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ORIGINAL ARTICLE

The topical application of Sphistin$_{12\text{-}38}$ in combination with sponge spicules for the acne treatment

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Abstract

We demonstrated for the first time that a marine-derived antimicrobial peptide (AMP), Sph$_{12\text{-}38}$, exhibit high antimicrobial activity against *P. acnes* with a minimum bactericidal concentration (MBC) value of 7 μM. Meanwhile, Sph$_{12\text{-}38}$ has no significant cytotoxicity to human keratinocytes (HKs) at its high concentration (33.5 μM). The topical application of sponge *Haliclona sp*. spicules (SHS) dramatically enhanced the skin penetration of Sph$_{12\text{-}38}$ up to 40.9 ± 5.9% ($p < 0.01$), which was 6.1 ± 0.9-fold higher than that of Sph$_{12\text{-}38}$ alone. Further, SHS resulted in the accumulation of most Sph$_{12\text{-}38}$ in viable epidermis and dermis. Further, the combined use of Sph$_{12\text{-}38}$ and SHS resulted in a cure rate of 100% for rabbit ear acne treatment in vivo for two weeks, while the one induced by other groups was 40%, 0% and 0% for SHS alone, Sph$_{12\text{-}38}$ alone and control group, respectively. The strategy of combined using AMP and SHS can also be applied in a rational designed topical delivery system for the management of other deep infection of the skin. The effectiveness of SHS by itself on the treatment of acne was also demonstrated by clinical trials. After 14 days of treatment by 1% SHS gel. The number of skin lesions decreased by 51.4%.

Keywords *Acne vulgaris* · Antimicrobial peptides · Sponge spicules · Topical delivery

Introduction

Acne vulgaris is a common skin condition caused by many factors and accompanied by inflammation, which can affect 11% of adults and 85% of teenagers [1, 2]. The exact role of *Propionibacterium acnes* (*P. acnes*) in formation and acne formation remains controversial. However, it is regarded as a prime predisposing cause of acne. So far, all most chemotherapies against *P. acnes* have various side effects, including local irritation and erythema [3] from the use of benzoyl peroxide; the liver damage and teratogenic effects [4] from the long term use of retinoids; dermatitis and infection from the use of salicylic acid [5], and among others. Antibiotics have been mainstay drugs for acne treatment for more than 40 years [6], which however results in more than 50% of *P. acnes* strains resistant to antibiotics because of the long-term extensive use of antibiotics [7, 8]. Therefore, it is necessary to develop a novel topical antimicrobial treatment for acne which also does not cause *P. acnes* resistance.

Antimicrobial peptides (AMPs) have been extensively studied due to their broad-spectrum antimicrobial activity and low drug resistance rate [9]. In our previous study,

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Sphistin (Fig. 1a), a histone H2A identified from hemocytes of *Scylla paramamosain*, its two truncated fragments Sph_{12-38} (Fig. 1b), Sph_{20-38} (Fig. 1c), and AS-hepc3 (Fig. 1d) that is a hepcidin-like gene cloned from black porgy of *Acanthopagrus schlegelii*, exhibit high antimicrobial activity against bacteria and yeast [10, 11]. However, their potential antimicrobial activity against *P. acnes* has not been investigated. Moreover, the topical application of AMPs for acne treatment has rarely been reported. The treatment of subcutaneous diseases infected by bacteria or fungi have always been a worldwide medical challenge. The pathogen like *P. acnes* generally resides deeply at the sebaceous gland in the mid-dermis, AMP as a class of hydrophilic macromolecular drug, can not reach the deep skin layers at their therapeutic concentrations because of the excellent defensive function of the stratum corneum (SC). Sponge *Haliclona sp.* spicules (SHS, Fig. 1e) have been proved that it can be applied topically as novel silicon microneedles in the field of skin drug delivery [12]. SHS can create plenty of long-lasting micro-channels on the skin [13]. Moreover, SHS can be flexibly applied to any desired skin location. In combination with SHS, AMPs might penetrate into deep layers of the skin through these micro/nano-channels caused by SHS and then kill pathogens effectively.

In this work, we investigated the potential antimicrobial activity against *P. acnes* of four AMPs, including Sphistin, Sph_{12-38}, Sph_{20-38} and AS-hepc3. More importantly, we showed the effect of AMPs in combination with SHS on the Acne treatment. This study demonstrated a potential strategy to exploit the antimicrobial properties of AMPs by topical delivery using SHS, thus pushing on their translation from lab to clinic.

Material and method

Chemicals and animals

All AMPs were synthesized by GLORY CHEMISTRY Co., Ltd. (Shanghai, China). The strain of *P. acnes* (ATCC6919) was purchased from Guangdong microbial culture collection center (Guangzhou, China). Clindamycin and Doxycycline Hyclate were purchased from Sigma (St. Louis, MO, USA). Human keratinocytes (HKs) from foreskin were purchased from PromoCell (Heidelberg, Germany). The porcine skin was purchased from YinXiang Group Co., Ltd. (Xiamen, China). 1% clindamycin gel was purchased from SuZhou Fourth Pharmaceutical Factory Co., LTD. (SuZhou, China). AnaeroPack was purchased from Mitsubishi Gas chemical company (Tokyo, Japan). The Scotch® Transparent Tape was purchased from 3 M Corporation (Saint Paul, MN, USA). Tissue OCT-Freeze Medium was purchased from Sakura (Torrance, CA, USA). And all other chemicals used in this study were of analytical grade and were purchased from Sinopharm Group Co. Ltd. (Shanghai, China).

New Zealand white rabbits were purchased from Vital-river Laboratory Animal Technology Co., Ltd. (Beijing,

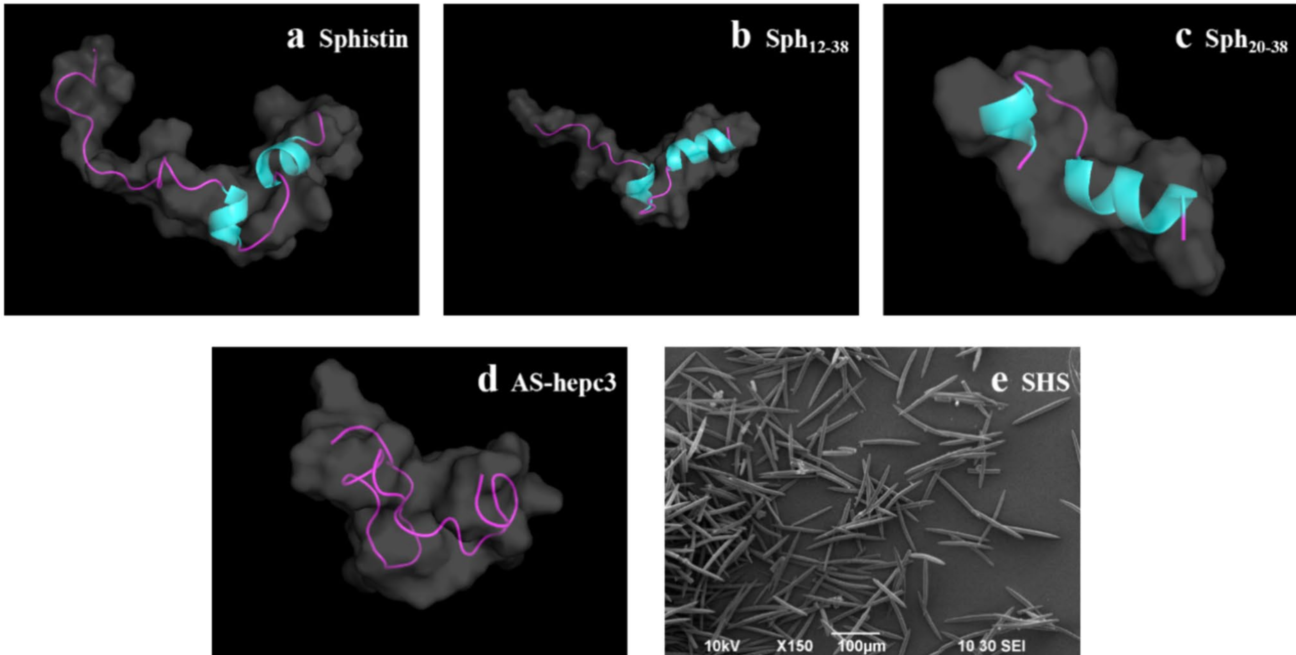

Fig. 1 Visualization of AMPs and SHS. **a** Sphistin; **b** Sph_{12-38}; **c** Sph_{20-38}; **d** AS-hepc3. 3D protein structures of AMPs were predicted by I-TASSER website and built by PyMol 2.4; **e** SHS by SEM observation

China). All animal experiments were conducted according to the guidelines of “Regulations on the administration of laboratory animals of Xiamen University” and approved by the Institutional Animal Care and Use Committee of Xiamen University (ethics approval number XMULAC20190002).

Bacterial culture, antimicrobial activitystudy, in vitro cytotoxicity study, stability study of $Sph_{12\text{-}38}$

All AMPs were verified by mass spectroscopy, respectively (Supplemental Figure S1). These experimental procedures are described in detail in the Supplementary Materials.

Skin penetration study in vitro

This part of experiment was performed according to the published experimental method [13]. In details, the porcine skin (YinXiang Group Co., Ltd., Xiamen, China) was removed subcutaneous fatty tissue and hair shaft carefully. Skin was rounded by the punch (36 mm in diameter). The conductivity through the skin was measured to ensure its integrity with a multimeter (FLUKE, Shanghai, China) [14].

Franz diffusion cell system (YuYan SV12, Shanghai, China) was used for in vitro skin penetration study. PBS (0.05 M) was added to receptor compartment. Skin disk was fixed on receptor compartment under occlusive condition. For SHS group, 10 mg of SHS was massaged on the skin by a handheld massager (300 r/min) at 0.3N pressure. Then 150 µL of PBS or FITC- $Sph_{12\text{-}38}$ solution (1 mg/mL) was added onto the skin surface. All the skin samples on the Franz diffusion cells were immersed in a 37 °C water circulating pump (YuYan SV12, Shanghai, China) for 16 h. The penetration of FITC- $Sph_{12\text{-}38}$ in different skin layers was analysed with the tape-stripping method [13, 15]. Briefly, the stratum corneum (SC) was stripped 10 times consecutively. The stripped tapes were collected according to the following scheme: SC 1 = first strip, SC 2 = second to fifth strips, and SC 3 = sixth to 10th strips. After tape-stripping, the viable epidermis was separated carefully from the dermis layer with a surgical scalpel. The dermis was then cut into small pieces. Then, FITC-$Sph_{12\text{-}38}$ was extracted from separated skin layers by using a mixture of methanol and PBS pH 7.4 (1:1, V/V) overnight at room temperature. Afterward the dispersions were centrifuged (5000 r/min for 5 min) to pellet skin tissue. The supernatants were withdrawn and diluted if necessary for the next measurement. The FITC-$Sph_{12\text{-}38}$ concentration was measured by a fluorescence spectroscopy (Thermo Fisher Varioskan LUX, MA, USA) with an excitation of 485 nm and an emission of 520 nm. The method was validated for linearity, accuracy and precision. The linear range of FITC-$Sph_{12\text{-}38}$ during the measurements was from 0.01 µg/mL to 10 µg/mL ($r^2 = 0.9999$).

Confocal microscopy study

The skin samples were cut into small pieces and imbedded in optimal cutting temperature compound (Sakura, Tokyo, Japan) then frozen in liquid nitrogen for 60 s. The porcine skin was sectioned with a thickness of 20 µm on a Cryomicrotome (Thermo Fisher, CryoStar NX50, MA, USA). All samples were visualized with confocal microscope (Carl Zeiss, LSM780NLO, Jena, Germany) with an excitation of 485 nm and an emission of 520 nm.

Acne treatment study in vivo

Twenty New Zealand white rabbit(female, 12 weeks old, weighting 2 kg to 2.5 kg) were used in this study. The modeling method of rabbit ear acne was performed as described in Kligman method [16, 17]. Briefly, the whole experiment period was 28 days. On the day 7 ~ 14, each rabbit ear was injected with 50 µl of the suspension of *P. acnes*. Oleic acid was applied to each rabbit ear daily during this 28-day period [18]. 1% clindamycin gel was used as a positive control for this experiment. All rabbits were randomly divided into 4 groups, 5 in each group. For SHS and $Sph_{12\text{-}38}$ + SHS group, SHS (5.6 mg/ cm^2) was applied to rabbit ear surface and massaged (0.3 N, 60 r/min) every 2 days. Saline (blank and SHS group), clindamycin gel (0.1 g/cm^2, clindamycin group) or $Sph_{12\text{-}38}$ solution (1 mg/ml, 84.7 ul/cm^2, $Sph_{12\text{-}38}$ and $Sph_{12\text{-}38}$ + SHS group) was applied to rabbit ear surface daily for 14 days. Each rabbit ear was photographed every day. After treatment, all animals were sacrificed by air injection. The number, diameter, quantity and height of rabbit ear acnes were recorded to value the treatment effect. The calculation formula was as Eq. 1 [19].

$$\text{The clearance rate of lesions }(\%) = \frac{\text{the number of skin lesions before treatment} - \text{the number of skin lesions after treatment}}{\text{the total number of skin lesions before treatment}} \times 100\% \tag{1}$$

Table 1 Bioactivities against *P. acnes* of Antimicrobial peptides and antibiotics

AMPs & Antibiotics	Amino acid sequence (N→C) Or molecular structure	MBC (μM) *P. acnes* ATCC6919
Sph_{12-38}	KAKAKAVSRSARAGLQFPVGRIHRHLK	7
Sphistin	AGGKAGKDSGKSKAKAVSRSARAGLQFPVGRI HRHLK	12
Sph_{20-38}	RSARAGLQFPVGRIHRHLK	24
AS-hepc3	SPAGRNSRRRRCRFCCGCCPDMIGCGTCCKF	18
Clindamycin		0.2
Doxycycline Hyclate	HCl	0.2

MBC minimum bactericidal concentration

Therapeutic efficacy of rabbit ear acnes was assessed as Eq. 2 [20]:

Cure: ≥ 90% clearance; Excellent response: 60 ~ 89% clearance; Good response: 20 ~ 59% clearance; Poor response: < 19% clearance or no significant response.

Finally, the effectiveness rate was calculated according to the following formula:

$$\text{The effectiveness rate}(\%) = \frac{\text{cure cases} + \text{excellent response cases}}{\text{total cases}} \times 100\% \quad (2)$$

Histopathology study of rabbit ear acne

Rabbit ear tissue was cut off and imbedded in optimal cutting temperature compound and frozen in liquid nitrogen for 60 s. Rabbit ear tissue was sliced into 10 μm on a Cryomicrotome. Each sample was stained with hematoxylin and eosin and then imaged under the microscope (Carl Zeiss, Axio Imager A2, Oberkochen, Germany).

Clinical study of SHS for acne treatment

Trial 1: This experiment was conducted by Xiang 'an Hospital of Xiamen University approved by Medical Ethics Committee of Xiamen University (Ethic Number: XDYX202305K24). Thirty subjects with grade I-II facial acne were recruited for the study and signed an informed consent form. SHS was made into a carbomer gel with a concentration of 1%. Designated area of skin was cleansed with 75% ethanol. 0.5 g SHS gel was applied to the affected area and massaged for 4 min every two days. After 14 days, ISGA (Investigators's static global assessment) grade and number of lesions was assessed by physicians to determine the efficacy of treatment.

Fig. 2 Morphological changes of *P. acnes* ATCC6919 observed with SEM induced by (**a**)$Sph_{12\text{-}38}$; **b** Sphistin; **c** $Sph_{20\text{-}38}$; **d** AS-hepc3; **e** Clindamycin; **f** Doxycycline hydrochloride; **g** PBS after a 2 h incubation

Trial 2: Frobupre® Hydratation Absorption Improving Massage Gel (FB gel, record number: 2023000354) is an anti-acne product based on SHS. The acne removal efficacy of the FB gel was clinically verified by SGS-CSTC Technical services Co., LTD. Thirty-three subjects with grade I-II facial acne were recruited for the study and signed an informed consent form. Designated area of skin was cleansed. 0.5 g FB gel (1% SHS) was applied to the affected area and massaged for 4 min every two days. After 14 days, Images of the subjects' faces were analyzed by VISIA (CANFIELD, USA). The number of lesions was assessed by physicians to determine the efficacy of treatment. And the treatment effect of the product was also scored by the subjects.

Statistical analysis

All experiments in this study were performed in triplicate at least. All data were expressed as the mean value ± S.D. Two-tailed and unpaired Student's t test were performed and $p < 0.05$ is considered to be significant. All image data was analyzed by Image J software.

Results

In vitro antimicrobial activity of AMPs against *P. acnes*

The antimicrobial activity of AMPs and antibiotics against *P. acne* were evaluated in vitro in terms of minimum bactericidal concentrations (MBCs). $Sph_{12\text{-}38}$ exhibited the lowest MBC values (7 μM) among all AMPs, which is approximately half that of Sphistin (12 μM). The MBC values of other AMPs were listed in Table 1. Both antibiotics, Clindamycin and Doxycycline Hydrochloride as positive controls, presented much lower MBC values (0.2 μM) compared to all AMPs.

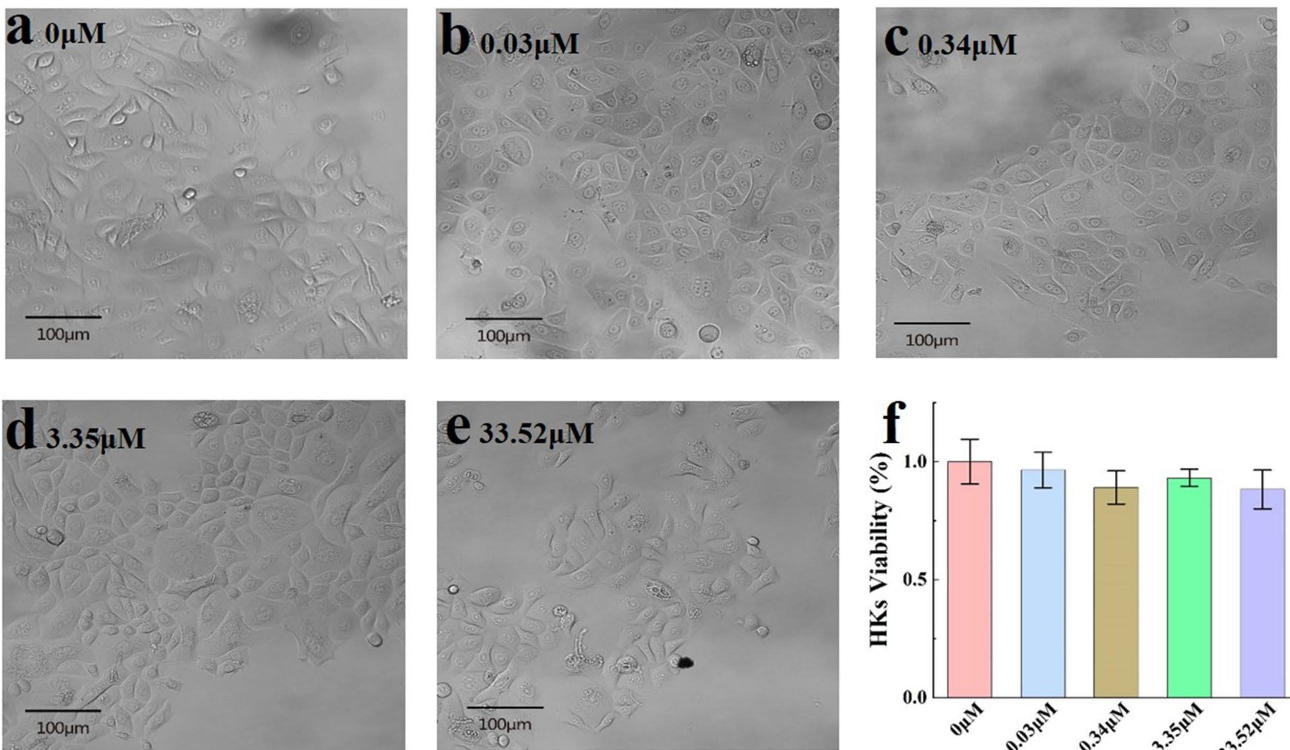

Fig. 3 $Sph_{12\text{-}38}$ had no significant cytotoxicity to human keratinocyte cell line. **a-e** Morphological changes of HKs after incubation with Sph12-38 at different concentrations for 24 h. The dosages were 0 μM, 0.03 μM, 0.34 μM, 3.35 μM, 33.52 μM, respectively. **f** Histogram of HKs survival rate at different $Sph_{12\text{-}38}$ concentrations after a 24 h incubation. *p*-values compared to control group: 0.03 μM group ($p=0.66$), 0.34 μM group ($p=0.48$), 3.35 μM group ($p=0.36$), 33.52 μM group ($p=0.46$)

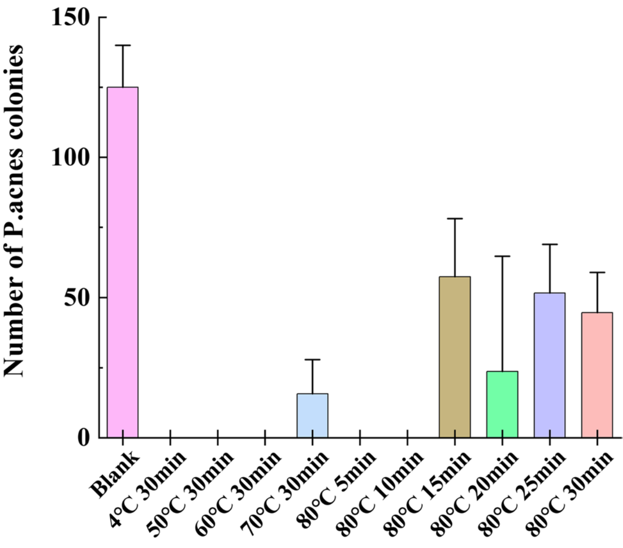

Fig. 4 The antibacterial ability of $Sph_{12\text{-}38}$ at its MBC under various temperatures and incubation times

P. acnes morphological changes caused by AMPs and antibiotics

The *P. acnes* morphological changes caused by these AMPs and antibiotics at their MBCs in 2 h have been investigated by using scanning electron microscopy (SUPRA 55, ZEISS, Germany). After 20 min incubation (37℃, anaerobic conditions), $Sph_{12\text{-}38}$ led to obvious bubbly protuberances on the surface of *P. acnes* (Supplemental Figure S2a), while all other testing groups did not result in obvious damage to the morphological structure and integrity of the bacterial. After 2 h incubation (37℃, anaerobic conditions), while *P. acnes* in PBS still had glossy and intact cell surface without any cell lysis or debris (Fig. 2g), *P. acnes* treated with $Sph_{12\text{-}38}$ (Fig. 2a) displayed obviously coarse cell membrane surfaces with a high degree of cell lysis and debris, which was comparable with *P. acnes* incubated with clindamycin (Fig. 2e). In contrast, other AMPs had a relatively weak impact on the morphological shape of (Fig. 2b-d). $Sph_{12\text{-}38}$ as AMP of choice has been selected for further studies.

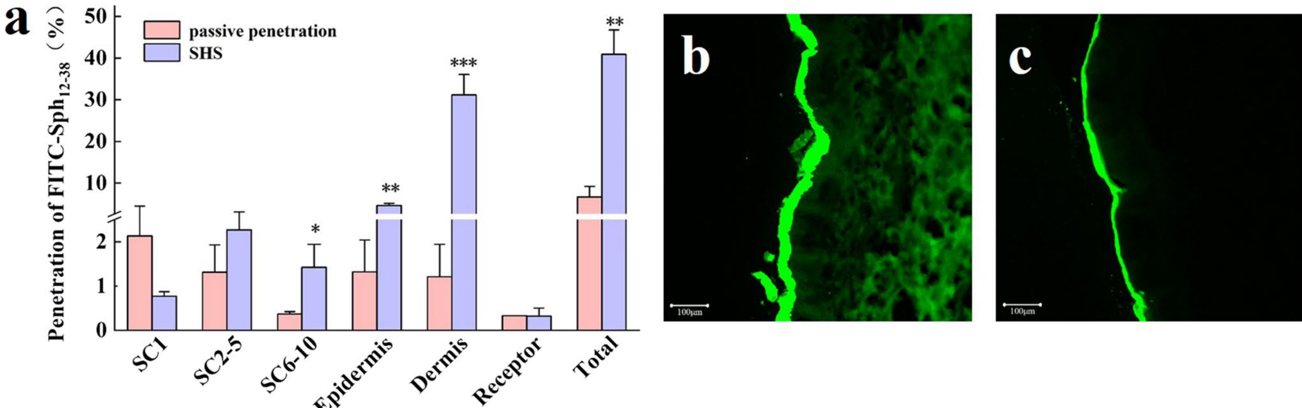

Fig. 5 SHS enhanced the skin absorption of FITC-$Sph_{12\text{-}38}$ in vitro. **a** skin absorption of FITC-$Sph_{12\text{-}38}$ in different skin layers. SC1 is the first strip, SC2-5 is the second to fifth strips, and SC6-10 is the sixth to tenth strips. **b** Skin penetration of FITC-$Sph_{12\text{-}38}$ after SHS treatment in 16 h. **c** Skin penetration of FITC-$Sph_{12\text{-}38}$ alone in 16 h. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Values represent mean ± SD ($n=3$)

The cytotoxicity of $Sph_{12\text{-}38}$ in human keratinocytes (HKs) in vitro

MTT assay has been performed to evaluate the cytotoxicity of $Sph_{12\text{-}38}$ in human keratinocyte cell line at different $Sph_{12\text{-}38}$ concentrations in 24 h incubation. The morphological changes of HKs induced by $Sph_{12\text{-}38}$ at different concentrations (Fig. 3) have been observed by confocal microscope. The results showed that $Sph_{12\text{-}38}$ had no significant cytotoxicity to human keratinocyte cell line at its high concentration (33.5 μM, $p=0.46$).

Thermal stability of $Sph_{12\text{-}38}$

The antibacterial activity of $Sph_{12\text{-}38}$ in terms of MBC at different temperatures was measured. $Sph_{12\text{-}38}$ at its MBC with

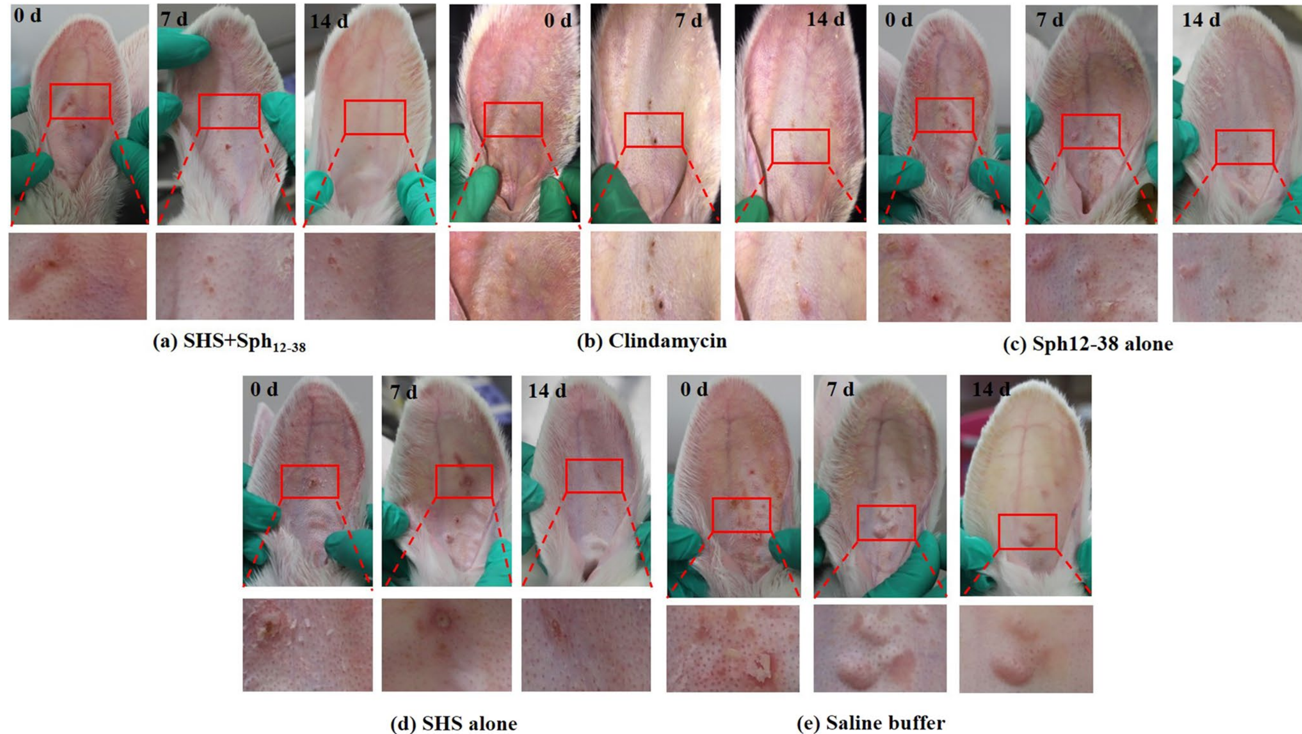

Fig. 6 The treatment on rabbit ear acne in vivo for 14 days by using (**a**) the combination of SHS and $Sph_{12\text{-}38}$, **b** Clindamycin alone, **c** $Sph_{12\text{-}38}$ alone, **d** SHS alone and (**e**) saline buffer

a

SHS+Sph12-38 treatment group

Clindamycin treatment group

SHS treatment group

Sph12-38 treatment group

Saline group

number of acne

diameterand thickness of acne (mm)

number(B) number(A) diameter(B) diameter(A) thickness(B) thickness(A)

b

Effectiveness rate

SHS+ Sph12-38 SHS Clindamycin Sph12-38 Saline

c

SHS+ Sph$_{12-38}$

Clindamycin

SHS

Sph$_{12-38}$

Saline

cure

excellent

good

poor

◂**Fig. 7** The treatment of rabbit ear acne by using $Sph_{12\text{-}38}$ in combination with SHS. **a** Characterization of rabbit ear acnes before (**B**) and after (**A**) treatment. **b** The effectiveness rate of acne after 14 days treatment from different strategies. **c** Therapeutic effect of different strategies on rabbit ear acne. Values represent mean ± SD, n = 10. * represents $p < 0.05$, *** represents $p < 0.001$

up to 60℃ over 30 min can effectively inhibit the growth of *P. acnes* (Fig. 4). Even heated to 80 °C for 10 min, $Sph_{12\text{-}38}$ was still thermally stable (Supplemental Figure S3), indicating a satisfying thermal stability of $Sph_{12\text{-}38}$.

Enhanced $Sph_{12\text{-}38}$ skin delivery by SHS topical application in vitro

The in vitro skin absorption of $Sph_{12\text{-}38}$ was investigated by using FITC-$Sph_{12\text{-}38}$. The skin absorption of $Sph_{12\text{-}38}$ was limited; only 6.7 ± 2.5% of FITC-$Sph_{12\text{-}38}$ was absorbed into skin in 16 h in vitro. In contrast, SHS can dramatically increase the skin absorption of $Sph_{12\text{-}38}$ in vitro; totally 40.9 ± 5.9% of the applied FITC-$Sph_{12\text{-}38}$ penetrated into the skin, which was approximately 6.1 ± 0.9-fold ($p < 0.01$) higher than $Sph_{12\text{-}38}$ alone. Further, SHS led to the significantly increased accumulation of $Sph_{12\text{-}38}$ in deep skin layers, including viable epidermis (4.6% ± 0.5%) and dermis (31.2% ± 4.9%), which were approximately threefold ($p < 0.01$) and 21-fold ($p < 0.01$) higher than those from $Sph_{12\text{-}38}$ alone, including 1.3 ± 0.7% and 1.2 ± 0.4% for viable epidermis and dermis deposition, respectively. The detailed skin penetration profiles of FITC-$Sph_{12\text{-}38}$ with or without SHS treatment were presented in Fig. 5a.

Further, the skin absorption of $Sph_{12\text{-}38}$ was studied with a confocal microscope. The deep skin penetration and homogenous skin distribution of FITC-$Sph_{12\text{-}38}$ in combined with SHS was observed (Fig. 5b), while FITC-$Sph_{12\text{-}38}$ alone was accumulated in the superficial layer of skin (Fig. 5c).

The treatment of acne in vivo by the combined use of $Sph_{12\text{-}38}$ and SHS

To investigate the therapeutic effect of $Sph_{12\text{-}38}$ on acne, rabbit ear acne models in vivo have been established by injecting 50 µL of *P. acnes* (10^7 CFU/ml) into rabbit auricles. During the acne model establishment, the representative symptoms of acne and its inflammation occur in rabbit ears increasingly, including local swelling, redness, papules, comedones, cysts, nodules, and pustules (Fig. 6). By macroscopic analysis, the combined use of $Sph_{12\text{-}38}$ and SHS showed the best therapeutic effect on acne compared to

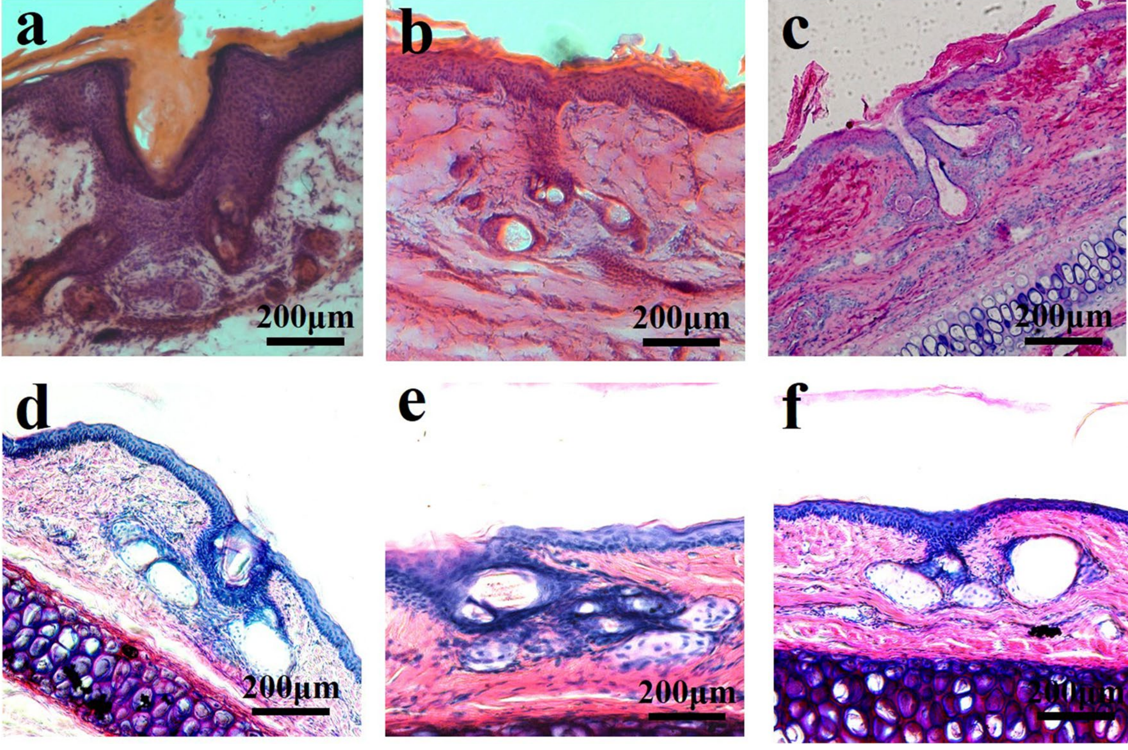

Fig. 8 Histopathological examination on the rabbit ear acnes before and after 14 days of different treatments. **a** before treatment; **b** the combined use of $Sph_{12\text{-}38}$ and SHS; **b** Clindamycin alone; **d** SHS alone; **e** $Sph_{12\text{-}38}$ alone; **f** Saline

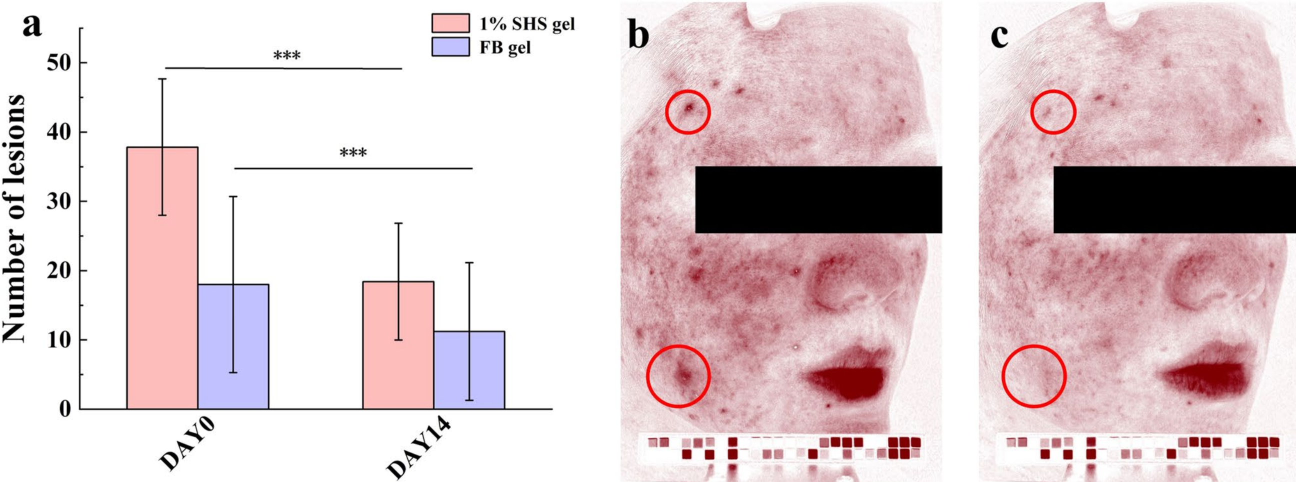

Fig. 9 SHS significantly cures human acne in vivo. **a** The number of skin lesions before and after SHS treatment. **b** The VISIA image before SHS treatment. **c** The VISIA image after SHS treatment. Values represent mean ± SD, n = 30. *** represents $p < 0.001$

$Sph_{12\text{-}38}$ or SHS alone. The symptom of acne or its inflammation in the ears disappeared after the 14 days treatment (Fig. 6a). In addition, decrescent follicular pores were distributed evenly in rabbit ears (Fig. 6a, 14 days treatment). In contrast, Clindamycin alone, SHS alone or $Sph_{12\text{-}38}$ alone led to much less effective therapeutic impact on acne after 14 days treatment; the papules, comedones and cysts in rabbit ears still existed (Fig. 6b, c, d). In the case of control group (treated with saline buffer), while the local swelling and redness disappeared gradually due to the skin self-healing capability, cysts, nodules and pustules still existed in rabbit ears after 14 days treatment (Fig. 6e).

Rabbit ear acnes have been characterized in terms of acnes number, diameter and thickness by using electronic Vernier caliper before and after 14 days treatment (Fig. 7a). Compared to all other groups, the combined use of $Sph_{12\text{-}38}$ and SHS significantly decreased 80.4% ($p < 0.001$) of the acnes number and 11.6% of acne thickness after14 days treatment. In contrast, while Clindamycin alone only significantly decreased 37.2% ($p < 0.001$) of the acnes number, SHS alone only significantly decreased 56.1% ($p < 0.001$) of the acnes number, $Sph_{12\text{-}38}$ alone only significantly decreased 20.9% ($p < 0.05$) of the acnes number. In the case of saline group, while there was no significant change in the acne thickness and number, the acnes diameter increased significantly during treatment due to growth of acnes tardily. In addition, the effectiveness rate of acne treatment induced by different methods have been calculated. The combined use of $Sph_{12\text{-}38}$ and SHS led to an effectiveness rate of 100% for acne treatment, which is obviously much higher than all other groups (Fig. 7b and c).

Moreover, the conditions of sebaceous gland, inflammatory cells and keratotic plug by histopathological examination have been evaluated. The destructive effect of acnes on rabbit ear tissues was observed with expended pilosebaceous unit, keratin filled follicle funnel and inflammatory cells infiltration (Fig. 8a). The combined use of $Sph_{12\text{-}38}$ and SHS obviously reduced the degree of inflammatory cell infiltration and expansion of pilosebaceous (Fig. 8b). In contrast, the infiltration of inflammatory cells and expansion of pilosebaceous still existed after 14 days treatment in other groups (Fig. 8c, d, e, f).

The treatment of acne in vivo human skin by SHS

Clinical evaluation results showed that the number of skin lesions of each subject were significantly reduced treated by SHS gel (SHS concentration 1%). The number of skin lesions (18.40 ± 8.43) decreased by 51.4% compared to the basal value (37.83 ± 9.83, $p < 0.001$, Fig. 9a). After the validation of clinical effect. Frobupre® Hydratation Absorption Improving Massage Gel (FB gel) with same concentration (1%) has been designed. As for FB gel, clinical evaluation results showed that the number of skin lesions (11.21 ± 9.95) decreased by 37.7% compared to the basal value (17.99 ± 12.71, $p < 0.001$, Fig. 9a). Figure 9b (Day 0) and Fig. 9c (Day 14) represent the treatment effect of one of the subjects. The results of the self-assessment showed that 94% of subjects were satisfied with the therapeutic effect of the product.

Discussion

Antimicrobial peptides (AMPs) are generally peptides with a broad spectrum of antibiotic activities against pathogenic microorganisms. AMPs have been the most promising alternative to antibiotics [21, 22]. AMPs derived from marine invertebrates are the main components of innate

immunity in marine invertebrates (including crustaceans) and have various bio-activities, including anti-bacterial, anti-fungal, anti-viral or anti-parasitic functions. In addition, some AMPs, such as $Sph_{12\text{-}38}$, have anti-inflammatory and immunomodulatory activities [23].

AMPs against pathogens are so far known to be involved in several mechanisms, among which the membrane permeabilization is one of main mechanisms. Besides, some others have been also described, including inhibition of DNA/RNA/protein synthesis, inhibition of other intracellular targets, influence of enzyme activity and so on [23–26]. As a cationic peptide, $Sph_{12\text{-}38}$ can adhere to the negatively charged *P. acnes* cell membranes by electrostatic attraction. $Sph_{12\text{-}38}$ led to obvious morphological changes of *P. acnes* in short time and subsequently resulted in cell lysis and debris in 20 min (Supplemental Figure S2a), suggesting that the primary mode of action of $Sph_{12\text{-}38}$ against *P. acnes* is the permeabilization of the bacterial cell membranes. On the other hand, the outer layer of keratinocytes does not carry a net charge since most lipids with negatively charged head groups are accumulated into the internal cytoplasm [27]. Thus, the cationic $Sph_{12\text{-}38}$ can selectively interact with negative charged *P. acnes* without binding to keratinocytes, which can eventually result in the rupture of *P. acnes* with no toxicity to keratinocytes (Fig. 3).

Although AMPs has the potential of wide bactericidal activity, rapid action and low resistance mechanism, there are still some problems in clinical application. Some antimicrobial peptides such as Chionodracine becomes to be unstable above 60 °C [28]. In contrast, $Sph_{12\text{-}38}$ as a small molecule peptide shows a satisfying thermal stability. Under the condition of 80 °C heating for 10 min. $Sph_{12\text{-}38}$ can still effectively inhibit the growth of *P. acnes* at MBC.

The toxicity of AMPs is also one of the problems that limit its application. Generally, positively charged AMPs will be combined with negatively charged bacterial cell membranes and selectively kill bacteria. However, mammalian cells may also be attacked by AMPs [29, 30]. Moreover, systemic administration of AMPs may affect the innate normal flora of the human body due to their broad-spectrum antimicrobial properties, which leads to an imbalance of human flora and the human body more susceptible to infection. Thus, topical administration by SHS is the most suitable application of AMPs, especially for local diseases such as acne.

As a hydrophilic biomacromolecule, $Sph_{12\text{-}38}$ alone can only penetrate into the superficial layer of skin (Fig. 5a). Thus, it cannot play an antimicrobial role in deep skin layers (Fig. 5c). To improve the topical and deep effect of $Sph_{12\text{-}38}$ against *P. acnes*, SHS was utilized topically to facilitate the accumulation of $Sph_{12\text{-}38}$ into deep skin layers (Fig. 5b). And our in vivo experiment further confirmed the effectiveness of the combination of $Sph_{12\text{-}38}$ and SHS on the treatment of acne (Fig. 6).

Generally, inflammatory acne is one of contraindications to traditional microneedles such as Dermaroller and Dermastamp [31]. In addition, the metastasis of *P. acnes* from the lesion to the undiseased skin area and layers could occur during the traditional microneedling treatment, causing the aggravation of acne. In contrast, all these problems seem to be avoided by the use of SHS as a novel silica microneedle. First, SHS can only penetrate into skin SC layer so that it does not lead to bleeding and infection. Further, SHS can retain within skin over 48–72 h and get eliminated by natural desquamation [12], which does not result in the metastasis of *P. acnes* during the treatment. Moreover, no side effect was observed in our in vivo experiment using SHS alone or combined mixture of SHS and $Sph_{12\text{-}38}$, suggesting the satisfying safety of SHS topical application for acne treatment. In addition, SHS can create plenty of continuous microchannels within skin deep to viable epidermis, which could possibly increase the oxygen penetration into skin to further inhibit the reproduction of anaerobiotic *P. acnes*. SHS could also promote the metabolism of the stratum corneum, thereby reducing the hyperplasia of the stratum corneum associated with acne to a certain extent. This could be beneficial for acne treatment. Further, SHS could stimulate subcutaneous blood and lymphatic circulation and accelerate the metabolism of *P. acnes* toxins. Moreover, we found that SHS can induce immune cells to gather under the treated skin [12] and may contribute to the immune regulation of acne. The exact mechanism of action of SHS on skin immunity should be investigated in detail in future studies. The effectiveness of SHS by itself on the treatment of acne was also demonstrated by our in vivo experiment (Fig. 7) and clinical study (Fig. 9). This shows that SHS as a new biomedical material has a promising application in the field of acne treatment. Compared the therapeutic effects of SHS gel (1%) and FB gel (1%). After 14 days of treatment, the number of skin lesions decreased by 51.4% and 37.7%, respectively. The favorable therapeutic effect has led to the successful commercialization of SHS. As a new drug formulation, the clinical study of $Sph_{12\text{-}38}$ required a longer period. Clinical studies on $Sph_{12\text{-}38}$ for acne treatment will be investigate in future studies.

Conclusion

The study demonstrated the antimicrobial activity of a marine-animal derived AMP, $Sph_{12\text{-}38}$, against *P. acnes*. at its low concentration without any cytotoxcity to human keratinocytes at its high concentrations. Topical application of SHS can facilitate the skin penetration and deposition of $Sph_{12\text{-}38}$ in skin deep layers in vitro. Further, the combined use of $Sph_{12\text{-}38}$ and SHS resulted in the satisfying therapeutic outcomes on rabbit ear acne model in vivo. The strategy in this study of using AMP and SHS would be applied in a rational designed topical delivery system for the management of other skin diseases or deep infection of the skin.

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Authors' contribution Conceptualization: M.C. and K.W.; Data Curation: H.W., C.Z., L.H.; Formal Analysis: H.W., C.Z.; Funding Acquisition: M.C., L.H., K.W.; Investigation: H.W., C.Z., L.H.; Methodology: H.W., C.Z., M.C. and K.W.; Project Administration: M.C., K.W.; Resources: G.P., M.X., H.P.; Software: G.P., M.X., H.P.; Supervision: M.C., K.W.; Validation: H.W., C.Z.; Visualization: H.W., C.Z.; Writing—Original Draft: H.W., C.Z.; Writing—Review & Editing: M.C., K.W. All authors agree to be accountable for all aspects of the work.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Declarations

Ethical approval All animal experiments were conducted according to the guidelines of "Regulations on the administration of laboratory animals of Xiamen University" and approved by the Institutional Animal Care and Use Committee of Xiamen University (ethics approval number XMU-LAC20190002). Because the structure of the sebaceous glands in rabbit ears is similar to that of humans, rabbits were selected as experimental animal model. All animals were raised in the Laboratory Animal Center of Xiamen University (Natural light, constant temperature 24℃). In order to reduce the pain of the rabbits, they were euthanized by intravenous air injection before sampling. Clinical study was conducted by Xiang 'an Hospital of Xiamen University approved by Medical Ethics Committee of Xiamen University (Ethic Number: XDYX202305K24). All authors have read and followed the ARRIVE guidelines (https:arriveguidelines.org).

Disclosure Prof. Dr. Ke-Jian Wang reports a patent ZL 202010101694.3 issued. Prof. Dr. Ming Chen reports two patents ZL 201610267764.6 and US 10,555,896 B2 issued.

Conflict of interest The authors declare no other conflict of interest.

References

1. Well D, BSN, MSN, APRN, FNP-BC. Acne vulgaris: a review of causes and treatment options. Nurse Pract. 2013;38(10):22–31.
2. Knutsen-Larson S, Dawson AL, Dunnick CA, Dellavalle RP. Acne vulgaris: pathogenesis, treatment, and needs assessment. Dermatol Clin. 2012;30(1):99–106 (**viii-ix**).
3. Thielitz A, Gollnick H. Overview of new therapeutic developments for acne. Expert Rev Dermatol. 2009;4(1):55–65.
4. Dawson AL, Dellavalle RP. Acne vulgaris. BMJ. 2013;346:f2634.
5. Arif T. Salicylic acid as a peeling agent: a comprehensive review. Clin Cosmet Investig Dermatol. 2015;8:455–61.
6. Tzellos T, Zampeli V, Makrantonaki E, Zouboulis CC. Treating acne with antibiotic-resistant bacterial colonization. Expert Opin Pharmacother. 2011;12(8):1233–47.
7. Kosmadaki M, Katsambas A. Topical treatments for acne. Clin Dermatol. 2017;35(2):173–8.
8. Schafer F, Fich F, Lam M, Gárate C, Wozniak A, Garcia P. Antimicrobial susceptibility and genetic characteristics of Propionibacterium acnes isolated from patients with acne. Int Soc Dermatol. 2013;52(4):418–25.
9. Zhang XG, Fang C, Bai H, Zhou Y, Hou Z. Research progress on mechanism of antimicrobial peptides. Prog Physiol Sci. 2011;42(1):11–5.
10. Yang M, Chen B, Cai JJ, Peng H, Ling C, Yuan JJ, Wang KJ. Molecular characterization of hepcidin AS-hepc2 and AS-hepc6 in black porgy (Acanthopagrus schlegelii): expression pattern responded to bacterial challenge and in vitro antimicrobial activity. Comp Biochem Physiol B Biochem Mol Biol. 2011;158(2):155–63.
11. Chen B, Fan DQ, Zhu KX, Shan ZG, Chen FY, Hou L, Cai L, Wang KJ. Mechanism study on a new antimicrobial peptide Sphistin derived from the N-terminus of crab histone H2A identified in haemolymphs of Scylla paramamosain. Fish Shellfish Immunol. 2015;47(2):833–46.
12. Zhang S, Ou H, Liu C, Zhang Y, Mitragotri S, Wang D, Chen M. Skin Delivery of Hydrophilic Biomacromolecules Using Marine Sponge Spicules. Mol Pharm. 2017;14:3188–200.
13. Zhang C, Zhang K, Zhang J, Ou H, Duan J, Zhang S, Wang D, Mitragotri S, Chen M. Skin delivery of hyaluronic acid by the combined use of sponge spicules and flexible liposomes. Biomaterials Science. 2019;7(4):1299–310.
14. Karande P, Jain A, Mitragotri S. Relationships between skin's electrical impedance and permeability in the presence of chemical enhancers. J Control Release. 2006;110(2):307–13.
15. Zhang S, Ou H, Liu C, Yuan Z, Mitragotri S, Wang D, Ming C. Skin Delivery of Hydrophilic Biomacromolecules Using Marine Sponge Spicules. Mol Pharm. 2017;14(9):3188.
16. Kligman AM, Kwong T. An improved rabbit ear model for assessing comedogenic substances. Br J Dermatol. 1979;100(6):699–702.
17. Kligman AM, Kwong T. An improved rabbit ear model for assessing comedogenic substances. British J Dermatol. 2010;100(6):699–702.
18. Kwon TR, Choi EJ, Oh CT, Bak DH, Im SI, Ko EJ, Hong HK, Choi YS, Seok J, Choi SY, Ahn GY, Kim BJ. Targeting of sebaceous glands to treat acne by micro-insulated needles with radio frequency in a rabbit ear model. Lasers Surg Med. 2017;49(4):395–401.
19. Wang Q, Jiang C, Liu W, Chen J, Lin X, Huang X, Duan X. A new optical intra-tissue fiber irradiation ALA-PDT in the treatment of acne vulgaris in rabbit model: improved safety and tolerability. An Bras Dermatol. 2017;92(3):350–5.
20. Ma L, Xiang LH, Yu B, Yin R, Chen L, Wu Y, Tan ZJ, Liu YB, Tian HQ, Li HZ, Lin T, Wang XL, Li YH, Wang WZ, Yang HL, Lai W. Low-dose topical 5-aminolevulinic acid photodynamic therapy in the treatment of different severity of acne vulgaris. Photodiagnosis Photodyn Ther. 2013;10(4):583–90.
21. Shabir U, Ali S, Magray AR, Ganai BA, Firdous P, Hassan T, Nazir R. Fish antimicrobial peptides (AMP"s) as essential and promising molecular therapeutic agents: A review. Microb Pathog. 2018;114:50–6.
22. Sierra JM, Fusté E, Rabanal F, Vinuesa T, Vi M. An overview of antimicrobial peptides and the latest advances in their development. Expert Opin Biol Ther. 2017;17(6):663–76.
23. Jenssen H, Hamill P, Hancock REW. Peptide Antimicrobial Agents. Clin Microbiol Rev. 2006;19(3):491–511.

24. Cudic LOJM. Intracellular Targets of Antibacterial Peptides. Curr Drug Targets. 2002;3(2):101–6.
25 Aguilar MI, Hall KN, Lee TH. Antimicrobial Peptide Structure and Mechanism of Action: A Focus on the Role of Membrane Structure. Curr Top Med Chem. 2016;16(1):25–39.
26. Rios AC, Moutinho C, Pinto FC, Fiol FDSD, Jozala AF, Chaud MV, Vila MMDC, Teixeira JA, Balcao VM. Alternatives to overcoming bacterial resistances: State-of-the-art. Microbiol Res. 2016;191:51–80.
27. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature. 2002;415(6870):389–95.
28. Wu Y, Zhang G. Inhibitory Effect of the Antimicrobial Peptide Chionodracine Against Propionibacterium Acnes and Its Anti-inflammatory Effect on Acne Vulgaris Guangdong chemical industry. 2021;48(4):26–28.
29. Zheng R, Yao B, Yu H, Wang H, Bian J, Feng F. Novel family of antimicrobial peptides from the skin of Rana shuchinae. Peptides. 2010;31(9):1674–7.
30. De Sa PB, Havens WM, Ghabrial SA. Characterization of a novel broad-spectrum antifungal protein from virus-infected Helminthosporium (Cochliobolus) victoriae. Phytopathology. 2010;100(9):880–9.
31. Tuanmahmood T, Mccrudden MTC, Torrisi BM, Mcalister E, Garland MJ, Singh TRR, Donnelly RF. Microneedles for intradermal and transdermal drug delivery. Eur J Pharm Sci. 2013;50(5):623–37.

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