the need for surgical intervention. Follow-up radiographs taken at 6 months post-treatment demonstrated significant healing of the periapical region. The patient remained asymptomatic, with no clinical complications. These findings suggest that MTA can be an effective and viable option for non-surgical treatment of radicular cysts. In conclusion, using MTA in the non-surgical management of radicular cysts may offer a promising approach to preserving the natural dentition while ensuring satisfactory clinical outcomes. Further long-term studies and clinical trials are needed to better understand the efficacy and limitations of MTA in the treatment of periapical cystic lesions.

Keywords: conservative, cysts, radicular, resolution, scalpel

Introduction

Mineral trioxide aggregate (MTA) is a bioactive, hydrophilic endodontic material known for its biocompatibility, as well as its ability to promote healing and osteogenesis. This material is composed of fine oxide powders, including tricalcium oxide, silicon oxide, and bismuth oxide, along with additional hydrophilic compounds such as tricalcium silicate and tricalcium aluminate. These components are responsible for the unique chemical and physical properties of MTA. The cement sets when exposed to moisture.⁽¹⁻⁵⁾ Upon hydration, the powder forms a colloidal gel with a pH of 12.5, which solidifies within 3 to 4 hours.^(6,7)

Dental trauma is an unpredictable event, and its management can often pose a challenge for clinicians. Dentists should be prepared to treat patients who have sustained dental injuries, as prompt action can significantly improve the overall prognosis. When a dental trauma results in pulp exposure, immediate intervention is necessary to address the exposed pulp. MTA is commonly used in such cases due to its high biocompatibility and mechanism similar to that of calcium hydroxide ($\text{Ca}(\text{OH})_2$), which is known for its strongly alkaline and antibacterial properties.

In contrast to $\text{Ca}(\text{OH})_2$, MTA hardens to a solid consistency, making it particularly effective for restorative procedures. Its rapid setting ability allows for definitive restoration after partial pulpotomies performed with MTA.⁽⁶⁾ Endodontic treatment is typically indicated when caries or trauma has caused irreversible damage to the pulp tissue, leading to necrosis.

The mechanism by which MTA functions is closely linked to the clinical characteristics of the human oral environment. When MTA comes into direct contact with oral tissues, it releases calcium ions that promote cell proliferation. Additionally, its alkaline pH helps create an antibacterial environment by regulating cytokine production. This further supports the migration and differentiation of cells responsible for forming hard tissue, leading to hydroxyapatite formation on the surface of MTA and providing a biological seal. MTA's advantages include its excellent biocompatibility, antibacterial properties, effective marginal adaptation, sealing ability, and its hydrophilic nature.⁽⁷⁾

Management of radicular cysts involves both non-surgical and surgical approaches, selected based on

lesion size, location, symptoms, and response to initial treatment. The primary goal is to eliminate the source of infection and allow for periapical healing. Non-surgical root canal therapy (NSRCT) is the first-line treatment for small to moderately sized cystic lesions. By disinfecting the root canal system and sealing it properly, many radicular cysts resolve without the need for surgery.⁽⁷⁾

When non-surgical management is insufficient—particularly in cases of large, symptomatic, or persistent cysts—surgical intervention is indicated. Enucleation is the most commonly employed surgical technique, involving complete removal of the cystic lining. It is typically chosen for accessible, well-demarcated cysts where surrounding structures are not at risk. In contrast, marsupialization (or decompression) is used when the cyst is large and enucleation could compromise adjacent anatomical structures such as nerves, sinuses, or permanent tooth buds. This technique reduces intra-cystic pressure by creating a surgical window, allowing gradual shrinkage of the lesion. Marsupialization may be followed by enucleation once the lesion is smaller and safer to remove.⁽⁷⁾

In some cases, a combined approach involving both decompression and later enucleation is necessary. Follow-up with clinical and radiographic evaluation is essential to confirm resolution.

Case presentation

A 17-year-old male patient had been referred to the department of endodontics with a chief complaint of severe pain in the upper front region. He gave a history of severe pain and swelling for which he had initiated treatment at a private clinic 9 months earlier but did not follow up there. Cone beam computed tomography examination suggested a radicular cyst in relation to maxillary left central incisor and maxillary left lateral incisor, with dimensions $11.4 \times 6.1 \times 8.8$ mm (Figure 1). However, the vitality tests revealed that maxillary left central incisor and maxillary left lateral incisor were non-vital. After the patient and his family had been informed about the treatment process, approval was obtained for the treatment.

Treatment Procedure

Local anesthesia was applied for patient comfort. The access cavity was opened. The old canal medication in the middle trio was removed using H files of ISO

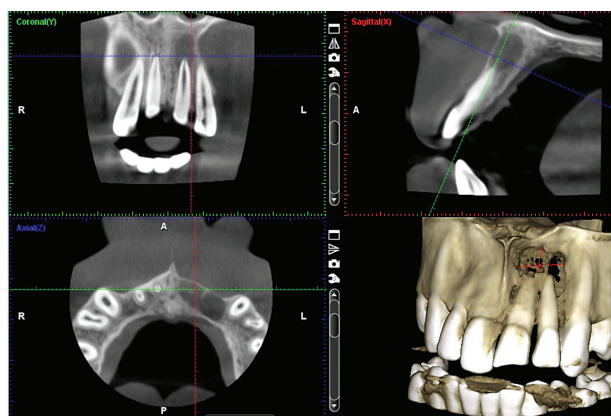


Figure 1: Showing pre-op CBCT image.

sizes 15, 20, and 25 in an up-and-down motion. Copious irrigation with 2.5% sodium hypochlorite was performed between the use of files. The H file was used again for the apical part. Working length was determined using an apex locator and was confirmed radiographically, as shown in Figure 2.

Later, the apical diameter was prepared with K and H files up to size #50 to clean the necrotic root structure. Copious irrigation was performed between each file. After the master apical diameter was prepared to size #50, root canal preparation was completed with the step-back technique. Serous exudate drained through the canal. After final irrigation, the tooth was closed with a temporary filling. Drainage did not stop, and dressing was continued for the next three sessions. The tooth remained asymptomatic during the sessions. At the fifth appointment, the tooth was ready for obturation. Furthermore, a conical shape was given to the root canal with the step-back technique. There were no irregularities in the root canal wall after preparation.

Plugger fit was checked, and the apical 3-4 mm, which consisted of maximum ramifications, was well sealed with MTA. A moist cotton pellet was placed, and the patient was recalled after 2 days. On the next visit, the remaining canal was obturated using thermoplasticized gutta-percha. Post-endodontic composite was performed with maxillary left central incisor and maxillary left lateral incisor.

Follow-up and outcomes

Follow-up via telephone communication was established regularly, revealing successful treatment with no complaints of pain, alleviation of symptoms, and preser-

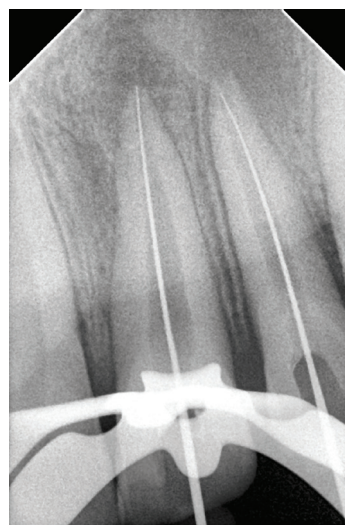


Figure 2: Showing confirmation of working length.

vation of tooth integrity. Figure 3 and 4 shows the results at the three-month and six-months follow-up respectively, also Figure 5 shows 3-dimensional post-operative CBCT where the lesion has healed and where we can visualize osteogenesis. This case highlights the efficacy of MTA as an end filling material healing and bone formation in periradicular involvement, offering promising outcomes

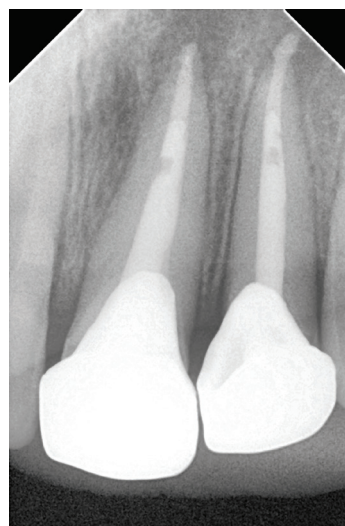


Figure 3: Showing the results at the three-month follow-up.

in challenging endodontic scenarios. Informed consent was obtained from the patient for publication.

Discussion

Osteoblast differentiation is influenced by various hormones and growth factors, including TGF- β (trans-

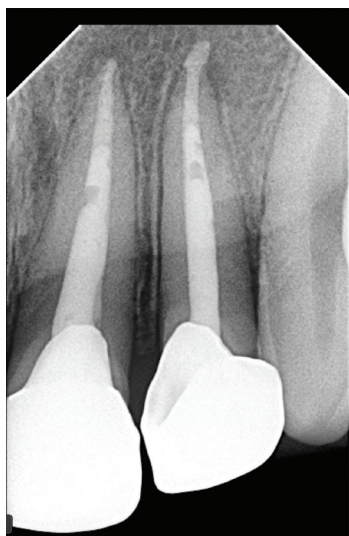


Figure 4: Showing the results at the six-month follow-up.

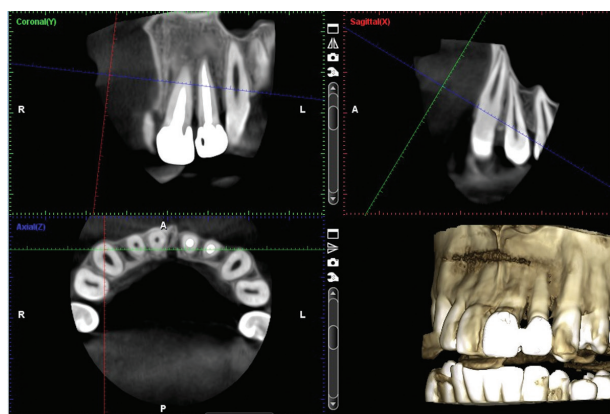


Figure 5: Showing six-month follow-up CBCT image.

forming growth factor- β), BMP-2, and bFGF (basic fibroblast growth factor).^(7,8) Among these, BMP-2 is considered the most potent promoter of osteoblast proliferation, differentiation, and mineralization.⁽⁹⁻¹¹⁾ Previous research has demonstrated that MTA not only promotes osteoblastic mineralization but also enhances BMP-2 mRNA expression.⁽¹²⁻¹⁴⁾

Nowicka *et al.*,⁽¹²⁾ demonstrated that the formation of reparative dentin bridges is influenced by the type of material used. Bonte *et al.*,⁽¹³⁾ found no significant statistical difference between $\text{Ca}(\text{OH})_2$ and MTA, but noted that MTA yielded superior dentin healing. They also suggested that apexification with MTA may lead to better outcomes compared to $\text{Ca}(\text{OH})_2$. Bernabé *et al.*,⁽¹⁴⁾ highlighted the utility of MTA as a root canal filling material following apicectomy, further noting that sonic vibration could enhance its sealing capacity. Sönmez

et al.,⁽¹⁵⁾ concluded that the sealing ability of AH Plus and ProRoot was comparable, but MTA Fillapex showed more microleakage when compared to the other two materials. Leye Benoist *et al.*,⁽¹⁶⁾ observed a higher success rate in the MTA group compared to $\text{Ca}(\text{OH})_2$. Hansen *et al.*,⁽¹⁷⁾ demonstrated that MTA results in a higher intracanal pH than Endodontic Sealer (ES), a finding that was also supported by Yildirim *et al.*⁽¹⁸⁾ MTA has been shown to be effective as an apical filling material in root canals, particularly in teeth requiring post-core restorations.

Recent studies highlight the remineralizing potential of nanosized materials, which can intercept the progression of early lesions. These materials include calcium phosphate, carbonate hydroxyapatite nanocrystals, nanoamorphous calcium phosphate, and nanoparticulate bioactive glass. Such materials, particularly when they facilitate the self-assembly of proteins, play a crucial role in biomimetic repair, even in the dental field. The small size of these nanomaterials makes them ideal carriers for dental products.^(17,19-24)

Consequently, it has been recently proposed that incorporating nanomaterials with biological benefits into adhesives not only enhances their mechanical and physical properties but also improves the durability and longevity of the adhesive bond.

A study by Karan and Aricioğlu in 2020⁽²⁵⁾, which assessed bone healing after the application of MTA and platelet-rich fibrin in periapical lesions using cone-beam computed tomography, found that MTA achieved high success rates in endodontic microsurgery for periapical lesions. MTA is a biocompatible material that offers superior sealing ability and promotes periapical tissue regeneration, outperforming conventional retrograde materials like gutta-percha (GP).^(26,27) Additionally, previous randomized clinical trials reported MTA success rates exceeding 80% after 12 months and over 90% after 24 months of follow-up.^(28,29)

Although MTA offers many advantages, it does have some drawbacks, including its relatively long setting time and challenges with proper application. If not carefully handled, the material can be easily displaced. Several factors can influence the setting time and physical properties of MTA, including the nanoparticle size and concentration, the powder-to-liquid ratio, environmental conditions such as pH and room temperature, and the air trapped during the mixing process.⁽³⁰⁾

The decision-making process between surgical and non-surgical management of periapical cysts requires a comprehensive evaluation of clinical, radiographic, and patient-related factors. Non-surgical root canal therapy (NSRCT) is widely accepted as the initial treatment of choice, particularly for lesions of endodontic origin that are asymptomatic, well-contained, and of small to moderate size. The rationale is based on the principle that periapical cysts, whether true or pocket cysts, often regress upon removal of the etiologic source—namely, the intraradicular infection. Healing is initiated through effective canal disinfection, reduction of intracanal pressure, and host-mediated immune response. While MTA is commonly highlighted for its bioactive properties, favorable outcomes in cyst resolution are not exclusively attributed to it. Rather, successful healing also occurs with other materials and techniques, provided that microbial elimination and apical sealing are achieved. $\text{Ca}(\text{OH})_2$, for example, promotes periapical healing by creating an alkaline pH that inhibits residual pathogens and encourages hard tissue deposition.⁽¹⁴⁾

However, non-surgical management has its limitations. In cases of persistent or large lesions unresponsive to endodontic therapy, or when there is cortical expansion, tooth displacement, or proximity to vital structures, surgical intervention may be warranted. Enucleation allows for complete removal of the cystic lining and histopathological confirmation, making it the preferred option for accessible and well-defined lesions. Marsupialization, though less definitive, is indicated for extensive cysts where enucleation may compromise anatomical structures. It allows gradual decompression, promoting reduction in cyst size and often followed by enucleation when safer.⁽¹⁴⁾

Expected outcomes differ: non-surgical treatment is conservative, cost-effective, and often sufficient, though it may require prolonged monitoring.⁽³¹⁾ Surgical approaches provide immediate lesion resolution but carry risks such as postoperative complications, longer recovery, and increased cost.⁽³²⁾ Ultimately, the choice between modalities should be individualized, taking into account the lesion's characteristics, patient preference, and clinician expertise. Regular radiographic follow-up is essential, regardless of the chosen method, to ensure complete resolution and prevent recurrence.^(33,34)

Conclusions

This case report highlights the successful management of a radicular cyst using MTA as the primary sealing material. The patient experienced notable clinical and radiographic improvements, with complete resolution of the cystic lesion and retention of the affected tooth. MTA's exceptional biocompatibility, sealing properties, and ability to stimulate tissue regeneration make it a promising material for treating radicular cysts. This case adds to the growing body of evidence suggesting that MTA can be a viable alternative to traditional surgical methods in managing periapical diseases, contributing to favourable healing outcomes. However, further research with larger sample sizes is necessary to validate its long-term effectiveness and potential benefits over conventional treatment approaches.

Ethical Considerations

Informed consent, with all the procedure mentioned was taken before delivering treatment.

Conflict of Interest

The authors declare no conflicts of interest.

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References

1. Lin YT, Lin YTJ. Success rates of mineral trioxide aggregate, ferric sulfate, and sodium hypochlorite pulpotomies: A prospective 24-month study. *J Formos Med Assoc.* 2020 ; 119(6):1080-5.
2. Liu Y, Liu XM, Bi J, Yu S, Yang N, Song B, *et al.* Cell migration and osteo/odontogenesis stimulation of iRoot FS as a potential apical barrier material in apexification. *Int Endodontic J.* 2020;53(4):467-77.
3. Lopes MB, Soares VCG, Fagundes FHR, Gonini-Junior A, Kaneshima RH, Guiraldo RD, *et al.* Analysis of molecular changes induced by mineral trioxide aggregate on sPLA2. *Braz Dent J.* 2019;30(5):453-8.
4. Serin Kalay T. Effects of intracoronary bleaching agents on the surface properties of mineral trioxide aggregate. *Odontology.* 2019;107(4):465-72.
5. Tu MG, Sun KT, Wang TH, He YZ, Hsia SM, Tsai BH, *et al.* Effects of mineral trioxide aggregate and bioceramics on macrophage differentiation and polarization *in vitro*. *J Formos Med Assoc.* 2019;118(10):1458–65.

6. Cervino G, Fiorillo L, Spagnuolo G, Bramanti E, Laino L, Lauritano F, *et al.* Interface between MTA and dental bonding agents: scanning electron microscope evaluation. *J Int Soc Prev Community Dent.* 2017;7(1):64-8.
7. Lapinska B, Klimek L, Sokolowski J, Lukomska-Szyman-ska M. Dentine surface morphology after chlorhexidine application—SEM study. *Polymers.* 2018;10(8):905.
8. Hurley MM, Abreu C, Gronowicz G, Kawaguchi H, Lorenzo J. Expression and regulation of basic fibroblast growth factor mRNA levels in mouse osteoblastic MC3T3-E1 cells. *J Biol Chem.* 1994;269(12):9392-6.
9. Higuchi C, Myoui A, Hashimoto N, Kuriyama K, Yoshioka K, Yoshikawa H, *et al.* Continuous inhibition of MAPK signaling promotes the early osteoblastic differentiation and mineralization of the extracellular matrix. *J Bone Miner Res.* 2002;17(10):1785-94.
10. Hiraki Y, Inoue H, Shigeno C, Sanma Y, Bentz H, Rosen DM, *et al.* Bone morphogenetic proteins (BMP-2 and BMP-3) promote growth and expression of the differentiated phenotype of rabbit chondrocytes and osteoblastic MC3T3-E1 cells *in vitro*. *J Bone Miner Res.* 1991;6(12):1373-85.
11. Ghasemi N, Rahimi S, Lotfi M, Solaimanirad J, Shahi S, Shafaie H, *et al.* Effect of Mineral trioxide aggregate, calcium-enriched mixture cement and mineral trioxide aggregate with disodium hydrogen phosphate on BMP-2 production. *Iran Endod J.* 2014;9(3):220-4.
12. Nowicka A, Wilk G, Lipski M, Kólecki J, Buczkowska-Radlińska J. Tomographic evaluation of reparative dentin formation after direct pulp capping with Ca(OH)₂, MTA, biodentine, and dentin bonding system in human teeth. *J Endod.* 2015;41(8):1234-40.
13. Bonte E, Beslot A, Boukpepsi T, Lasfargues JJ. MTA versus Ca(OH)₂ in apexification of non-vital immature permanent teeth: a randomized clinical trial comparison. *Clin Oral Invest.* 2015;19(6):1381-8.
14. Bernabé PFE, Gomes-Filho JE, Bernabé DG, Nery MJ, Otoboni-Filho JA, Dezan-Jr E, *et al.* Sealing ability of mta used as a root end filling material: effect of the sonic and ultrasonic condensation. *Braz Dent J.* 2013;24(2):107-10.
15. Sönmez IS, Oba AA, Sönmez D, Almaz ME. In vitro evaluation of apical microleakage of a new MTA-based sealer. *Eur Arch Paediatr Dent.* 2012;13(5):252-5.
16. Leye Benoist F, Gaye Ndiaye F, Kane AW, Benoist HM, Farge P. Evaluation of mineral trioxide aggregate (MTA) versus calcium hydroxide cement (Dycal®) in the formation of a dentine bridge: a randomised controlled trial. *Int Dent J.* 2012;62(1):33-9.
17. Hansen SW, Marshall JG, Sedgley CM. Comparison of intracanal endosequence root repair material and ProRoot MTA to induce pH changes in simulated root resorption defects over 4 weeks in matched pairs of human teeth. *J Endod.* 2011;37(4):502-6.
18. Yildirim T, Taşdemir T, Orucoglu H. The evaluation of the influence of using MTA in teeth with post indication on the apical sealing ability. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2009;108(3):471-4.
19. Culp DJ, Robinson B, Parkkila S, Pan P wen, Cash MN, Truong HN, *et al.* Oral colonization by *Streptococcus mutans* and caries development is reduced upon deletion of carbonic anhydrase VI expression in saliva. *Biochim Biophys Acta.* 2011;1812(12):1567-76.
20. Gibbins HL, Yakubov GE, Proctor GB, Wilson S, Carpenter GH. What interactions drive the salivary mucosal pellicle formation?. *Colloids Surf B Biointerfaces.* 2014;120(100):184-92.
21. Heller D, Helmerhorst EJ, Oppenheim FG. Saliva and serum protein exchange at the tooth enamel surface. *J Dent Res.* 2017;96(4):437-43.
22. Hannig M, Hannig C. Nanomaterials in preventive dentistry. *Nat Nanotechnol.* 2010;5(8):565-9.
23. Huq N, Myroforidis H, Cross K, Stanton D, Veith P, Ward B, *et al.* The Interactions of CPP-ACP with Saliva. *Int J Mol Sci.* 2016;17(6):915.
24. Kanaparthi R, Kanaparthi R. The changing face of dentistry: nanotechnology. *Int J Nanomedicine.* 2011;6:2799-804.
25. Karan NB, Aricioğlu B. Assessment of bone healing after mineral trioxide aggregate and platelet-rich fibrin application in periapical lesions using cone-beam computed tomographic imaging. *Clin Oral Invest.* 2020;24(2):1065-72.
26. Christiansen R, Kirkevang LL, Hørsted-Bindslev P, Wenzel A. Randomized clinical trial of root-end resection followed by root-end filling with mineral trioxide aggregate or smoothing of the orthograde gutta-percha root filling— 1-year follow-up. *Int Endod J.* 2009;42(2):105-14.
27. Torabinejad DrM, editor. Mineral Trioxide Aggregate: Properties and Clinical Applications [Internet]. 1st ed. Wiley; 2014 [cited 2024 Dec 26]. Available from: <https://onlinelibrary.wiley.com/doi/book/10.1002/9781118892435>
28. Chong BS, Pitt Ford TR, Hudson MB. A prospective clinical study of mineral trioxide aggregate and IRM when used as root-end filling materials in endodontic surgery. *Int Endod J.* 2003;36(8):520-6.
29. Lindeboom JAH, Frenken JWFH, Kroon FHM, Van Den Akker HP. A comparative prospective randomized clinical study of MTA and IRM as root-end filling materials in single-rooted teeth in endodontic surgery. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2005;100(4):495-500.
30. Kogan P, He J, Glickman GN, Watanabe I. The effects of various additives on setting properties of MTA. *J Endod.* 2006;32(6):569-72.
31. Bhojwani P, Ikhar A, Patel A, Chandak M, Bhopatkar J, Manik K, *et al.* Rooted in metal: a philosophical reflection on preservation. *Multidiscip Sci J.* 2024;7(6):2025298.
32. Bhojwani P, Ikhar A, Patel A, Chandak M, Bhopatkar J, Manik K, *et al.* Recent advances in antimicrobial and

- biosynthesis properties of bioglass and nanoparticles: a narrative review. *Multidiscip Rev.* 2024;7(12):2024286.
33. Bhojwani P, Ikhar A, Patel A, Chandak M, Sedani S, Bhop-takar J, *et al.* Optimizing cosmetic results in dental fluorosis: a case report of bleaching and microabrasion techniques. *Case Rep Dent.* 2025;2025(1):9972925.
34. Bhojwani PR, Ikhar A, Patel A, Chandak M, Sedani S. Microabrasion as an Effective Treatment for Severe Dental Fluorosis: Images in Medicine. *JCDR [Internet].* 2025 Jul 1 [cited 2025 Aug 12]; Available from: https://www.jcdr.net/article_fulltext.asp?issn=0973-709x&year=2025&month=-July&volume=19&issue=7&page=ZJ01-ZJ02&id=21206

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