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Biological Effects of Pulp Capping Material Containing Fluocinolone Acetonide on Human Dental Pulp Cells

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Abstract

Objective: The aim of this study was to test some biological properties of pulp capping material containing fluocinolone acetonide (PCFA).

Materials and Methods: The PCFA is a hard setting calcium hydroxide cement containing 50 mmol/L of fluocinolone acetonide (FA). Cytotoxicity and cell proliferation were evaluated by MTT assay. RT-PCR and Western blotting were used to study the effects of PCFA on RNA (dentin sialophosphoprotein: DSPP) and protein (dentin sialoprotein: DSP) synthesis. Anti-inflammatory effect of PCFA was determined by analysis of cyclooxygenase 2 (COX-2) expression. Dycal® was used as control.

Results: The diluted conditioned media from PCFA slightly increased cell proliferation, significantly increased DSPP expression and decreased COX-2 expression ($p<0.05$).

Conclusions: In term of biologic properties, the PCFA may promote cell proliferation, mineralization and decrease pre-existing inflammation in human dental pulp cells. It may be considered as an alternative pulp capping material in the treatment of inflamed dental pulp.

Keywords: dental pulp, fluocinolone acetonide, inflammation, *in vitro*

Introduction

Vital pulp therapy is a method of treatment in which the exposed dental pulp is covered with a material protecting the pulp from additional injury, permitting healing and repair. It is the best way to ensure the prevention of periradicular pathology, which is one of the major objectives in endodontic treatment.(1) Several pulp capping materials have been used for capping exposed dental pulps. Traditionally, calcium hydroxide has been successfully used for many decades and remained vital due to solid clinical documentations. Its high alkalinity provides the anti-bacterial property and encourages tissue repair.(2-5)

Success of a pulp capping procedure depends on many factors especially microbial infection. Following proper disinfection and debridement, soft and hard tissue healing occur at a very high rate.(6-9) Therefore, the pulp capping procedure was traditionally recommended only for the sterile or mechanical pulp exposures in mature teeth, and has remained controversial for the caries-exposed pulp. Although dental pulp itself has a great potential of healing, severe and long-lasting inflammatory reactions consistently induce irreversible tissue destruction due to lack of collateral blood circulation and low compliance environment in unyielding hard tissue.

Clinically, it is impossible to evaluate the extent of the exact inflammatory condition by current evaluation criteria such as sensitivity to electric pulp or cold test, spontaneous pain or pain characteristics, radiological signs of apical pathology. However, these criteria may not reflect the status of the pulp and dentin bridge formation.[10] Therefore, clinicians always have a chance to treat the inflamed pulp tissue which has unpredictable severity of the inflamed underlying tissue and may cause variable long-term clinical success.[11-14]

To achieve a goal of dental pulp healing in the unknown inflammatory condition, anti-inflammatory medicament mixed with a drug delivery vehicle would be an effective direct pulp-capping material.[15] Anti-inflammatory agents, such as corticosteroids, may be considered as candidates for reducing inflammation and stimulating healing. The use of topical corticosteroids in vital pulp treatment was first reported more than 50 years ago by Rapoport and Abramson[16], with 80-93% success in pulp capping procedures. However, a commercial product (Ledermix®; Lederle Pharmaceuticals, Division of Cyanamid, Wolfratshausen, Germany) containing both 1% triamcinolone acetonide and 3.21% demethylchlortetracycline in a zinc oxide-eugenol base, leads to unpredictable and frequently unfavorable results.[17-19] Despite the unpredictable long-term success, the short-term application of a corticosteroid to inflamed dental pulp can be considered to resolve inflammation and consequently promote healing.

Fluocinolone acetonide is a synthetic corticosteroid commonly used for topical application in the management of dermatologic disorders and oral vesiculoerosive lesions.[20-22] The effects of fluocinolone acetonide on skin treatment are concentration-dependent. High concentrations of fluocinolone acetonide inhibit epidermal mitotic activity, but low concentrations slightly increase this activity.[23] Increased proliferation of cultured human skin fibroblasts is noted over a wide range of concentrations[24] and some promising effects on human dental pulp cell proliferation, extracellular matrix formation and mineralization have been shown.[25,26] Recently, a pulp capping material containing fluocinolone acetonide (PCFA) was invented, which is made of two-paste, hard setting calcium hydroxide cement that can release a range of suitable concentrations fluocinolone acetonide. Some basic physical and mechanical properties are comparable to Dycal®.[27] The invention of the PCFA is to control the existing inflammation and facilitate the healing of the inflamed dental pulp. However, its biologic properties have not been studied. The purposes of this study were to test some of biologic effects of the experimental pulp capping material containing fluocinolone acetonide to human dental pulp cells using cell proliferation, mineralization markers and inflammatory marker.

Materials & Methods

1. Cell culture

Human dental pulp cells were obtained from caries-free lower third molars extracted for orthodontic reasons at the department of oral surgery, Faculty of Dentistry, Chulalongkorn University with patient's informed consent. The study protocol was approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand. The teeth were extensively washed with sterile phosphate-buffered saline solution (PBS) and cracked open along the longitudinal axis. The pulp was gently removed by forceps, minced into small pieces (1x1x1 mm^3) and seeded in 35-mm plastic tissue culture dishes (Nunc, Naperville, IL, USA). The explants were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-Glutamine, 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 0.25 μg/ml of amphotericin B and incubated at the condition of 5% CO_2, 37°C. The medium and supplements were purchased from Gibco (Gibco BRL, Grand Island, NY, USA). After the outgrowth of human cultured dental pulp cells reached confluence, they were subcultured into new culture dishes. The 3rd to 5th passages of three different donors were used in this study.

2. Preparation of test materials

The PCFA or Dycal® (Dentsply Sirona, Charlotte, NC, USA) was mixed and immediately put into cylindrical plastic molds (5 mm in height, 8 mm in diameter). Dycal® was mixed according to the manufacturers' recommendation. Before complete setting, 1 mL of culture media was poured on the surface of each specimen. All specimens were kept at 37°C, 100% humidity for 24 hours. Then, the specimen was removed and the conditioned medium was filtered by sterile 0.2 μm pore diameter filters. The

50- and 100-time dilutions of conditioned medium form PCFA was assigned as F50 and F100 groups, respectively. The same dilutions of conditioned media form Dycal® were used as control groups and were assigned as D50 and D100, respectively.

3. Colorimetric (MTT) assay for cytotoxicity and cell proliferation assay

The 5 x 10^4 primary human dental pulp cells were seeded in 24-well plates with DMEM containing 10% FBS and incubated for 24 hours. Then, the media was replaced twice by serum free medium at 3 hour intervals in order to wash out the serum. The cells were then treated with the prepared conditioned medium from PCFA or controls (F50, F100, D50, and D100) for 24, 48, and 72 hours. Serum free medium and DMEM containing 10% FBS was used as negative and positive controls, respectively. The viable cells were detected by using the MTT dye, which was deduced by the mitochondrial dehydrogenase, presented in living cells and formed blue formazan crystals. Two hundred microliters of MTT solution [3-(4,5,dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, (Sigma-Aldrich, St. Louis, MO, USA) was added to each well, and incubated for 4 hours. Subsequently, 900 μl of dimethyl sulfoxide and 125 μl of glycine buffer (0.1 M NaCl + 0.1 M Glycine, pH 10.5) were added into each well to dissolve the formazan crystal. The survival or proliferation rates of the cells were calculated from spectrophotometer measurement at 570 nm wavelength. Data obtained from the MTT assay showed a relative optical density by comparing with the negative control.

4. RNA and protein synthesis

1) Analysis of dentin sialophosphoprotein (DSPP) synthesis by RT-PCR technique:

The 1 x 105 primary human dental pulp cells were seeded in DMEM with 10% FBS in 6-well tissue culture plates, and incubated for 24 hours. Then, the cells were treated with the conditioned medium (F50, F100, D50, and D100) and supplemented with 50 μg/ml ascorbic acid and 10 mM β-glycerophosphate. The medium with and without 10 nM of dexamethasone were used as positive and negative controls. The medium was changed every 48 hours for 14 days in every group. The medium was removed, and total RNA was extracted using the Trizol reagent (Gibco BRL) according to the manufacturer's instructions. One microliter of each RNA sample was converted to cDNA by reverse transcription using ImProm-IITM Reverse Transcription System (Promega, Madison, WI, USA) for 90 minute at 42°C. Subsequent to the reverse transcription, polymerase-chain reaction (PCR) was performed to detect DSPP cDNA. The primers specific to DSPP and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were prepared following the reported sequences from GenBank. The oligonucleotide sequences of DSPP and GAPDH primers were:

DSPP	sense	5' AATGGGACTAAGGAAGCTG 3'
	antisense	5' AAGAAGCATCTCCTCGGC 3'
GAPDH	sense	5' TGAAGGTCGGAGTCAACGGAT 3'
	antisense	5' TCACACCCATGACGAACATGG 3'

The PCR was performed using Tag polymerase (Qiagen, Hilden, Germany) with 25 μl PCR volume. The reaction mixtures contained 25 pM of primers and 1 μl of RT reaction. The amplification profiles for DSPP (38 cycles) and GAPDH (22 cycles) were set at denaturation for 1 minute at 94°C, primer annealing for 1 minute at 60°C, and chain elongation for 2 minutes at 72°C in DNA thermal cycler (Biometra, Gottingen, Germany). The amplified DNA was then electrophoresed on a 1% agarose gel and visualized by ethidium bromide fluorostaining. The relative intensities of the gel bands were analyzed by Scion Image version Alpha 4.0.3.2 software (Scion corporation, Frederick, MD, USA). The experiments were performed from three different donors.

2) Analysis of dentin sialoprotein (DSP) synthesis by Western blot technique:

The 1 x 10^5 primary human dental pulp cells were seeded in DMEM with 10% FBS in 6-well tissue culture plates, and incubated for 24 hours. Then, the cells were treated with the conditioned medium and supplemented with 50 μg/ml ascorbic acid and 10 mM β-glycerophosphate. The medium with and without 10 nM of dexamethasone were used as positive and negative controls. The medium in each well was changed every 48 hours for 14 days. Then, the cells were washed three times with PBS. The cells were lysed with RIPA buffer (50 mmol/l TrisHCL, 150 mmol/l NaCl, 1 mmol/l EDTA, 1% NP-40, 0.25% Na-deoxycholate). The total amount of protein from each extract was determined using BCA protein assay kit (Pierce, Rockford, IL, USA). Equal amounts of

protein from each sample were mixed with running buffer and loaded onto a 12% polyacrylamide gel and separated by electrophoresis along with pre-stained high molecular weight standards (Bio-Rad, Hercules, CA, USA). The proteins were transferred to a nitrocellulose membrane (Immobilon-P, Millipore Corporation, Bedford, MA, USA) using a trans-blot cell (Gibco BRL) with a 180-mA current for 1.5 hours. Then, the nitrocellulose membrane was stained for DSP by using goat polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) diluted to 1/100, or β-actin by using mouse monoclonal antibody (Chemicon International, Temecula, CA, USA) diluted to 1/1000. After washing in PBS, the membrane was incubated with biotinylated-secondary antibody (Sigma Chemical Co.) for 50 minutes at room temperature, and peroxidase-conjugated streptavidin (Zymed, South San Francisco, CA, USA) for 50 minutes, respectively. The protein bands were detected using a Super-Signal® West Pigo Trial Kit (Pierce) and were exposed on CL-X Posture film (Pierce). The relative intensity of DSP-specific bands was digitalized and compared to the protein marker to indicate the type of protein. The band intensity was determined by scion image analysis software (Scion corporation) and optical density was adjusted to the percentage of expression by comparing with control. The intensity of protein bands indicated the relative amounts of protein in the samples. The experiments were performed from three different donors.

5. Test of anti-inflammatory effect

1) Induction of COX-2 mRNA expression

The 1.5 x 10^5 primary human dental pulp cells were seeded in DMEM with 10% FBS in 6-well tissue culture plates and incubated for 24 hours. Then, the medium was replaced twice by serum free medium at 3 hours intervals in order to wash out the serum before exposure of the conditioned medium. The cells were then exposed to 20 μg/ml of lipopolysaccharide (LPS) of Pseudomonas aeruginosa (Sigma Chemical Co.) for 3 hours to induce inflammation in the cell culture.[28] RNA isolation and RT-PCR were performed to confirm the expression of COX-2 mRNA, which is a gene encoding the key enzyme in the inflammatory process.

2) Determination of anti-inflammatory effect

After treatment with LPS for 3 hours, the cells were washed 2 times with serum free medium. The cells were then treated with conditioned medium (F50, F100, D50, and D100) for 24 hours. Serum free medium with and without LPS treatment were used as controls. At the end of culture, the medium was removed, and total RNA was extracted using the Trizol reagent (Gibco BRL) according to the manufacturer's instructions. One microliter of each RNA sample was converted to cDNA by reverse transcription using ImProm-IITM Reverse Transcription System (Promega, Madison, WI, USA) for 90 minute at 42°C. Subsequent to the reverse transcription, polymerase-chain reaction (PCR) was performed for detection of COX-2 cDNA. The primers specific to COX-2 and GAPDH were prepared following the reported sequences from GenBank. The oligonucleotide sequences of COX-2 and GAPDH primers were:

COX-2	sense	5' TTCAAATGAGATTGTGGGAAAATTGCT 3'
	antisense	5' AGATCATCTCTGCCTGAGTATCTTT 3'
GAPDH	sense	5' TGAAGGTCGGAGTCAACGGAT 3'
	antisense	5' TCACACCCATGACGAACATGG 3'

The PCR was performed using Tag polymerase (Qiagen, Hilden, Germany) with 25 μl PCR volume. The reaction mixtures contained 25 pM of primers and 1 μl of RT reaction. The amplification profiles for COX-2 (27 cycles) and GAPDH (22 cycles) were set at denaturation for 1 minute at 94°C, primer annealing for 1 minute at 60°C, and chain elongation for 2 minute at 72°C in DNA thermal cycler (Biometra, Gottingen, Germany). The amplified DNA was then electrophoresed on a 1% agarose gel and visualized by ethidium bromide fluorostaining. The relative intensities of the gel bands were measured by imaging software analysis (Scion Image version Alpha 4.0.3.2; Scion, Frederick, MD, USA). The experiments were performed from three different donors.

6. Statistics

All experiments were conducted at least in triplicates. The results were reported as mean ± standard deviations. Data were analyzed by using SPSS program version 19 (IBM Corporation, Armonk, NY, USA) and Stats Direct software (StatsDirect Ltd, Altrincham Cheshire, UK). Several types of statistical tests were selected to determine statistically significant differences from the data. For MTT assay, all data were normal distributed, but the equal variances of 72-hour group was not assumed. Therefore,

one-way ANOVA with Post hoc multiple comparisons were used for the 24- and 48-hour groups, but Kruskal Wallis test with pairwise comparisons (Conover-Inman method) was used for 72-hour group. The Kruskal Wallis tests with pairwise comparisons (Conover-Inman method) were also used for the RNA and protein analysis, and test of anti-inflammatory effect because the data was not normal distributions and sample sizes was limited. All statistical significance was determined at $p<0.05$.

Results

1. Cytotoxicity and cell proliferation assay

Full concentration of the conditioned medium from both Dycal® and PCFA had toxicity to the HDPCs. The conditioned medium was, therefore, diluted before evaluation of cytotoxicity and cell proliferation. The diluted conditioned medium from PCFA (F50 and F100) slightly increased the cell proliferation with increasing time. At 48 hours, the culture media with 10% FBS significantly ($p<0.05$) increased cell proliferation when compared to the negative control (SFM). At 72 hours, the culture media with 10% FBS, F50, and F100 significantly increased cell proliferation when compared to the media obtained from the other groups. The effect of culture media with 10% FBS and F50 was not significantly different ($p<0.05$) (Figure 1).

2. RNA and protein synthesis

The effects of diluted condition media from Dycal® and PCFA on DSPP expression and DSP synthesis were shown in Figure 2a and 2b, respectively. The gene expression resulted from RT-PCR revealed that all experimental and positive control (dexamethasone) groups enhanced DSPP expression compared to the negative control. The conditioned medium form the PCFA increased the expression of DSPP approximately 2.5 folds when compared to the negative control. The positive control and F50 groups significantly ($p<0.05$) increased the gene expression when compared to the negative control and D50 (Figure 2c). For protein analysis, the results from Western blotting revealed the conditioned medium form the PCFA increased the synthesis of DSP approximately 1.7 to 1.8 fold when compared to the negative control. However, the effect was not significantly different ($p<0.05$) among the experimental and control groups (Figure 2c).

3. Test of anti-inflammatory effect

The results showed that the HDPCs were induced to express the COX-2 gene expression after the treat-

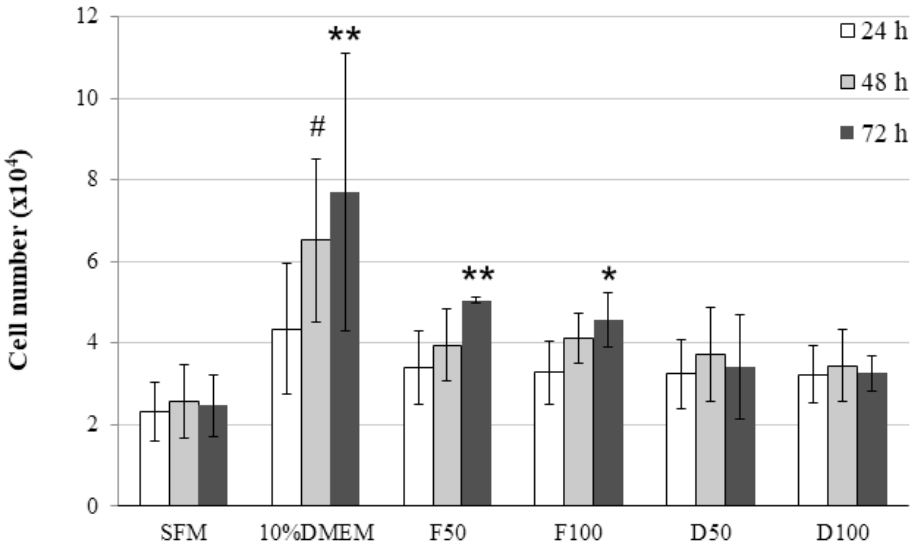

Figure 1: Effects of conditioned medium from Dycal® and PCFA on cytotoxicity and cell proliferation of HDPCs at 24, 48, and 72 hours. SFM was used as negative control. F50 and F100 were the conditioned medium from PCFA diluted to 50 and 100 times, respectively. D50 and D100 were the conditioned medium from Dycal® diluted to 50 and 100 times, respectively. The results presented as cell numbers (mean ± SD). #Statistically significant difference ($p<0.05$) compared with the negative control group at 48 hours.
*,**Statistically significant difference ($p<0.05$) compared with the other groups at 72 hours.

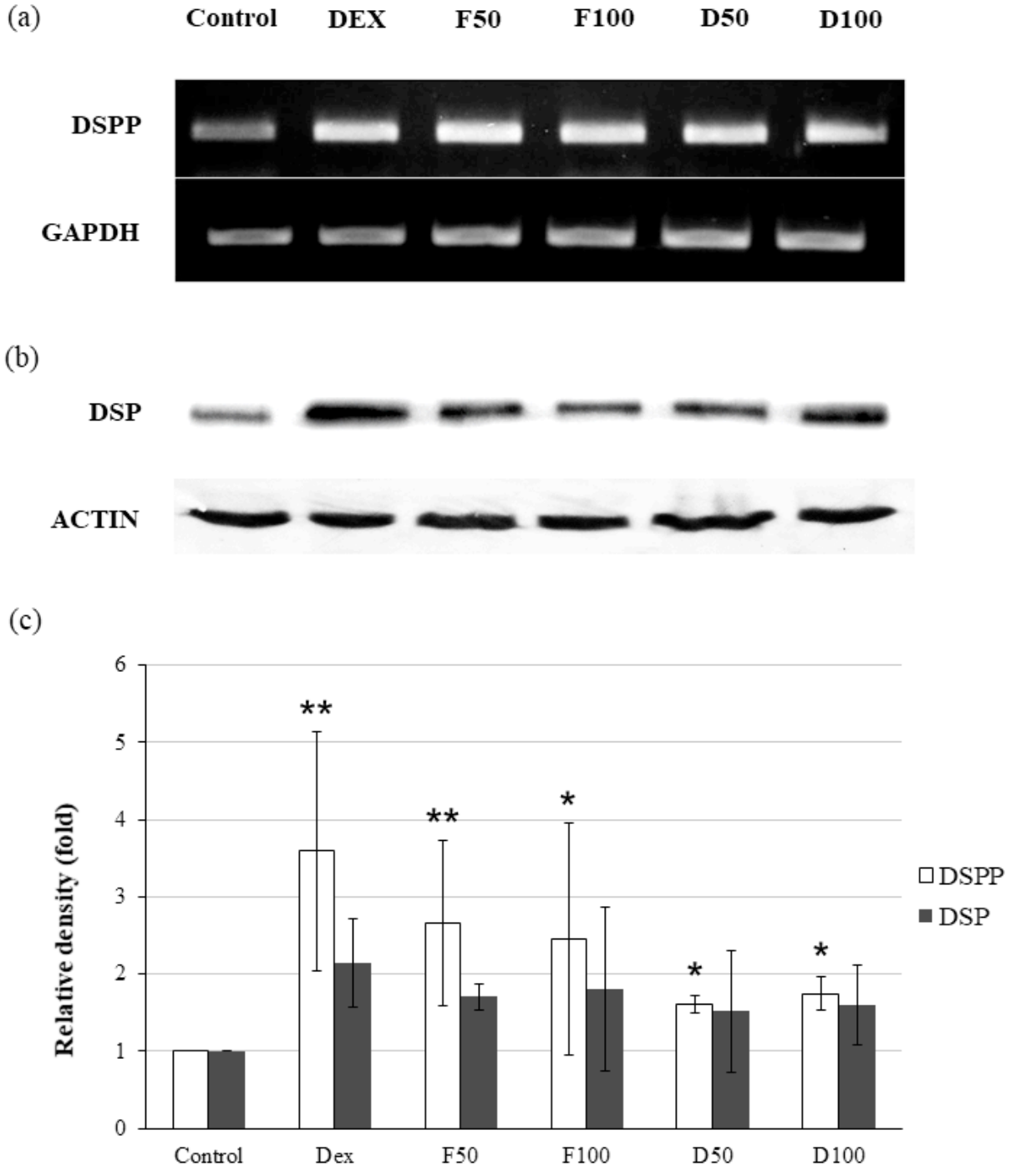

Figure 2: Effects of conditioned medium from PCFA and Dycal® on DSPP expression and DSP synthesis of HDPCs. The results from RT-PCR and Western blotting showed the effects of the conditioned medium on (a) DSPP, (b) DSP in HDPCs, respectively, (c) The graph shows mean ± standard deviation of band density from three separate experiments.

*,**Statistically significant difference was found in DSPP expression compared with the other groups ($p<0.05$). No significant difference was found in DSP synthesis.

ment with LPS at 3, 6 and 24 hours. Therefore, three-hour treatment of LPS was done prior to the experiment. The conditioned medium from F50 and F100 of PCFA could approximately reduce the expression of COX-2 to 43% and 59%, respectively, which were significantly decreased COX-2 expression ($p<0.05$). In contrast to the conditioned media form Dycal®, It did not significantly affect the COX-2 expression (Figure 3a, b).

Discussion

Various techniques have been used for treatment of the inflamed dental pulp such as direct pulp capping, partial or full pulpotomy. Success rate of calcium hydroxide pulp capping is fairly high at short-term follow-up period but decreased at long-term follow-ups.[29] Therefore, the direct pulp capping procedure has been considered as controversy by many clinicians. To increase

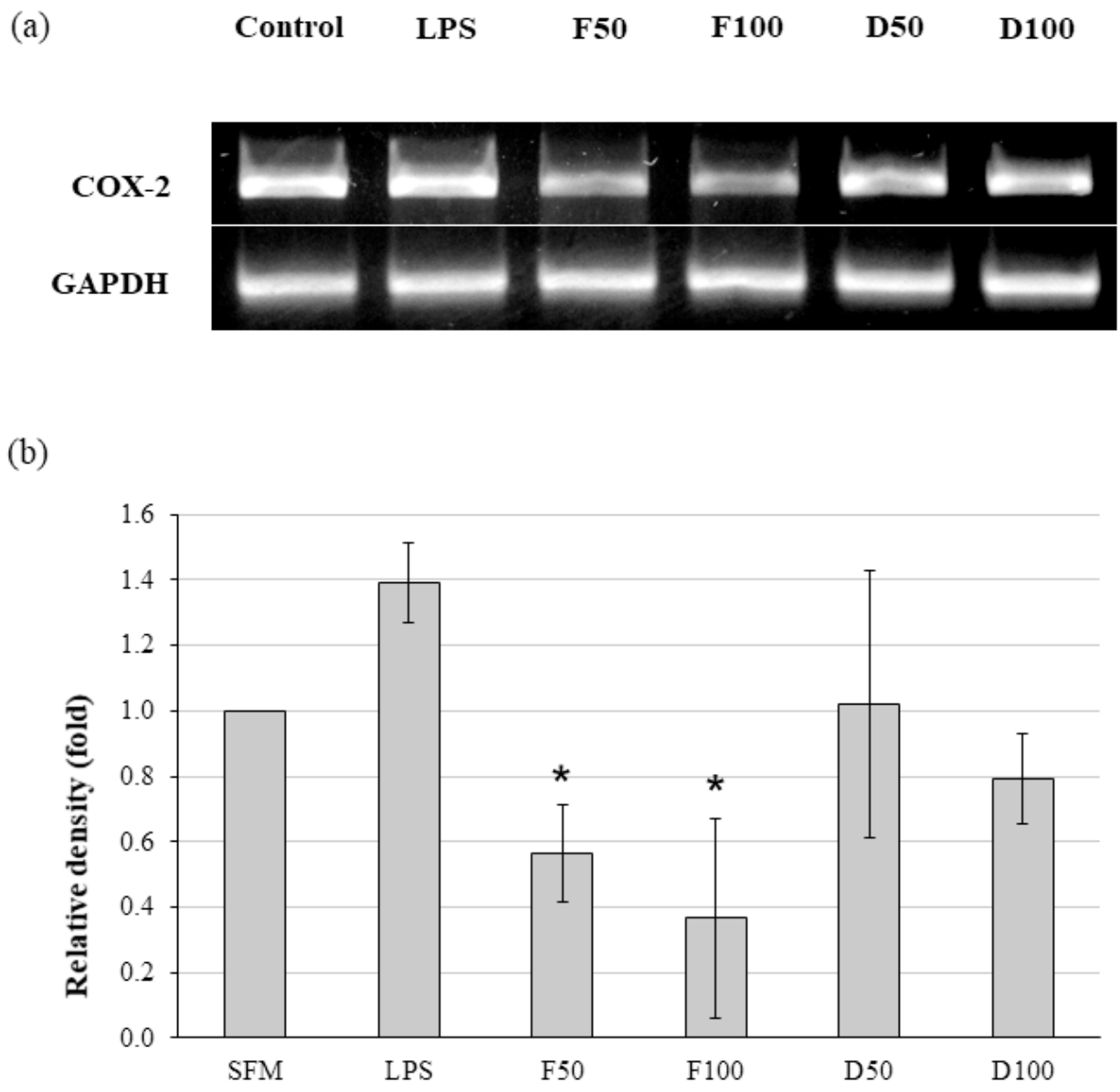

Figure 3: Effects of conditioned medium from PCFA and Dycal® on COX-2 expression from HDPCs. (a) At 24 hours, the results from RT-PCR showed the anti-inflammatory effects of the conditioned medium on COX-2 mRNA expression in HDPCs. (b) The graph shows mean ± standard deviation of band density from three separate experiments.

*Statistically significant difference was found in COX-2 expression compared with the other groups ($p<0.05$).

the success rate, development of new biologically based therapeutics that reduce pulp inflammation, promote the continued formation of new dentin-pulp complex, and restore vitality by stimulating the regrowth of pulpal tissue may be essential.[15] Recently, a wide range of concentrations of fluocinolone acetonide (0.1-10 μmol/l) demonstrated positive effects on human dental pulp cell proliferation, and both fibronectin and type I collagen synthesis.[26] With known anti-inflammatory effects, it may have some potential in stimulation of early phase of healing of dental pulp tissue. Therefore, the novel calcium hydroxide-based pulp capping material containing fluocinolone acetonide (PCFA) was developed.[27] The expected benefits of PCFA may be combination of high alkalinity of calcium hydroxide, control of pre-existing inflammation and stimulatory effect of fluocinolone acetonide.

In this study, we diluted the conditioned media obtained from both PCFA and Dycal® prior to use in the experiments. Our previous *in vitro* model revealed that the PCFA could release 50 μmol/l of fluocinolone acetonide, so the conditioned medium from the PCFA was diluted 50 times in order to obtain the conditioned medium containing 1 μmol/l of fluocinolone acetonide.[26,27] Moreover, high pH of the conditioned media at full concentration might be toxic to the HDPCs.[27] The conditioned media was also diluted 100 times to simulate in case that released fluocinolone acetonide was diluted by fluid in the pulpal tissue. Therefore, the 50- and 100- times dilutions were used in this study. A slight increase in the cell viability in the experimental groups was in accordance with previous studies.[25,26]

Dentin bridge formation is one of the criteria for successful treatment of exposed dental pulp. In the absence of inflammation, the dental pulp itself can repair and form hard tissue to seal off the underlying tissue.[30-33] The DSPP and DSP were selected to examine as mineralization markers in this study because they are important markers of dentin formation.[34-37] Increase of DSPP and DSP activities were demonstrated in all experimental groups. These results may be related to the high pH of calcium hydroxide.[30-33,38] In this study, PCFA significantly increased the DSPP expression which corresponded to the earlier studies.[25,39] HDPCs also exhibited slight increase in DSP synthesis, but the results were not statistically significant. The results confirmed that hard tissue formation was not inhibited by PCFA which confirms our previous histologic study.[40]

Induced inflamed human dental pulp cells can be induced by many substances such as bacteria, lipopolysaccharide, or proinflammatory cytokines.[41] LPS of *P. aeruginosa* was selected in this study because of its effectiveness in stimulation of COX-2 gene expression.[28] The result showed that the PCFA decreased COX-2 gene expression which confirmed the availability and anti-inflammatory effect of fluocinolone acetonide. The overall results from these *in vitro* experiments imply that PCFA may cause superficial pulp necrosis, stimulate early phase of healing in the underlying tissue, decrease pre-existing inflammation, and not interfere with the hard tissue formation.

Overall, PCFA is a cement containing 50 mmol/l fluocinolone acetonide that can release fluocinolone acetonide at a concentration of 50 μmol/l from 50 mm^2 surface area, which is equal to 1 μmol/l/mm^2. Both concentrations of the diluted conditioned media (50 and 100 times) might have therapeutic and anti-inflammatory effects from calcium hydroxide and fluocinolone acetonide. However, it also has some disadvantages. Since the disintegration and leakage of the calcium hydroxide base/liner material has been a concern[27], further studies are also required to improve its mechanical properties. Addition of fillers, micro- or nano-fillers, may improve the strength and disintegration of this material. The adhesive property of the material to dentin was important to prevent microleakage and increase the retention of the material. Some studies have attempted to add polyacrylic acid or adhesive monomers to increase this property.[42-46]

Cell culture experiment has several limitations including the finite doubling potential of most normal cells. Some cultured cells have a tendency to change their morphology, functions, or the range of gene expressions. Furthermore, the mechanisms of dental pulp healing *in vivo* are more complex, involving both cellular and extracellular events which cannot be completely simulated in the cell culture system. However, the results from this study confirmed our previous *in vivo* study. Our experiment in rat's teeth showed that the PCFA group can decrease inflammation in inflamed dental rat pulps after capping for 8 and 30 days and stimulate hard tissue formation after 30 days[40]. Although both *in vitro* and *in vivo* studies showed the effectiveness of the PCFA, a clinical trial is mandatory because the methods of application and

time used in these experiments were totally different to a real clinical situation.

Conclusions

PCFA slightly increased the cell proliferation, promoted DSPP and reduced COX-2 gene expressions in human dental pulp cells. PCFA might promote cell proliferation, mineralization and decrease pre-existing inflammation in human dental pulp cells and be considered alternative pulp capping material in the treatment of inflamed dental pulp.

References

1. Trope M. The vital tooth - its importance in the study and practice of endodontics. Endod Topics. 2003;5:1.
2. Fitzgerald M, Heys RJ. A clinical and histological evaluation of conservative pulpal therapy in human teeth. Oper Dent. 1991;16:101-12.
3. Kozlov M, Massler M. Histologic effects of various drugs on amputated pulps of rat molars. Oral Surg Oral Med Oral Pathol. 1960;13:455-69.
4. Tronstad L. Reaction of the exposed pulp to dycal treatment. Oral Surg Oral Med Oral Pathol. 1974;38:945-53.
5. Zander HA. Reaction of the pulp to calcium hydroxide. J Dent Res. 1939;18:373-9.
6. Baume LJ, Holz J. Long term clinical assessment of direct pulp capping. Int Dent J. 1981;31:251-60.
7. Cvek M. A clinical report on partial pulpotomy and capping with calcium hydroxide in permanent incisors with complicated crown fracture. J Endod. 1978;4:232-7.
8. Kakehashi S, Stanley HR, Fitzgerald RJ. The Effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol. 1965;20:340-9.
9. Mejare I, Cvek M. Partial pulpotomy in young permanent teeth with deep carious lesions. Endod Dent Traumatol. 1993;9:238-42.
10. Olsson H, Petersson K, Rohlin M. Formation of a hard tissue barrier after pulp cappings in humans. A systematic review. Int Endod J. 2006;39:429-42.
11. Barthel CR, Rosenkranz B, Leuenberg A, Roulet JF. Pulp capping of carious exposures: treatment outcome after 5 and 10 years: a retrospective study. J Endod. 2000;26:525-8.
12. Dammaschke T, Leidinger J, Schafer E. Long-term evaluation of direct pulp capping--treatment outcomes over an average period of 6.1 years. Clin Oral Investig. 2010;14:559-67.
13. Tronstad L, Mjor IA. Capping of the inflamed pulp. Oral Surg Oral Med Oral Pathol. 1972;34:477-85.
14. Trope M, McDougal R, Levin L, May KN, Jr., Swift EJ, Jr. Capping the inflamed pulp under different clinical conditions. J Esthet Restor Dent. 2002;14:349-57.
15. Komabayashi T, Zhu Q. Innovative endodontic therapy for anti-inflammatory direct pulp capping of permanent teeth with a mature apex. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109:e75-81.
16. Rapoport L, Abramson II. Application of steroid hormones in pulp-capping and pulpotomy procedures; a preliminary report. Oral Surg Oral Med Oral Pathol. 1958;11:545-8.
17. Barker BC, Ehrmann EH. Human pulp reactions to a glucocorticosteroid-antibiotic compound. Aust Dent J. 1969;14:104-19.
18. Clarke NG. The corticosteroid-antibiotic dressing as a capping for inflamed dental pulps. Aust Dent J. 1971;16:71-6.
19. Ulmansky M, Sela J, Langer M, Yaari A. Response of pulpotomy wounds in normal human teeth to successively applied Ledermix and Calxyl. Arch Oral Biol. 1971;16:1393-8.
20. Buajeeb W, Pobrurksa C, Kraivaphan P. Efficacy of fluocinolone acetonide gel in the treatment of oral lichen planus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;89:42-5.
21. Hooley JR, Hohl TH. Use of steroids in the prevention of some complications after traumatic oral surgery. J Oral Surg. 1974;32:864-6.
22. Lozada F, Silverman S, Jr. Topically applied fluocinonide in an adhesive base in the treatment of oral vesiculoerosive diseases. Arch Dermatol. 1980;116:898-901.
23. Fisher LB, Maibach HI. The effect of corticosteroids on human epidermal mitotic activity. Arch Dermatol. 1971;103:39-44.
24. Kirk D, Mittwoch U. Effects of topically potent glucocorticoids on human diploid fibroblasts *in vitro*. Br J Dermatol. 1977;97:69-75.
25. Liu Z, Jiang T, Wang Y, Wang X. Fluocinolone acetonide promotes the proliferation and mineralization of dental pulp cells. J Endod. 2013;39:217-22.
26. Muincharern W, Louwakul P, Pavasant P, Lertchirakarn V. Effect of fluocinolone acetonide on human dental pulp cells: cytotoxicity, proliferation, and extracellular matrix formation. J Endod. 2011;37:181-4.
27. Louwakul P, Lertchirakarn V. Incorporation of anti-inflammatory agent into calcium hydroxide pulp capping material: an *in vitro* study of physical and mechanical properties. Dent Mater J. 2012;31:32-9.
28. Chang YC, Huang FM, Yang SF, Liu CM, Lai CC, Chan Y, *et al*. Induction of cyclooxygenase-2 mRNA and protein expression in human pulp cells stimulated with black-pigmented bacteroides. J Endod. 2003;29:240-3.
29. Cushley S, Duncan H, Lappin M, Chua P, Elamin A, Clarke M, *et al*. Efficacy of direct pulp capping for management of cariously exposed pulps in permanent teeth: a systematic review and meta-analysis. Int Endod J. 2021;54:556-71.

30. Fitzgerald M. Cellular mechanics of dentinal bridge repair using ^{3}H-thymidine. J Dent Res. 1979;58:2198-206.
31. Heys DR, Heys RJ, Cox CF, Avery JK. The response of four calcium hydroxides on monkey pulps. J Oral Pathol. 1980;9:372-9.
32. Kitasako Y, Shibata S, Arakawa M, Cox CF, Tagami J. A light and transmission microscopic study of mechanically exposed monkey pulps: dynamics of fiber elements during early dentin bridge formation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;89:224-30.
33. Mjor IA, Dahl E, Cox CF. Healing of pulp exposures: an ultrastructural study. J Oral Pathol Med. 1991;20:496-501.
34. Goldberg M, Smith AJ. Cells and extracellular matrices of dentin and pulp: A biological basis for repair and tissue engineering. Crit Rev Oral Biol Med. 2004;15:13-27.
35. Suzuki S, Sreenath T, Haruyama N, Honeycutt C, Terse A, Cho A, *et al*. Dentin sialoprotein and dentin phosphoprotein have distinct roles in dentin mineralization. Matrix Biol. 2009;28:221-9.
36. Wei X, Ling J, Wu L, Liu L, Xiao Y. Expression of mineralization markers in dental pulp cells. J Endod. 2007;33: 703-8.
37. Wei X, Wu L, Ling J, Liu L, Liu S, Liu W, *et al*. Differentially expressed protein profile of human dental pulp cells in the early process of odontoblast-like differentiation *in vitro*. J Endod. 2008;34:1077-84.
38. Fitzgerald M, Chiego DJ, Jr., Heys DR. Autoradiographic analysis of odontoblast replacement following pulp exposure in primate teeth. Arch Oral Biol. 1990;35:707-15.
39. Alliot-Licht B, Bluteau G, Magne D, Lopez-Cazaux S, Lieubeau B, Daculsi G, *et al*. Dexamethasone stimulates differentiation of odontoblast-like cells in human dental pulp cultures. *Cell Tissue Res.* 2005;321:391-400.
40. Louwakul P, Lertchirakarn V. Response of inflamed pulps of rat molars after capping with pulp-capping material containing fluocinolone acetonide. J Endod. 2015;41:508-12.
41. Kim YS, Min KS, Lee HD, Oh HW, Kim EC. Effect of cytosolic phospholipase A2 on proinflammatory cytokine-induced bone resorptive genes including receptor activator of nuclear factor kappa B ligand in human dental pulp cells. J Endod. 2010;36:636-41.
42. Cohen BD, Combe EC. Development of new adhesive pulp capping materials. Dent Update. 1994;21:57-62.
43. Katoh Y, Suzuki M, Kato C, Shinkai K, Ogawa M, Yamauchi J. Observation of calcium phosphate powder mixed with an adhesive monomer experimentally developed for direct pulp capping and as a bonding agent. *Dent Mater J.* 2010;29:15-24.
44. Niinuma A. Newly developed resinous direct pulp capping agent containing calcium hydroxide (MTYA1-Ca). Int Endod J. 1999;32:475-83.
45. Shinkai K, Taira Y, Suzuki M, Kato C, Ebihara T, Wakaki S, *et al*. Dentin bond strength of a new adhesive system containing calcium phosphate experimentally developed for direct pulp capping. Dent Mater J. 2009;28:743-9.
46. Tziafas D, Koliniotou-Koumpia E, Tziafa C, Papadimitriou S. Effects of a new antibacterial adhesive on the repair capacity of the pulp-dentine complex in infected teeth. Int Endod J. 2007;40:58-66.