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ORIGINAL ARTICLE/**ARTICOLO ORIGINALE**

Antibacterial effects of two synthetic peptides against *Enterococcus faecalis* biofilms: a preliminary *in vitro* study

Effetti antibatterici di due peptidi sintetici nei confronti di biofilm di Enterococcus faecalis: studio preliminare in vitro

KEYWORDS
Antimicrobial peptides, Biofilms, Confocal laser scanner microscopy, Enterococcus faecalis

PAROLE CHIAVE
Peptidi antimicrobici, Biofilm, Microscopia a scansione laser, Enterococcus faecalis

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Abstract

Aim: *Current endodontic techniques are unable to fully eradicate intracanal bacteria. Thus, new agents that effectively eliminate endodontic pathogens are needed. The aim of this study was to assess the antibacterial properties of two synthetic peptides, namely KP and L18R, against planktonic cells and biofilms of the endodontic pathogen Enterococcus faecalis.*
Methodology: *KP and L18R bactericidal activity against E. faecalis ATCC 29212 was evaluated by colony forming unit assays and the half maximal effective concentration (EC_{50}) was calculated. The effect of peptides on E. faecalis biofilm formation onto polystyrene plates was also assessed by the crystal violet assay. Confocal laser scanning microscopy (CLSM) analysis was carried out to compare the effects of KP, L18R and a $Ca(OH)_2$ saturated solution in an in vitro model of dental infection consisting in 2-day-old E. faecalis biofilms grown on hydroxyapatite disks.*
Results: *Both KP and L18R showed strong bactericidal activity against planktonic E. faecalis. L18R proved to be 10-folds more effective than KP (KP and L18R EC_{50} values=4.520×10^{-6} M and 3.624×10^{-7} M, respectively). Peptides inhibited E. faecalis biofilm formation in a dose-dependent manner and L18R resulted more effective*

Obiettivo: *le tecniche tradizionali di disinfezione endodontica non sono in grado di eliminare completamente i microrganismi del sistema canalare e, pertanto, si rende necessario lo sviluppo di nuovi agenti antimicrobici efficaci nei confronti dei microrganismi endodontici. Lo scopo di questo studio è la valutazione in vitro delle proprietà antibatteriche di due peptidi sintetici, denominati KP e L18R, nei confronti di cellule in sospensione e biofilm del patogeno endodontico Enterococcus faecalis.*
Materiali e metodi: *l'attività battericida di KP e L18R nei confronti di E. faecalis ATCC 29212 in forma planctonica è stata valutata mediante saggi convenzionali di determinazione di unità formanti colonia, stabilendo la concentrazione in grado di inibire il 50% della crescita batterica (EC_{50}), mentre l'effetto dei peptidi sulla formazione di biofilm in piastre di polistirene è stato studiato mediante saggio con il cristal violetto. È stata condotta, inoltre, un'analisi al microscopio confocale a scansione laser (MCSL) per valutare l'azione di KP, L18R e di una soluzione satura di $Ca(OH)_2$ in un modello in vitro di infezione endodontica rappresentato da biofilm di E. faecalis cresciuti per 2 giorni su dischetti di idrossiapatite.*
Risultati: *KP e L18R hanno mostrato un'efficace attività battericida nei confronti di E. faecalis allo stato planctonico e L18R si è mostrato 10 volte più efficace rispetto KP (EC_{50}=$4,520\times10^{-6}$ M e $3,624\times10^{-7}$ M, rispettivamente, per KP e L18R). Entrambi i peptidi hanno causato una riduzione dose-dipendente della*

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Abstract

than KP. CLSM images showed that $Ca(OH)_2$, KP and L18R remarkably impaired *E. faecalis* biofilms pre-grown on hydroxyapatite.
Conclusions: KP and L18R effectively inhibited *E. faecalis*, both in planktonic and biofilm form. L18R demonstrated a more potent antibacterial activity than KP. These preliminary results suggest that antimicrobial peptides may represent a promising new strategy for endodontic infection control.

formazione del biofilm e L18R è risultato più efficace rispetto KP. Le immagini ottenute mediante analisi al MCSL hanno mostrato una alterazione della struttura di biofilm di *E. faecalis* pre-formato su dischetti di idrossiapatite in presenza di $Ca(OH)_2$, KP e L18R.
Conclusioni: entrambi i peptidi analizzati sono in grado di inibire *E. faecalis*, sia in forma planctonica che di biofilm e L18R si è dimostrato più efficace rispetto a KP. Questi risultati preliminari suggeriscono che i peptidi antimicrobici possono rappresentare una promettente strategia per il controllo delle infezioni endodontiche.

Introduction

Endodontic treatment aims to resolve or prevent apical periodontitis eliminating the microorganisms from the root canal system (1). Unfortunately, current antimicrobial chemo-mechanical techniques have shown several limitations in microbial eradication (2, 3) and inter-appointment intracanal medications are often necessary (4). Treatment with calcium hydroxide, $Ca(OH)_2$, is the most widely used (5). $Ca(OH)_2$ antimicrobial properties depend on the release and diffusion of hydroxyl ions (OH^-) that cause a strongly alkaline environment and inhibit the growth of many endodontic pathogens (6). In clinical conditions, the activity of $Ca(OH)_2$ is limited by the inherent buffering capacity of the dentine (7). *Enterococcus faecalis*, a Gram-positive facultative anaerobic species frequently found in endodontic treatment failures, showed a relative resistance to $Ca(OH)_2$ (8, 9). One of the strategies adopted by *E. faecalis* to resist to antimicrobials is the formation of biofilms on the canal surfaces, alone or in combination with other species (10). Biofilms are formed by bacterial cells grown on a solid surface and enveloped in a self-produced polysaccharide and protein extracellular matrix. Eradication of microrganisms in biofilms is much more difficult in comparison to planktonic cells. Given these considerations, the exploitation of new effective antibiofilm substances would be beneficial to improve endodontic treatment success.

Antimicrobial peptides (AMPs) are a group of short, low-molecular-weight peptide sequences with a wide spectrum of antimicrobial activities (11). Their mechanism of action has not been fully elucidated, but for many of them seems to be mediated by a cell membrane damage finally leading to cell death (12). Other proposed mechanisms of killing are not related to membrane permeabilization. Some AMPs can cross the cell membrane through direct penetration or by a transporter mediated mechanism and interact with intracellular targets inducing several toxic effects, including enzyme inhibition, DNA degradation, formation of reactive oxygen species and ATP leakage (13).

AMPs could be distinguished in natural and synthetic ones. The former are produced by most living organisms, plants and animals, from mammals to insects, as a component of their innate immune system, and even bacteria and fungi. Examples of natural peptides are nisin synthesised by *Lactobacillus lactis* (14) or cathelicidin LL-37, expressed by human neutrophils and epithelial tissues (15). Beside natural AMPs, a wide library of synthetic peptides has been created in order to improve antimicrobial activities, to optimize pharmacological properties and to reduce production costs. Among synthetic peptides, two recently described AMPs, namely KP and L18R, have shown promising antimicrobial activities.

KP (killer peptide) is a decapeptide derived from a recombinant anti-idiotypic antibody, which is the internal image of a wide-spectrum antimicrobial yeast toxin (16). KP proved to be effective against protozoa, fungi, bacteria and viruses (17). L18R, derived from a gene (IGHJ2) encoding a human immunoglobulin heavy chain, displayed a strong fungicidal activity *in vitro* and *in vivo* (18). Both peptides did not show detectable toxicity to different human cells, including erythrocytes, epithelial cells and peripheral blood mononuclear cells (17-19).

The aim of this preliminary study was to assess the antibacterial properties of KP and L18R against planktonic cells and biofilms of the endodontic pathogen *E. faecalis*.

Materials and Methods

Peptides

KP (AKVTMTCSAS, molecular weight 998.2) and L18R (LLVLRSLGPWHPGHCLLR, molecular weight 2068.1) were synthesised at the CRIBI Biotechnology Center (University of Padua, Italy) with the solid phase peptide synthesis method using a multiple peptide synthesiser (SyroII, MultiSynTech GmbH, Witten, Germany). Then, the peptides were solubilised in dimethylsulphoxide at a concentration of 20 mg/ml. For the experiments, KP and L18R were diluted in sterile distilled water to the suitable concentrations.

Bactericidal activity against planktonic E. faecalis cells

The reference *E. faecalis* ATCC 29212 strain was grown in Brain Heart Infusion Agar (BHA; Sigma-Aldrich, St. Louis, USA) plates and maintained by biweekly passages. Peptide antibacterial activity was evaluated by colony forming unit (CFU) assay, as previously described (20). Briefly, 100 µl of a suspension of *E. faecalis* ATCC 29212 at a concentration of approximately 10^4 cells/ml were incubated in a 96-well microplate in the absence (control) or presence of each peptide, at decreasing concentrations. After 5 h at 37 °C, bacterial suspensions were seeded on BHA plates and colonies were counted after 24 hours of incubation at 37 °C. Killing percentage was calculated in relation to the number of colonies in controls. Three independent experiments were performed. The half maximal effective concentration (EC_{50}) was calculated by nonlinear regression analysis using Prism 4.01 (Graph Pad software, San Diego, USA).

Inhibition of E. faecalis biofilm formation on polystyrene surfaces

KP and L18R effects on early stages of *E. faecalis* ATCC 29212 biofilm formation were investigated as follows. Overnight cultures in Brain Heart Infusion Broth (BHI; Sigma-Aldrich, St. Louis, USA) supplemented with 0.25% glucose (BHIg) were diluted to 7.5×10^6 cells/ml in fresh medium and 200 µl of the suspension were transferred into wells of polystyrene flat-bottom 96-well plates (Corning Incorporated, New York, USA). After 90 min at 37 °C, non-adherent bacterial cells were washed off. Decreasing concentrations of peptides in 200 µl of sterile distilled water were added to the wells while controls were incubated in 200 µl of water for 5 h at 37 °C. Subsequently, the wells were washed and 200 µl/well of BHIg were added and incubated at 37 °C for 24 h.

After 48 h, biofilm mass was assessed by the crystal violet assay. The medium was removed and the wells were dried at 80 °C for 15 minutes and then stained using 200 µl/well of 0.25% crystal violet (Sigma-Aldrich, St. Louis, USA) for 15 min. Then the wells were washed three times with phosphate-buffered saline (PBS) and dried for 12 hours in a dark room. Then, 200 µl/well of 85% ethanol was added and the absorbance at 540 nm was measured using a microplate reader (Multiskan Ascent Microplate Reader, Thermo Electron, Waltham, USA). The results were expressed as percentage of biofilm mass reduction in relation to untreated controls.

Inhibition of E. faecalis biofilm on hydroxyapatite disks

In order to evaluate the antibiofilm properties, an *in vitro* model of root canal infection was employed using hydroxyapatite (HA) disks. HA disks were prepared according to Lagori et al. (21). Briefly, 0.15 g of HA powder (Sigma Aldrich, St. Louis, USA) were placed in a 12.891-mm diameter mould (Specac Inc., Fort Washington, USA). HA powder was compressed using a hydraulic press (Eurocem EPI 1, Settimo Milanese, Italy) at the pressure of 50 bars. Disks were heated under vacuum at 980 °C. Sterilized HA disks were incubated with 500 µl of a 7.5×10^6 cells/ml *E. faecalis* ATCC 29212 suspension for 48 h at 37 °C in a flat-bottom 24-well plate. After incubation, the medium was washed off and the disks were treated (500 µl/well) with a saturated solution of $Ca(OH)_2$ endodontic dressing, KP (100 µg/ml) or L18R (50 µg/ml) for 24 h. Control samples were incubated with sterile water. The saturated solution of $Ca(OH)_2$ was previously prepared by mixing 38 mg of Calxyl (OCO, OCO Präparate GmbH, Dirmstein, Germany) with 10 mL of sterile distilled water. The preparation was centrifuged at 3000 g and aqueous supernatant was filtered aseptically using a sterile 25 mm diameter (0.22 µm) syringe filter (Millex®, Merck Millipore, Burlington, USA).

After treatment, the disks were processed for confocal laser scanning microscopy (CLSM) by washing with sterile water and staining with 500 µl/well of a fluorescent staining solution containing 0.3% SYTO9 and 0.3% propidium iodide (LIVE/DEAD FilmTracer™ LIVE/DEAD® Biofilm Viability Kit, Invitrogen, Paisley, UK). SYTO 9 and propidium iodide selectively stain alive cells in green and dead cells in red, respectively. A LSM 510 Meta scan head integrated with the Axiovert 200 M inverted microscope (Carl Zeiss, Jena, Germany) using a 40×NA1.3 oil immersion lens was employed for observation. The excitation/emission wavelengths were 480/500 nm for SYTO 9 and 490/635 nm for propidium iodide. A stack of 80-100 slices was captured along the Z-axis of the biofilm. CLSM images were acquired and 3D reconstructed with Imaris 9.5.0 software (Bitplane AG, Zurich, Switzerland). Each experiment was performed in duplicate.

Results

KP and L18R bactericidal activity against planktonic cells

CFU assays were carried out on planktonic *E. faecalis* ATCC 29212. Both peptides showed a significant activity against the endodontic pathogen (figure 1), with EC_{50} values in the micromolar range, 4.520×10^{-6} M and 3.624×10^{-7} M for KP and L18R, respectively. Notably, EC_{50} values for L18R were approximately 10-fold lower.

Inhibitory effects of KP and L18R on early stages of E. faecalis biofilm development

The ability of peptides to inhibit *E. faecalis* biofilm formation onto polystyrene plates was investigated by crystal violet assay. Both KP and L18R proved to notably reduce the biofilm mass in a dose-dependent manner (figure 2). In particular, KP at 50 µg/ml concentration caused 35% biofilm mass reduction. Conversely, at the same concentration, L18R determined a 73% biofilm mass reduction. As for the bactericidal activity against planktonic cells, L18R resulted more effective in biofilm inhibition in comparison to KP.

KP and L18R inhibition of E. faecalis biofilm on hydroxyapatite disks

Antibiofilm properties of KP and L18R against 2 day-old *E. faecalis* ATCC 29212 biofilms grown on HA disk were assessed using CLSM in comparison with samples treated with a saturated $Ca(OH)_2$ endodontic dressing solution. 3D CLSM image reconstructions of control samples (water incubation) showed a homogeneous and robust biofilm layer with a majority of cells alive (figure 3A). Instead, HA disks exposed to saturated $Ca(OH)_2$ solution, KP (100 µg/ml), and L18R (50 µg/ml)

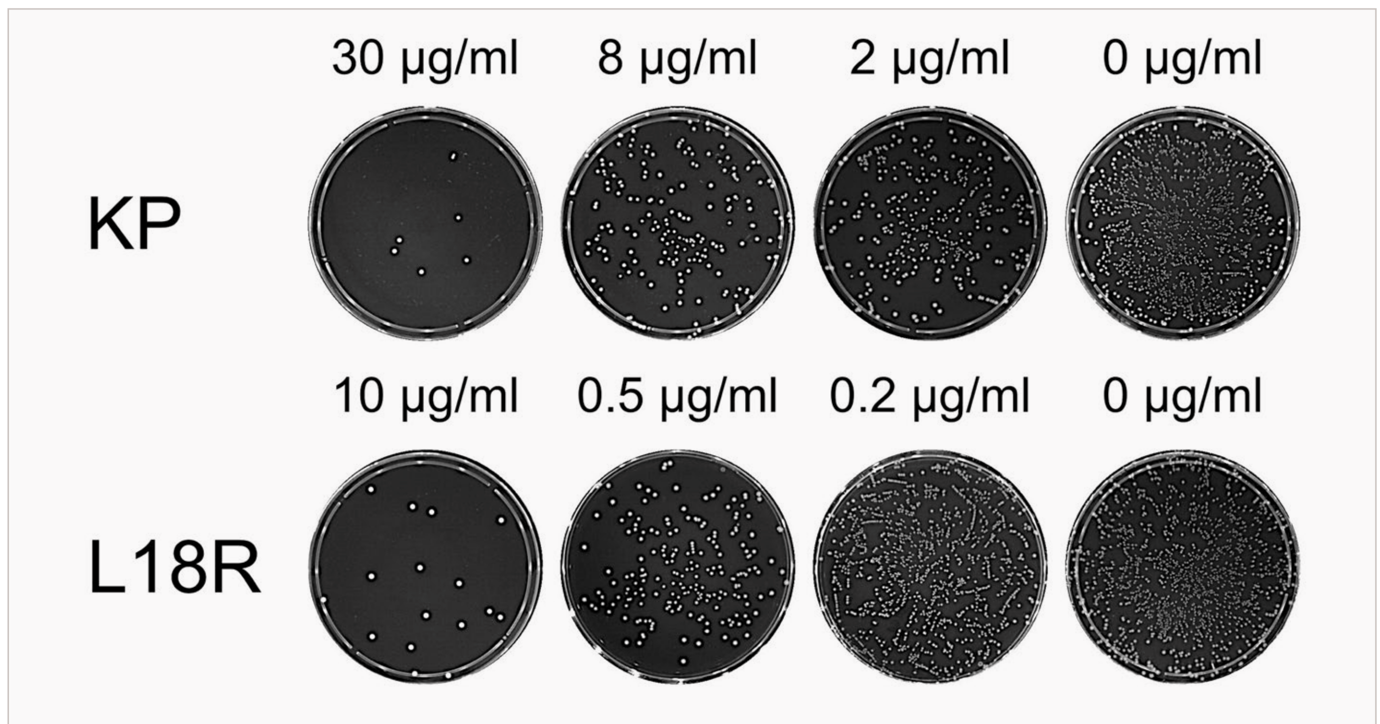

Figure 1
KP and L18R effects against *E. faecalis* ATCC 29212 planktonic cells determined by CFU assay. Representative plates show *E. faecalis* ATCC 29212 inhibition at different KP and L18R concentrations.

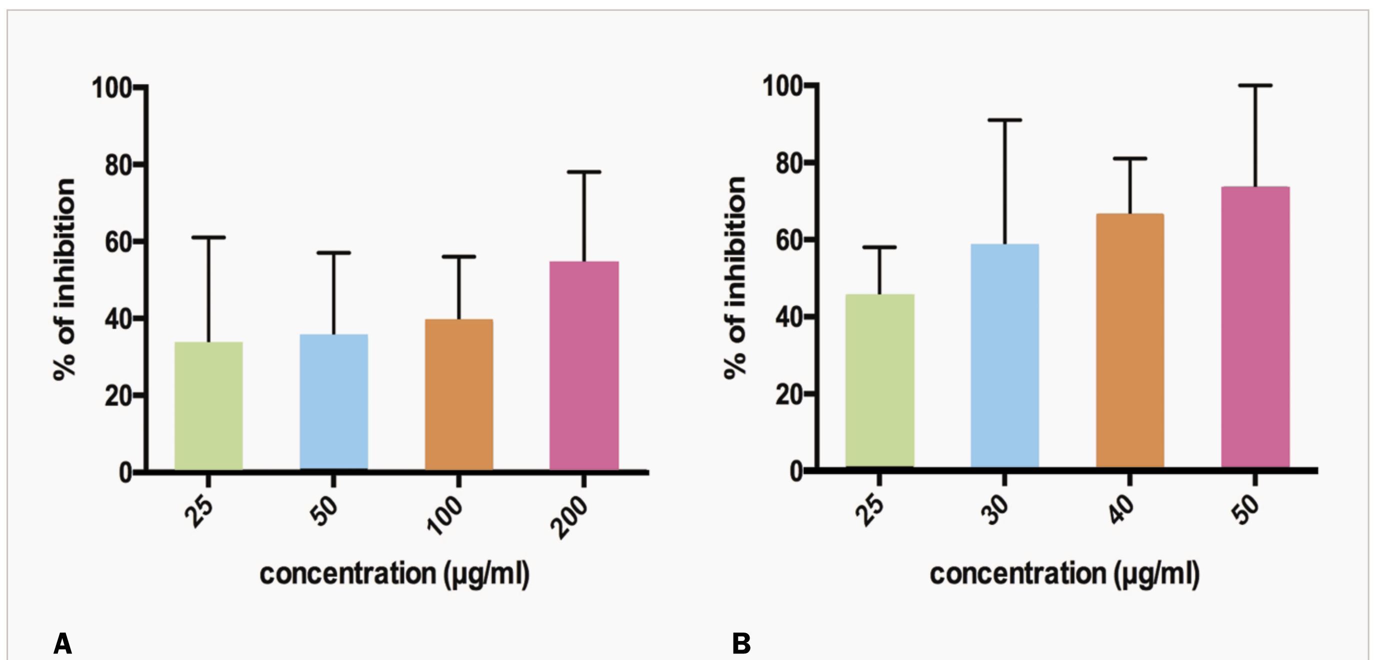

Figure 2
KP **(A)** and L18R **(B)** effects against *E. faecalis* ATCC 29212 biofilm. Biofilm mass was determined by crystal violet assay, comparing samples treated with different peptide concentrations to untreated samples. Data are presented as mean ± SD of at least three independent experiments.

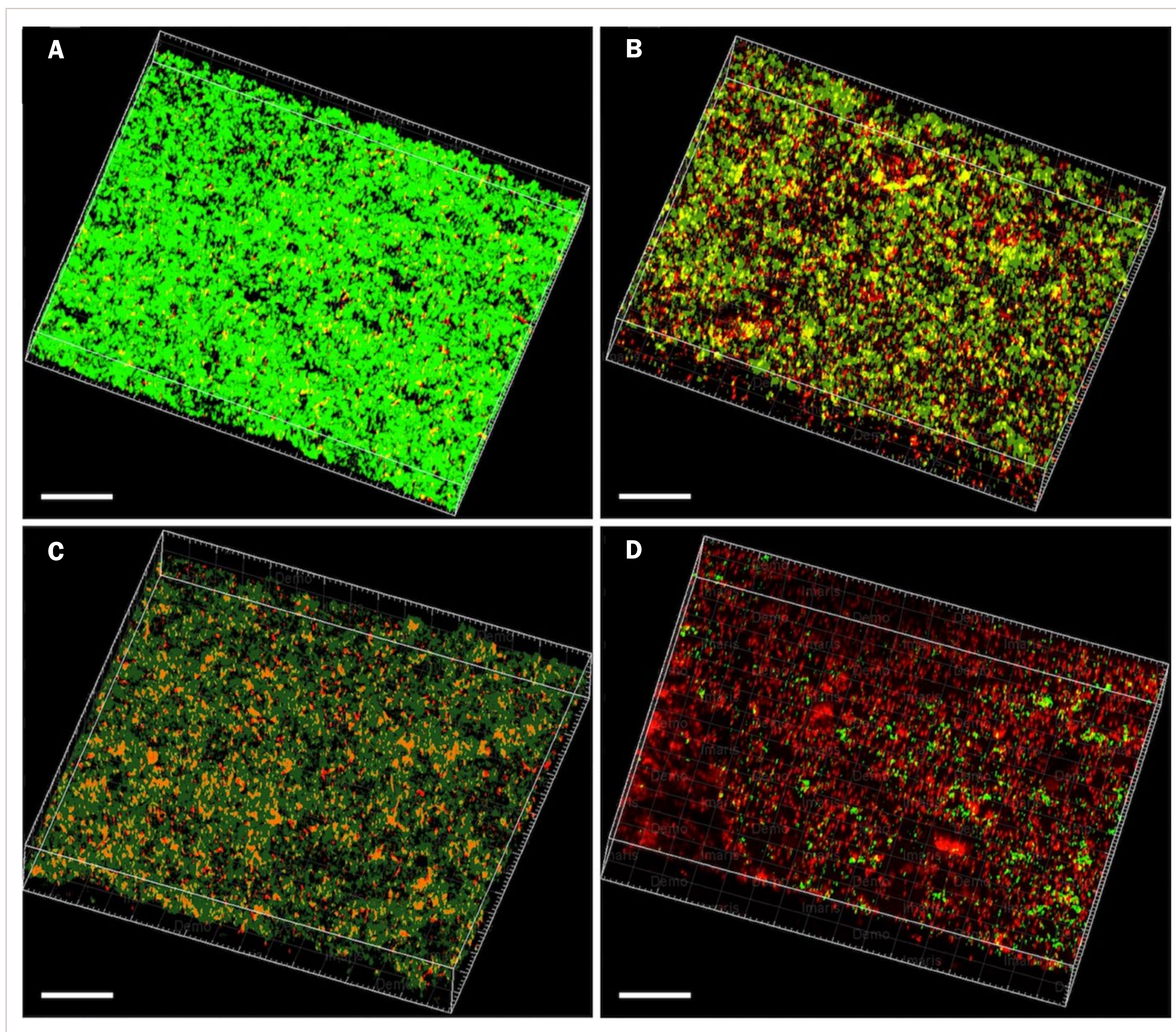

Figure 3
Representative 3D reconstruction of confocal laser scanning microscopy (CLSM) images of *E. faecalis* biofilms on HA disks. Images represent 2 day-old *E. faecalis* ATCC 29212 biofilms exposed for 24 h to H_2O (control, A), $Ca(OH)_2$ saturated solution (B), 100 µg/ml KP (C) and 50 µg/ml L18R (D) and stained with SYTO9 and propidium iodide. Green, live cells; red, dead cells. Bars=50 µm.

showed an impaired biofilm layer, with a consistent number of dead cells (figure 3B-D). Overall, the qualitative analysis of the images revealed a similar effect of $Ca(OH)_2$ solution and KP treatments, while L18R appeared as the most effective in reducing both the total biomass and the viability of the biofilm.

Discussion

The persistence of *E. faecalis* in the root canal system after conventional decontamination is considered a possible cause of endodontic treatment failure (22). Compared to other endodontic pathogens found in infected root canals, this Gram-positive facultative anaerobic species showed some resistance to sodium hypochlorite (23), a commonly used endodontic irrigant, and proved to be less susceptible to calcium hydroxide (24, 25), which is widely employed as intracanal medication. Since *E. faecalis* ability to form biofilm on root canal surfaces contributes to its resistance, the search for new alternative antibacterial substances able to inhibit *E. faecalis* both in planktonic and biofilm form is encouraged.

The present study evaluated *in vitro* the effects of two synthetic immunoglobulin-derived peptides against *E. faecalis* ATCC 29212. The peptide L18R resulted more effective than KP against the bacterium, both in planktonic and biofilm form. In particular, the EC_{50} of L18R against planktonic cells resulted 10-fold lower as compared to KP. Moreover, L18R exhibited stronger inhibition of biofilm formation as compared to KP. The effect of both peptides were also evaluated in a model of established dental infection consisting of a 2 day-old *E. faecalis* biofilm grown on HA disks in comparison to the effect of a saturated $Ca(OH)_2$ endodontic dressing solution.

The qualitative analysis of CLSM images demonstrated that both KP and L18R were able to impair the biofilm structure, reducing the number of attached and viable cells. The partial resistance of *E. faecalis* to $Ca(OH)_2$, as reported by other authors (24, 25), was confirmed. L18R was associated to the highest proportion of dead cells and to the lowest cell density compared to the other experimental groups, demonstrating a strong detaching effect of the peptide.

The mechanism of KP antibacterial activity is still not fully understood. In previous studies on yeasts, it was demonstrated that the physico-chemical properties of KP and its interaction with superficial cell-wall glucan-like structures were at the basis of the antifungal effects (17). It is possible to speculate that interactions with the surface of the bacterial cells may be involved in the antimicrobial action of the peptide. For L18R, an interaction with the cell membrane of yeasts followed by direct penetration via an energy independent pathway was hypothesised and made plausible by the hydrophobic face of the peptide (18).

As demonstrated in previous studies (17-19) an important feature of KP and L18R is the low toxicity, that could be partially explained by the cationic property of their molecules. Indeed, the peptides can easily interact with the negatively charged membranes of bacterial cells, while do not bind to the zwitterionic membranes of mammalian cells (26). This feature may represent an important advantage over other treatments, such as $Ca(OH)_2$, for which a certain degree of toxicity against eukaryotic cells was demonstrated (27).

The results reported in this study are the first investigations on KP and L18R effects against an endodontic pathogen. Prior to consider the use of these peptides as a strategy for endodontic infection control, it will be necessary to evaluate the effects against other microorganisms and employ multi-species biofilm models for a better simulation of the clinical scenario.

Conclusions

Both KP and L18R demonstrated marked inhibitory abilities against planktonic cells and biofilms of the endodontic pathogen *E. faecalis* in the experimental conditions adopted. L18R showed better performances as compared to KP and its possible role as endodontic disinfectant should be further investigated.

Clinical Relevance

For their antibiofilm activity, AMPs may be promising agents for root canal infection control in the future.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

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References

1. Østavik D, Pitt Ford T. Apical periodontitis: microbial infection and host responses. In: Ørstavik D, Pitt Ford T, ed. Essential Endodontology Prevention and Treatment of Apical Periodontitis, (2007), 2nd edn; pp. 179–191. London, UK: Wiley-Blackwell.
2. Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981;89:321-8.
3. Siqueira JF Jr, Machado AG, Silveira RM et al. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of Enterococcus faecalis from the root canal, in vitro. Int Endod J 1997;30:279-82.
4. Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985;18:35-40.
5. Estrela C, Holland R. Calcium hydroxide: study based on scientific evidences. J Appl Oral Sci 2003;11:269-82.
6. Siqueira JF Jr, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J 1999;32:361-9.
7. Wang JD, Hume WR. Diffusion of hydrogen ion and hydroxyl ion from various sources through dentine. Int Endod J 1988;21:17-26.
8. Siqueira JF Jr, de Uzeda M. Disinfection by calcium hydroxide pastes of dentinal tubules infected with two obligate and one facultative anaerobic bacteria. J Endod 1996;22:674-6.
9. Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod 2002;28:689-93.
10. Stuart CH, Schwartz SA, Beeson TJ et al. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32:93-8.
11. Marr AK, Gooderham WJ, Hancock RE. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Curr Opin Pharmacol 2006;6:468-72.
12. Wimley WC, Hristova K. Antimicrobial peptides: successes, challenges and unanswered questions. J Membr Biol 2011;239:27-34.
13. Ageitos JM, Sanchez-Perez A, Calo-Mata P et al. Antimicrobial peptides (AMPs): ancient compounds that represent novel weapons in the fight against bacteria. Biochem Pharmacol 2017;133:117-38.
14. Rogers LA, Whittier EO. Limiting factors in the lactic fermentation. J Bacteriol 1928;16:211-29.
15. Tjabringa GS, Rabe KF, Hiemstra PS. The human cathelicidin LL-37: a multifunctional peptide involved in infection and inflammation in the lung. Pulm Pharmacol Ther 2005;18:321-7.
16. Polonelli L, Magliani W, Ciociola T et al. From Pichia anomala killer toxin through killer antibodies to killer peptides for a comprehensive anti-infective strategy. Antonie Van Leeuwenhoek 2011;99:35-41.
17. Magliani W, Conti S, Ciociola T et al. Killer peptide: a novel paradigm of antimicrobial, antiviral and immunomodulatory auto-delivering drugs. Future Med Chem 2011;3:1209-31.
18. Polonelli L, Ciociola T, Sperinde M et al. Fungicidal activity of peptides encoded by immunoglobulin genes. Sci Rep 2017;7:10896.
19. Giovati L, Santinoli C, Mangia C et al. Novel activity of a synthetic decapeptide against Toxoplasma gondii tachyzoites. Front Microbiol 2018;9:753.
20. Ciociola T, Giovati L, Giovannelli A et al. The activity of a mammalian proline-rich peptide against Gram-negative bacteria, including drug-resistant strains, relies on a nonmembranolytic mode of action. Infect Drug Resist 2018;11:969-79.
21. Lagori G, Fornaini C, Rocca JP et al. Use of photo-Fenton's reaction by 400-nm LED light for endodontic disinfection: a preliminary in vitro study on Enterococcus faecalis. J Photochem Photobiol B 2017;171:85-9.
22. Sundqvist G, Figdor D, Persson S et al. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:86-93.
23. Rosen E, Tsesis I, Elbahary S et al. Eradication of Enterococcus faecalis biofilms on human dentin. Front Microbiol 2016;7:2055.
24. Bystrom A, Claesson R, Sundqvist G. The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. Endod Dent Traumatol 1985;1:170-5.
25. Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol 1990;6:142-9.
26. Matsuzaki K. Control of cell selectivity of antimicrobial peptides. Biochim Biophys Acta 2009;1788:1687-92.
27. Ehlinger C, Dartevelle P, Zaet A et al. A new combination with D-cateslytin to eradicate root canal pathogens. Int J Pept Res Ther 2019;25:1679-87.