COMPOSITIONS AND USES OF MATERIALS WITH HIGH ANTIMICROBIAL ACTIVITY AND LOW TOXICITY

Cross-reference to Prior Applications

[0001] This application claims the benefit and priority of U.S. Provisional Patent Application Serial No. 61/376,195, filed 23 August 2010, which is
to the extent permitted by applicable law.

U.S. Government Support

[0002] Not Applicable.

Background of the Invention

Field of the Invention

[0003] The current invention relates to compositions of matter that are able to kill (or inhibit) microbes, and have low mammalian toxicity. The current invention also relates to certain compositions and their uses in a variety of settings including but not limited to preservatives, antiseptics, and the prevention and treatment of wound infections, as well as other infectious diseases.

Discussion of Related Art

[0004] Cationic antimicrobials have demonstrated utility; toxicity is a problem. For over half a century, cationic (positively charged) antimicrobials have been used in a variety of medical and non-medical settings, ranging from systemic antibiotics to industrial cleansers. Cationic antimicrobials bind preferentially to bacterial membranes, which typically display more negative charge than mammalian membranes. This interaction can disrupt membrane function and potentially lead to bacterial cell death. Cationic antimicrobial compounds include certain antibiotics (e.g., polymyxins), bisbiguanides (e.g., chlorhexidine), polymeric biguanides (e.g., polyhexamethylene biguanide), and quaternary ammonium compounds (QAC) (e.g., benzalkonium chloride), as well as natural antimicrobial peptides (AMPs) (e.g., defensins). While each class of cationic antimicrobial compounds has demonstrated antimicrobial activity in one or more settings, toxicity has been a consistent problem.[1-12]

[0005] Polymyxins, produced by *Bacillus polymyxa,* are cyclic peptides with hydrophobic tails.[6, 7] The cyclic peptide portion (approx. 10 amino acid residues; positively charged) interacts strongly with negatively charged lipopolysaccharide (LPS) found on the outer membrane of Gram-negative bacteria. The hydrophobic tail is thought to interact with, and

What is claimed is:

1. An antimicrobial composition comprising:
at least one species of synthetic copolypeptide of at least forty amino acid residues comprising:
at least one hydrophilic segment containing at least five contiguous cationic amino acid residues; and
at least one hydrophobic segment containing at least five contiguous hydrophobic amino acid residues;
wherein the hydrophilic segment contains a larger number of amino acid residues than the hydrophobic segment; and
water;
wherein said at least one species of synthetic copolypeptide forms multimeric structures in aqueous media;
wherein said at least one species of synthetic copolypeptide inhibits or kills microbes in aqueous media; and
wherein the composition inhibits or kills microbes.

2. The composition as described in claim 1, wherein said at least one species of synthetic copolypeptide comprises substantially only natural amino acids.

3. The composition as described in claim 1, wherein the ratio of the number of amino acids in said hydrophilic segment to the number of amino acids in said hydrophobic segment is at least 1.8 to 1.

4. The composition as described in claim 1, wherein the multimeric structures formed in aqueous media are selected from the group consisting of multimers in solution, micelles, sheets, vesicles, and fibrils.

5. The composition as described in claim 1, wherein said at least one species of synthetic copolypeptide is characterized by the ability to form mixtures in water without visible precipitate at room temperature at concentrations up to 10 fold above the critical aggregation concentration (CAC).

6. The composition as described in claim 1, wherein said at least one species of synthetic copolypeptide has a critical aggregation concentration (CAC) in water that is at least 1 log lower than that of a random sequence copolypeptide of the same amino acid composition.

7. The composition as described in claim 1 characterized in that it kills or inhibits microbes in vitro at a lower concentration than it kills mammalian cells in vitro.

8. The composition as described in claim 1 characterized in that it kills or inhibits microbes in or on mammalian tissues in vivo at concentrations that show low toxicity for those tissues.

9. The composition as described in claim 1 characterized in that said at least one species of synthetic copolypeptide forms mixtures in water without visible precipitate at room temperature at concentrations of at least 1000 μg/mL.

10. The composition as described in claim 1 characterized in that it kills or inhibits microbes in vitro as measured by greater than 3 logs killing of *Staphylococcus epidermidis* and *Escherichia coli* in standard 60 minute time-kill assays at concentrations of said at least one species of synthetic copolypeptide of 100 μg/mL or less.

11. The composition as described in claim 1 having a storage modulus of at least 50 Pa at a concentration of said at least one species of synthetic copolypeptide of less than 40 mg/mL.

12. The composition as described in claim 1, wherein said at least one species of synthetic copolypeptide promotes platelet aggregation.

13. The composition as described in claim 1, wherein said at least one species of synthetic copolypeptide inhibits fibrinolysis.

14. The composition as described in claim 1 further comprising a combination of immiscible phases in a dispersed mixture or emulsion.

15. The composition as described in claim 1 formulated as a solution, a gel, a cream, a foam, or a dressing.

16. The composition as described in claim 1, further comprising an added active pharmaceutical ingredient (API) selected from steroids, pro-inflammatory agents, anti-inflammatory agents, anti-acne agents, preservatives, hemostatic agents, angiogenic agents, wound healing agents, anti-cancer agents and other antimicrobial agent.

17. The use of the composition according to any one of claims 1 to 18 in the manufacture of a medicament for preventing or treating infection; for topical anti-infection; for microbial decolonization; for wound treatment; for surgical site treatment; for trauma treatment; for burn treatment; for treatment of diabetic foot ulcers; for eye treatment; for vaginal infections; or for urinary tract infections.

18. The use of an antimicrobial composition in the manufacture of a medicament for the prevention or treatment of infection in a mammal, wherein said composition comprises:

at least one species of a synthetic copolypeptide of at least forty amino acid residues comprising:

at least one hydrophilic segment containing at least five contiguous cationic amino acid residues; and

at least one hydrophobic segment containing at least five contiguous hydrophobic amino acid residues;

wherein the hydrophilic segment contains a larger number of amino acid residues than the hydrophobic segment; and

water;

wherein said at least one species of synthetic copolypeptide forms multimeric structures in aqueous media;

wherein said at least one species of synthetic copolypeptide inhibits or kills microbes in aqueous media; and

wherein the composition inhibits or kills microbes.

19. The use of a hemostatic composition in the manufacture of a medicament for the promotion of hemostasis in a mammal, wherein said composition comprises:

at least one species of a synthetic copolypeptide of at least forty amino acid residues comprising:

at least one hydrophilic segment containing at least five contiguous cationic amino acid residues; and

at least one hydrophobic segment containing at least five contiguous hydrophobic amino acid residues; and

wherein the hydrophilic segment contains a larger number of amino acid residues than the hydrophobic segment; and

water;

wherein the composition of said copolypeptide forms multimeric structures in aqueous media; and

wherein the composition promotes hemostasis.

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at least one hydrophilic segment containing at least five contiguous cationic amino acid residues; and

at least one hydrophobic segment containing at least five contiguous hydrophobic amino acid residues;

wherein the hydrophilic segment contains a larger number of amino acid residues than the hydrophobic segment; and

water;

wherein said at least one species of synthetic copolypeptide demonstrates a critical aggregation concentration (CAC) that is below that of a random copolypeptide of similar amino acid composition; and

wherein said at least one species of synthetic copolypeptide in water inhibits or kills microbes; and

wherein the composition inhibits or kills microbes.

2. The composition as described in claim 1, wherein the said at least one species of synthetic copolypeptide demonstrates a critical aggregation concentration (CAC) that is below 500 µg/mL.

3. The composition as described in claim 1, wherein the composition inhibits or kills microbes at concentrations of less than 500 µg/mL of the said at least one species of synthetic copolypeptide.

4. The composition as described in any one of claims 1 to 3, wherein the synthetic copolypeptide is comprised of naturally occuring amino acids.

5. The composition as described in any one of claims 1 to 4, wherein the ratio of the number of amino acids in the hydrophilic segment to the number of amino acids in the hydrophobic segment is at least 1.8 to 1.

6. The composition as described in any one of claims 1 to 5, wherein the copolypeptide is characterized by the ability to form mixtures in water without visible precipitate at room temperature at concentrations up to 10 fold above the critical aggregation concentration (CAC).

7. The composition as described in any one of claims 1 to 5, wherein the copolypeptide is characterized by the ability to form mixtures in water without visible precipitate at room temperature at concentrations up to 100 fold above the critical aggregation concentration (CAC).

8. The composition as described in any one of claims 1 to 7, wherein the copolypeptide has a critical aggregation concentration (CAC) in water that is at least 1 log lower than that of a random copolypeptide of the same amino acid composition.

9. The composition as described in any one of claims 1 to 8, wherein the composition kills or inhibits microbes *in vitro* at a lower concentration than it kills mammalian cells *in vitro*.

10. The composition as described in any one of claims 1 to 8, wherein the composition kills or inhibits microbes in or on mammalian tissues *in vivo* at concentrations that show low toxicity for those tissues.

11. The composition as described in any one of claims 1 to 10, wherein the copolypeptide forms mixtures in water without visible precipitate at room temperature at concentrations of at least 1000 μg/mL.

12. The composition as described in any one of claims 1 to 11, wherein the composition kills or inhibits microbes *in vitro* as measured by greater than 3 logs killing of *Staphylococcus epidermidis* and *Escherichia coli* in standard 60 minute time-kill assays at copolypeptide concentrations of 100 μg/mL or less.

13. The composition as described in any one of claims 1 to 10, wherein the copolypeptide forms mixtures in water without visible precipitate at room temperature at

concentrations at least 10 fold above the concentration required to inhibit or kill microbes in vitro as measured by greater than 3 logs killing of *Staphylococcus epidermidis* and *Escherichia coli* in standard 60 minute time-kill assays.

14. The composition as described in any one of claims 1 to 13 having a storage modulus of at least 50 Pa at a concentration of the copolypeptide of less than 40 mg/mL.

15. The composition as described in any one of claims 1 to 14, wherein the composition promotes platelet aggregation.

16. The composition as described in any one of claims 1 to 14,, wherein the composition inhibits fibrinolysis.

17. The composition as described in any one of claims 1 to 16, wherein the copolypeptide forms mixtures that are a combination of immiscible phases in a dispersed mixture or emulsion.

18. The composition as described in any one of claims 1 to 17, wherein the composition is formulated as a solution, a gel, a cream, a foam, or a dressing.

19. The composition as described in any one of claims 1 to 18, further comprising an added active pharmaceutical ingredient (API) selected from steroids, pro-inflammatory agents, anti-inflammatory agents, anti-acne agents, preservatives, hemostatic agents, angiogenic agents, wound healing agents, anti-cancer agents and other antimicrobial agents.