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Song, X., Pan, H., Wang, H., Liao, X., Sun, D., Xu, K., Chen, T., Zhang, X., Wu, M., Wu, D., & Gao, Y. (2020). Identification of new dermaseptins with self-assembly tendency: membrane disruption, biofilm eradication and infected wound healing efficacy. *Acta Biomaterialia*. <https://doi.org/10.1016/j.actbio.2020.03.024>

Published in:
Acta Biomaterialia

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
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Identification of new dermaseptins with self-assembly tendency: membrane disruption, biofilm eradication and infected wound healing efficacy

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Graphic Abstract

Abstract

Severe infections associated with antibiotic-resistant bacteria and biofilms have attracted increasing interest as these diseases are difficult to treat with current antibiotics. Typical cationic antimicrobial peptides dermaseptins are considered to be the most promising next-generation antibiotics because of their broad-spectrum antimicrobial activities and minor side effects. Two new dermaseptin peptides, DMS-PS1 and DMS-PS2 have been identified by “shotgun” molecular cloning of encoding cDNAs in the crude skin secretions of the waxy monkey tree frog, *Phyllomedusa sauvagei*. The mature peptide sequences predicted from the cloned cDNAs were separated from crude skin secretions and confirmed by mass spectrometry. Chemically synthetic replicates were assessed for a variety of biological activities. Both dermaseptins were potently effective against a broad spectrum of microorganisms including antibiotic-resistant bacteria and displayed significant potency against Gram-positive and Gram-negative bacterial biofilms with low toxicity towards mammalian red blood cells. Remarkably, DMS-PS2 was effective against infections in murine skin caused by methicillin-resistant *Staphylococcus aureus* as a result of an induced wound. The actions of DMS-PS2 were with a membrane permeabilization mode. Overall, the data provided convincing evidence for the development of anti-infectious agents and/or biomaterials as a new therapeutic approach against bacterial infections.

1 Introduction

With the threat of antibiotic-resistant bacteria increasing, antimicrobial peptides (AMPs) have the potential to become a new therapeutic approach to address the serious difficulties against persistent bacterial infections, resistant bacteria infection, and medical device infection. The rate of the mutation of the microorganisms to develop resistance increases since most of the conventional antibiotics could act on a single intracellular target. However, AMPs could work on multiple targets simultaneously to interfere with the integrity of bacterial membranes, resulting in cell lysis. Among all the targets of antimicrobial peptides, bacterial membranes are an outstanding target because bacteria need to alter their overall membrane rather than modifying specific intracellular

targets to require resistance characteristics; thus, it hardly evokes bacterial resistance ¹. Besides, the advantages of specifically targeting the pathogenic bacteria instead of human cells are created by the difference in the cell membrane between the eukaryotes and prokaryotes. Therefore, the development of AMP with explicit targets could enhance the discovery of new antibiotics, contributing to alleviating the pressures of conventional antibiotics.

To date, following the increasingly important role of biomaterials in medical treatment, bacterial biofilm has also been a common cause of persistent implant-related infections. Irritation, infection, and even biofilm formation would be caused by the invasive medical device implantation. Biofilm infections could induce chronic clinical infections on biotic and abiotic sites; implanted biomaterials provide suitable surfaces for its formation. Bacteria embedded with biofilm fail to be killed by conventional antibiotic therapy because the interactions between deleterious agents and bacteria are blocked by the hydrated polymeric matrix. Moreover, sessile bacterial cells of biofilms release antigens and planktonic cells to cause immune complex damage and expansion of infections. Consequently, the antimicrobial coating on the biomaterial surface is a feasible way for the biofilm inhibitory device development, such as the combination of antibiotics/silver and polymers/hydrogels ^{2,3}. The excellent properties of peptides such as chemical versatility, biocompatibility, and biodegradability made them promising biomaterial for application ⁴. Furthermore, it was reported that AMPs could collapse the biofilm polymeric matrix by interacting with anionic LPS in the biofilm ⁵. Accordingly, a new field of treatment for bacterial infections would be produced through AMPs, which have additional antimicrobial effects with suppressing the biofilm formation or dispersing the existing biofilms by interfering with small signal molecules in terms of working at a molecular level ⁶.

Amphibian skin secretion is recognised as an under-utilised and an important resource for drug discovery in the pharmaceutical industries, especially for providing diverse bioactive components. Due to the advantages of potent efficacy, broad-spectrum, and abundant structures, abundant broad-spectrum AMPs that have been discovered from skin secretions of anurans have been considered to be the most promising drug candidates. Dermaseptins are a significant family of antimicrobial peptides that have genetic correlations and a remarkable identity in their sequences that contain a conserved Trp3 and a conserved motif (AA (A/G) KAAL (G/N) A) in mid regions ⁷. Dermaseptins are prototypical cationic α -helical amphipathic peptides (K-rich) and possess broad-spectrum antimicrobial activities ⁸⁻¹⁰. After the electrostatic interaction between dermaseptins and bacterial cells, partial dermaseptins of antimicrobial actions focus on membrane-damaging processes through the coil-to-helix translation upon binding to lipid bilayers in the mode of the “carpet” model ^{11,12}.

In this paper, the identification of two dermaseptin peptides (DMS-PS1 and DMS-PS2) is reported through isolating and characterizing from the crude skin secretions of *P. sauvagei*. The primary structures were identified by “shotgun” molecular cloning from a cDNA library constructed from crude skin secretions; besides, they were concurrently validated by electrospray ion-trap MS/MS in the effluents of skin secretions. In a range of bioassays, their antimicrobial activity against both planktonic cells and sessile cells embedded in biofilm, and the toxicity for mammalian red blood cells were determined. In various experimental and bioinformatic methods, the membrane-disrupting mechanism and the aggregating properties were assessed. Generally, the two dermaseptins exhibited broad-spectrum antimicrobial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, and methicillin-resistant *Staphylococcus aureus* (MRSA), which are most commonly biofilm-forming pathogens ¹³. The

mode of DMS-PS2 action that has a better potency between the two dermaseptins was determined. The insight into microbial cell membrane disruption by DMS-PS2 was provided.

In addition to the activities and mechanism studies, the peptide aggregating property was also assessed. Compared with a single peptide molecule, peptides also display an ability to self-assemble into supramolecular structures in certain conditions, including certain pH, ionic strength, temperature, and enzymatic degradation. Therefore, a new way of developing antimicrobial coating biomaterial for implanting devices is provided by these features. Intriguingly, the oligomerization of the hydrophobic DMS-PS2 was observed within 3 hours; the intensity was increased with the constant incubation. The small-sized aggregation may be the initial step of more organised assembled particles.

Moreover, the evidence for further development of inherently antimicrobial biomaterial with DMS-PS2 sequence as the backbone and with side-chain functionalization is provided by these data.

2 Materials and Methods

2.1 Acquisition of skin secretions

The skin secretion of *P. sauvagei* was obtained from captive-bred frogs from a commercial source. The frogs were bred by feeding with multivitamin crickets in a 12 h light/dark circle at 22°C for 4 months. Two methods were utilised to obtain skin secretions by a mild electrical stimulation (15 V) of the dorsal skin with a duration of 5 s or by giving a gentle massage on the dorsal surface¹⁴. The visible milky skin secretions were rinsed off by a stream of deionised water; then, they were immediately freeze-dried and stored at -20°C for future work. All procedures had been approved by appropriate national licensing and local ethics authorities and permits to operate with live animals.

2.2 Molecular cloning of antimicrobial peptide biosynthetic precursor-encoding cDNAs from a secretion-derived cDNA library from a secretion-derived cDNA library

The pure and intact polyadenylated mRNA was extracted from crude skin secretions by hybridizing to the magnetic oligo-dT beads (Dynal Biotech, Wirral, UK). The isolated mRNA was employed for downstream reverse transcription and synthesis of the first-strand cDNA. The cDNA was used to perform 3'-rapid amplification of cDNA ends to construct integrated cDNAs by a SMART-RACE kit (Clontech, Oxford, UK). The process was realised by employing a nested universal primer (supplied with the kit) and a sense primer (S1: 5'-TCTGAATTRYAAGMSCARACATG-3', R = A + G, Y = C + T, M = A + C, S = C + G), which were designed from a highly conserved domain in the 5'-untranslated region of previously characterised antimicrobial peptide encoding cDNAs from *P. sauvagei*. Besides, the PCR samples were purified by rapid PCR purification systems (Omega Bio-Tek, Norcross, GA, USA) and cloned with the pGEM-T easy vector system (Promega Corporation, Madison, WI, USA). Moreover, the sequenced samples were analysed by an ABI 3100 automated sequencer. Finally, the putative peptide homologue structures were analysed by bioinformatics using NCBI-BLAST.

2.3 Isolation and structural characterization of antimicrobial peptides from lyophilised skin secretions

The prepared skin secretion of 5 mg was reconstituted in 1 mL trifluoroacetic acid (TFA)/water (0.05/99.95; v/v) and clarified by centrifugation. The supernatant was analysed by revers-phase HPLC (RP-HPLC) with an analytical column (Phenomenex C-18; 5 µm, 10.0 mm x 250 mm). The

gradient elution programme was from TFA/water (0.05/99.95; v/v) to TFA/water/acetonitrile (0.05/19.95/80.00; v/v/v), with a flow rate of 1 mL/min during 240 min. The linear gradient process was constantly monitored by dual-wavelength at 214 nm and 280 nm, respectively. The effluents were continuously collected at 1 min intervals. The molecular masses of contents in each fraction were detected by matrix-assistant laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) on a linear time-of-flight Voyager DE mass spectrometer (Perseptive Biosystems, Foster City, CA, USA). Each sample was covered with α -cyano-4-hydroxycinnamic acid as the matrix for crystallization and detected in positive mode. An LCQ Fleet electrospray ion-trap mass spectrometer was used to further analyse fractions containing peptides with the same molecular weight as the new putative cDNA-encoded peptides masses by MS/MS fragmentation sequencing (Thermo Fisher Scientific, Foster City, CA, USA).

2.4 Peptide synthesis

Each confirmed primary structured peptide was chemically synthesised by solid-phase synthesis with an automatic PS4 peptide synthesiser (Protein Technologies, Tucson, AZ, USA). The process was achieved by a repeated cycle of deprotection-wash-coupling-wash. The products were deprotected and cleaved from the resin; then, they were subjected to purification by reverse-phase HPLC and confirmed by MALDI-TOF mass spectrometry. The following experiments all used synthesised peptides.

2.5 Secondary structure determination by circular dichroism (CD) and peptide aggregation determination by dynamic light scattering (DLS) and atomic force microscope (AFM)

The secondary structure of peptide was determined by an Applied Photophysics qCD spectrometer (Applied Photophysics, UK). The peptide was determined as 1 nm bandwidth and 0.5 nm data pitch at 50 μ M in 10 mM ammonium acetate, 10 mM ammonium acetate with 50% 2,2,2-trifluoroethanol (TFE), 10 mM sodium dodecyl sulfonate (SDS), and 100 mM SDS. Besides, the interactions of peptide with bacterial cells were also investigated by CD¹⁵. Mid-logarithmic phase *P. aeruginosa* culture was centrifuged and washed with sterilized PBS. Bacteria suspension was further diluted to 1×10^7 CFU/mL; peptide was dissolved in sterilised PBS at 20 μ M. Equal volume solutions were subjected to mix; moreover, the variation in peptide secondary structure during the time was monitored every hour. The peptide (10 μ M) and bacteria suspension (5×10^6 CFU/mL) were recorded. The wavelength was detected from 200 nm to 260 nm. The predicted α -helicity was predicted by the CDNN program provided by the manufacturer.

The average hydrodynamic diameters and the aggregation were detected by the dynamic light scattering (DLS). The stock solutions were prepared by dissolving samples in the phosphate buffer solution PBS (pH 7.2-7.4). Following filtration, samples were diluted to 50 mM by PBS and further incubated at 37°C for 3 h, 24 h, and 72 h. Size distribution was measured using the same NanoZS90 device (Malvern Instrument Ltd., UK) to investigate the ability of the peptide aggregation. Then, samples were further detected by the AFM (Shimadzu SPM 9600) for tapping mode with the force constant of 42 N/m and a resonant frequency of 320 kHz. Finally, samples (20 μ L) were dropped on to the mica plate surface and naturally dried out at ambient temperature. All images were recorded as 512×512 pixel images.

2.6 Physicochemical property prediction, secondary structure visualization, and molecular docking

In this study, the property of peptides was elucidated using different methods. In the first instance, the physicochemical parameters, including hydrophobicity, hydrophobic moment, net charge at neutral pH, were predicted using the Heliquist server¹⁶. The Helical Wheel Projection Server (<http://rزلab.ucr.edu/scripts/wheel/wheel.cgi>) was employed to visualize the α -helicity, hydrophobicity of the peptides. I-TASSER online server was employed to predict and infer the 3-D structural models¹⁷. Besides, the model quality was inspected by Ramachandran plots using RAMPAGE¹⁸ and by z-scores using ProSA¹⁹. Molecular docking was performed using AutoDock Tools and AutoDock Vina in order to simulate the AMP-membrane interaction²⁰. The docking studies were described in our previous work in detail²¹. Generally, the Gram-negative bacterial cell membrane model and peptide model were prepared; the grid box and size were determined; the docking was performed. The fast Fourier transform (FFT)-based protein docking server M-ZDOCK was employed to perform a symmetric multimer docking²². The calculated top prediction with the best affinity score was rendered with the PyMol (PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC).

2.7 Antimicrobial minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays

The MIC and MBC of each synthetic peptide were determined by incubating the antimicrobial agents with the separate species of cultured microbes. The microbial strains were commercially obtained from ATCC (American Type Culture Collection, USA), NCTC (National Type Culture Collection, UK) and NCYC (National Collection of Yeast Cultures, UK), including *Staphylococcus aureus* (NCTC 10788), methicillin-resistant *Staphylococcus aureus* (NCTC 12493), methicillin-resistant *Staphylococcus aureus* (ATCC 43300), *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (NCYC 1467). Before testing, the tested microbes were warmed up in Mueller-Hinton Broth (MHB) for 16-20 h at 37°C, while *C. albicans* was cultured in Yeast Extract Peptone Dextrose (YPD) medium. After confirming that the growth had reached their respective logarithmic growth phases, the optical density (OD) of inoculum was measured at 550 nm. The suspension culture was diluted to 5×10^5 CFU/mL. A sequence of peptide concentrations was transferred into a microtiter plate and incubated with the microbial suspension at final concentrations of 1-512 μ g/mL. Following overnight incubation, the OD of each well was determined by a plate reader (EL808, Biolise BioTek, Winooski, VT, USA) at 550 nm. The MIC was the lowest concentration with no visible growth. Then, 10 μ L of culture from each clear well were added into a Mueller-Hinton agar (MHA) or YPD agar plate and incubated overnight to measure the lowest concentration without subsequent generation of colonies (MBC).

2.8 Anti-biofilm activity of peptides

Anti-biofilms activity of the peptides was conducted by quantifying the minimal biofilm inhibition (MBIC) and the minimal biofilm eradication concentrations (MBEC) against *S. aureus* (NCTC 10788), *E. coli*, and *P. aeruginosa*. The inhibition of bacterial initial attachment (IA) was performed against *P. aeruginosa*. The anti-biofilm studies were according to our previous work with minor modifications²¹. Generally, for the IA assay, the *P. aeruginosa* strain was cultured in MHB at 37°C for 16-20 h. The overnight bacteria were collected and washed by the pre-warmed sterile PBS and resuspended with fresh MHB to 5×10^6 CFU/mL. A volume of 200 μ L culture with a series of peptide dilutions (1-512 μ g/mL) was transferred to each well of the microtiter plate and incubated at 37°C for 1 h without agitation to facilitate bacterial binding. After incubation, each well received sterile PBS to wash twice; this was completely decanted to remove the planktonic

cells. Methanol of 200 μ L was added to the prewashed wells and fixed for 30 min. Following fixation, the plate was decanted cleanly and air-dried. Sessile bacteria and biofilm were stained with 0.1% crystal violet (CV) for 30 min and washed twice with water to remove extra dye; then, they were air-dried at room temperature. The plate contents were dissolved with 33% acetic acid and measured by a plate reader (EL808, Biolise BioTek, Winooski, VT, USA) at 550 nm.

The inhibition of biofilm formation was performed using MBIC assay. After overnight incubation of the microbes in the wells of a plate, the plate was washed twice with sterile PBS and decanted completely. Each well received 200 μ L of fresh medium with 2% glucose and was stained with 50 μ L of 1% 2, 3, 5- Triphenyl tetrazolium chloride (TTC (g/v)) solution per well for 5 h. Following sufficient incubation, 200 μ L supernatant from each well was measured at 470 nm by a plate reader. The biofilm eradication activity was assessed using MBEC assay. The same diluted inoculum of 200 μ L was dispersed into each well in a plated bottom plate and incubated for 48 h to form mature biofilms. Afterwards, mature biofilms were washed twice to remove planktonic cells and incubated with the tested peptide at serial dilutions and fresh medium overnight. The washing, staining, and detecting processes were performed as described in the same way as with the MBIC assay.

2.9 Live/Dead staining for the evaluation of the effect of peptides on bacterial growth and localization of FITC-labelled peptides in bacteria.

MRSA (ATCC 43300) and *P. aeruginosa* were cultured overnight separately in MHB. Following subculture, the fresh bacteria culture (10^8 CFU/mL) was centrifuged and gently washed with 0.9% (w/v) NaCl twice; then, they were incubated with different tested peptide concentrations for 1 h at 37°C. The mixtures were subsequently stained with the Live/Dead BacLight™ kit (SYTO9 and propidium iodide (PI); Thermo Fisher Scientific Inc., Waltham, MA, USA). Fluorescence emission was detected by the SP8 Lighting confocal microscope (Leica, Germany). The localization of samples on the bacteria cell membranes was detected by the fluorescent confocal microscope. Mid-logarithmic phase *P. aeruginosa* culture was centrifuged and washed with 0.9% (w/v) NaCl. The bacterial culture (10^7 CFU/mL) was incubated with FITC-labeled DMS-PS2 at a concentration of 32 μ g/mL. After the incubation of 30 min, samples were counterstained with DAPI for 10 min and observed by the fluorescent confocal laser microscope with a 63 $\times$ magnification objective.

2.10 High-resolution scanning electron microscopy (SEM)

Bacterial samples, MRSA (ATCC 43300) and *P. aeruginosa*, were cultured overnight separately in MHB; then, they were harvested by centrifuging at 5000 rpm for 15 min. Different concentrations of peptides were incubated subsequently with bacteria suspension at 37°C for 1 h. Afterwards, bacteria were washed twice with PBS; samples were fixed with 2.5% glutaraldehyde at 4°C overnight. Samples were washed twice with PBS and then fixed in 1% OsO₄ for 1 h. After dehydration with gradient ethanol, samples were dehydrated through critical point drying and coating with gold. Micrographs were recorded by a Gemini 300 scanning electron microscope (Zeiss, Germany).

2.11 Membrane Permeability Assay

The membrane permeability assay was assessed using the SYTOX™ green nucleic acid stain (Life Technologies) method, which was previously published with minor modifications²³. MRSA (ATCC 43300) bacteria were incubated with gradient peptide solutions at 37°C for 1 h. After the

mixture was stained with the SYTOX Green stain, the fluorescence emission was measured at 523 nm with excitation at 485 nm using a Synergy HT plate reader (Biotech, Minneapolis, MN, USA).

2.12 Haemolysis assay

The haemolysis of peptides was assessed by incubating with fresh murine red blood cells. The fresh murine blood was collected from the mice eye socket. A 2% (v/v) suspension of murine red blood cells in PBS was prepared by multiple suspensions and centrifugations. Tested peptide dilutions (1-512 $\mu\text{g/mL}$) were incubated with red cell suspensions at 37°C for 2 h. The lysis of eukaryotic cells was detected by measuring the absorbance of haemoglobin released at 550 nm with an ELISA plate reader (BioTek, USA). PBS was used as the negative control while the non-ionic detergent (1% Triton-X 100) was used as the positive control. HC_{50} (the peptide concentration that caused 50% red blood cell lysis) was calculated to assess the haemolytic activity of the peptides. The therapeutic index (TI) recommended by the FDA of the US to indicate the safe-efficacy window was calculated²⁴. TI is the ratio of the HC_{50} to the geometric mean of MICs, larger values indicate greater cell selectivity.

2.13 In vivo treatment of MRSA infection in murine epidermis

ICR mice (10 weeks) weighing 18-22 g were kindly provided by the Laboratory Animals Center of Wenzhou Medical University and treated strictly in accordance with international ethical guidelines and the National Institutes of Health Guide concerning the Care and Use of Laboratory Animals. All animals were housed at the constant room temperature with a 12h/12 h light/dark cycle, fed the standard rodent diet and given water ad libitum. The mice that acclimatized to the laboratory for 7 days before the in vivo study. The method was utilized in previous publications with minor modifications^{25,26}. After anesthesia with 4% chloral hydrate, mice were cleaned and depilated; then, silicone rings (inner diameter: 8 mm $\times$ height: 0.5 mm) were sutured on the back skin. Two circular wounds were excised in the silicone rings and immediately infected with bacteria suspension (MRSA, ATCC 43300, 10^8 CFU/mL). After one day of infection, the viable bacterial counts were detected according to previously described methods. Mice had been constantly infected until the viable counts were stable and larger than the number of inoculations. After the infection was established, mice were randomly separated into different groups (n = 7 per group). On the basis of the experimental design, topical antimicrobial agents were applied twice every day by treatment via gauze pad (8 mm $\times$ height: 0.5 mm) and the gauze was held in place by the application of a patch. The groups consisted of MRSA-infection control group (CON), DMS-PS2 treatment of MRSA-infection group (DMS10.24: DMS-PS2 10.24 $\mu\text{g/day}$) and a second DMS-PS2 treatment of MRSA-infection group (DMS20.48: DMS-PS2 20.48 $\mu\text{g/day}$). The photographs of each wound were taken every 12 h and the viable bacterial counts were detected depending on the methods described previously before the medication process.

2.14 Statistic analysis.

Statistical analysis was performed using Student's t-test. Differences were considered statistically significant with a probability of $P < 0.05$.

3 Results

3.1 Molecular cloning of new AMP precursor-encoding cDNAs from the library constructed from the skin secretion of *P. sauvagei* and prediction of the primary structure of new AMPs

Two transcripts were reduplicatively obtained by molecular cloning and their nucleic acid sequence; the corresponding translated amino acid sequences were illustrated in Figure 1. Both translated open-reading frames (ORFs) consisted of 76 amino acid residues, which were composed of a canonical pro-sequence with highly conserved 22-residue signal peptides, Asp- and Glu-rich acidic intervening sequences, and highly identical mature peptides (28 residues) (Figure 1A, 1B). The processing site was at a typical prohormone convertase site Lys-Arg for the conversion of the precursors to the active forms. Alignments of peptides precursors (Figure 1C) exhibited a high degree of primary structural identity, for the mature peptides, they were differing only at positions of 1, 5, 7, and 18 in the sequence. It was speculated that both DMS-PS1 and DMS-PS2 have a C-terminal amidation modification. The C-terminal residue was followed by a C-terminal Gly residue, which acted as an amide donor to generate a C-terminal amide; the endopeptidase removed the C-terminal basic residues²⁷.

Figure 1. Molecular cloning of the naturally occurring AMPs DMS-PS1 and DMS-PS2. Nucleotide sequences and translated ORF amino acid sequences of cDNAs encoding DMS-PS1 (A) and DMS-PS2 (B) cloned from a secretion-derived library. Putative signal peptides, mature peptides and stop codons are double-underlined, single-underlined and indicated by asterisks, respectively; (C) Sequence alignment of precursors of DMS-PS1 and DMS-PS2. ¹Putative signal peptide; ²Acidic spacer peptide region; ³Propeptide convertase processing site; ⁴Mature peptide; ⁵Tripeptide (Gly-Glu-Gln) residues amide donor. Conserved residues are indicated in yellow;

Moreover, a complex chromatogram in 240 min (Figure 2A) was provided by the crude skin secretion analysis performed by RP-HPLC. Following MS/MS fragmentation (Figure 2B, 2C), both dermaseptins were detected by MS/MS in the #117 elute sample, and most b-ions and y-ions of target fractions were mapped with the calculated value; then, the glycine residue at the C-terminal end was served as the amide donor and the amidation modification of C-terminus was conformed (Figure S1). Therefore, two 28-residue truncated isoforms were identified by mass spectrometric analysis in primary structures; they were named dermaseptin-PS1 (DMS-PS1) and dermaseptin-PS2 (DMS-PS2). The validated structures of these two natural peptides were successfully chemically synthesised; then, they were confirmed to have the purity of >95%.

Figure 2. Identification of DMS-PS1 and DMS-PS2. (A) Reverse-phase HPLC chromatogram of *P. sauvagii* skin secretion. Both dermaseptin peptides were detected in the same fraction (#117, indicated with an arrow); MS Ion spectra of DMS-PS1 (B) and DMS-PS2 (C).

3.2 Secondary structure and physicochemical property of DMS-PS1 and DMS-PS2

Dermaseptins are a large class of cationic peptides with α -helical amphipathic structures. The properties of α -helices in the peptides were illustrated by simulating both DMS-PS1 and DMS-PS2 with the helical wheel plots (Figure 3A, 3B). These clearly revealed that the hydrophobic faces of both peptides consist of hydrophobic amino acid residues, which orientate towards the polar face. The physicochemical parameters of the two peptides were similar (Table 1). The hydrophobic moment that was a measurement of the amphipathicity of the helix and overall net charges was basically identical for both peptides. However, the hydrophobicity of DMS-PS2 was slightly more than the propensity of DMS-PS1. Then, the 3-D models of the two peptides were predicted by the I-TASSER server and verified by ProSA and RAMPAGE server (Figure S2). The verified models revealed that the α -helical conformation should be adopted in both DMS-PS1 and DMS-PS2 (Figure 3C, 3D). In addition to the molecular simulation, CD assays were also used to further experimentally determine the secondary structure of DMS-PS2. DMS-PS2 existed as a random coil in aqueous solution (10 mM ammonium acetate buffer) but displayed a typical α -helical structure in membrane mimic environment with helicity of 13.2% (50% TFE), 42.7% (10 nM SDS) and 34.9% (100 nM SDS) (Figure 3E). Furthermore, the CD spectra of DMS-PS2 was obviously induced to a typical α -helical conformation by bacteria (*P. aeruginosa*) after incubated together for 1 h or more; however, it could not be induced immediately (Figure 3F). Additionally, it was found that DMS-PS2 molecule was spread on the surface of the Gram-negative bacterial membrane adopting an α -helical conformation verified by based on the molecular docking results (Figure 3G).

Figure 3. Simulation and determination of the secondary structures of peptides. Helical wheel plots of DMS-PS1 (A) and DMS-PS2 (B), the hydrophobic moment direction was indicated by an arrow and the different residues on the hydrophobic face were framed in red colour; The predicted 3-D models of DMS-PS1 (C) and DMS-PS2 (D), the amino acid residues which are different from each other are represented in sticks and labelled, Val 18 in DMS-PS2 is shown in yellow; (E) CD spectra of DMS-PS2 (50 μ M) in 10 mM ammonium acetate water solution, in 50% TFE/10 mM ammonium acetate solution, in 10 nM and 100 nM SDS solution; (F) CD spectra of DMS-PS2 (10 μ M) induced by bacteria suspension (*P. aeruginosa*, 5×10^6 CFU/mL) in different time durations; (G) Theoretical docking study performed with DMS-PS2 and the inner cytoplasmic membrane of Gram-negative bacterial model. Balls represent the polar heads of the phospholipids and green sticks represent their hydrophobic tails.

Table 1. The physicochemical properties of DMS-PS1 and DMS-PS2

Peptide	Primary Structures	H ¹	< μ H> ²	Net Charge
DMS-PS1	GLWKSFLKENVGKAAGKAALNAVTDNVNQ-NH ₂	0.323	0.267	+4
DMS-PS2	ALWKTLKENVGKAAGKAVLNAVTDNVNQ-NH ₂	0.374	0.269	+4

¹H: Hydrophobicity

²< μ H>: Hydrophobic moment

3.3 Self-aggregation of DMS-PS2

The rigid symmetric multimer docking of two DMS-PS2 molecules indicated that the two peptides form an approximately anti-parallel form; the hydrophobic surface is exposed rather than being buried inside of the dimer interface (Figure 4A). It was hypothesized in this study that the hydrophobic DMS-PS2 molecules may have a self-assembly tendency; then, this was investigated using dynamic light scattering (DLS) assays. An average diameter of fresh DMS-PS2 was approximately 15 nm in phosphate buffer (pH 7.2) while DMS-PS2 started to aggregate (average diameter: 60 nm) by the incubation of 3 h in physiological condition (Figure 4B). The aggregation effect was time-dependently increased but without concentration-dependent and without pH-dependent (Figure S3). Besides, the distribution of particle size gradually tended to be balanced

homogeneous. The average diameter of DMS-PS2 after the incubation of 72 h was significantly increased (average diameter: 150 nm), with the size of 10-fold larger than that of the fresh sample. The AFM images indicated that after the incubation of 72 h, DMS-PS2 formed a self-assembled structure compared to the freshly prepared DMS-PS2 (Figure 4C). Besides, the diameters of the major nanoparticles were consistent with the result of DLS.

Figure 4. Aggregation of peptide DMS-PS2. (A) The anti-parallel symmetric dimer of DMS-PS2. The molecules are representing in cartoon and the hydrophobic surfaces are rendering. (B) The DLS spectrum of DMS-PS2 fresh prepared (black), incubated for 3 h (red), 24 h (green) and 72 h (blue). (C) AFM micrographs of the morphology of fresh DMS-PS2 and the aggregated DMS-PS2 incubated for 72 h.

3.4 Inhibition of mobile and sessile microbial growth and haemolytic activity

Five key species of microbes, including Gram-positive bacteria (sensitive/resistant), Gram-negative bacteria, and yeast, were selected in order to assess the antimicrobial ability of peptides.

Both DMS-PS1 and DMS-PS2 displayed potent activity against all planktonic microorganisms at the concentrations tested in both MIC and MBC assays (Table 2). Similarly, these two peptides exhibited potent broad-spectrum antimicrobial activities against the reference microbes, but DMS-PS2 possessed an overall stronger antimicrobial activity no matter against sensitive strains or resistant strains. For *P. aeruginosa*, which is easy to form biofilms and difficult to eliminate, DMS-PS2 also displayed potent bacteriostatic and bactericidal activity, inhibiting the growth of this bacterium at the concentrations ranging from 16 to 32 µg/mL.

Therefore, the minimum biofilm inhibitory concentration (MBIC), minimum biofilm eradication (MBEC), and initial attachment inhibitory concentration (IA) were applied to evaluate the actions of peptides affecting bacterial biofilm (Table 3). Two synthetic peptides displayed concentration-dependently potent inhibition of the sessile bacterial cells. Both dermaseptins maintained strong inhibition of the biofilm formation against *S. aureus* (NCTC 10788) (MBIC of DMS-PS1 32 µg/mL, DMS-PS2 16 µg/mL) together with significant increases in MBEC values (128 and 64 µg/mL, respectively). DMS-PS1 and DMS-PS2 were maintained equal activity to inhibit the immobilised bacteria cells (16 µg/mL against *E. coli*, 256 µg/mL against *P. aeruginosa*) for Gram-negative bacteria. Besides, DMS-PS1 and DMS-PS2 had identical potencies in order to avoid initial attachment on the flat-bottomed plate against *P. aeruginosa* at 128 µg/mL. Furthermore, DMS-PS2 was able to eradicate the mature biofilm (MBEC of 512 µg/mL against *E. coli* and *P. aeruginosa*).

DMS-PS1 exhibited an extremely low haemolytic activity while DMS-PS2 induced a stronger increase in haemolysis of the murine erythrocytes. For DMS-PS1, less than 50% haemolysis was observed at 512 µg/mL, its HC₅₀ was indicated as > 512 µg/mL causing the TI of DMS-PS1 was higher than 16, while the HC₅₀ of DMS-PS2 was 210.7 µg/mL and the TI was 18.6. Even though DMS-PS1 only possessed an overall weaker antimicrobial and haemolytic activity compared to DMS-PS2, DMS-PS2 displayed a moderate cell selectivity. Importantly, the haemolysis of the fresh murine erythrocytes induced by the concentration was significantly larger than the concentration of the antimicrobial effect.

Table 2. MICs, MBCs, HC₅₀, and TI of DMS-PS1 and DMS-PS2

Strains	MIC/MBC (µg/mL)	
	DMS-PS1	DMS-PS2
<i>S. aureus</i> (NCTC 10788)	16/32	8/16
MRSA (NCTC 12493)	64/128	16/32
MRSA (ATCC 43300)	32/64	8/16
<i>C. albicans</i> (NCYC 1467)	32/32	16/32
<i>E. coli</i> (NCTC 10418)	16/16	8/16
<i>P. aeruginosa</i> (ATCC 27853)	64/128	16/32
HC ₅₀ ¹	> 512	210.7
TI ²	> 16	18.6

¹The peptides concentration causing 50% haemolysis of the red bloods cells. When less than 50% haemolysis was observed at 512 µg/mL, HC₅₀ was indicated as > 512 µg/mL.

²Therapeutic index (TI) is the ratio of the HC₅₀ to the geometric mean of MIC.

Table 3. Anti-biofilm activity of DMS-PS1 and DMS-PS2

	<i>S. aureus</i> ¹ (µg/mL)		<i>E. coli</i> (µg/mL)		IA ²	<i>P. aeruginosa</i> (µg/mL)	
	MBIC	MBEC	MBIC	MBEC		MBIC	MBEC
DMS-PS1	32	128	16	>512	128	256	>512
DMS-PS2	16	64	16	512	128	256	512

¹ *S. aureus*: NCTC 10788

²IA is the minimum initial attachment inhibitory concentration

3.5 Inducement of permeability of the bacterial cell membrane and damage to bacterial morphology

The effects of DMS-PS1 and DMS-PS2 treatments on MRSA (ATCC 43300) were studied by the membrane permeability assay. Both peptides exhibited the concentration-dependent effect of membrane integrity. DMS-PS1 partially induced membrane permeabilization even at the highest tested concentration (256 µg/mL) while structurally similar DMS-PS2 displayed a more potent membrane permeabilizing activity at the concentration of 32 µg/mL and higher tested concentrations (Figure 5A).

The antimicrobial activity and membrane permeabilizing activity of DMS-PS2 was more potent than the activity of DMS-PS1 and both dermaseptins share conserved sequences, therefore, the antimicrobial actions were investigated using DMS-PS2 as the representative of these two peptides. Peptide localization experiment was performed by observing FITC-labeled peptides (Sub-MIC) using confocal microscopy. DMS-PS2 was observed to predominantly localize on the cell membrane for *P. aeruginosa* (Figure S2).

It can be illustrated from the results that DMS-PS2 localized on the cell membrane and affected the membrane integrity, thus the effect on the bacteria following treatment with the DMS-PS2 was further investigated in this study. All the bacterial cells could be stained with green color by the Syto 9 dye while the bacteria with disrupted cell membrane showed the red color by staining with the propidium iodide. Comparing to control, the permeability of the cell membrane of MRSA (ATCC 43300) and *P. aeruginosa* was significantly affected by the treatment of DMS-PS2 at the MIC concentration because many bacterial cells captured the red color. Besides, bacteria cells displayed permeabilized membrane at MBC concentration since most of the bacteria were stained with red colours (Figure 5B).

Furthermore, scanning electron microscopy (SEM) was performed to investigate the effect of DMS-PS2 on the cell membrane. The control was observed with the smooth and intact surface and completely separated. By comparison, numerous cells were evidently lysis followed by fusing, clumping, and disintegration by treating with DMS-PS2 for 1 h. Abundant cell clusters were evidently observed in the environment. Additionally, *P. aeruginosa* incubated with DMS-PS2 displayed aggregation, shrinking, and collapse. Both the planktonic bacteria cells and the adherent cells embedded in the biofilm had been damaged in the existence of the DMS-PS2 (MBC) (Figure 5C).

Figure 5. The antimicrobial actions of DMS-PS2. (A) The effect of membrane permeability of DMS-PS1 and DMS-PS2 against *S. aureus* (ATCC 43300). (B) The effect of DMS-PS2 on bacterial viability and membrane integrity, green fluorescence indicates the total bacteria cells, while red fluorescence indicates the membrane-impaired bacterial cells. (C) Evaluation of the effect of DMS-PS2 on bacterial morphology, MRSA (ATCC 43300) (left half panel) and *P. aeruginosa* (right half panel).

3.6 Assessment of wound healing of DMS-PS2 on MRSA infection in the murine epidermis

The efficacy of DMS-PS2 on infected skin wound healing was evaluated with a murine skin MRSA (ATCC 43300) infection model (Figure 6A). The treatment of the infection model was assessed by the validation of the proliferating and stable bacterial viable counts. DMS-PS2 potentially inhibited the MRSA growth in the infected murine epidermis. The viable count results of DMS-PS2 treatment groups (9.6×10^5 CFU/mL for DMS10.24 group; 5.6×10^4 CFU/mL for DMS20.48 group) displayed significantly decrease compared to the saline control samples (2.5×10^7 CFU/mL for CON group) on day 1 (Figure 6B). After the third-day treatment, the viable count remained at a low level (< 100 CFU/mL) and the wound surface started to scab with shrinking. Compared to CON group that failed to control the growth of MRSA, the DMS-PS2 treated infected wound was efficient to decrease the bacterial viable counts and showed distinctly wound-healing. The DMS-PS2 treated wound initiated to scab 3 days post-infection while CON

group appeared to scab 7 days post-infection. Moreover, escharotic exfoliation gradually appeared after 7-day medication (Figure 6C).

Figure 6. Assessment of wound healing of DMS-PS2 on MRSA infection in the murine epidermis. (A) The murine epidermal MRSA infection wound model; the validation of the proliferating and stable bacterial viable counts within the yellow and purulent wound was performed. **(B)** The colony count of MRSA in the infected wounds after first day treatment with DMS-PS2 (10.24 μ g and 20.48 μ g dosage), in comparison with the control group (MRSA+ PBS). **(C)** Evaluation of the effect of DMS-PS2 (DMS20.48; DMS-PS2 20.48 μ g/day) on MRSA infected murine skin.

4 Discussion

Frogs usually live in an environment that is dominated by different microflora, parasites, predators and physical factors, and therefore, their skin secretions are unique to defence, containing diverse chemicals that could be exploited. The process associated with the skin secretion consists of a holocrine mechanism that involves the self-lysis of gland cells and the release of their contents upon stimulation by external stimuli. Therefore, bioactive components of frog skin sections, especially biologically active peptides, have been extensively studied. In this study, two new antimicrobial peptides from the skin secretion of *P. sauvagei* have been isolated and identified. The two AMPs exhibit featured characteristics of dermaseptin family peptides, including a conserved Trp at position 3 and a consistent internal sequence (AAGKAA(A/V)LN) and a C-terminal amidation modification as prevalent hallmark of dermaseptin. Interestingly, most of the dermaseptin peptides contain the consensus motif (AA(A/G)KAAL(G/N)A) exactly as the DMS-

PS1, while DMS-PS2 contains a hydrophobic valine (Val) residue at 18 position. The helical wheel projection indicated that the existence of Val extended the hydrophobic face while other different amino acid residues share similar properties at other sites. Moreover, considering the same net charge and almost the same hydrophobic moment of them, only the total hydrophobicity of DMS-PS2 is higher than DMS-PS1 (0.374 vs 0.323). These results may be largely relevant to the increase of the efficacy and stronger antimicrobial actions of DMS-PS2, with the increasing the haemolytic effect as well. In addition to higher molecular diversity and complexity, an extra positive charge to enhance antimicrobial activities can be provided by the amidated post-translational modification^{15,16}.

The rich dermaseptin family peptides have attracted much attention from scientists due to the broad-spectrum antimicrobial activities against multiple opportunistic pathogens^{23–26}. In this study, the two dermaseptins, especially DMS-PS2, displayed more antimicrobial potency than many of the reported natural dermaseptins against a variety of microorganisms including antibiotic-resistant bacteria MRSA (NCTC 12493, ATCC 43300) and a devastating chronically infecting bacterium *P. aeruginosa* (ATCC 27853). The process of biofilm formation is complicated, generally, it consists of initial attachment, microcolony formation, and attached bacteria that mature into a differentiated biofilm²⁸. Both DMS-PS1 and DMS-PS2 dramatically inhibit the formation of biofilms and induce eradication of mature biofilms of three pathogens tested. Moreover, both peptides are strikingly effective in avoiding the *P. aeruginosa* cell clustering into microcolonies on the plastic surface, which is the initial step of biofilm formation. Therefore, the data in this study have revealed that these could be new anti-infective agents against persistent infections caused by planktonic microorganisms and sessile bacterial biofilms^{6, 23, 29}. Most of cationic antimicrobial peptides are more efficient to Gram-positive than the Gram-negative bacteria. It is believed that difference in the structures of outer membranes of bacteria can reduce differential susceptibilities³⁰. In particular, the outer membrane of Gram-negative bacteria could trap the AMPs to prevent their further translocation thereby protecting the cytoplasmic membrane. Interestingly, most of the dermaseptin superfamily peptides display the potent and broad-spectrum antimicrobial activity especially against Gram-negative bacteria. One hypothesis holds that the extent of the initial interaction with the cell membrane could also be modulated by cell-wall components, which can either entrap AMPs or promote their accumulation on the cytoplasmic membrane³¹. Gram-negative bacteria have an additional outer membrane barrier to cross, but still many cationic peptides are more selective against Gram-negative than Gram-positive bacteria. This was hypothesised that the electrostatic interactions on outer membrane of Gram-negative bacteria becomes stronger as the anionic glycolipid lipopolysaccharide fills the outer monolayer of the outer membrane. While the outer-membrane interaction is not immediate cause of cell death, having diffused through the outer membrane, the accumulation approach of peptides on the cytoplasmic membranes result in final cell death. All this evidence could be used to hypothesize that the antimicrobial activity is related to the evolutionary of the *Phyllomedusa* and *Agalychnis* frogs that are the major resources of dermaseptins⁷.

It is generally assumed that cationic AMPs could interact with negatively charged head groups of the cytoplasmic membrane and cause the membrane damage to exert antimicrobial activity. In addition, the α -helices of AMPs play an important role in the activity. DMS-PS2 displays a random coil conformation but could be induced to an α -helical structure in the membrane-mimic condition. Especially in the 10 nM SDS solution, the α -helical content could be induced to 42.7%.

Furthermore, the secondary structure of DMS-PS2 in bacterial membrane was also investigated. A typical α -helical conformation was induced by the incubation with gram-negative bacteria *P. aeruginosa*. The fact that the FITC-labelled DMS-PS2 localized around the cell membrane of *P. aeruginosa* further experimentally confirmed the peptide-membrane interaction, and the interaction was visualized by molecular docking.

Therefore, the antimicrobial mechanism of DMS-PS2 was investigated in this study, deducing that the antimicrobial effects are probably caused by the impact of membrane permeability and integrity. Compared to DMS-PS1, DMS-PS2 exhibited a more potent membrane permeabilizing effect with a typical dose-dependent manner. Followed by the visualization through confocal laser scanning microscopy, it was found that the bacterial membrane permeability was largely increased under the treatment of DMS-PS2, especially at the MBC level. We deduced that DMS-PS2 could alter the permeability of cell membrane at MIC concentration to exert inhibitory activity but not lethal. Although it was indicated that DMS-PS2 could rapidly affect the permeability and depolarization of cytoplasmic membranes, whether the appeared increased membrane permeability was caused by the cell membrane disruption still needed to be further investigated. This was answered by the SEM results. In contrast to the smooth and intact morphology of the tested bacteria without peptide treatment, the DMS-PS2 treated groups exhibited devastating membrane disruption, which could also explain the potent antimicrobial activity of DMS-PS2 and its membrane permeabilizing effect. Besides, bacteria cells from the biofilm-forming strain *P. aeruginosa* bacterium interact more with one another to form an initial biofilm compared with separated MRSA individual bacterium. What need to be pointed out is that the size difference between MRSA and *P. aeruginosa* is not significant, as revealed in Figure 5C, however, this phenomenon has also been reported in previous studies^{32,33}. This phenomenon might because that the three-dimensional effect and different viewing angle of bacillus morphology make them look smaller than the actual sizes comparing to the coccus morphology. Nevertheless, DMS-PS2 could not only destroy the individual planktonic bacterium but also roll the embedded bacterial groups into a mass. The micrographs indicate that the cell membranes were rapidly affected and disrupted by DMS-PS2, resulting in cell death. This is correlated with the anti-biofilm activity of DMS-PS2. Additionally, both dermaseptins exhibit no significant toxicity for mammalian blood red cells at the effective antimicrobial concentrations. However, DMS-PS2 still exhibited a moderate toxicity towards the mouse fibroblast cells, as revealed by the MTT assay (Figure S4). The cell toxicity indicated that the peptide was not only toxic to the eukaryotic fungal membranes but also affected eukaryotic cell membranes. Furthermore, the result of the acute toxicity assay was investigated and the result demonstrated that all the mice could survive the injected dose of DMS-PS2 (5 mg/kg, data not shown), which was about 5 times than the dose administrated to the skin. It was supposed to be reasonable to detect the toxic effect of DMS-PS2 on non-infected wound, while the results of cell viability and the acute toxicity assays, to some extent, indicated that DMS-PS2 might not be toxic at tested dose on the skin. Although DMS-PS2 came at the cytotoxic effect, it qualifies as a possible drug candidate, especially given its potent and broad-spectrum activity. Pexiganan, which is modified from magainin, has been used to treat diabetic foot infections in clinical trials³⁴. Maganin was discovered in the skin secretion of the frog *Xenopus laevis*, but it has significant toxicity toward mammalian cells.

Compared with the action of a single peptide molecule, it is reported that some engineered antimicrobial peptides were shown to self-assemble into stable supramolecular structures and these

peptides with assembled status exhibited significantly increased antimicrobial activities and biofilm inhibitory and eradicating activities³⁵. An effective way to design a self-assembly peptide is to enhance the hydrophobicity of it. As mentioned above, the total hydrophobicity of DMS-PS2 is relatively higher. Moreover, the CD spectra of DMS-PS2 displayed a typical strong random coil structure in aqueous solution. Together with its potent antimicrobial and anti-biofilm activities, these facts attracted our interest in investigating the self-assembling property of DMS-PS2. The molecular simulated α -helical structure and the α -helical wheel projection of DMS-PS2 clearly exhibited an amphipathic feature. It can be illustrated from the rigid self-docking results that the binding poses of two DMS-PS2 molecules and the two DMS-PS2 molecules presented as an anti-parallel form. Generally, the hydrophobic residues have to be buried inside to form hydrophobic binding and to avoid contact with water molecules. What beyond our expectation was that the hydrophobic residues of DMS-PS2 are exposed outward when DMS-PS2 exist as the dimer. For certain antimicrobial peptides, the experimental studies proposed that the α -helical structure undertake a transition to aggregated supramolecules^{13,31,36}. This phenomenon was also confirmed by DLS and AFM. The aggregation of DMS-PS2 was observed by incubating in a physiological solution for 3 hours. Furthermore, the intensity of the aggregation of the peptide was significantly augmented with the constant incubation. In line with the observation, the conformation of DMS-PS2 in PBS solution was constantly changed. Therefore, the antimicrobial effect of DMS-PS2 in solution was predicted as a dynamically changing process, ranging from unimolecular to oligomerization, in order to associate with the sustained and potent antimicrobial effects. The process of peptide self-assembly into amyloid-like fibrils is a two-step process, that is, from single peptide molecule to small amyloid oligomers and from the developed oligomers to assembled fibrils³⁷. Apparently, our DMS-PS2 formed small-sized aggregates with oligomerization at the tested condition, indicating that the persistent aggregation might play a part in killing the bacterial cells or penetrating the bacterial biofilms. The aggregation effect was time-dependently increased but without concentration-dependent and without pH-dependent, consistently with the aggregating process of other natural antimicrobial peptides³⁸. The hypothesis holds that amyloid propensity and antimicrobial activity are related in the sense that aggregation-prone regions may have served as templates from which AMPs were evolutionarily derived. In addition, the aggregation of peptides and proteins can be divided into two main categories: those associated with pathological processes, and those with functional implications in vivo³⁸. It has reported that peptides are specialized role of AMPs in amphibian skin, where aggregation/concentration effects are less harmful considered the aggregation effect in most organisms³⁹.

It is profoundly understood that the clinical application of peptide drugs has been restricted due to their poor absorption, susceptibility, the largest obstacle, and rapid proteolytic degradation. For DMS-PS2 we identified, in addition to the clarified antimicrobial mode of action, the efficacy of the peptide treatment against the murine skin MRSA infection model makes the most sense. DMS-PS2 demonstrated the potent efficiency of bacterial inhibition on the infected wound and its obvious effect on wound healing. Under the daily topical dosage of only 10.24 μg and 20.48 μg ,

the wound treated DMS-PS2 initiated to scab 3 days post-infection and escharotic exfoliation gradually appeared after 7-day medication.

5 Conclusion

In conclusion, two new AMPs from the skin secretion of *P. sauvagii* have been identified in this study. Cationic dermaseptins exhibited significant broad-spectrum antimicrobial activities; activities against bacterial biofilms of persistent infections are associated with weak toxicity for mammalian red blood cells. The membrane permeabilizing ability of DMS-PS2 was confirmed; importantly, it demonstrated potent efficiency of the treatment of MRSA infected murine skin model. Furthermore, what beyond our expectation is that DMS-PS2 displayed a self-aggregating property, which also makes it a promising suggestion for infection-proof biomaterial development.

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