measured by cytotoxicity assays. While US Patent No. 9,017,730 describes local application of a number of synthetic cationic copolypeptide preparations, it does not provide evidence of toxicities that may result from the use of these preparations *in vivo*.

[0006] U.S. Patent No. 9,446,090 describes synthetic cationic polypeptide(s) along with mutually water-miscible mixtures that contain such a polypeptide and a second pharmaceutically acceptable polymer. Specific examples describe antimicrobial activity against certain bacteria using particular mixtures of synthetic cationic polypeptide(s) with second polymers such as polyethylene glycol (PEG), hydroxyethylcellulose (HEC), and Poloxamer 407. Even though the second polymers alone were not known to have significant antimicrobial activity, the data indicated that in the mixtures, addition of the second polymer maintained the antimicrobial activity of the synthetic cationic polypeptide(s) *in vitro* and in some cases, enhanced overall antimicrobial performance *in vivo*. However, US Patent No. 9,446,090 does not provide evidence of toxicities that may result from the use of these mixtures of synthetic cationic polypeptide(s) with other pharmaceutically-acceptable polymers *in vivo*. Further, it leaves open the possibility that the second polymer may increase the toxicity of the mixtures, as compared to the synthetic cationic polypeptide(s) alone.

[0007] While U.S. Patent Nos. 9,017,730 and 9,446,090 describe significant advances in the art, a number of challenges remain, particularly with respect to developing pharmaceutically acceptable preparations of locally applied cationic antimicrobials that have both high efficacy and high safety *in vivo*.

SUMMARY

[0007a] In one aspect, the present disclosure refers to an antimicrobial pharmaceutical composition, comprising:

an aqueous carrier; and

an antimicrobial synthetic cationic polypeptide(s) dispersed in the aqueous carrier at a concentration in the range of about 0.01% to about 5%, by weight based on total weight of the antimicrobial pharmaceutical composition;

wherein:

the antimicrobial synthetic cationic polypeptide(s) comprises a plurality of positively charged amino acid units at neutral pH;

the antimicrobial synthetic cationic polypeptide(s) at a concentration of 2 wt% in deionized water has a viscosity at 37°C of 2 centistokes (cSt) or greater;

the aqueous carrier, containing the antimicrobial synthetic cationic polypeptide(s) at 2 wt%, has a viscosity at 37°C that is greater than that of the aqueous carrier containing albumin at 2 wt% in place of the antimicrobial synthetic cationic polypeptide(s); and

the antimicrobial pharmaceutical composition has a low toxicity after being infused into the peritoneal cavity of a plurality of healthy, young adult mice at a dose of 10 mL/kg, as measured by a mouse survival rate of 50% or greater at 72 hours.

[0007b] In another aspect, the present disclosure refers to a use of an antimicrobial pharmaceutical composition disclosed herein in the manufacture of a medicament for preventing microbial contamination of tissues other than intact, healthy skin, wherein

a mammalian subject having a tissue site other than intact, healthy skin that is at risk of microbial contamination is identified; and

the antimicrobial pharmaceutical composition is to be administered to the site in an amount effective to at least partially protect the tissue site from becoming contaminated with microbes.

[0007c] In yet another aspect, the present disclosure refers to a use of an antimicrobial pharmaceutical composition disclosed herein in the manufacture of a medicament for reducing microbial load in or on tissues other than intact, healthy skin, wherein

a mammalian subject having a tissue site other than intact, healthy skin that has a microbial load is identified; and

the antimicrobial pharmaceutical composition is to be administered to the tissue site in an amount effective to at least partially reduce the microbial load.

[0007d] In a further aspect, the present disclosure refers to an antimicrobial pharmaceutical composition disclosed herein for use in preventing microbial contamination

of tissues other than intact, healthy skin, or for use in reducing microbial load in or on tissues other than intact, healthy skin.

[0008] We have developed cationic antimicrobial pharmaceutical compositions and methods of use that allow local applications *in vivo* of doses that provide antimicrobial effectiveness with low risk of local tissue toxicities and/or low risk of systemic / distant organ toxicities.

[0009] In developing the present disclosure, we found that the risk of toxicities after local application of cationic antimicrobials *in vivo* are particularly relevant when they are applied in abundance to tissue sites, or in pathophysiological settings, other than healthy intact skin. Such sites and pathophysiological settings may include open wounds, body

cavities, body orifices, diseased skin, and others. These tissue sites and pathophysiological settings may demonstrate higher local sensitivities and / or higher absorption and systemic distribution of the locally applied antimicrobial agents or excipients, as compared to healthy, intact skin. Greater local tissue toxicities and greater systemic / distant organ toxicities may follow.

[0010] Further, we made a series of discoveries that helped guide the development of the present disclosure. First, we discovered that changes in the molecular design of synthetic cationic polypeptide(s) can have different effects on antimicrobial efficacy and toxicities *in vivo*; second, we discovered that changes in formulation of synthetic cationic polypeptide(s) can have different effects on antimicrobial efficacy and toxicities *in vivo*; and third, we discovered that sterilization of synthetic cationic polypeptide(s) preparations can adversely affect molecular integrity and biophysical properties, and thereby affect antimicrobial effectiveness, toxicities, or both. Thus, we discovered a combination of problems for which solutions have now been developed.

[0011] In various examples, pharmaceutical compositions and methods of use are provided that allow local applications of effective doses of antimicrobial synthetic cationic polypeptide(s) to various tissue sites and/or in various pathophysiological settings with low risk of local and/or systemic toxicities. Without limiting the scope of the present disclosure, in various examples this is achieved by one or more of (A), (B) and (C), as follows:

[0012] (A) Design of the antimicrobial synthetic cationic polypeptide(s), especially hydrophobic content and sequence arrangement, to promote increased viscosity in water. Unexpectedly, this design feature was found to enhance safety *in vivo*.

[0013] (B) Formulating antimicrobial synthetic cationic polypeptide(s) into pharmaceutical compositions in a way that allows them to exhibit viscosity-enhancing properties. Without limiting the scope of the present disclosure, this approach is believed to have the effect of improving tissue coverage and/or enhancing retention time, yielding greater effectiveness for an applied dose. In addition, such improvements in formulation were found unexpectedly to enhance safety. Without limiting the scope of the present disclosure, excipients may include salts such as sodium chloride and potassium chloride, sugars and sugar alcohols such as dextrose, mannitol, glycerol, xylitol, and sorbitol, surfactants, and combinations thereof.

[0014] (C) Sterilization of the pharmaceutical compositions in a way that allows the sterilized pharmaceutical composition to exhibit a viscosity comparable to the viscosity of the unsterilized pharmaceutical composition. Without limiting the scope of the present disclosure, this approach is believed to have the effect of protecting the molecular structure and function of the antimicrobial synthetic cationic polypeptide(s), as well as decreasing the risk of product contamination by certain microbial organisms (e.g., fungal dermatophytes) that may exhibit resistance to effects of the antimicrobial synthetic cationic polypeptide(s).

[0015] An example provides an antimicrobial pharmaceutical composition, comprising: an aqueous carrier; and an antimicrobial synthetic cationic polypeptide(s) dispersed in the aqueous carrier at a concentration in the range of about 0.01% to about 5%, by weight based on total weight of the antimicrobial pharmaceutical composition. In an example, the antimicrobial synthetic cationic polypeptide(s) comprises a plurality of positively charged amino acid units at neutral pH. In an example, the antimicrobial synthetic cationic polypeptide(s) at a concentration of 2 wt% in deionized water has a viscosity at 37°C of 2 centistokes (cSt) or greater. In an example, the aqueous carrier, containing the antimicrobial synthetic cationic polypeptide(s) at 2 wt%, has a viscosity at 37°C that is greater than that of the aqueous carrier containing albumin at 2 wt% in place of the antimicrobial synthetic cationic polypeptide(s). In an example, the aqueous carrier, containing the antimicrobial synthetic cationic polypeptide(s) at 2 wt%, has a viscosity at 37°C that is at least about 20% greater than that of the aqueous carrier containing albumin at 2 wt% in place of the antimicrobial synthetic cationic polypeptide(s). In an example, the aqueous carrier, containing the antimicrobial synthetic cationic polypeptide(s) at 2 wt%, has a viscosity at 37°C that is at least about 50% greater than that of the aqueous carrier containing albumin at 2 wt% in place of the antimicrobial synthetic cationic polypeptide(s). In an example, the aqueous carrier, containing the antimicrobial synthetic cationic polypeptide(s) at 2 wt%, has a viscosity at 37°C that is at least about 100% greater than that of the aqueous carrier containing albumin at 2 wt% in place of the antimicrobial synthetic cationic polypeptide(s). In an example, the antimicrobial pharmaceutical composition has a low toxicity after being infused into the peritoneal cavity of a plurality of healthy, young

adult mice at a dose of 10 mL/kg, as measured by a mouse survival rate of 50% or greater at 72 hours.

[0016] Another example provides a method of preventing microbial contamination of tissues other than intact, healthy skin, comprising: identifying a mammalian subject having a tissue site other than intact, healthy skin that is at risk of microbial contamination; and administering an antimicrobial pharmaceutical composition as described herein to the site in an amount effective to at least partially protect the tissue site from becoming contaminated with microbes.

[0017] Another example provides a method of reducing microbial load in or on tissues other than intact, healthy skin, comprising: identifying a mammalian subject having a tissue site other than intact, healthy skin that has a microbial load; and administering an antimicrobial pharmaceutical composition as described herein to the tissue site in an amount effective to at least partially reduce the microbial load.

[0018] These and other examples are described in greater detail below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **Figure 1**. Schematic illustration of synthetic cationic polypeptide(s) with various amino acid sequence arrangements. Certain arrangements may be referred to as blocks or segments.

[0020] **Figure 2**. Synthetic cationic polypeptide(s) categorized into three groups based on cationic segment lengths (number of amino acid units).

[0021] **Figure 3**. Examples of synthetic cationic polypeptide(s) categorized into groups (I) Long Cationic Segment (≥ 200 units) Polypeptide(s), (II) Medium Cationic Segment (100 to 199 units) Polypeptide(s), and (III) Short Cationic Segment (10-99 units) Polypeptide(s). The cationic segment contains a plurality of cationic amino acid units, but need not be composed entirely of cationic amino acid units. The hydrophobic segment contains a plurality of hydrophobic amino acid units, but need not be composed entirely of hydrophobic amino acid units.

[0022] **Figure 4**. Example synthetic cationic polypeptide(s) with cationic-hydrophobic block sequence arrangement based on lysine and enantiopure L-leucine amino

WHAT IS CLAIMED IS:

1. An antimicrobial pharmaceutical composition, comprising:

an aqueous carrier; and

an antimicrobial synthetic cationic polypeptide(s) dispersed in the aqueous carrier at a concentration in the range of about 0.01% to about 5%, by weight based on total weight of the antimicrobial pharmaceutical composition;

wherein:

the antimicrobial synthetic cationic polypeptide(s) comprises a plurality of positively charged amino acid units at neutral pH;

the antimicrobial synthetic cationic polypeptide(s) at a concentration of 2 wt% in deionized water has a viscosity at 37°C of 2 centistokes (cSt) or greater;

the aqueous carrier, containing the antimicrobial synthetic cationic polypeptide(s) at 2 wt%, has a viscosity at 37°C that is greater than that of the aqueous carrier containing albumin at 2 wt% in place of the antimicrobial synthetic cationic polypeptide(s); and

the antimicrobial pharmaceutical composition has a low toxicity after being infused into the peritoneal cavity of a plurality of healthy, young adult mice at a dose of 10 mL/kg, as measured by a mouse survival rate of 50% or greater at 72 hours.

2. The antimicrobial pharmaceutical composition of claim 1, wherein the plurality of positively charged amino acid units at neutral pH is at least 10.

3. The antimicrobial pharmaceutical composition of claims 1 or 2, wherein the aqueous carrier is water or an aqueous solution of a pharmaceutically acceptable salt.

4. The antimicrobial pharmaceutical composition of any one of claims 1 to 3, wherein the aqueous carrier further comprises a non-ionic additive(s).

5. The antimicrobial pharmaceutical composition of claim 4, wherein the non-ionic additive(s) is present in an amount effective to increase the osmotic concentration of the antimicrobial pharmaceutical composition to a value that is at least 10% greater than that of the antimicrobial pharmaceutical composition without said additive(s).

6. The antimicrobial pharmaceutical composition of claim 4 or 5, wherein the non-ionic additive is selected from the group consisting of dextrose, mannitol, glycerol, xylitol, sorbitol, surfactant(s), and combinations thereof.

7. The antimicrobial pharmaceutical composition of any one of claims 1 to 6, wherein the aqueous carrier further comprises an additive in an amount that increases the viscosity of the antimicrobial pharmaceutical composition.

8. The antimicrobial pharmaceutical composition of any one of claims 1 to 7, wherein the aqueous carrier further comprises an additive in an amount that decreases the viscosity of the antimicrobial pharmaceutical composition.

9. The antimicrobial pharmaceutical composition of any one of claims 1 to 8, wherein the antimicrobial pharmaceutical composition is sterilized by a sterilization technique(s) configured to achieve a sterilized antimicrobial pharmaceutical composition with the antimicrobial synthetic cationic polypeptide(s) having a weight average molecular weight comparable to that of the antimicrobial synthetic cationic polypeptide(s) of the antimicrobial pharmaceutical composition without sterilization by said sterilization technique(s).

10. The antimicrobial pharmaceutical composition of any one of claims 1 to 9, wherein the antimicrobial pharmaceutical composition is sterilized by a sterilization technique(s) configured to achieve a sterilized antimicrobial pharmaceutical composition with the antimicrobial synthetic cationic polypeptide(s) having a weight average molecular weight and dispersity comparable to that of the antimicrobial synthetic cationic polypeptide(s) of the antimicrobial pharmaceutical composition without sterilization by said sterilization technique(s).

11. The antimicrobial pharmaceutical composition of any one of claims 1 to 10, wherein the antimicrobial pharmaceutical composition is sterilized by a sterilization technique(s) configured to achieve a sterilized antimicrobial pharmaceutical composition having a viscosity level at 37°C that is comparable to that of the antimicrobial pharmaceutical composition without sterilization by said sterilization technique(s).

12. The antimicrobial pharmaceutical composition of claim 11, wherein the viscosity of the sterilized antimicrobial pharmaceutical composition at 37°C is in the range of 20% to

200% of the viscosity of an otherwise comparable unsterilized antimicrobial pharmaceutical composition.

13. The antimicrobial pharmaceutical composition of any one of claims 1 to 12, wherein the antimicrobial pharmaceutical composition has a microbiocidal activity that is comparable to that of the otherwise comparable unsterilized antimicrobial pharmaceutical composition, wherein the microbiocidal activity is determined by a 60 minute time-kill assay against at least one bacteria selected from the group consisting of *S. aureus*, *S. epidermidis*, *P. aeruginosa*, and *E. coli*.

14. The antimicrobial pharmaceutical composition of any one of claims 1 to 13, wherein the antimicrobial synthetic cationic polypeptide(s) further comprises a plurality of hydrophobic amino acid units.

15. The antimicrobial pharmaceutical composition of claim 14, wherein the plurality of hydrophobic amino acid units comprises at least 5 hydrophobic amino acid units selected from leucine (L), isoleucine (l), valine (V), phenylalanine (F) or alanine (A).

16. The antimicrobial pharmaceutical composition of claim 14, wherein the plurality of hydrophobic amino acid units comprises at least 10 hydrophobic amino acid units selected from leucine (L), isoleucine (l), valine (V), phenylalanine (F) or alanine (A).

17. The antimicrobial pharmaceutical composition of claim 14, wherein the plurality of hydrophobic amino acid units comprises at least 15 hydrophobic amino acid units selected from leucine (L), isoleucine (l), valine (V), phenylalanine (F) or alanine (A).

18. The antimicrobial pharmaceutical composition of any one of claims 14 to 17, wherein the hydrophobic amino acid units comprise leucine units.

19. The antimicrobial pharmaceutical composition of any one of claims 1 to 18, wherein the plurality of positively charged amino acid units comprises lysine units.

20. The antimicrobial pharmaceutical composition of any one of claims 1 to 19, wherein the antimicrobial synthetic cationic polypeptide(s) is a block copolypeptide that comprises hydrophobic leucine units and positively charged lysine units.

21. The antimicrobial pharmaceutical composition of any one of claims 1 to 20, wherein the antimicrobial synthetic cationic polypeptide(s) comprises at least 40 amino acid units.

22. The antimicrobial pharmaceutical composition of any one of claims 1 to 21, wherein the antimicrobial synthetic cationic polypeptide(s) comprises a sequence arrangement of hydrophobic amino acid units and positively charged amino acid units that is configured to promote self-assembly of the antimicrobial synthetic cationic polypeptide(s) into multimeric structures.

23. The antimicrobial pharmaceutical composition of claim 22, wherein the antimicrobial synthetic cationic polypeptide(s) self-assembles into multimeric structures, as measured by a critical aggregation concentration that is below 1000 μg/mL at 37°C in deionized water.

24. The antimicrobial pharmaceutical composition of any one of claims 1 to 23, wherein the antimicrobial synthetic cationic polypeptide(s), in deionized water at 37°C at a concentration of 3 wt%, forms a self-supporting hydrogel.

25. The antimicrobial pharmaceutical composition of any one of claims 1 to 24, wherein the antimicrobial synthetic cationic polypeptide(s) displays surfactant activity in deionized water at 37°C at a concentration of 1 wt%, as measured by a decrease in surface tension of at least 10% as compared to deionized water alone.

26. The antimicrobial pharmaceutical composition of any one of claims 1 to 25, further comprising an anti-inflammatory compound.

27. The antimicrobial pharmaceutical composition of claim 26, wherein the anti-inflammatory compound is selected from the group consisting of a corticosteroid, a histamine inhibitor and a cytokine inhibitor.

28. Use of an antimicrobial pharmaceutical composition of any one of claims 1 to 27 in the manufacture of a medicament for preventing microbial contamination of tissues other than intact, healthy skin, wherein

a mammalian subject having a tissue site other than intact, healthy skin that is at risk of microbial contamination is identified; and

the antimicrobial pharmaceutical composition is to be administered to the site in an amount effective to at least partially protect the tissue site from becoming contaminated with microbes.

29. The use of claim 28, wherein the tissue site is selected from diseased skin, a surgical site, a traumatic wound, debrided tissues, peritoneal cavity, a pulmonary airway, a sinus, and urinary tract.

30. The use of claim 28 or 29, wherein the tissue site is to be sampled to assess the microbial load.

31. The use of any one of claims 28 to 30, wherein the antimicrobial pharmaceutical composition is to be administered intraoperatively to a surgical site.

32. The use of any one of claims 28 to 31, wherein the antimicrobial pharmaceutical composition is to be administered to the tissue site in abundance.

33. Use of an antimicrobial pharmaceutical composition of any one of claims 1 to 27 in the manufacture of a medicament for reducing microbial load in or on tissues other than intact, healthy skin, wherein

a mammalian subject having a tissue site other than intact, healthy skin that has a microbial load is identified; and

the antimicrobial pharmaceutical composition is to be administered to the tissue site in an amount effective to at least partially reduce the microbial load.

34. The use of claim 33, wherein the tissue site is selected from diseased skin, a surgical site, a traumatic wound, debrided tissue, abscess, peritoneal cavity, a pulmonary airway, a sinus, and urinary tract.

35. The use of claim 33 or 34, wherein the tissue site is to be sampled to assess the microbial load.

36. The use of any one of claims 33 to 35, wherein the antimicrobial pharmaceutical composition is to be administered intraoperatively to a tissue site that is microbially contaminated, infected or both.

37. The use of any one of claims 33 to 36, wherein the antimicrobial pharmaceutical composition is to be administered to the tissue site in abundance.

38. An antimicrobial pharmaceutical composition of any one of claims 1 to 27 for use in preventing microbial contamination of tissues other than intact, healthy skin, or for use in reducing microbial load in or on tissues other than intact, healthy skin.