skin grafting are considered as gold standard in chronic wound management. However, skin grafting is limited by the quality and quantity of donor skin, which does not solve the problem of bacterial clearance either. Similarly, other new procedures such as delivery of angiogenesis-inducing growth factors and cytokines and hyperbaric oxygen therapy do not resolve the fundamental issue of infections. As an alternative, anti-microbial peptides (AMPs) are at the limelight due to the less likelihood to induce bacterial resistance. Yet most of the anti-microbial peptides (AMPs) failed at preclinical stages due to the following reasons: (i) limited broad-spectrum anti-microbial activity (works only on certain types of bacteria); (ii) targeting of eukaryotic cells, (iii) hemolytic and/or cytotoxic effects at anti-microbial concentrations; (iv) low anti-microbial activity in clinically relevant environments; and (v) proteolytic degradation. Regarding the targeting of eukaryotic cells, such targeting with substances can carry certain risks due to cytotoxic effects. Such targeting substances can damage or kill cells, affecting not just targeted cells but also surrounding healthy cells, leading to broad cellular harm. Additionally, targeting of eukaryotic cells raises concerns about acute toxicity, which results from short-term exposure and can vary from mild to severe symptoms, and systemic toxicity, which impacts multiple organ systems, causing widespread health issues. Such toxicity risks are significant for both topical applications, applied directly to the skin and systemic applications, meant to affect the body more broadly.

[0005] Therefore, what is needed is an anti-microbial agent effective in treating chronic wounds, and method of treating chronic wounds using the anti-microbial agent. Furthermore, other desirable features and characteristics will become apparent from the subsequent detailed description and the appended claims, taken in conjunction with the accompanying drawings and this background of the disclosure.

SUMMARY

[0006] In one aspect, the present disclosure provides a peptide comprising the sequence of KKLLKKX$_1$X$_2$KLX$_3$KKX$_4$L (SEQ ID NO: 49), wherein: X$_1$ is S, W, L, K, 4-biphenyl-L-alalnine, 1-naphthyl-L-alanine, or 2-naphthyl-L-alanine; X$_2$ is V, L, or K; X$_3$ is S, L, or K; and X$_4$ is A, L, K, or 2-aminoisobutyric acid. In another aspect, the present disclosure provides a peptide comprising the sequence of KKLLKKX$_1$X$_2$KLX$_3$KKX$_4$L (SEQ ID NO: 49), wherein: X$_1$ is S, W, L, K, 4-biphenyl-L-alalnine, 1-naphthyl-L-alanine, or 2-naphthyl-L-alanine; X$_2$ is V, L, or K; X$_3$ is S, L, or K; and X$_4$ is A, K, or 2-aminoisobutyric acid.

[0007] In another aspect, the present disclosure provides a peptide comprising the sequence of X$_1$X$_2$KLX$_3$KKX$_4$LKLX$_5$X$_6$X$_7$L (SEQ ID NO: 50), wherein: X$_1$ is S, W, L, K, 4-biphenyl-L-alalnine, 1-naphthyl-L-alanine, or 2-naphthyl-L-alanine; X$_2$ is V, L, or K; X$_3$ is S, L, or K; X$_4$ is A, L, K, or 2-aminoisobutyric acid; X$_5$ is A, L, K, or 2-aminoisobutyric acid; X$_6$ is Q, L, or K; and X$_7$ is S, L, or K. In another aspect, the present disclosure provides a peptide comprising the sequence of X$_1$X$_2$KLX$_3$KKX$_4$LKLX$_5$X$_6$X$_7$L (SEQ ID NO: 50), wherein: X$_1$ is S, W, L, K, 4-biphenyl-L-alalnine, 1-

naphthyl-L-alanine, or 2-naphthyl-L-alanine; X_2 is V, L, or K; X_3 is S, L, or K; X_4 is A, L, K, or 2-aminoisobutyric acid; X_5 is A, L, K, or 2-aminoisobutyric acid; X_6 is Q, L, or K; and X_7 is S, or L.

[0008] In another aspect, the present disclosure provides a peptide comprising the sequence of $KKLLKKX_1X_2KLX_3KKX_4LKLX_5X_6X_7L$ (SEQ ID NO: 51), wherein: X_1 is S, W, L, K, 4-biphenyl-L-alalnine, 1-naphthyl-L-alanine, or 2-naphthyl-L-alanine; X_2 is V, L, or K; X_3 is S, L, or K; X_4 is A, L, K, or 2-Aminoisobutyric acid; X_5 is A, L, K, or 2-Aminoisobutyric acid; X_6 is Q, L, or K; and X_7 is S, L, or K. In another aspect, the present disclosure provides a peptide comprising the sequence of $KKLLKKX_1X_2KLX_3KKX_4LKLX_5X_6X_7L$ (SEQ ID NO: 51), wherein: X_1 is S, W, L, K, 4-biphenyl-L-alalnine, 1-naphthyl-L-alanine, or 2-naphthyl-L-alanine; X_2 is V, L, or K; X_3 is S, L, or K; X_4 is A, L, K, or 2-Aminoisobutyric acid; X_5 is A, L, K, or 2-Aminoisobutyric acid; X_6 is Q, L, or K; and X_7 is S, or L.

[0009] In another aspect, the present disclosure provides a composition comprising one or more of the peptides as disclosed herein.

[00010] In another aspect, the present disclosure provides a pharmaceutical composition comprising the peptide as disclosed herein, or the composition as disclosed herein, and a pharmaceutically acceptable carrier or excipient.

[00011] In another aspect, the present disclosure provides a wound dressing comprising the peptide as disclosed herein, the composition as disclosed herein, or the pharmaceutical composition as disclosed herein.

[00012] In another aspect, the present disclosure provides a nucleic acid composition encoding the peptide as disclosed herein.

[00013] In another aspect, the present disclosure provides an expression vector comprising the nucleic acid composition as disclosed herein.

[00014] In another aspect, the present disclosure provides a host cell comprising one or more of the nucleic acid compositions as disclosed herein, or one or more of the expression vectors as disclosed herein.

[00015] In another aspect, the present disclosure provides a method of treating or preventing the recurrence of a bacterial infection in a subject, comprises administering to the subject a pharmaceutically effective amount of the peptide as disclosed herein, the composition as disclosed herein, the pharmaceutical composition as disclosed herein, or the wound dressing as disclosed herein. In another aspect, the present disclosure provides a use of a pharmaceutically effective amount of the peptide as disclosed herein, the composition as disclosed herein, the pharmaceutical composition as disclosed herein, or the wound dressing as disclosed herein in the manufacture of a medicament for treating or preventing the recurrence of a bacterial infection.

[00016] In another aspect, the present disclosure provides a method of promoting wound healing and tissue regeneration in a subject, wherein the method comprises administering to the subject a pharmaceutically effective amount of the peptide as disclosed herein, the composition as disclosed herein, the pharmaceutical composition as disclosed herein, or the wound dressing as disclosed herein. In another aspect, the present disclosure provides a use of a pharmaceutically effective amount of a peptide as disclosed herein, the composition as disclosed herein, the pharmaceutical composition as disclosed herein, or the wound dressing as disclosed herein in the manufacture of a medicament for promoting wound healing and tissue regeneration.

[00017] In another aspect, the present disclosure provides a method of reducing biofilm/preventing formation of biofilm in a subject, wherein the method comprises administering to the subject a pharmaceutically effective amount of the peptide as disclosed herein, the composition as disclosed herein, the pharmaceutical composition as disclosed herein, or the wound dressing as disclosed herein. In another aspect, the present disclosure provides a use of a pharmaceutically effective amount of a peptide as disclosed herein, the composition as disclosed herein, the pharmaceutical composition as

disclosed herein, or the wound dressing as disclosed herein in the manufacture of a medicament for reducing biofilm/preventing formation of biofilm.

FIGURE DESCRIPTION

[00018] **Figure 1** provides a demonstration of the development process for peptide variants starting from the base sequence of E1A, which is a 21-residue short region within peptide ENO3 (SEQ ID NO: 52). The variants are generated by separating the distribution of basic amino acid (Lysine) and hydrophobic residues that align on opposing faces, forming a unique cationic arm which can interact with the negatively charged bacterial membrane, thereby disrupting the membrane of the bacteria. **Figure 1A** shows a Helical Wheel of the peptide sequence of E1A and **Figure 1B** shows a Helical Wheel of the exemplary peptide sequence of E1AM1. Helical Wheels predict protein sequence

CLAIMS

What is claimed is:

1. A peptide comprising the sequence of KKLLKKX_1X_2KLX_3KKX_4L (SEQ ID NO: 49), wherein:

X_1 is S, W, L, K, 4-biphenyl-L-alanine, 1-naphthyl-L-alanine, or 2-naphthyl-L-alanine;

X_2 is V, L, or K;

X_3 is S, L, or K; and

X_4 is A, K, or 2-aminoisobutyric acid.

2. The peptide of claim 1, wherein the peptide sequence comprises the sequence KKLLKKWVKLSKKAL (SEQ ID NO: 16).

3. The peptide of claim 1, wherein the peptide sequence consists of any one of the following:

(a) KKLLKKWVKLSKKALKLAQS (SEQ ID NO: 11);

(b) KKLLKKWVKLSKKALKLAQ (SEQ ID NO: 12);

(c) KKLLKKWVKLSKKALKLA (SEQ ID NO: 13);

(d) KKLLKKWVKLSKKALKL (SEQ ID NO: 14);

(e) KKLLKKWVKLSKKALK (SEQ ID NO: 15);

(f) KKLLKKWVKLSKKAL (SEQ ID NO: 16); or

(g) KKLLKK(2-Nal)LKLSKKALKLAL (E1AM2 W7(2-Nal) V8L Q19L C-2, SEQ ID NO: 47).

4. A peptide comprising the sequence of X_1X_2KLX_3KKX_4LKL$X_5X_6X_7$L (SEQ ID NO: 50), wherein:

X_1 is S, W, L, K, 4-biphenyl-L-alalnine, 1-naphthyl-L-alanine, or 2-naphthyl-L-alanine;

X_2 is V, L, or K;

X_3 is S, L, or K;

X_4 is A, L, K, or 2-Aminoisobutyric acid;

X_5 is A, L, K, or 2-Aminoisobutyric acid;

X_6 is Q, L, or K; and

X_7 is S, or L.

5. The peptide of claim 4, wherein the peptide sequence comprises the sequence of WVKLSKKALKLAQSL (SEQ ID NO: 10).

6. The peptide of claim 4, wherein the peptide sequence consists of any one of the following:

(a) KLLKKWVKLSKKALKLAQSL (SEQ ID NO: 5);

(b) LLKKWVKLSKKALKLAQSL (SEQ ID NO: 6);

(c) LKKWVKLSKKALKLAQSL (SEQ ID NO: 7);

(d) KKWVKLSKKALKLAQSL (SEQ ID NO: 8);

(e) KWVKLSKKALKLAQSL (SEQ ID NO: 9); or

(f) WVKLSKKALKLAQSL (SEQ ID NO: 10).

7. A peptide comprising the sequence of KKLLKKX_1X_2KLX_3KKX_4LKL$X_5X_6X_7$L (SEQ ID NO: 51), wherein:

X_1 is S, W, L, K, 4-biphenyl-L-alalnine, 1-naphthyl-L-alanine, or 2-naphthyl-L-alanine;

X_2 is V, L, or K;

X_3 is S, L, or K;

X_4 is A, L, K, or 2-aminoisobutyric acid;

X_5 is A, L, K, or 2-aminoisobutyric acid;

X_6 is Q, L, or K; and

X_7 is S, or L.

8. The peptide of claim 7, wherein the peptide sequence consists of any one of the sequences of (i) KKLLKKWVKLSKKALKLAQSL (E1AM2, SEQ ID NO: 2);
(ii) KKLLKKWVKLSKKALKLALSL (E1AM2 Q19L, SEQ ID NO: 27);
(iii) KKLLKKWLKLSKKALKLAQSL (E1AM2 V8L, SEQ ID NO: 19);
(iv) KKLLKKWLKLSKKALKLALSL (E1AM2 V8L Q19L, SEQ ID NO: 39); or
(v) KKLLKKWLKLSKKALKLALSL (E1AM2 V8L Q19L C-2, SEQ ID NO: 44).

9. The peptide of any one of the claims 1, 2, 4, 5, and 7, wherein the peptide further comprises a partial sequence from ENO3, wherein the peptide has less than 22 amino acid residues.

10. A composition comprising one or more of the peptides of any one of claims 1-9.

11. A pharmaceutical composition comprising the peptide of any one of claims 1-9, or the composition of claim 10, and a pharmaceutically acceptable carrier or excipient.

12. A wound dressing comprising the peptide of any one of claims 1-9, the composition of claim 10, or the pharmaceutical composition of claim 11.

13. A nucleic acid composition encoding the peptide of any one of claims 1-9.

14. An expression vector comprising the nucleic acid composition of claim 13.

15. A host cell comprising one or more of the nucleic acid compositions of claim 13, or one or more of the expression vectors of claim 14.

16. Use of a pharmaceutically effective amount of the peptide of any one of claims 1-9, the composition of claim 10, the pharmaceutical composition of claim 11, or the wound dressing of claim 12 in the manufacture of a medicament for treating or preventing the recurrence of a bacterial infection.

17. Use of a pharmaceutically effective amount of a peptide of any one of claims 1-9, the composition of claim 10, the pharmaceutical composition of claim 11, or the wound dressing of claim 12 in the manufacture of a medicament for promoting wound healing and tissue regeneration.

18. Use of a pharmaceutically effective amount of a peptide of any one of claims 1-9, the composition of claim 10, the pharmaceutical composition of claim 11, or the wound dressing of claim 12 in the manufacture of a medicament for reducing biofilm/preventing formation of biofilm.