

TITLE: CYTOCOMPATIBILITY AND SYNERGY OF EGCG AND CATIONIC PEPTIDES AGAINST BACTERIA RELATED TO ENDODONTIC INFECTIONS, IN PLANKTONIC AND BIOFILM CONDITIONS

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ABSTRACT:

Phytochemicals are promising molecules that have been studied in the prevention and treatment of oral diseases, since they act both in the elimination of pathogens and in the control of the inflammatory response of the organism. The aim of the study was to evaluate the cytotoxic, antimicrobial/antibiofilm effect of the flavonoid epigallocatechin-3-gallate (EGCG) alone or in combination with peptides LL-37 or KR-12-a5. Minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC), and Fractional inhibitory concentration (FIC) of EGCG, LL-37, and KR-12-a5 were determined against *Streptococcus mutans*, *Enterococcus faecalis*, *Actinomyces israelii*, and *Fusobacterium nucleatum* by microdilution and checkerboard methods, after 24h of treatment. L-929 fibroblasts were exposed to combinations of EGCG with peptides in different concentrations and cell metabolism evaluated by MTT assays. The effect of compounds for 24-36h, alone or in combination, was also evaluated on 48h single biofilm or dual-species biofilms with *E. faecalis* formed in polystyrene plates by means of bacterial counting. *E. faecalis* biofilms were also grown in dentinal tubules for 2 weeks, treated with EGCG + KR-12-a5 and percentage of dead cells was determined by analysis of images using Confocal Microscopy. The data were evaluated by ANOVA / Tukey ($p < 0.05$). The combination of EGCG + KR-12-a5 showed synergistic or additive effect under planktonic conditions. Combinations were not toxic to fibroblasts. Under simple biofilm conditions, EGCG + KR-12-a5 eliminated *S. mutans* and *A. israelii* and reduced *E. faecalis* and *F. nucleatum*. In dual-species biofilms of *E. faecalis* + *S. mutans*, the combination eliminated *S. mutans* and reduced *E. faecalis*. In biofilms of *E. faecalis* + *A. israelii* or *F. nucleatum*, the combination eliminated *E. faecalis*. The combination reduced 88.04 % of *E. faecalis* biofilms within dentinal tubules. The association of EGCG and KR-12-a5 is cytocompatible and promotes a synergistic effect against bacteria associated with endodontic infections under planktonic and biofilm conditions.

Keywords: EGCG, Cationic Antimicrobial Peptides, Synergism, Biofilms, Cytotoxicity

Development Agency: CNPq (# 140467/2018-1); CAPES-PROCAD (# 88881.068437/2014-01).