A.I. assisted rational design of antimicrobial peptides based on human endogenous proteins and their applications for cosmetic preservative system optimization

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Introduction:

The disinfectants and preservatives are common ingredients in cosmetic products to inhibit the growth of microbes. The skin infection, such as ecthyma and acne vulgaris caused by *Staphylococcus aureus* and *Cutibacterium acnes* respectively, can be so severe as to leave scars, which requires proper treatments.[1] However, disinfectants like alcohols and benzoyl peroxide, have the limitations of instability and health concerns. The common preservatives include parabens, formaldehyde releasers, phenoxyethanol and organic acids, all of whose application, however, are limited by non-broad-spectrum properties, irritation, endocrine disruption or other health concerns.[2] With that natural organic products are becoming mainstream, “Preservative-free” is becoming a popular selling proposition. Thus, a safe but powerful, broad-spectrum antimicrobial is in high demand.

Mesenchymal stem cells (MSCs) secrete numerous factors to regulate cell metabolism as well as controlling homeostasis. Natural antimicrobial peptides (AMPs) generated by MSCs, such as LL-37, β-defensin and lactoferricin, act as an important part in human innate system and have a broad-spectrum antimicrobial activity, thus regarded as potential antimicrobial agent because of safety and efficacy.[3] However, natural AMPs are large protein molecules and unstable in varied solution environment *in vitro* so their applications are limited. The development of biomimetic AMPs with better effect, more stability and lower cost is challenging.

With the increasing understanding of antimicrobial mechanism, the sequence and structure information are gathered for machine learning to speed up the AMP screening and design.[4] We utilized computational simulation and A.I. machine learning to rationally design biomimetic short AMPs derived from natural full-length AMP in order to significantly enhance stability and reduce the manufacturing cost.[5] These AMPs would be promising alternatives of disinfectants and preservatives in cosmetic products.

Materials & Methods:

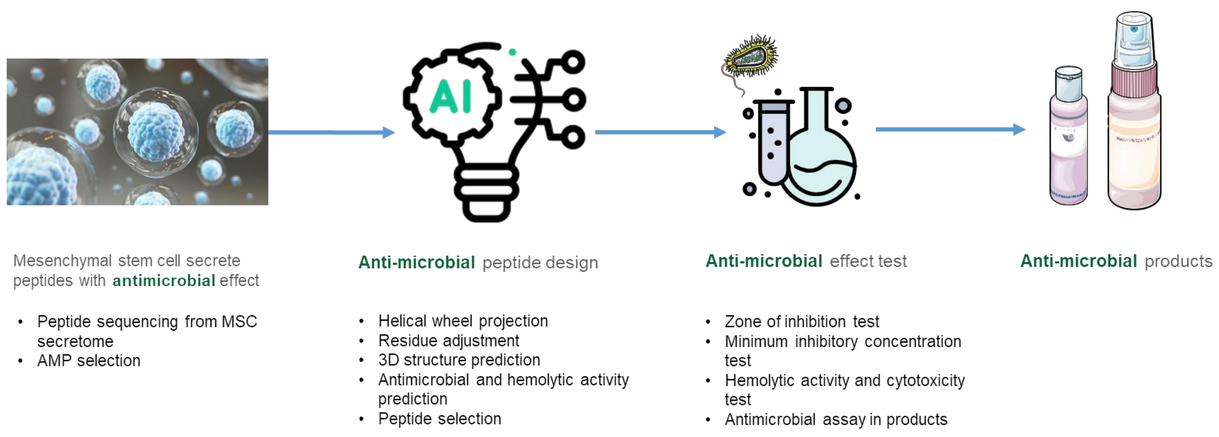

Figure. A.I. assisted rational design of antimicrobial peptides based on human endogenous proteins. β-defensin is selected from MSC secretome as the template. After the calculation of helical wheel projection, the residues of short analogs of β-defensin are adjusted based on molecular properties. The *ab-initio* three-dimensional structures of peptides are analyzed for supporting the α-helical conformations. The hemolytic activity are also predicted. All top scored novel biomimetic peptides are synthesized and screened for antimicrobial activity as well as safety assays. Product mixed with designed AMPs are subsequently tested for the further application.

Results & Discussion:

The antimicrobial peptides were selected from MSC secretome as the templates. IK-16-1 and IK-16-2 were derived from beta-defensin 103 (Table. 1) with predicted α-helix structures (Fig. 1). Antimicrobial activity was predicted by Antimicrobial Peptide Scanner version 2[6], while the hemolytic activity was predicted by HAPPENN[7]. All analogs were scored highly and less likely to be hemolytic (Table. 2). IK-16-1 had a broad-spectrum inhibitory effect though antifungal activity was not so strong as antibacterial activity. IK-16 and IK-16-2, though, did not show any inhibitory activity within the concentration range here (Table. 3).

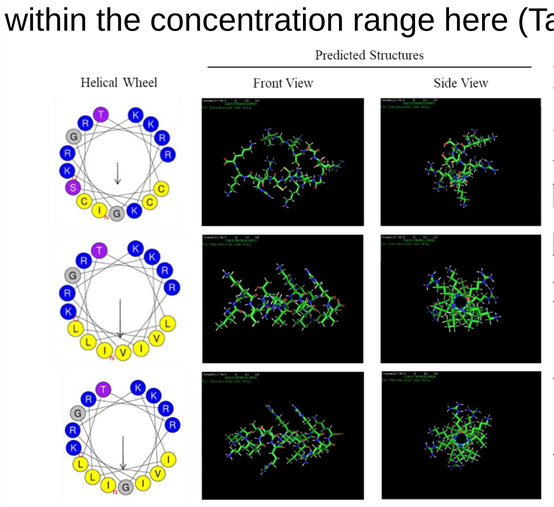

Fig1: The results of Helical Wheel Projection and predicted structures of beta-defensin analogs.

Table 1. The sequence of beta-defensin and modified analogs

origin	Sequences	Modified peptides
Beta-Defensin 103 (30-45)	IGKCSTRGRKCCRRKK (IK-16)	IGKVLTRVRKLLRRIK (IK-16-1)
		IGKVLTRGRKILRRIK (IK-16-2)

Table 2. The scores of antimicrobial and hemolytic activity prediction

Sequence ID(s)	sequences	Predicted Class	Probability as AMPs	Probability to be hemolytic
IK-16	IGKCSTRGRKCCRRKK	AMP	0.9999	0.004
IK-16-1	IGKVLTRVRKLLRRIK	AMP	1.0	0.006
IK-16-2	IGKVLTRGRKILRRIK	AMP	0.9999	0.004

Table 3. MIC of AMPs against different species

	species	MIC (μg/mL，18 h)			MIC (μg/mL，18 h)
IK-16-1	*Candida albicans*	125.0	125.0	125.0	125.0 ± 0.0
	Escherichia coli	125.0	125.0	62.5	104.2 ± 36.1
	Pseudomonas aeruginosa	125.0	62.5	62.5	83.3 ± 36.1
	Staphylococcus aureus	125.0	62.5	62.5	83.3 ± 36.1
IK-16	*Candida albicans*	> 125.0	> 125.0	> 125.0	-
	Escherichia coli	> 125.0	> 125.0	> 125.0	-
	Pseudomonas aeruginosa	> 125.0	> 125.0	> 125.0	-
	Staphylococcus aureus	> 125.0	> 125.0	> 125.0	-
IK-16-2	*Candida albicans*	> 125.0	> 125.0	> 125.0	-
	Escherichia coli	> 125.0	> 125.0	> 125.0	-
	Pseudomonas aeruginosa	> 125.0	> 125.0	> 125.0	-
	Staphylococcus aureus	> 125.0	> 125.0	> 125.0	-

To validate the application of IK-16-1 in cosmetic products as an alternative of preservatives, we combined a common preservative formulation with IK-16-1. IK-16-1 could largely inhibit the growth of microbes at very early stage (Table. 4).

Table 4. The CFU calculation in cosmetic products at different time

sample	species	0 days (cfu/mL)	3 days (cfu/mL)	7 days (cfu/mL)	14 days (cfu/mL)	22 days (cfu/mL)
IK-16-1	*Escherichia coli*	<10	<10	<10	<10	<10
	Staphylococcus aureus	<10	<10	<10	<10	<10
	Pseudomonas aeruginosa	<10	<10	<10	<10	<10
	Candida albicans	1.95×10^3	<10	<10	<10	<10
IK-16	*Escherichia coli*	$>3\times10^3$	7×10^2	<10	<10	<10
	Staphylococcus aureus	$>3\times10^3$	60	<10	<10	<10
	Pseudomonas aeruginosa	$>3\times10^3$	$>3\times10^3$	<10	<10	<10
	Candida albicans	1.7×10^3	<10	<10	<10	<10

Hemolytic activity was also tested. All peptides has no hemolytic effect on rabbit erythrocytes, indicating the safety of AMPs (Fig. 2).

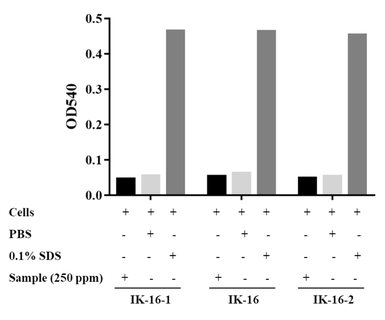

Fig2: The hemolytic results of AMPs at the concentration 250 μg/mL. NC, negative control, PBS; PC, positive control, 0.1% SDS.

Fig3: Zone of inhibition of 10 mg/mL IK-16-1 against *C. acnes*. the mean diameter is 3.35 cm.

Inhibitory effect on *C. acnes* was also tested with a high concentration of IK-16-1 (Fig. 3). IK-16-1 could inhibit the growth of *C. acnes* and further tests were in progress.

In our work, peptides with helical structures are adjusted to separate hydrophobic and hydrophilic residues on the surface and theoretically the ability of pore formation of AMPs will be enhanced. Results confirm with our hypothesis and the workflow to design AMPs is feasible. IK-16-1 can inhibit the growth of *E. coli*, *S. aureus*, *P. aeruginosa* and *C. albicans*, making it possible to be an alternative of disinfectant. Besides, the test as a preservative confirmed that IK-16-1 can effectively inhibit the growth of microbes in products. It can be used to reduce the application of disinfectants and preservatives in cosmetic products.

Above all, though not perfect, AI assisted rational design of antimicrobial peptides derived from human endogenous proteins is feasible. More AMPs can be obtained from this workflow to function as safe and powerful alternatives of preservatives.

Conclusions:

In our work, AI assisted rational design of antimicrobial peptides based on human endogenous proteins was extensively demonstrated. The results confirmed with our hypothesis and the combination of computational design and AI prediction to design AMPs was feasible. We identified an AMP named IK-16-1 based on native beta-defensins and found it potent as an alternative of preservatives in cosmetic products.

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