Research Square

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A novel antimicrobial peptide targeting porphyromonas gingivalis that can reduce inflammation in a rat periodontitis model

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Abstract

Objectives

This study aimed to develop a novel antimicrobial peptide that can inhibit the growth of Porphyromonas gingivalis (P. gingivalis) and potentially reduce periodontal inflammation in a rat ligature-induced periodontitis model.

Methods

This study introduces a novel antimicrobial peptide, PFK, designed to target P. gingivalis and its associated biofilms. We evaluated its antibacterial activity, biofilm disruption capability, safety and biocompatibility in vitro, as well as its therapeutic potential in a rat model of periodontitis. Periodontitis was induced in Sprague-Dawley rats (n = 15) via ligature placement around the mandibular first molars, combined with oral inoculation of P. gingivalis. Rats were divided into three groups: (1) untreated control, (2) treated with minocycline hydrochloride ointment Palio, and (3) treated with PFK (topical application, 10 μg/mL). Gingival tissues and alveolar bone were analyzed after 4 weeks.

Results

PFK demonstrated potent antibacterial activity against P. gingivalis and effectively disrupted biofilms in vitro. In the rat periodontitis model, topical PFK treatment reduced inflammatory cell infiltration in gingival tissues. Safety and biocompatibility assays indicated that PFK was biologically safe and exhibited low cytotoxicity.

Conclusions

These findings suggest that PFK is a promising candidate for periodontitis treatment and may address the limitations of current therapies.

1. INTRODUCTION

Periodontitis is a chronic inflammatory disease that affects the supporting structures of teeth, including the gingiva, periodontal ligament, and alveolar bone[1]. If untreated, it can lead to tooth loss and systemic complications such as cardiovascular disease and diabetes[2, 3]. *P. gingivalis* is a keystone pathogen in periodontitis, capable of disrupting the host immune response and promoting dysbiosis within the oral microbiome[4]. Current therapeutic options include mechanical debridement and the use of systemic or local antibiotics. However, the increasing prevalence of antibiotic resistance[5], combined with the protective nature of bacterial biofilms[6], limits the effectiveness of conventional treatments.

Antimicrobial peptides (AMPs) are naturally occurring or synthetic short peptides that exhibit broad-spectrum antimicrobial activity and immunomodulatory properties. Their unique mechanisms of action make them promising alternatives to conventional antibiotics and anti-inflammatory drugs in treating infections, including periodontal diseases. AMPs not only kill bacteria but also modulate host immune responses, reducing excessive inflammation. For example, human cathelicidin (LL-37) suppresses pro-inflammatory cytokines (e.g., IL-1β, TNF-α) while promoting tissue repair[7]. This dual action is particularly beneficial in chronic inflammatory conditions like periodontitis. Due to their membrane-disrupting mechanism, bacteria struggle to develop resistance against AMPs compared to conventional antibiotics (which target single enzymes or pathways). Some AMPs also inhibit biofilm formation, further reducing bacterial persistence[8].

In this study, we designed and synthesized a novel antimicrobial peptide PFK, specifically targeting *P. gingivalis*. We assessed its efficacy in vitro and in vivo, focusing on its ability to inhibit bacterial growth, disrupt biofilms, and reduce inflammation in a rat model of periodontitis.

2. MATERIALS AND METHODS

2.1. Peptide design and synthesis

Antimicrobial peptides PFK, PVK and PIK were designed based on the amphipathic structure and cationic properties known to enhance interactions with bacterial membranes. The peptide sequence was synthesized using solid-phase peptide synthesis and purified via high-performance liquid chromatography (HPLC) to > 95% purity.

2.2. Bacterial strain and culture

P. gingivalis W83 was cultured anaerobically in brain heart infusion (BHI) broth supplemented with hemin and vitamin K. Biofilm formation was induced on polystyrene plates under anaerobic conditions.

2.3. In vitro antibacterial assay

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of PFK against *P. gingivalis* were determined using a microdilution method. *P. gingivalis* stored at -80°C were inoculated on blood agar plates. *P. gingivalis* was cultured in an anaerobic environment at 37°C for 4 days. *P. gingivalis* colonies were resuspended in BHI liquid medium and the bacterial solution concentration was adjusted to about 10^5 CFU/mL for use.

Antimicrobial peptides PFK, PVK and PIK were diluted to 512 μg/mL, and the above samples (antimicrobial peptides PFK, PVK and PIK) were diluted 2 times in a gradient manner using BHI medium, for a total of 8 gradient dilutions. 50 μL of sample dilution and test bacterial solution were added to 96-well plates and cultured at 37°C for 24 h. *P. gingivalis* was cultured in an anaerobic environment at 37°C for 72 h. 10 μL of resazurin dye was added to each well of the 96-well plate to observe the MIC results.

2.4. Biofilm inhibition assay

The ability of PFK to disrupt pre-formed *P. gingivalis* biofilms was evaluated using a microtitre plate assay where biofilms are formed on the bottom of a microtitre plate and are typically stained with crystal violet to assay biofilm biomass[9]. Biofilm biomass was quantified by measuring absorbance at 570 nm.

2.5. Hemolytic toxicity test

Sterile defibrinated horse blood samples (Solarbio, TX0040) were collected in centrifuge tubes and centrifuged at 3000 rpm for 5 min to remove impurities. The cells were then washed three times with 10 mM PBS (pH 7.4), and the red blood cells were collected and diluted with 10 mM PBS (pH 7.4) to a final concentration of 4% (i.e., the red blood cell volume fraction was 4%). Thereafter, 100 μL of antimicrobial peptides of different concentrations were mixed with 100 μL of 4% red blood cells, incubated in a 37°C water bath for 30 min, and finally centrifuged at 10000 rpm for 5 min at room temperature. The absorbance of the supernatant at 540 nm was measured using a UV spectrophotometer. Red blood cells were suspended in PBS and 1% Triton X-100 as negative and positive controls, respectively. The experiment was repeated three times, and the standard deviation was calculated using the STDEV function.

The formula for calculating the hemolysis rate is: Hemolysis rate (%) = (Dt-Dnc)/(Dpc-Dnc) × 100%. Where Dt represents the absorbance of the test sample treated with different concentrations of antimicrobial peptides at 540 nm, and Dpc and Dnc refer to the absorbance of the positive control and negative control at 540 nm, respectively.

2.6. Cytotoxicity assay

Mouse lung fibroblast L929 cells stored in liquid nitrogen were subcultured. 100 μL of cell suspension (about 5000 cells per well) was added to a 96-well plate and pre-incubated in a cell culture incubator (37°C, 5% CO_2) for 24 hours. 10 μL of antimicrobial peptides of different concentrations were added to the cell suspension and cultured in the incubator for 24 hours. 10 μL of CCK-8 solution was added to each well and cultured in the incubator for 1 hour. The absorbance value OD450 at 450 nm was measured using a microplate reader. The calculation formula for the survival rate of CCK-8 cells is: survival rate (100%) = (OD value of the experimental group - OD value of the blank control group) / (OD value of the control group - OD value of the blank control group) × 100%. The OD value of the experimental group is the absorbance value of the cells after antimicrobial peptide treatment, the OD value of the control group is the absorbance value of the untreated cells, and the OD value of the blank control group is the absorbance value of the culture medium.

2.7. Preparation of ointment

Prepare 100 mL of biological antibacterial preparation. Prescription includes poloxamer 407 18g, HPMC (4000) 0.5g, glycerol 2g, antimicrobial peptide 0.1-10mg, purified water 100 mL (100g), citric acid monohydrate appropriate amount.

100 mL purified water was heated to 60–70℃, then the weighed HPMC was dispersed into the water, cooled to room temperature for use. 18g poloxamer 407 was added to the above dispersion and

refrigerated overnight at 2–8℃ until the poloxamer was fully dissolved. 2g glycerol was added to the above mixed solution, stirred to dissolve, and placed at room temperature to form a gel. 0.1–10 mg of PFK, PVK or PIK was added to the matrix gel. The pH was adjusted to 7 with citric acid monohydrate. After the ointment is prepared, it is dispensed into medical syringes and injected into the pockets formed by periodontitis.

2.8. Rat model of periodontitis

Rats were divided into three groups: (1) untreated control, (2) treated with minocycline hydrochloride ointment Palio, and (3) treated with PFK (topical application, 10 μg/mL).

Male Sprague–Dawley rats (n = 15, 8 weeks old) were obtained from the Experimental Animal Center of Naval Medical University (Shanghai, China). Periodontitis was induced in Sprague-Dawley rats (n = 15, male, 8 weeks old) via ligature placement around the mandibular first molars[10], combined with oral inoculation of *P. gingivalis*[11]. Isoflurane (3–5% induction, 1–3% maintenance via nose cone) was applied for inhalational anesthesia. Rats were placed in lateral recumbency on a heated surgical pad (37°C). The mouth was opened using a rodent mouth gag or gentle manual retraction. The maxillary or mandibular second molars were identified. The ligature was passed around the tooth cervix (gingival sulcus) using sterile forceps. A firm but non-strangulating knot was tied to avoid immediate tissue necrosis. Excess ligature ends were trimmed to prevent oral irritation. Control rats received no ligation. Euthanasia was performed by inhalation of overdose isoflurane (5%). The jaw was dissected, and gingival tissues and alveolar bone were fixed in 10% neutral buffered formalin for histology.

2.9. Paraffin sections

Paraffin sections were dewaxed to water. The baked slices were immersed in fresh xylene and then immersed in anhydrous ethanol, and the slices were washed until no other residue.

2.10. Immunohistochemistry

Pancreatic enzyme repair solution was added to tissue sections, placed at 37° for 15 min, then the slides were placed in PBS (PH7.4) and shake on a decolorizing shaker for 3 times, 5 min each time. The sections were put into 3% hydrogen peroxide solution, incubated at room temperature in the dark for 25 min, the slides were placed in PBS (PH7.4) and shake on a decolorizing shaker for 3 times, 5 min each time. 3% BSA was added in the tissue circle to evenly cover the tissue, and blocked at room temperature for 30 min. The blocking solution was gently shake off, PBS was added to the slices according to a certain ratio of primary antibody (IL-1β), and the slices were laid flat in a humidified box and incubated overnight at 4°C. The slides were placed in PBS (PH7.4), shake and washed on a decolorizing shaker 3 times, 5 minutes each time. After the slices were slightly dried, the secondary antibody (HRP labeled) of the corresponding species was added to the primary antibody in the circle to cover the tissue and incubated at room temperature for 50 minutes. The slides were placed in PBS (PH7.4), shake and washed on a decolorizing shaker 3 times, 5 minutes each time. After the slices were slightly dried, freshly prepared DAB color development solution was added in the circle, the color development time

was controlled under a microscope, positive is brown-yellow, and the slices were rinsed with tap water to stop color development. Cell nuclei were re-stained with Harris hematoxylin re-staining for about 3 minutes, washed with water, differentiation with 1% hydrochloric acid alcohol for a few seconds, washed with tap water, blued with ammonia water, and washed with running water. Dehydration and sealing: The slices were put into 75% alcohol for 5 minutes, 85% alcohol for 5 minutes, 100% ethanol for 5 minutes-absolute ethanol for 5 minutes, xylene for 5 minutes to dehydrate and made transparent. The slices were taken out of xylene and dried them slightly, and sealed with neutral gum.

2.11. Statistical analysis

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test. A P value < 0.05 was considered statistically significant.

3. RESULTS

3.1. Design of antimicrobial peptides targeting *P. gingivalis*

The antimicrobial peptides designed in this study were shortened from the original 35 amino acids to 20 amino acids based on the structural analysis of the naturally discovered antimicrobial peptide Cecropin B sequence (KWKVFKKIEKMGRNIRNGIVKAGPAIAVLGEAKAL), reducing its molecular weight of 3.8 kDa to below 3 kDa, thereby reducing the difficulty of synthesizing antimicrobial peptides. Through amino acid polarity analysis and peptide isoelectric point analysis, the proportion of positively charged amino acids was increased, thereby increasing the binding ability of antimicrobial peptides to bacterial cell membranes. By replacing amino acids of the same nature, the α-helical structure of the antimicrobial peptide sequence remained unchanged after the shortening of the antimicrobial peptide sequence. Finally, three new antimicrobial peptides PFK, PVK and PIK were designed, and their three-dimensional structures were displayed using Alphafold 3 protein structure prediction software (Fig. 1). The three new sequences of the designed antimicrobial peptides PFK, PVK, and PIK have hydrophilic and lipophilic properties.

3.2. Antibacterial activity of antimicrobial peptide PFK against *P. gingivalis*

To determine the antibacterial activity of antimicrobial peptide PFK against *P. gingivalis*, a minimum inhibition assay was performed. The result of the test shows that the antimicrobial peptide PFK exhibits stronger antibacterial activity (Fig. 2). The minimum inhibitory concentration for the common oral pathogen *P. gingivalis* is 16 μg/mL; the antibacterial effect of the antimicrobial peptide PVK on *P. gingivalis* is relatively weak, higher than 200 μg/mL; the antibacterial effect of the antimicrobial peptide PIK and Cecropin B on *P. gingivalis* is relatively weak, higher than 500 μg/mL.

3.3. Inhibitory effect of antimicrobial peptide PFK on biofilm

The ability of PFK to disrupt *P. gingivalis* biofilm was evaluated by a microtiter plate assay, in which biofilms were grown on the bottom of microtiter plates for 3 days. The biofilm biomass was quantified by

measuring the absorbance at 492 nm using crystal violet staining. The results showed that at 12.4 μg/mL, the antimicrobial peptide PFK could inhibit the biofilm to less than 50%, at 124 μg/mL, the antimicrobial peptide PFK could inhibit the biofilm to less than 40%, and at 1.24 mg/mL, and the antimicrobial peptide PFK could inhibit the biofilm to less than 20% (Fig. 3). At 1.24 mg/mL, Student's *t* test for biomass of PFK treated biofilm versus biomass of minocycline hydrochloride treated biofilm yielded *P* values <0.001, suggesting significant difference.

3.4. Safety and biocompatibility

To test the safety and biocompatibility of antimicrobial peptide PFK, hemolytic toxicity test and cytotoxicity assay were performed. The results of the determination in Fig.4A show that the antimicrobial peptides PFK, PVK and PIK all have low hemolysis and high safety. The hemolysis rate of the antimicrobial peptide PFK was 64.4% at a high concentration of 10 mg/mL, but it dropped to 1.7% at a concentration of 1 mg/mL. When the concentration was less than or equal to 100 μg/mL, the antimicrobial peptide PFK had no hemolysis. The antimicrobial peptide PVK had the lowest hemolysis rate at a high concentration of 10 mg/mL, which was only 18.9%; at 1 mg/mL, the hemolysis rate was 7.1%; at 100 μg/mL, the hemolysis rate was 4.2%; when the concentration was less than or equal to 10 μg/mL, the antimicrobial peptide PVK had no hemolysis. The antimicrobial peptide PIK had a hemolysis rate of 51.6% at a high concentration of 10 mg/mL. As the concentration decreased, the hemolysis gradually decreased, but it still had weak hemolysis. In summary, when the concentration is 10 mg/mL, the antimicrobial peptide PVK has the lowest hemolytic activity and is the safest; when the concentration is less than or equal to 1 mg/mL, the antimicrobial peptide PFK has the lowest hemolytic activity and is the safest.

The results of the determination show that the antimicrobial peptides have low cytotoxicity and high safety when the concentration is less than or equal to 64 μg/mL (Fig. 4B). At a concentration of 256 μg/mL, the antimicrobial peptides PFK, PVK and PIK all exhibit high cytotoxicity. When the concentration of the antimicrobial peptides dropped to 64 μg/mL or below, the survival rate of the cells increased significantly, among which the survival rates of PFK and PVK were higher than those of PIK, indicating that PFK and PVK are safer as antimicrobial ingredients.

3.5. Preparation of antibacterial ointment and its antibacterial effect *in vitro*

Based on the above results, PFK is the most preferred antimicrobial peptide, so it is used as the main antibacterial ingredient to prepare the ointment. Antimicrobial peptide ointment formula: 5.4% octadecyl alcohol, 5.4% glyceryl monostearate, 26.9% liquid paraffin, 2.69% white vaseline, 5.4% glycerol, 0.51% hydroxyethyl cellulose, 53.7% water, and 0.05% betaine. After the ointment is prepared, it is divided into medical syringes and injected into the pockets formed by periodontitis during use. It can inhibit the growth of oral pathogens such as *P. gingivalis*, thereby slowing down or eliminating periodontitis.

The ointment is configured into three concentrations: low, medium and high. The antibacterial effect of the ointment is evaluated by carrier immersion sterilization experiments (Fig. 5). The results show that

the antibacterial effect of low-concentration PFK ointment on *P. gingivalis* is higher than 98%, and the antibacterial effect of medium and high-concentration PFK ointment on *P. gingivalis* is higher than 99%.

3.6. Therapeutic effects in a rat periodontitis model

In order to evaluate the in vivo therapeutic effect of PFK ointment, a rat periodontitis model was constructed by ligation. The drug administration group was divided into three concentrations: low, medium and high. The administration period was divided into 1 week, 2 weeks and 3 weeks. The periodontitis model without drug administration was used as blank control, and the periodontitis drug Palio was used as positive control. After the rats were killed, the maxillary bones of different samples were sampled for paraffin section immunohistochemical analysis, and the antibody used was IL-1β (Fig. 6A). The inflammatory sites in the gingival area were analyzed using's IHC Profiler of ImageJ[12], and the percentage of positive inflammatory cells was obtained to evaluate the intensity of the inflammation (Fig. 6B).

The results showed that the gingival inflammation of rats without drug administration was the most severe, with the percentage of positive inflammatory cells occupying more than 40%. After 1 week of drug administration, the percentage of positive inflammatory cells in the medium concentration PFK sample was the lowest, less than 30%. After 2 weeks of drug administration, the percentage of positive inflammatory cells in all drug administration groups decreased. After 3 weeks of administration, the percentage of positive inflammatory cells in all drug groups decreased significantly, among which the percentage of positive inflammatory cells in low-concentration PFK samples was close to that of the positive drug, falling below 20%.

4. DISCUSSION

The development of novel antimicrobial peptides (AMPs) represents a promising strategy to overcome the limitations of conventional periodontal therapies. Our findings demonstrate that the engineered peptide PFK exhibits potent antimicrobial activity against *P. gingivalis* (MIC = 16 μg/mL), effectively disrupts biofilms, and reduces inflammation in a rat periodontitis model. These results align with recent advances in peptide-based therapeutics for oral infections[13, 14].

The antimicrobial efficacy of PFK against *P. gingivalis* compares favorably with other recently developed AMPs. For instance, the synthetic peptide GH12 showed MIC values of 8–32 μg/mL against periodontal pathogens[15], while the natural peptide LL-37 exhibited MICs ranging from 10–50 μg/mL[16, 17]. PFK's superior performance over control peptides PVK and PIK likely stems from its optimized amphipathic structure and increased cationic charge density, which enhances membrane interactions[18, 19].

PFK's biofilm disruption capability is particularly noteworthy, as biofilms represent a major therapeutic challenge in periodontitis[17]. At 12.4 μg/mL, PFK reduced *P. gingivalis* biofilm by 50%, outperforming minocycline hydrochloride in our assays. This aligns with findings that certain AMPs can penetrate biofilm matrices more effectively than conventional antibiotics[20, 21]. The mechanism may involve

interference with quorum sensing systems and disruption of extracellular polymeric substances (EPS) [22, 23].

The anti-inflammatory effects of PFK observed in our rat model add another dimension to its therapeutic potential. Periodontal inflammation involves complex host-microbe interactions[24], and PFK's ability to reduce IL-1β expression suggests immunomodulatory properties similar to other host defense peptides[25, 26]. This dual antimicrobial/anti-inflammatory action could provide advantages over current monotherapeutic approaches[27].

Our safety assessments indicate that PFK has acceptable hemolytic activity (< 2% at ≤ 100 μg/mL) and cytotoxicity profiles for periodontal applications. These findings are consistent with recent developments in peptide engineering strategies that enhance specificity for microbial membranes over mammalian cells[28, 29]. The successful formulation of PFK into a topical ointment builds upon previous work demonstrating the feasibility of local peptide delivery for periodontal therapy[30, 31].

The current limitations of periodontal treatment highlight the need for innovative approaches like PFK. Mechanical debridement often fails to completely remove subgingival biofilms, while antibiotic use is constrained by resistance concerns[32, 33]. PFK's membrane-targeting mechanism may reduce resistance development compared to conventional antibiotics, though long-term monitoring would be required[34].

5. Conclusion

In conclusion, PFK demonstrated antimicrobial and biofilm-disrupting activity against P. gingivalis, reduced periodontal inflammation in a ligature-induced rat model, and exhibited acceptable safety within the therapeutic concentration range. These findings support PFK as a promising non-antibiotic adjunctive candidate for periodontal therapy. Further studies are required to assess long-term safety, peptide stability, and translational feasibility in more clinically relevant models.

Declarations

Ethics approval and consent to participate

All animal experiments were approved by the Ethics Committee of Changhai Hospital, Naval Medical University (Approval No. CHEC2023-217). The animals used in this study were obtained from an institutional experimental animal center and were not privately owned; therefore, informed consent from animal owners was not applicable.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

S.W., X.M., and Q.H. designed the study. X.M., J.X., and J.P. developed the methodology and conducted the experiments. X.M. curated and analyzed the data. Q.H. and Y.W. acquired the funding. Y.W. and Q.H. supervised the research. X.M. prepared the figures and visualizations. X.M. drafted the manuscript. S.W., Y.W., and Q.H. critically reviewed and revised the manuscript. All authors read and approved the final

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Figures

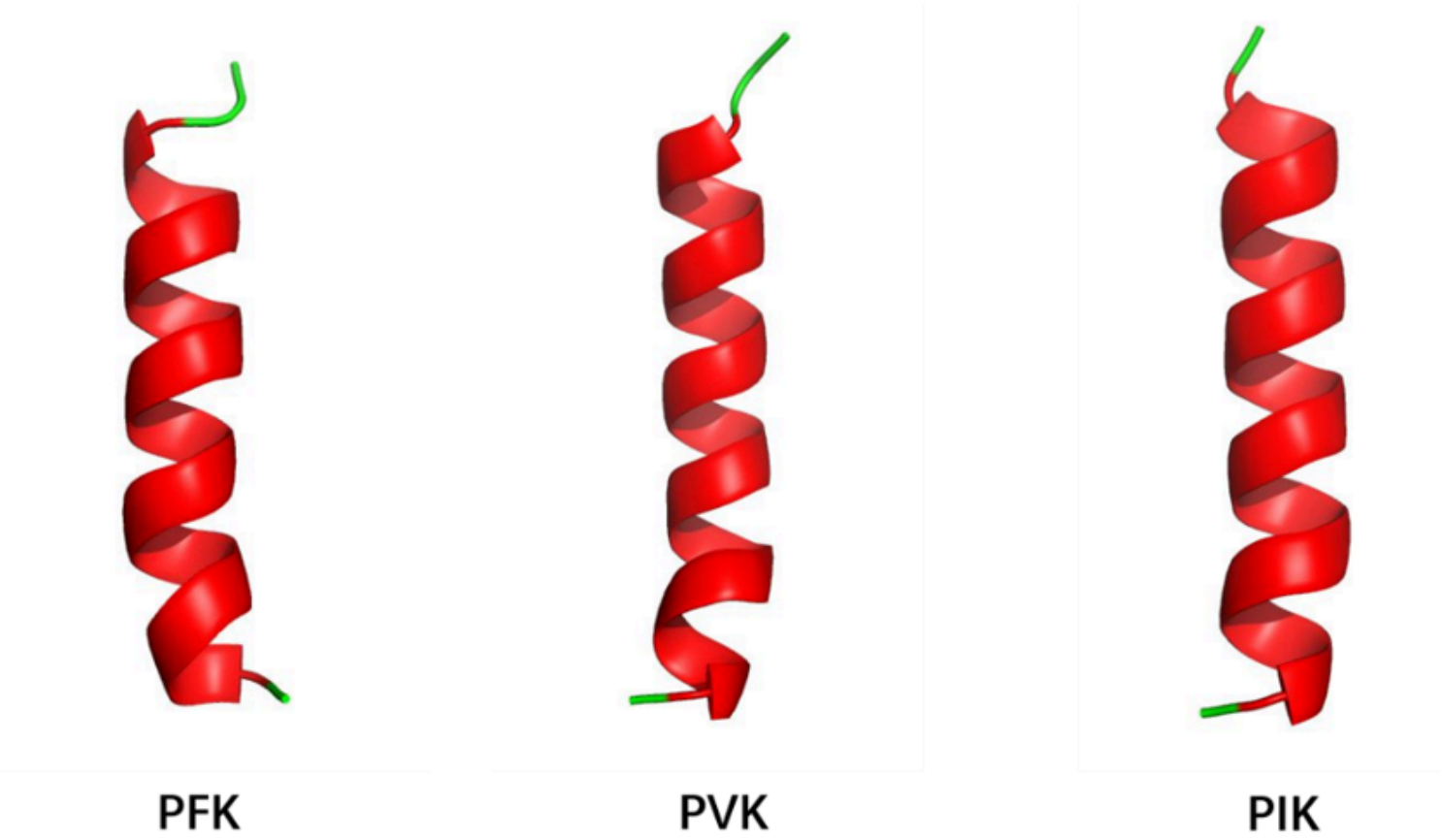


Figure 1

AlphaFold3 predicted structures of antimicrobial peptides PFK, PVK and PIK. PFK, PVK and PIK all form α -helical structures. Sequence of PFK: FKKASHLFKKTLKKIFSKWK, Sequence of PVK: IVAVRIRWQVTLKKIFSKWK, Sequence of PIK: VQWRIRVAVIRKTLKKIFSKWK

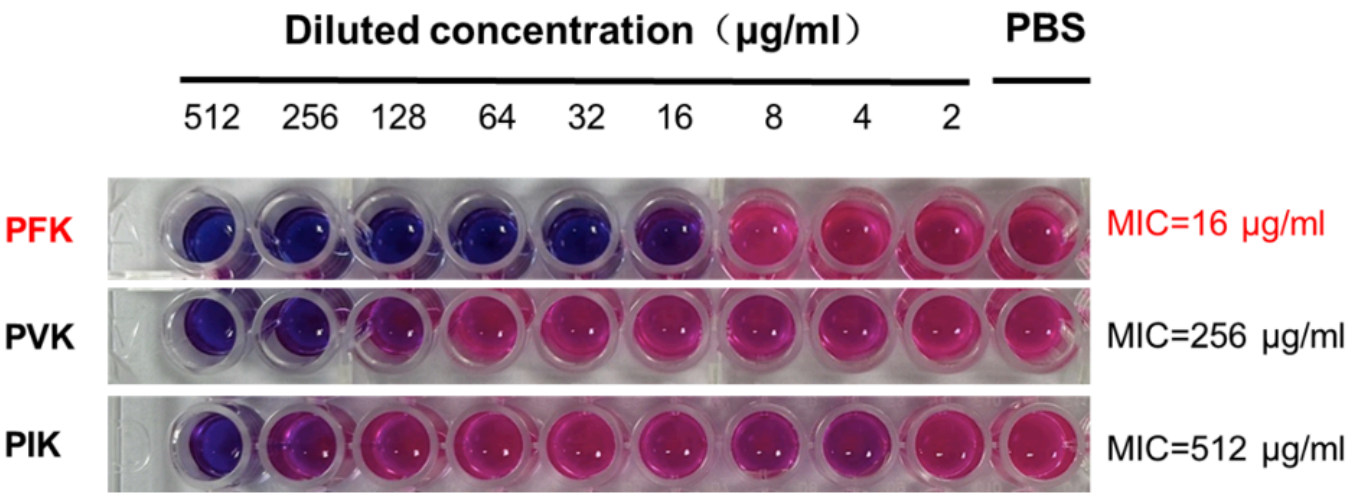


Figure 2

Minimum inhibition assay of antimicrobial peptide PFK against *P. gingivalis*. Bacteria culture was treated with 512 μg/mL, 256 μg/mL, 128 μg/mL, 64 μg/mL, 32 μg/mL, 16 μg/mL, 8 μg/mL, 4 μg/mL, 2 μg/mL and 0 μg/mL (PBS) of PFK, PVK, PIK or Cecropin B. Viable bacteria were stained as purple by resazurin

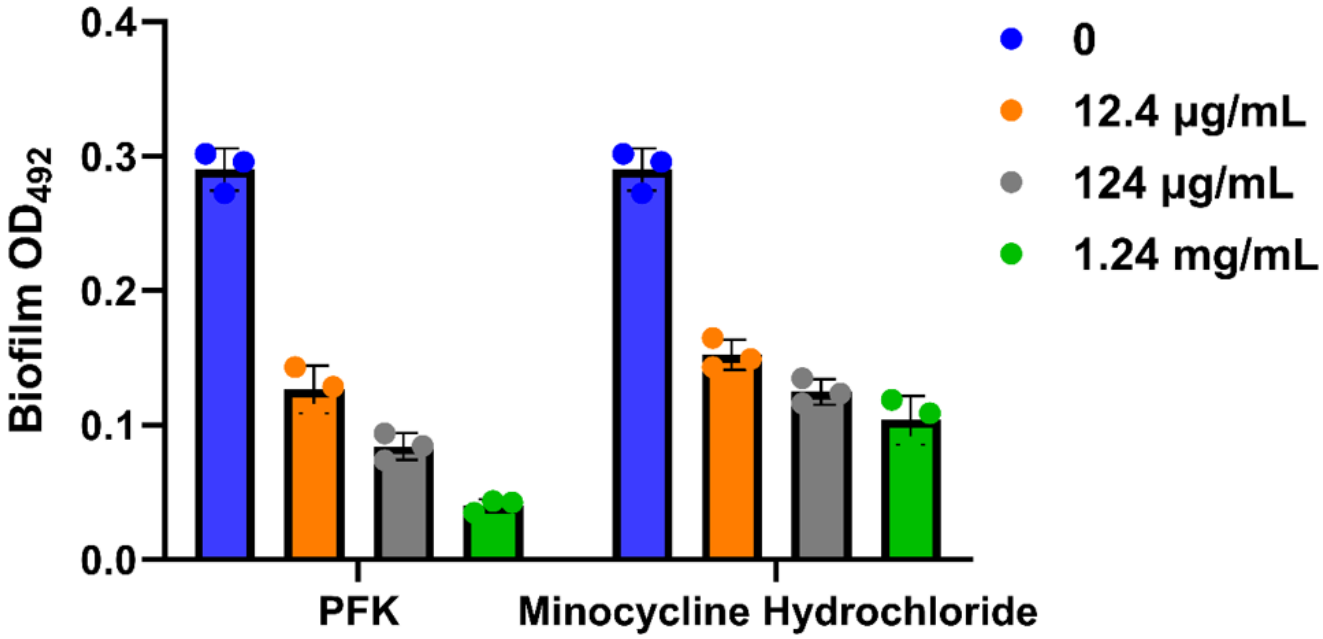


Figure 3

P. gingivalis biofilm inhibition assay of antimicrobial peptide PFK. *P. gingivalis* biofilm was treated with 0 μg/mL, 12.4 μg/mL, 124 μg/mL, and 1.24 mg/mL of PFK or minocycline hydrochloride, respectively. Biofilm biomass was quantified by measuring absorbance at 492 nm. At 1.24 mg/mL, Student's *t* test for biomass of PFK treated biofilm versus biomass of minocycline hydrochloride treated biofilm yielded *P* values <0.001, suggesting significant difference

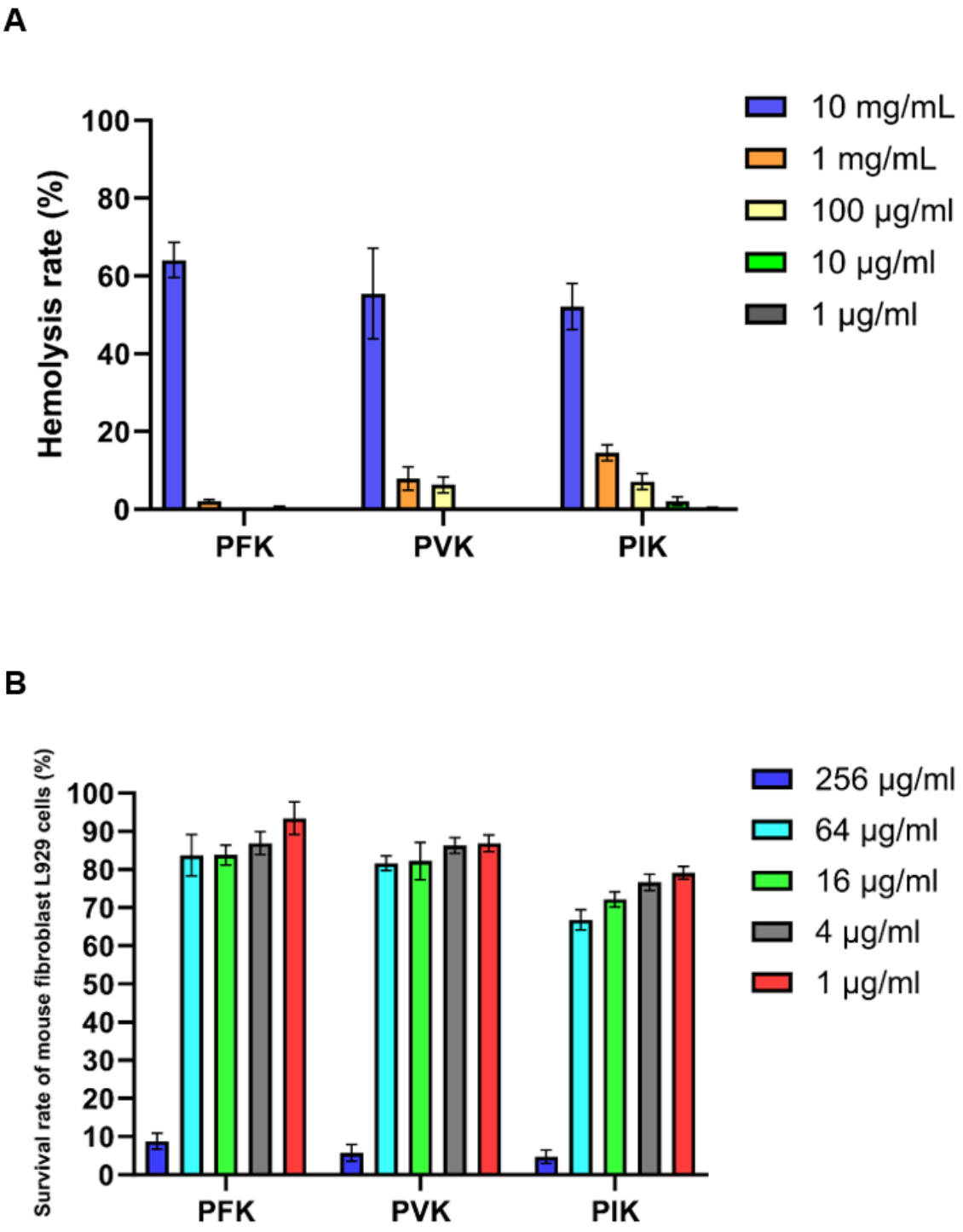


Figure 4

Safety and biocompatibility assessments of PFK. (A**)** hemolytic toxicity test. At 1 mg/mL, 100 μg/mL, 10 μg/mL and 1 μg/mL, student's *t* test for hemolysis rate of PFK versus PVK and PIK yielded *P* values <0.001, suggesting significant difference. (B) cytotoxicity assay. At 1 mg/mL, 100 μg/mL, 10 μg/mL and 1 μg/mL, student's *t* test for cell survival rate of PFK versus PVK and PIK yielded *P* values >0.05, suggesting no significant difference

Ointment Type	Colony count (mean of three replicates)	Antibacterial rate
Ointment base	>1000	
add PFK 0.1 mg/ml （10 min treatment）	14	>98.6%
add PFK 0.25 mg/ml （10 min treatment）	6	>99.4%
add PFK 0.5 mg/ml （10 min treatment）	0.67	>99.9%

Figure 5

Antibactericidal rate of antimicrobial peptide PFK ointment. 0.1 mg/mL, 0.25 mg/mL and 0.5 mg/mL PFK were added into ointment base as active ingredient

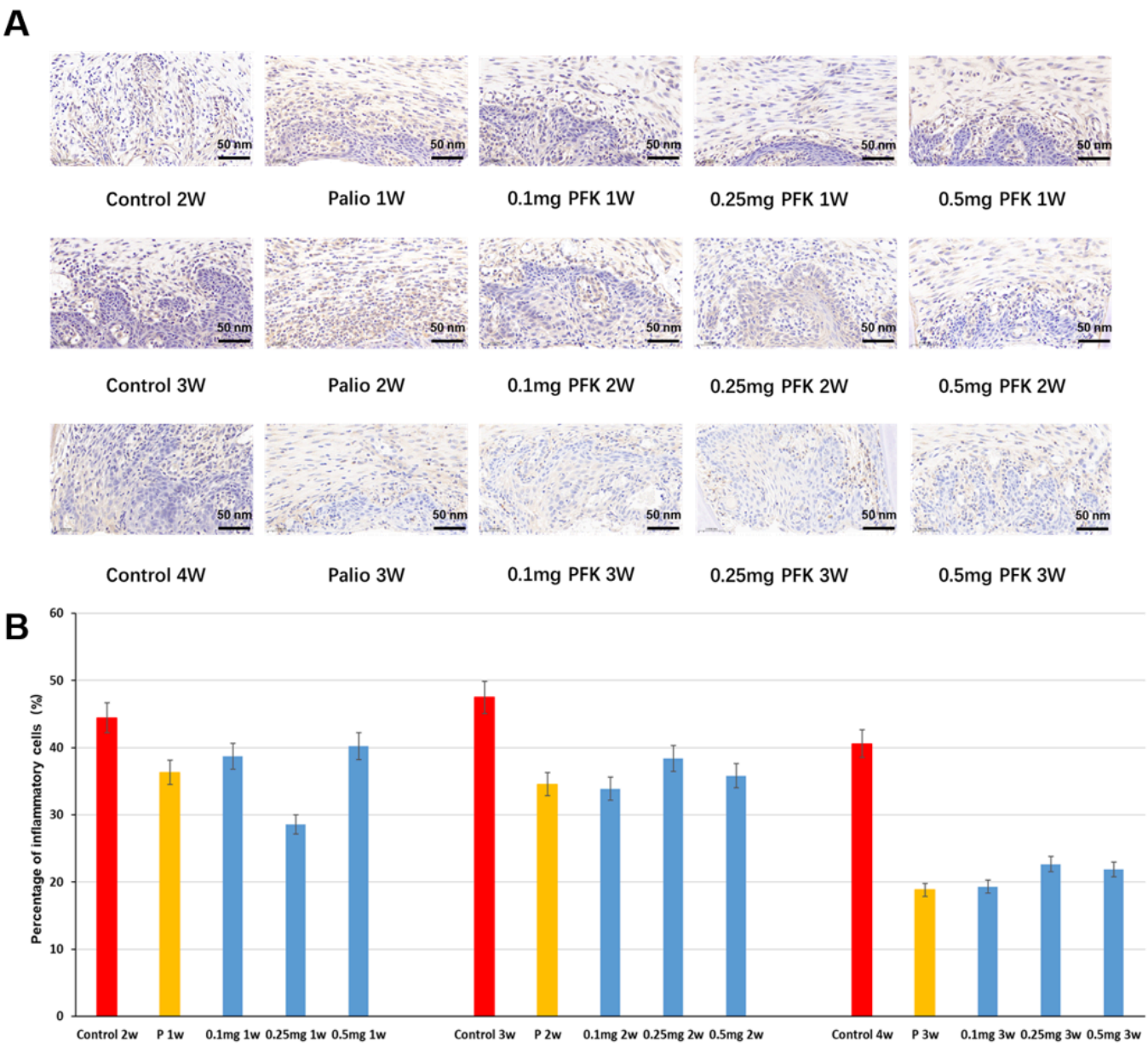


Figure 6

Immunohistochemical experiment of antimicrobial peptide PFK efficacy. (A) immunohistochemical analysis of rat alveolar bone samples, (B**)** inflammatory cell analysis using ImageJ. Rats were divided into five groups: group 1: negative control, group 2: positive control, treated with 0.1 mg Palio, group 3: treated with 0.1 mg PFK ointment, group 4: treated with 0.25 mg PFK ointment, group 5: treated with 0.5 mg PFK ointment. Alveolar bone samples were collected at 2 weeks, 3 weeks and 4 weeks. At 4 weeks, Student's *t*test for PFK treated groups (0.1 mg, 0.25 mg and 0.5 mg) versus control group yielded *P* values <0.001, suggesting significant difference