Novel dentotropic antimicrobial peptide to prevent dental caries: abridged secondary publication

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KEY MESSAGES

1. GA-KR12 can be successfully synthesised with high purity (98.98%).
2. GA-KR12 is a biocompatible stable peptide with antimicrobial and remineralising properties.
3. GA-KR12 can significantly reduce the *Streptococcus mutans* mono-species biofilm and inhibit its metabolism and growth.
4. GA-KR12 prevents the demineralisation of tooth hard tissue and enhance the remineralisation of artificial caries on enamel and dentine.
5. Grafting a mineralising molecule and an antimicrobial peptide to develop a novel peptide against caries is a viable strategy.

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Introduction

Dental caries is perhaps the most prevalent chronic disease worldwide; most carious teeth are left untreated.[1] Controlling oral microbial biofilms and maintaining tooth minerals are equally important to prevent/control caries. Antimicrobial peptides are the first line of defence against infection of multicellular organisms and a potential therapeutic strategy for managing oral diseases.[2] Gallic acid is a component for inducing and accelerating mineralisation owing to its pyrogallol moiety.[3] KR12 is an ideal template peptide derived from LL-37.[4] KR12 is small size and has low toxicity and antimicrobial properties against cariogenic species. We developed a novel dentotropic antimicrobial peptide using gallic acid and KR12 and evaluated its antimicrobial effect against cariogenic biofilm and its remineralisation effect on enamel and dentine caries.

Methods

The novel dentotropic antimicrobial peptide was created by grafting gallic acid as a mineralising domain to the N-terminal of peptide KR12 as an antimicrobial domain (Fig 1). The peptide GA-KR12 was synthesised using standard fluorenylmethoxycarbonyl synthesis via standard solid-phase peptide synthesis. After the synthesis, high-performance liquid chromatography and mass spectrum and circular dichroism spectroscopy were

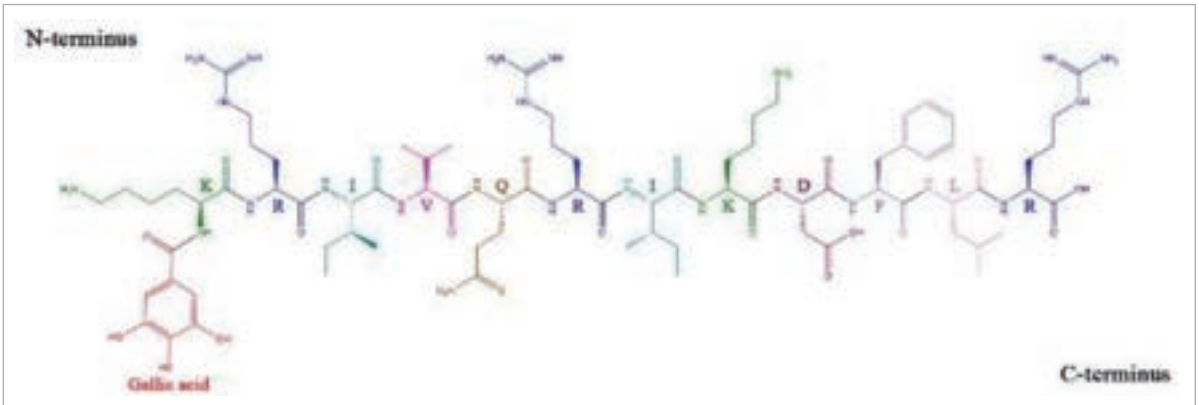

FIG 1. The structure of GA-KR12

used to evaluate the purity, molecular weight, and secondary structure of GA-KR12. Human gingival fibroblasts were used to evaluate the cytotoxicity of GA-KR12 by mitochondrial dehydrogenase activity assay. Six cariogenic species were used to evaluate the antimicrobial properties of GA-KR12 by minimum inhibitory concentration and minimum bactericidal/fungicidal concentration. The morphology of cariogenic species was analysed by transmission electron microscope. The architecture, viability, and growth kinetics of the cariogenic biofilm (*Streptococcus mutans*) were determined by scanning electron microscopy (SEM), confocal laser scanning microscopy, and culture colony-forming units (CFUs), respectively. The mineral loss, calcium-to-phosphorus ratio, surface morphology, and crystal characteristics of the enamel surface were determined by micro-computed tomography, energy dispersive spectroscopy, SEM, and X-ray diffraction, respectively. The mineral loss, changes in chemical structure, surface morphology, and crystal characteristics of the dentine surface were determined by micro-computed tomography, Fourier transform infrared, SEM, and X-ray diffraction, respectively.

TABLE. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) / minimum fungicidal concentration (MFC) of GA-KR12, KR12, and chlorhexidine against cariogenic species

Cariogenic species	GA-KR12	KR12	Chlorhexidine
Streptococcus mutans			
MIC, μM	160	320	1.25
MBC, μM	320	1280	10
Streptococcus sobrinus			
MIC, μM	320	No activity	5
MBC, μM	1280	No activity	20
Lactobacillus acidophilus			
MIC, μM	320	40	2.50
MBC, μM	1280	160	40
Lactobacillus rhamnosus			
MIC, μM	320	No activity	2.50
MBC, μM	1280	No activity	20
Actinomyces naeslundii			
MIC, μM	160	320	0.16
MBC, μM	640	1280	0.32
Candida albicans			
MIC, μM	10	10	0.16
MFC, μM	20	20	0.32

Results

GA-KR12 was successfully synthesised with high purity (98.98%). The molecular weight of GA-KR12 was 1724.04. GA-KR12 was biocompatible to human gingival fibroblast. The minimum inhibitory concentration and minimum bactericidal concentration / minimum fungicidal concentration against the tested species were 10 to 320 μM and 20 to 1280 μM, respectively (Table). GA-KR12 induced remarkable morphological defects in the tested species.

SEM showed confluent growth of *S mutans* in the water group but not in the GA-KR12-treated group (Fig 2). The live-to-dead ratios and log CFUs of the GA-KR12-treated group were lower than those of the water group. The mineral loss of the GA-KR12-treated group was lower than that of the water group. The calcium-to-phosphorus molar ratios of the GA-KR12-treated group were higher than that of the water group. A uniformly remineralised prismatic pattern on enamel blocks was observed in the GA-KR12-treated group. The hydroxyapatite on the enamel surface in the GA-KR12-treated group was better crystallised than that in the water group.

The surface of the dentine blocks *S mutans* partially covered the GA-KR12-treated group with a damaged cell structure (Fig 3). The live-to-dead ratios and log CFUs of the GA-KR12-treated group were lower than those of the water group. The mineral loss and amide I-to-hydrogen ratio of the GA-KR12-treated group were lower than that of the water group. SEM images showed that the GA-KR12-treated group had less exposed dentine collagen fibres than the water group.

Discussion

GA-KR12 is a biocompatible and stable peptide with antimicrobial and remineralising properties. It inhibits the growth of cariogenic species and promotes the remineralisation of caries on enamel and dentine. This is the first study to graft gallic acid to an antimicrobial peptide to achieve a dentotropic antimicrobial peptide. Gallic acid with a pyrogallol moiety has the same mechanism as tunicate does to induce mineralisation.[3] KR12 is an ideal template peptide because of its small size, low toxicity, and antimicrobial properties against cariogenic species.[4]

In the present study, GA-KR12 was synthesised by standard solid-phase peptide synthesis. GA-KR12 had similar percentages of the α-helical and β-sheet structures with those obtained in a previous study for KR12.[5] GA-KR12 was biocompatible to human gingival fibroblast and safe for dental use. GA-KR12 treatment changed the morphology of the bacteria and fungi cells. Because of the interaction between the negatively charged cell membrane and the

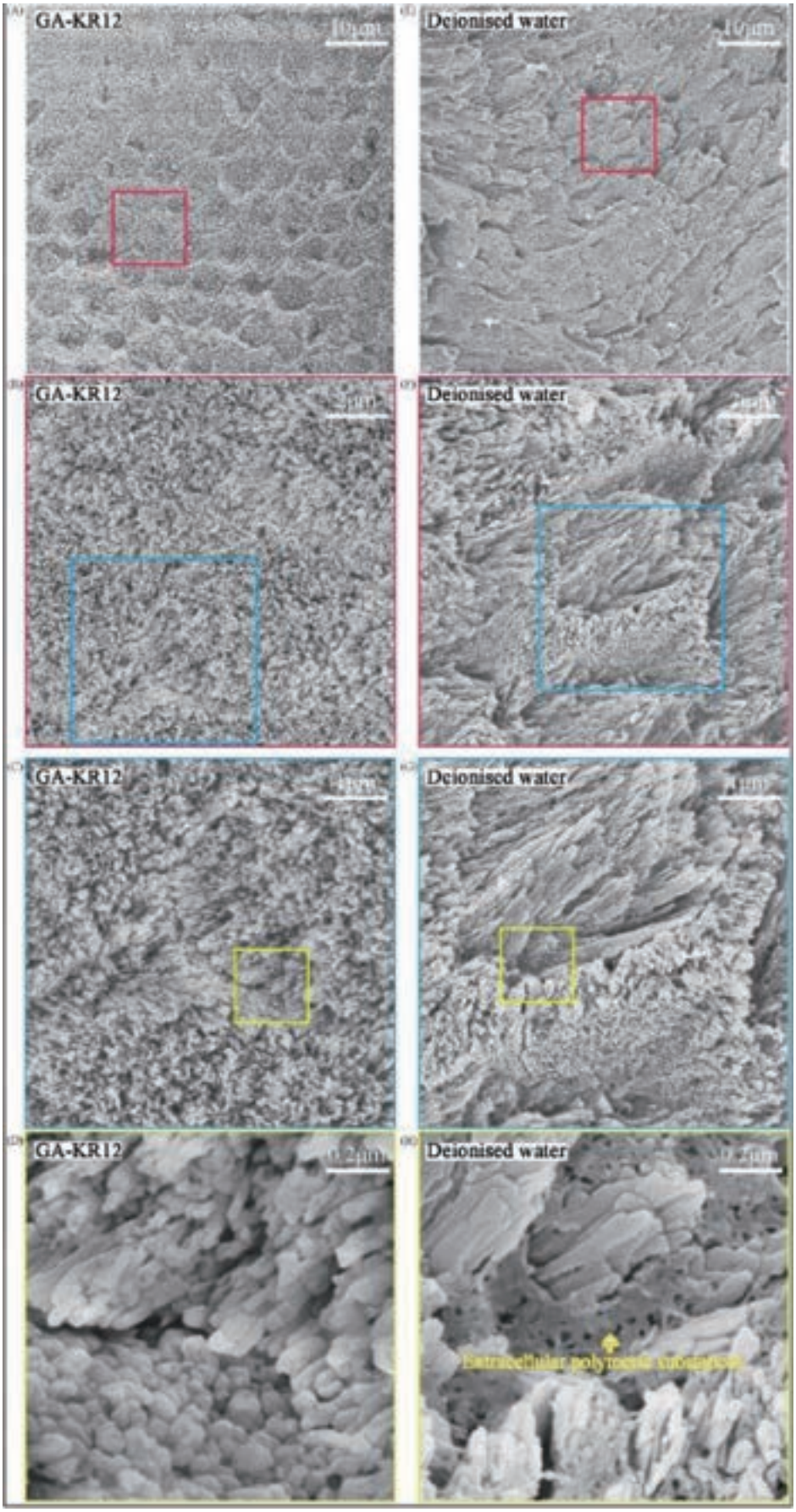

FIG 2. Scanning electron microscopy images of enamel surface morphology of the GA-KR12 and the deionised water groups (1000×, 5000×, 10 000×, and 50 000×)

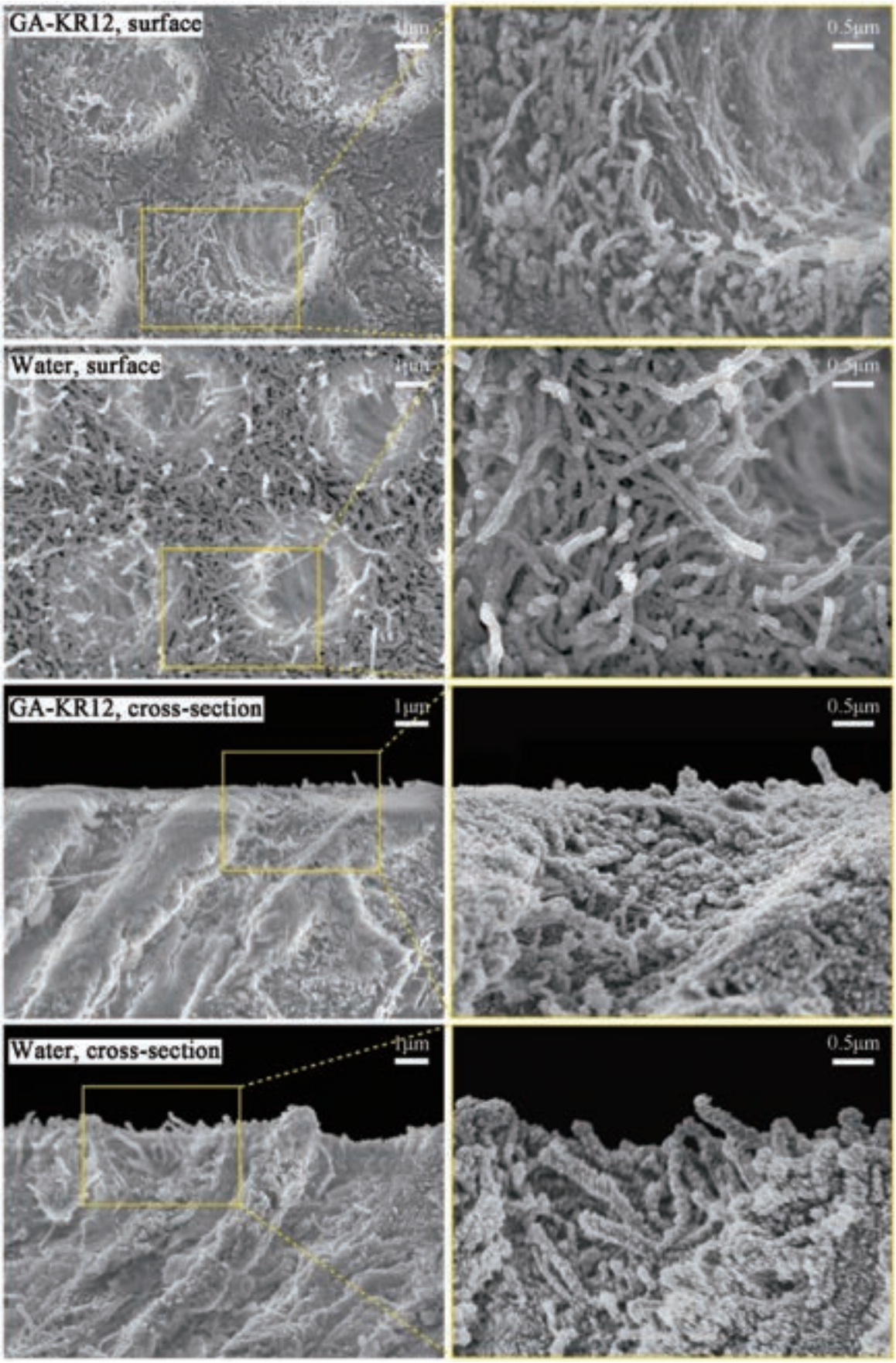

FIG 3. Scanning electron microscopy images of dentine morphology in the GA-KR12 and the deionised water groups (7000× and 20 000×)

positively charged peptide, the cells showed an irregular cell shape and an abnormal membrane curvature. Subsequently, the cell membrane was disrupted by the inserting of the peptides, which caused cytoplasmic content to be leaked. Furthermore, GA-KR12 could significantly reduce the *S mutans* mono-species biofilm and inhibit its metabolism and growth. SEM images indicated that the affluence of the *S mutans* biofilm was less in the GA-KR12-treated group. In addition, some *S mutans* cells lost their regular cell shape.

Hard tissues of the tooth include enamel and dentine. The mineral structures of enamel and dentine are different. In the present study, the remineralising effect of GA-KR12 to enamel and dentine caries was evaluated separately using an *S mutans* biofilm–remineralisation cycling model, which combined biological and chemical factors to provide periodic pH alternation and a microbiological environment for bacterial impact. GA-KR12 effectively promoted the remineralisation of both enamel and dentine caries and the formation of an extra-fibrillar mineral. The probable mechanisms could be that GA-KR12 prevent the degradation of collagen scaffold and acidic dissolution of mineral crystal by inhibition of *S mutans* biofilm. Moreover, the pyrogallol group of GA-KR12 can attract calcium ions from the remineralising solution to promote the remineralisation of the caries.

Conclusion

GA-KR12 is a biocompatible stable peptide with antimicrobial and mineralising properties. GA-KR12 can significantly reduce the *S mutans* mono-species biofilm and inhibit its metabolism and growth. GA-KR12 can prevent the demineralisation of tooth hard tissue and enhance the remineralisation of artificial caries on enamel and dentine. Grafting a mineralising molecule and an antimicrobial peptide to develop a novel peptide against caries is a viable strategy.

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Disclosure

The results of this research have been previously published in:

1. Niu JY, Yin IX, Wu WKK, Li QL, Mei ML, Chu CH. Efficacy of the dual-action GA-KR12 peptide for remineralising enamel caries: an in vitro study. Clin Oral Investig 2022;26:2441-51.
2. Niu JY, Yin IX, Wu WKK, Li QL, Mei ML, Chu CH. Remineralising dentine caries using an artificial antimicrobial peptide: an in vitro study. J Dent 2021;111:103736.

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