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Original Article

Antimicrobial activity of cationic peptides in endodontic procedures

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ABSTRACT

Objectives: The present study aimed to investigate the antimicrobial and biofilm inhibition activity of synthetic antimicrobial peptides (AMPs) against microbes such as *Enterococcus faecalis, Staphylococcus aureus*, and *Candida albicans* which are involved in endodontic infections. **Materials and Methods:** Agar diffusion test was done to determine the activity of peptides. The morphological changes in *E. faecalis* and reduction in biofilm formation after treatment with peptides were observed using scanning electron microscope. The efficacy of peptides using an *ex vivo* dentinal model was determined by polymerase chain reaction and confocal laser scanning microscopy. Platelet aggregation was done to determine the biocompatibility of peptides. **Results:** Among 11 peptides, two of the amphipathic cationic peptides were found to be highly active against *E. faecalis, S. aureus*, *C. albicans*. Efficacy results using dentinal tubule model showed significant reduction in microbial load at 400 µm depth. The peptides were also biocompatible. **Conclusion:** These results suggest that synthetic AMPs have the potential to be developed as antibacterial agents against microorganisms involved in dental infections and thus could prevent the spread and persistence of endodontic infections improving treatment outcomes and teeth preservation.

Key words: Antimicrobial peptides, biocompatibility, endodontic treatment, minimal inhibitory concentration, high pressure liquid chromatography, molecular weight

INTRODUCTION

The emergence of pathogenic bacterial resistance to available conventional antibiotics is a major threat in treatment of infectious diseases.[1] It is known that more than 70% of the clinical isolates of dental infection are resistant to existing antibiotics.[4] The resistance developed by bacteria is mainly due to alteration of target site, inactivation of drugs, or changes in the metabolic pathway. Peptides comprising about or less than 50 amino acids and possessing a net positive charge are grouped as AMPs. An overall positive charge in AMPs is due to the presence of related large numbers of lysine and arginine residues.[7] AMPs exhibit a broad spectrum of antimicrobial activity similar to that of naturally occurring peptides and hence resistance to such agents is relatively rare[18,8] Synthetic AMPs also offer the flexibility for inclusion of unnatural amino acids such as α,β didehydrophenylalanine (ΔPhe), D-amino acids, aminoisobutyric acid (Aib), N-methylated amino acids during the synthesis and such modifications leads to better stability of these peptides toward proteolysis. AMPs with modified amino acids are thus reported to have enhanced antimicrobial efficacy for potential clinical application.[6,19]

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Endodontic infections resulting in tooth decay are characterized by the presence of dental plaque and biofilm comprising multiple species of microbes.[11] Several studies have shown that microorganisms which are involved in biofilm evades the host defence and also withstands the antimicrobial chemotherapy.[29] Hence, search for new agents which will eradicate those microbes becomes very essential. The current treatment of eradicating microbes in endodontic infection involves irrigating the root canal with powerful irrigants like chlorhexidine (CHX) and sodium hypochlorite (NaOCl).[10] These agents, owing to their chemical properties, can damage surrounding tissues over long residence in root canal.[26,27] Several reports have shown that despite the use of such strong antiseptics, treatment failures occur. Furthermore, only 33% of teeth refilled had showed endodontic success with a 24-77% of root canal treatment failure cases. Among several described reasons for the failure of conservative treatment models using the powerful irrigants includes their above said toxicity resulting in improper cleaning of root canal; anatomic complexity preventing irrigation of root canal in its entirety leaving resident bacteria in root canal for reinfection.[3] As *Enterococcus faecalis*, the predominant microbe in 4-40% of endodontic infections can also penetrate deep into the tubule,[20,28] its complete removal from root canal is expected to provide good results in combating majority of root canal infection.

The main advantage of AMPs resides in the mechanism of their action,[5] which is different from that of conventional antibiotics currently used in endodontic treatment.[15] As AMPs demonstrate enhanced antimicrobial activity by disrupting bacterial membrane integrity along with attacking cytoplasmic targets,[13,26] we hypothesized that AMPs could probably be a better agent in eradicating resident bacteria thereby preventing the possibility of re infection. Specifically with better tissue diffusion, we hypothesized that AMPs could kill *E. faecalis* at low μM concentration in a short period of time and also lack of microbial resistant mechanisms is an added advantage.[2,23]

In the present work, we synthesized eleven AMPs and screened them for activity against a panel of organisms such as *E. faecalis*, *Staphylococcus aureus*, *Streptococcus spp., Candida albicans* which are reported in endodontic infections. Results showed that VSL2 and VS2 possessed significant activity against the microbe particularly *E. faecalis.* These peptides also proved to be biocompatible. The killing kinetics and the analysis of the mechanism of action of these chosen peptides against the target organism (*E. faecalis)* demonstrated significant and fast efficacy.

MATERIALS AND METHODS

Peptide synthesis

The peptides were synthesized using standard Fmoc (9-fluorenylmethoxycarbonyl) chemistry on rink amide MBHA (4-methylbenzhydrylamine hydrochloride salt) resin with DIPCDI (Diisopropylcarbodiimide) and HoBT (Hydroxybenzotriazole) as coupling agents. Fmoc group was removed by 20% piperidine in DMF (Dimethyl formamide). The coupling of amino acids and deprotection was monitored by Kaiser test. Once the coupling of amino acid was completed the amino termini of the peptide was acetylated using 20% acetic anhydride in dichloromethane (DCM); the resin was washed with DMF, methanol, DCM, and dried in a desiccator. Peptides were cleaved finally from the resin by stirring in a cleavage mixture containing 95% trifluoroacetic acid (TFA), 2.5% H_20 and 2.5% triisopropylsilane for 3h and TFA was evaporated, using high vacuum followed by precipitation of peptides by adding cold dry ether. Then the ether was filtered through a sinter funnel and the peptides were dissolved in 10% acetic acid and lyophilized. Same methodology was adopted to synthesize and characterize all the peptides.[18]

Purification and characterization of peptide

Crude peptides were purified by reverse-phase high-performance liquid chromatography on a Deltapac C18 column (15-μm inside diameter [i.d.], 300 by 19 mm) using an acetonitrile-water linear gradient of 5-65% acetonitrile (0.1% TFA)-water (0.1% TFA) with a flow rate of 5 mL/min for 60 min, with detection at 214 and 280 nm. The purified peptides were reinjected into an analytical reversed-phase C18 column (Phenomenex; C18; 5-μm i.d; 250 by 4.6 mm) using an acetonitrile-water linear gradient of 5 to 65% acetonitrile (0.1% TFA)-water (0.1% TFA) at a flow rate of 1 mL/min over 60 min and were found to be 98% pure. Peptide mass was confirmed by electro spray ionization mass spectrometry at International Centre for Genetic Engineering and Biotechnology, New Delhi, India.

Bacterial strains and growth

Microbial strains were obtained from clinical laboratory at Sri Ramachandra University. *E. faecalis*, *S. aureus*, *Streptococcus spp.,* were maintained in brain heart infusion medium and *C. albicans* in sucrose dextrose medium at 37° under aerobic condition.

Agar diffusion test

The microbial suspension (*E. faecalis*, *S. aureus*, *Streptococcus spp., and C. albicans)* were swabbed on the agar plate and uniform wells were drilled on the agar plate to load the peptides of varying concentrations (10, 30, 50, and 100 μM) and incubated at 37°C for 24 h to observe the zone of inhibition. Ampicillin served as positive control. Eleven peptides were screened to determine their antibacterial activity.

Morphological changes and biofilm assay by scanning electron microscopy

E. faecalis was grown on Whatman No: 1 filter paper strips[31] and incubated overnight at 37°C. After incubation the stripes were treated with peptides at their MICs for 24 h and followed by immersion in neutralizing broth to stop the action of peptides. The strips were dried and coated with gold. The observations were made on supra 55, Carl Zeiss scanning electron microscope.

Quantitative assessment of bacterial viability in dentin by confocal laser scanning microscopy

The dentin block with a length of 4 mm was horizontally sectioned from each tooth at 1mm below the cementoenamel junction. The root canals inside the blocks were enlarged to a size of gates glidden drill #6. Each dentin blocks were fractured by making a thin groove in the middle of the specimen by using low speed hand piece with a small bur and then the specimens were fractured by a blade and hammer into semicylindrical halves. The processed dentin was infected with *E. faecalis* for 7 days. The contaminated specimens were washed with sterile water and were randomly divided into four groups: CHX, VSL2, VS2 and untreated. The specimens were disinfected with each drug at their MIC concentrations and incubated for 24 hours at 37°C. After the disinfection the dentin specimens were stained with fluorescein diacetate (FDA) which emits green fluorescence in live cells and propidium iodide (PI) emits red fluorescence in dead cells whose membranes are disrupted and binds to deoxyribonucleic acid (DNA). The uninfected specimens were stained under the same protocol and used as negative control. Fluorescence from the stained cells were viewed by using confocal laser scanning microscopy (CLSM) and by staining with FDA and PI the % of live and dead cells were computed. *Post hoc* multiple comparisons were used to compare the results at a significance level of $P > 0.05$.

Quantification of microbial load on *ex vivo* dentinal tubule model by real-time polymerase chain reaction

Preparation of tooth samples

Freshly extracted central, tooth samples were collected (IEC approval No: IEC-NI/09/DEC/13/37) and immersed in 5.25% sodium hypochlorite to remove soft surface tissues and organic debris. The tooth samples were horizontally sectioned into coronal, middle, and apical sections using a carbide disc.[12] The root canal of each specimen was enlarged with a No: 10 round bur. The smear layer including organic and inorganic debris was removed by placing the samples in an ultrasonic bath containing 17% ethylenediaminetetraacetic acid, followed by rinsing with 5.25% NaOCl each for 5 min. The processed tooth samples were then sterilized by autoclaving at 121°C for 30 min.

Contamination of blocks

Sterilized tooth samples were immersed in *E. faecalis* suspension and incubated for 7 days at 37°C. Samples which were not incubated with organisms served as negative control to confirm the sterility of samples. Medium was changed every day. After incubation, the samples were gently washed with saline to remove the excess culture from the surface of the sample. Subsequently, 10 samples were used for each group and the inoculated samples were grouped as follows:

Group 1: Tooth samples treated with peptides

Group 2: Tooth samples treated with positive control (vancomycin)

Group 3: Tooth samples without any treatment served as negative control

Group 4: Tooth samples treated with positive control (2% CHX).

After the incubation, the dentinal chips were harvested at 400 μm depth and the microbial load was quantified using real-time (RT) polymerase chain reaction (PCR) from the chips.

Real-time PCR

DNA was isolated from the harvested dental chips by phenol chloroform isoamyl alcohol method. All the PCR reactions were done in 20 μl on detection system. Microbial load of *E. faecalis* present in dentinal tubule was determined using real time PCR (ABI 7900 HT). The assay is based on the detection of the 16s rRNA gene. Universal 16s rRNA primers were used for the amplification. The forward primer (5′ GATTAGATACCCTGGTAGTCC 3′) reverse primer (5′ CCCGGGAACGTATTCACCG 3′) sequence were used for quantification of

E. faecalis. The assay was performed by using SYBR green.[14] The following PCR conditions were adopted to amplify target: To amplify the product, 50°C for 2 min, 95°C for 10 min, 95°C for 15 s, and 60°C for 1 min for 35 cycles. The efficacy of the compounds against *E. faecalis* was calculated based on the cycle threshold (CT) value.[30]

Percentage of reduction = CT value of treated sample/ CT value of untreated Sample ×100

Platelet aggregation assay

Human blood sample from healthy volunteer was obtained in vacutainers containing the anticoagulant sodium citrate (IEC approval No: IEC-NI/09/ DEC /13/37). The citrated blood was centrifuged at 1000 rpm for 10 min and the supernatant was again centrifuged at 4000 rpm for 10 min to get platelet-rich plasma (PRP). 100 µL of PRP was treated with peptides (VSL2 and VS2) of varying concentrations (10, 50, and 100 µM). Collagen served as positive control.[16,32]

Statistical analysis

Comparison between treatments groups were conducted using the Kruskal-Wallis/analysis of variance analysis of variance on rank. *Post hoc* multiple comparison was done using Student-Newman-Keuls test. Comparison within each group was conducted by using Wilcoxon signed-rank test. Values of $P > 0.05$ were considered to be not significant.

RESULTS

Zone inhibition assay carried out by agar diffusion test

Zone inhibition assay carried out on agar plates for 24 h showed that out of the 11 peptides screened [Table 1], only two peptides, VSL2 and VS2, showed high activity against *E. faecalis*, *S. aureus S. spp., and C. albicans*, implicated in endodontic treatment failure shown in Figure 1. The MIC for both the peptides was found to be 10 µM. The standard deviations of the zone size of both the peptides against various organisms are shown in Table 2 and the sequence of peptides in Table 3.

Determination of the effect of peptides on microbe biofilm formation and cell lysis as observed by electron microscopy

Biofilm assay showed that treatment with the AMPS (VSL2 and VS2) lead to significant reduction in microbial load as compared with the untreated control. SEM analysis showed that peptide treatment significantly decreased the degree of attachment of viable bacterial cells to matrix (Whatman paper) demonstrating their potency to inhibit biofilm formation. The morphological changes in the organisms are shown in Figure 2 and the reduction in biofilm formation is shown in Figure 3.

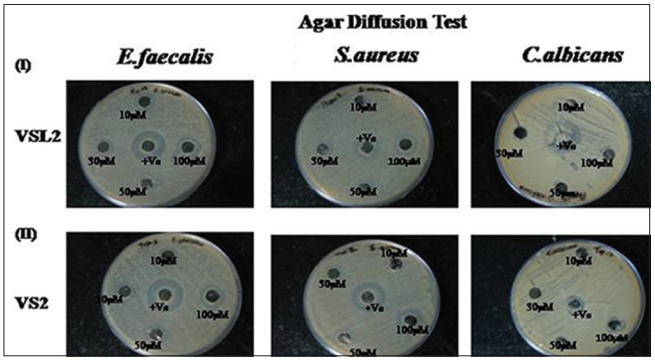

Figure 1: (I) Confocal laser scanning microscopy of Enterococcus faecalis infected dentinal tubules treated by different antibacterial agents after viability staining (a) Untreated control, (b) Chlorhexidine, (c) VSL2, and (d) VS2; (II) The percentage of live and dead cells distributed in dentinal tubule

Table 1: Screening activity of peptides on various organisms

Peptide/organism	*E. faecalis*	*S. aureus*	*E. coli*	*P. mirabilis*	*S. typhi*	*A. baumannii*	*C. albicans*
VSL2	+	+	+	-	-	-	+
VS2	+	+	+	-	-	-	+
TrpΔPhe	-	-	-	-	-	-	-
ArgΔPhe	-	-	-	-	-	-	-
Pep-1	-	-	-	-	-	+	-
Pep-2	-	-	-	-	-	-	-
Pep-3	-	-	-	-	-	-	-
Pep-4	-	-	-	-	-	-	+
Pep-6	-	-	-	-	-	-	-
Pep-7	-	-	-	-	-	-	-
Pep-8	-	-	-	-	-	-	-

A.baumannii: Acinetobacter baumannii, S.typhi: E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, P. mirabilis: Proteus mirabilis, S. aureus: Staphylococcus aureus, C. albicans: Candida albicans (- indicates no activity and + indicates activity)

Quantitative assessment of bacterial viability in dentin by CLSM

The efficacy of AMPS against *E. faecalis* penetrated into the dentinal tubules were assessed by CLSM [Figure 4a]. The results shows 58% dead cells in VSL2 treated tooth samples and 70% dead cells after VS2 treatment when compared with untreated samples which showed 6% of dead cells [Figure 4b]. The antibacterial effect of all the compounds was not statistically different from each other ($P > 0.05$).

Quantification of microbial load on *ex vivo* dentinal tubule model by real-time PCR

Antibacterial activity of VSL2 and VS2 tested against *E. faecalis* in dentinal tubule model by real-time PCR. The activity of peptides was comparable to vancomycin and the peptides high inhibitory activity at 400 µm after 24 h of treatment. VSL2 showed 97%, vancomycin and VS2 showed 98% reduction, and CHX showed 99% of reduction of *E. faecalis* after 24 h treatment.

Platelet aggregation assay

Platelet aggregation assay revealed that the AMPs did not cause aggregation of platelets even at 10 × MICs (100 µM) [Figure 5] and hence they are biocompatible.

DICSUSSION

Endodontic treatment involves eradication of microbes invading the root canal and thereby teeth conservation. Use of high concentration irrigants and packaging of medicaments into the root canal are the two main modes of treatments of root canal infection. Irrigants usage is limited due to their toxicity profile. Calcium hydroxide, the most widely used intracanal medicament, leads to incomplete eradication of the infecting microbes causing reinfection and tooth decay. Medicaments that are used commonly for root canal treatment also get distributed to the whole body from the root apex and hence may cause allergic

Table 2: Zone of inhibition of peptides against *Enterococcus faecalis, Staphylococcus aureus,* and *Candida albicans*

Peptide concentration (µM)	Mean±SD of zone of inhibition in mm		
	E. faecalis	*S. aureus*	*C. albicans*
VSL2			
10	10±0.57	0±0	0±0
30	12±0	10±0.57	0±0
50	11±0.57	12±0.57	0±0
100	14±0.57	10±0	8±1.73
VS2			
10	11±0	8±1.73	0±0
30	12±0.57	10±0.57	0±0
50	12±1.15	12±0	0±0
100	14±1.15	14±1.15	9±0

SD: Standard deviation

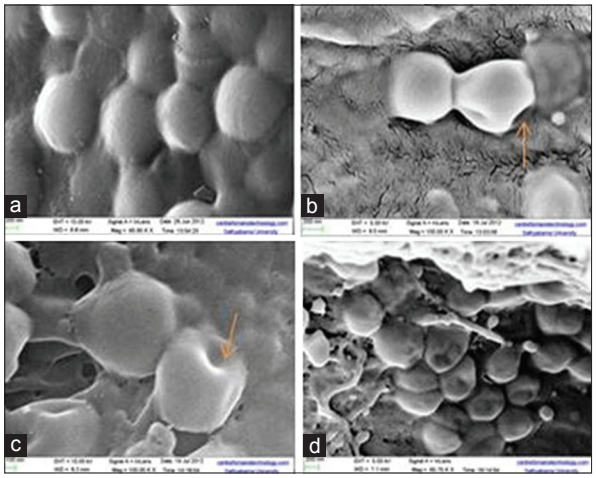

Figure 2: The zone of inhibition in the agar diffusion assay indicated the microbial susceptibility to the drugs in the VSL2 treated plates (Figure 1) and VS2 (Figure 2)

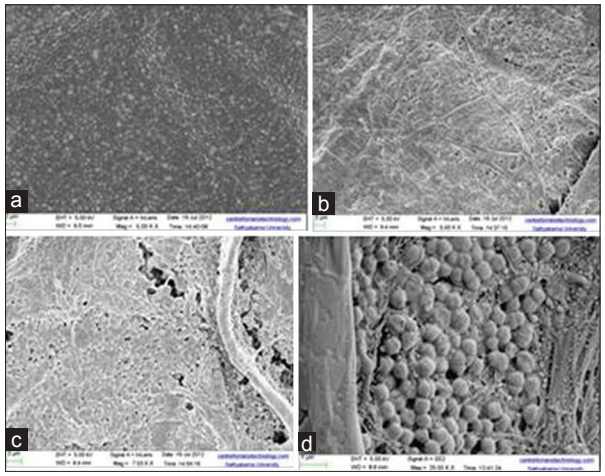

Figure 3: Morphological changes (indicated by arrows) were observed when microbes treated with peptides VSL2 (c) and VS2 (d) also with ampicillin treated (b) as compared with untreated control (a). Arrows indicates the site of damage in the cell membrane at the magnification of 100 KX

Table 3: Amino acid sequence of the designed antimicrobial peptides

Peptide	Amino acid sequence	Length (residue)	HPLC (RT in min)	MW (calculated)	MW (observed)
VSL2	Ac-A-ΔF-K-A-ΔF-W-K-ΔF-V-K-ΔF-V-K-NH2	13	30	1679.8	1679
VS2	Ac-K-W-ΔF-W-K-ΔF-V-K-ΔF-V-K-NH2	11		1577.6	1575

HPLC: High performance liquid chromatography, MW: Molecular weight, RT: Retention time

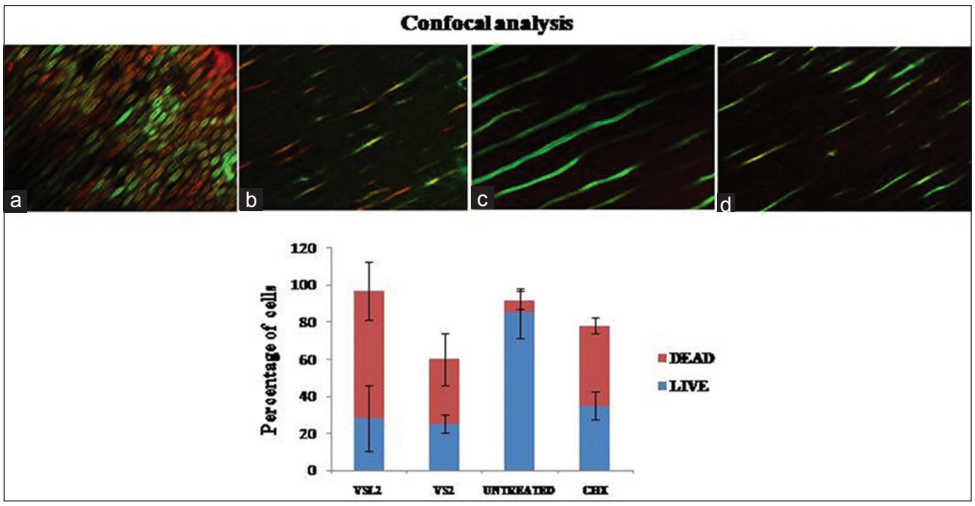

Figure 4: Efficacy of AMPs in reducing biofilm formation by Enterococcus faecalis. Biofilm formation was reduced after treatment with ampicillin, VSL2 and VS2 (b-d) when compared with the untreated control (a) at the magnification of 10 KX

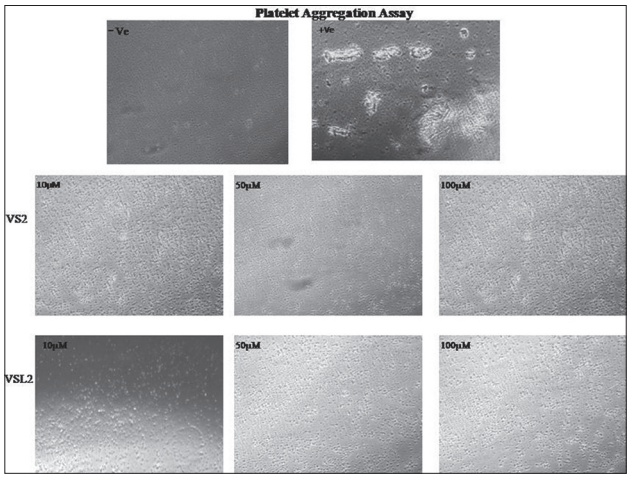

Figure 5: Platelet aggregation property of peptides at MIC 10 µM. Platelet aggregating property of peptides were determined by incubating platelet rich plasma with the AMPs at three different concentrations (10, 50, and 100 µM). Collagen served as positive control

reactions. Furthermore, few medicaments are potent carcinogens and hence use of such medicaments is prohibited in modern endodontic treatments.[25] Based on these observations from clinics, we tested our hypothesis that AMPs owing to their peptidic nature and hence biocompatibility could serve as useful intracanal medicament for eradication of microbes from infected root canal and hence (AMPs) could be one of the best possible alternatives.[17,22]

We showed that designed AMPs could serve as useful intracanal medicament and eradicate *E. faecalis* at 400 µm depth. Our initial screening study showed that out of 11 peptides tested, two demonstrated significant activity against *E. faecalis* the most important pathogen involved in root canal infections. These peptides (VSL and VS2) showed activity against *E. faecalis*, *S. aureus*, *and C. albicans* but not against *Streptococcus* which was confirmed thrice by repeating the experiments. Recent studies on vancomycin resistant *Streptococcus* have shown the presence of vanB gene in clinical isolates to be responsible; probably, these factors could be a reason for null activity of our peptides on *Streptococcus*.[33] Biofilm is a structured consortium of bacteria embedded in a self-produced polymer matrix consisting of polysaccharide, protein and DNA[9] and this biofilm reduction as determined by SEM showed that peptides prevented the aggregation of *E. faecalis*. This assay is significant since formation of biofilm, an important causative factor in dental infection was inhibited by the tested AMPs. The morphological changes documented by SEM analysis revealed that microbe death by cell membrane damage could probably be the major mechanism of action for the given antibacterial peptides. Similar observations were also reported earlier for other AMPs.[21,24] The efficacy of AMPS against the organisms invading the root canal was also proved by CLSM.

Ex vivo study on dentinal tubule model showed a drastic reduction in microbial load at different depths in root canal. Real-time PCR for 16s rRNA quantification asserted the efficacy of the AMPs as an intracanal medicament. We also observed that the AMPS needed to be in place for only 12 h, beyond which permanent dentures could be affixed, showing high efficacy and fast response of the medicament. Since our intention is to develop a safe intracanal medicament which can eradicate root canal pathogens, we also studied biocompatibility of the AMPs and these peptides did not cause platelet aggregation.

The cytotoxicity of these peptides have already been tested against human cell lines and both the peptides were found to be non-cytotoxic.[18] Taken together, these AMPs were not cytotoxic and also did not cause platelet aggregation.

CONCLUSION

The AMPs VSL2 and VS2 showed potent activity against *E. faecalis* majorly involved in root canal infections. The AMPS were also found to be biocompatible. All these properties prove that these AMPs can be developed as worthy candidates to treat endodontic infections.

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REFERENCES

1. Andersson DI, Hughes D. Antibiotic resistance and its cost: Is it possible to reverse resistance? Nat Rev Microbiol 2010;8:260-71.
2. Batoni G, Maisetta G, Brancatisano FL, Esin S, Campa M. Use of antimicrobial peptides against microbial biofilms: Advantages and limits. Curr Med Chem 2011;18:256-79.
3. 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.
4. Coates AR, Hu Y. Targeting non-multiplying organisms as a way to develop novel antimicrobials. Trends Pharmacol Sci 2008;29:143-50.
5. Feder R, Dagan A, Mor A. Structure-activity relationship study of antimicrobial dermaseptin S4 showing the consequences of peptide oligomerization on selective cytotoxicity. J Biol Chem 2000;275:4230-8.
6. Fernandez-Lopez S, Kim HS, Choi EC, Delgado M, Granja JR, Khasanov A, *et al*. Antibacterial agents based on the cyclic D, L-alpha-peptide architecture. Nature 2001;412:452-5.
7. Hancock RE, Brown KL, Mookherjee N. Host defence peptides from invertebrates: Emerging antimicrobial strategies. Immunobiology 2006;211:315-22.
8. Hancock RE, Chapple DS. Peptide antibiotics. Antimicrob Agents Chemother 1999;43:1317-23.
9. Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int J Antimicrob Agents 2010;35:322-32.
10. Kawashima N, Wadachi R, Suda H, Yeng T, Parashos P. Root canal medicaments. Int Dent J 2009;59:5-11.
11. Kolembrander PE. Multispecies communities: Interspecies interactions influence growth on saliva as sole nutritional source. Int J Oral Sci 2011;3:49-54.
12. Krause TA, Liewehr FR, Hahn CL. The antimicrobial effect of MTAD, sodium hypochlorite, doxycycline, and citric acid on Enterococcus faecalis. J Endod 2007;33:28-30.
13. Lee MT, Chen FY, Huang HW. Energetics of pore formation induced by membrane active peptides. Biochemistry 2004;43:3590-9.
14. Mirhendi SH, Makimura K. PCR detection of Candida albicans in blood using a new primer pair to diagnosis of systemic candidiasis. Iran J Public Heath 2006;32:1-5.
15. Mohan KV, Rao SS, Atreya CD. Antiviral activity of selected antimicrobial peptides against vaccinia virus. Antiviral Res 2010;86:306-11.
16. Yallapu MM, Ebeling MC, Chauhan N, Jaggi M, Chauhan SC. Interaction of curcumin nanoformulations with human plasma protein and erythrocytes. Int J Nanomedicine 2011;6:2779-90.
17. O'Toole G, Kaplan HB, Kolter R. Biofilm formation as microbial development. Annu Rev Microbiol 2000;54:49-79.
18. Pathak S, Chauhan VS. Rationale-based, de novo design of dehydrophenylalanine-containing antibiotic peptides and systematic modification in sequence for enhanced potency. Antimicrob Agents Chemother 2011;55:2178-88.
19. Reddy KV, Yedery RD, Aranha C. Antimicrobial peptides: Premises and promises. Int J Antimicrob Agents 2004;24:536-47.
20. Rôças IN, Siqueira JF Jr, Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod 2004;30:315-20.
21. Straus SK, Hancock RE. Mode of action of the new antibiotic for Gram-positive pathogens daptomycin: Comparison with cationic antimicrobial peptides and lipopeptides. Biochim Biophys Acta 2006;1758:1215-23.
22. Strøm MB, Haug BE, Skar ML, Stensen W, Stiberg T, Svendsen JS. The pharmacophore of short cationic antibacterial peptides. J Med Chem 2003;46:1567-70.
23. Tao R, Tong Z, Lin Y, Xue Y, Wang W, Kuang R, *et al*. Antimicrobial and antibiofilm activity of pleurocidin against cariogenic microorganisms. Peptides 2011;32:1748-54.
24. Vaara M. Agents that increase the permeability of the outer membrane. Microbiol Rev 1992;256:395-411.
25. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002;415:389-95.
26. Ringel AM, Patterson SS, Newton CW, Miller CH, Mulhern JM. *In vivo* evaluation of chlorhexidine gluconate solution and sodium hypochlorite solution as root canal irrigants. J Endod 1982;8:200-4.
27. Pashley EL, Birdsong NL, Bowman K, Pashley DH. Cytotoxic effects of NaOCl on vital tissue. J Endod 1985;11:525-8.
28. Williams JM, Trope M, Caplan DJ, Shugars DC. Detection and quantitation of E. faecalis by real-time PCR (qPCR), reverse transcription-PCR (RT-PCR), and cultivation during endodontic treatment. J Endod 2006;32:715-21.
29. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet 2001;358:135-8.
30. Endo MS, Ferraz CC, Zaia AA, Almeida JF, Gomes BP. Quantitative and qualitative analysis of microorganisms in root-filled teeth with persistent infection: Monitoring of the endodontic retreatment. Eur J Dent 2013;7:302-9.
31. Chai WL, Hamimah H, Cheng SC, Sallam AA, Abdullah M. Susceptibility of Enterococcus faecalis biofilm to antibiotics and calcium hydroxide. J Oral Sci 2007;49:161-6.
32. Weber M, Gerdsen F, Gutensohn K, Schoder V, Eifrig B, Hossfeld DK. Enhanced platelet aggregation with TRAP-6 and collagen in platelet aggregometry in patients with venous thromboembolism. Thromb Res 2002;107:325-8.
33. Poyart C, Pierre C, Quesne G, Pron B, Berche P, Trieu-Cuot P. Emergence of vancomycin resistance in the genus Streptococcus: Characterization of a vanB transferable determinant in Streptococcus bovis. Antimicrob Agents Chemother 1997;41:24-9.

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