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Original Article

Antibiofilm Effect of Citric Acid-modified Chlorhexidine Gluconate on a Dual-species Biofilm

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Abstract

Objective: The purpose of this study was to evaluate the antibiofilm effect of chlorhexidine gluconate (CHX) added with citric acid (CA) on bacterial-fungal biofilm.

Methods: Dentin slices were sectioned from the crown of extracted human third molars. After sterilization, samples were inoculated with Enterococcus faecalis *(E. faecalis)* and Candida albicans *(C. albicans)* for 14 days to establish a bacterial-fungal biofilm. All samples were randomly divided into four treatment groups: phosphate-buffered saline (PBS) (negative control), 2% CHX, 10% CA, and 10% citric acid-modified 2% chlorhexidine gluconate (CAmCHX). Each dentin slice was treated with one of the selected solutions for 1 minute. Following treatment, samples were labeled with a fluorescent viability stain to identify live and dead cells. The proportion of dead cells to total cells was analyzed with confocal laser scanning microscopy (CLSM), and biofilm removal efficacy was evaluated by scanning electron microscopy (SEM).

Results: 10% CA and CAmCHX groups demonstrated a higher proportion of dead cells to total cells than the PBS group ($p<0.05$). No significant difference was observed between the 10% CA and CAmCHX groups ($p>0.05$). SEM images revealed less remaining biofilm in 10% CA and CAmCHX groups. Whereas, in 2% CHX and PBS groups, the biofilm structure was still intact.

Conclusions: Within the limitations of this study, 10% CA and CAmCHX demonstrated an antibiofilm effect against *E. faecalis* and *C. albicans* biofilm on the surface of dentin slices. CAmCHX can be thought as an alternative choice for irrigation to remove the biofilm. Future study should focus on the cytotoxicity of this agent prior to clinical used.

Keywords: biofilms, *Candida albicans*, chlorhexidine, citric acid, *Enterococcus faecalis*