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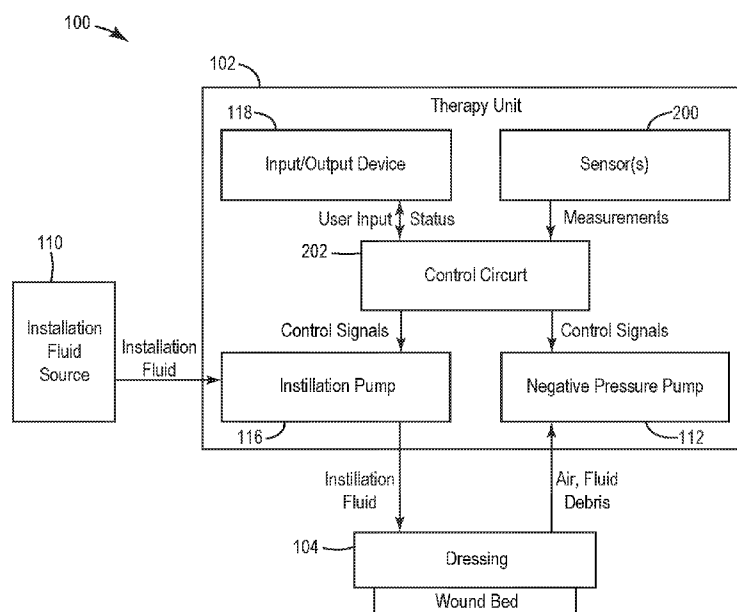


FIG. 8

(57) Abstract: The present disclosure provides non-silver instillation fluid compositions that are useful in negative pressure wound therapy systems involving instillation therapy. The non-silver instillation fluid compositions of the present technology are suitable for deep tissue delivery and effectively reduce bio-film formation without the need to open a closed wound.

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NON-SILVER WOUND INSTILLATION FLUID WITH BIO-FILM REDUCTION PROPERTIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of priority to U.S. Provisional Application No. 63/046,869, filed on July 1, 2020, which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[002] The present technology relates to non-silver instillation fluid compositions that are useful in negative pressure wound therapy systems involving instillation therapy.

BACKGROUND

[003] The following description of the background of the present technology is provided simply as an aid in understanding the present technology and is not admitted to describe or constitute prior art to the present technology.

[004] Infections can retard wound healing and, if left untreated, can result in tissue loss, systemic infections, septic shock, and death. Moreover, in addition to vegetative or free-floating bacteria present in a wound, bacterial biofilms may also form in a wound (*e.g.*, chronic wounds) presenting further challenges in wound therapy. A biofilm is an association of microorganisms that can adhere to a surface forming three-dimensional microbial communities. The ability of bacteria to form these complex biofilms can impede a host's defense mechanisms against pathogens.

[005] Negative pressure wound therapy (NPWT) is a type of wound therapy that involves applying negative pressure to a wound site to promote wound healing. Clinical studies have shown that providing reduced pressure in proximity to a wound site can assist in wound healing by promoting blood flow to the wound, stimulating the formation of granulation tissue, and encouraging the migration of healthy tissue over the wound. NPWT involves placement of a porous, foam interface into the wound and a semi-occlusive dressing that overlays the interface and seals the wound. However, microbial infection and subsequent biofilm formation impairs the efficacy of NPWT.

SUMMARY OF THE PRESENT TECHNOLOGY

[006] In one aspect, the present disclosure provides an apparatus comprising: (a) a wound dressing configured to be applied on to a wound surface, (b) an instillation fluid source comprising a wound instillation fluid composition that includes about 0.1% to about 20% citric acid by weight and a solvent, wherein the instillation fluid source is configured to be coupled to the wound dressing through a first tube connection, (c) an instillation pump configured to selectively deliver the wound instillation fluid composition from the instillation fluid source to the wound dressing, and (d) a vacuum source for applying negative pressure to the wound dressing, wherein the vacuum source is

configured to be coupled to the wound dressing through a second tube connection. In some embodiments, the wound instillation fluid composition comprises 25 mM to 800 mM citric acid, or about 50 mM to 400 mM citric acid. The wound instillation fluid composition may have a pH in the range of 1-5 or 2-3, and/or may be isotonic. Additionally or alternatively, in some embodiments, the solvent comprises water or phosphate-buffered saline (PBS).

[007] In certain embodiments, the wound instillation fluid composition further comprises about 0.1% to about 20% or about 0.25% to about 1% acetic acid by weight. Additionally or alternatively, in some embodiments, the wound instillation fluid composition further comprises about 0.1% to about 20% of one or more organic acids by weight, wherein the one or more organic acids are selected from the group consisting of malic acid, gallic acid, ascorbic acid, boric acid, alginic acid, tartaric acid, and formic acid.

[008] Additionally or alternatively, in some embodiments, the wound dressing comprises a collagen, an oxidized cellulose, and optionally silver. In some embodiments, the oxidized cellulose comprises oxidized regenerated cellulose (ORC). The first tube connection and/or the second connection may be composed of polyvinyl chloride, polyethylene, polypropylene, or any combination thereof. In any and all embodiments of the apparatus disclosed herein, the vacuum source for applying negative pressure and/or the instillation pump is a vacuum pump, a suction pump, a micro-pump, or a wall vacuum port.

[009] In any of the preceding embodiments of the apparatus described herein, exposure of a wound to the wound instillation fluid compositions of the present technology results in the killing of at least 90%-99.9% of microbes in the wound within 24 hours. Additionally, or alternatively, in some embodiments, exposure of a wound to the wound instillation fluid compositions disclosed herein results in partial or complete removal of a microbial biofilm present in the wound and/or prevents the formation of a microbial biofilm in the wound. In some embodiments, the microbial biofilm comprises Gram-positive bacteria (*e.g.*, *Staphylococcus aureus* and *Streptococcus mutans*), Gram-negative bacteria (*e.g.*, *Pseudomonas aeruginosa*), a fungus (*e.g.*, yeast), or any combination thereof.

[0010] In another aspect, the present disclosure provides a negative pressure and instillation wound therapy (NPIWT) system, comprising: (a) a wound dressing configured to be applied on to a wound surface, (b) an instillation pump configured to (i) be fluidly coupled to the wound dressing through a first connection and (ii) provide a wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprising about 0.1% to about 20% citric acid by weight and a solvent, (c) a negative pressure pump for applying negative pressure to the wound dressing, wherein the negative pressure pump is configured to be coupled to the wound dressing through a second connection, and (d) a control circuit that is communicably coupled to the instillation pump and the negative pressure pump and is configured to: (i) control the instillation pump to provide an amount of the wound instillation fluid composition to the dressing; (ii) provide a dwell time; and (iii)

control the negative pressure pump to provide a cyclic variation of negative pressure at the wound dressing. In some embodiments of the NPIWT system, the wound instillation fluid composition comprises 25 mM to 800 mM citric acid, or about 50 mM to 400 mM citric acid. Additionally or alternatively, in some embodiments, the solvent comprises water or phosphate-buffered saline (PBS).

5 **[0011]** In certain embodiments of the NPIWT system, the wound instillation fluid composition further comprises about 0.1% to about 20% or about 0.25% to about 1% acetic acid by weight. Additionally or alternatively, in some embodiments, the wound instillation fluid composition further comprises about 0.1% to about 20% of one or more organic acids by weight, wherein the one or more organic acids are selected from the group consisting of malic acid, gallic acid, ascorbic acid, boric
10 acid, alginic acid, tartaric acid, and formic acid.

[0012] Additionally or alternatively, in some embodiments of the NPIWT system, the wound dressing comprises a collagen, an oxidized cellulose, and optionally silver. In some embodiments, the oxidized cellulose comprises oxidized regenerated cellulose (ORC). The first connection and/or the second connection may be a tube composed of polyvinyl chloride, polyethylene, polypropylene, or
15 any combination thereof. In any and all embodiments of the NPIWT system disclosed herein, the negative pressure pump and/or the instillation pump is a vacuum pump, a suction pump, a micro-pump, or a wall vacuum port.

[0013] In any of the preceding embodiments of the NPIWT system described herein, exposure of a wound to the wound instillation fluid compositions of the present technology results in the killing of
20 at least 90%-99.9% of microbes in the wound within 24 hours. Additionally, or alternatively, in some embodiments, exposure of a wound to the wound instillation fluid compositions disclosed herein results in partial or complete removal of a microbial biofilm present in the wound and/or prevents the formation of a microbial biofilm in the wound. In some embodiments, the microbial biofilm comprises Gram-positive bacteria (*e.g.*, *Staphylococcus aureus* and *Streptococcus mutans*), Gram-negative bacteria (*e.g.*, *Pseudomonas aeruginosa*), a fungus (*e.g.*, yeast), or any combination thereof.
25

[0014] In any and all embodiments of the NPIWT system of the present technology, the amount of the wound instillation fluid composition provided to the dressing is about 1 ml to about 20 ml. In some embodiments of the NPIWT system described herein, the dwell time is about 10 seconds to about 30 minutes. Additionally or alternatively, in some embodiments, the negative pressure applied
30 to the wound dressing is about -5 mm Hg to about -500 mm Hg, or about -75 mm Hg to about -300 mm Hg.

[0015] In one aspect, the present disclosure provides a method for treating a wound in a subject in need thereof, comprising (a) providing a device to the wound, wherein the device comprises: a wound dressing, optionally a retainer layer, a drape, an instillation pump configured to instill a wound
35 instillation fluid composition to the wound dressing, and a vacuum source for applying negative pressure to the wound, wherein each of the vacuum source and the instillation pump are fluidly

connected to the drape through tubing; (b) administering to the wound the wound dressing; (c) optionally applying the retainer layer over the wound dressing; (d) applying the drape over the wound dressing and/or the retainer layer, wherein the drape is configured to seal the wound dressing and/or the retainer layer and the wound site; (e) instilling the wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprises about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent; (f) soaking the wound in the wound instillation fluid composition for a first temporal interval; (g) applying negative pressure on the wound for a second temporal interval; and (h) repeating steps (e)-(g) at least once. In another aspect, the present disclosure provides a method for treating or preventing biofilm formation in a wound in a subject in need thereof, comprising (a) providing a device to the wound, wherein the device comprises: a wound dressing, optionally a retainer layer, a drape, an instillation pump configured to instill a wound instillation fluid composition to the wound dressing, and a vacuum source for applying negative pressure to the wound, wherein each of the vacuum source and the instillation pump are fluidly connected to the drape through tubing; (b) administering to the wound the wound dressing; (c) optionally applying the retainer layer over the wound dressing; (d) applying the drape over the wound dressing and/or the retainer layer, wherein the drape is configured to seal the wound dressing and/or the retainer layer and the wound site; (e) instilling the wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprises about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent; (f) soaking the wound in the wound instillation fluid composition for a first temporal interval; (g) applying negative pressure on the wound for a second temporal interval; and (h) repeating steps (e)-(g) at least once. In some embodiments of the methods disclosed herein, steps (e)-(g) are repeated for about 2 to about 1000 cycles. Additionally or alternatively, in some embodiments of the methods disclosed herein, the tubing comprises polyvinyl chloride, polyethylene, polypropylene, or any combination thereof.

[0016] In one aspect, the present disclosure provides a method for treating or preventing biofilm formation in a wound in a subject in need thereof, comprising (a) providing an apparatus comprising a wound dressing, an instillation pump configured to instill a wound instillation fluid composition to the wound dressing, and a vacuum source for applying negative pressure to the wound, wherein the vacuum source is fluidly connected to the wound dressing through a first tube connection and the instillation pump is fluidly connected to the wound dressing through a second tube connection; (b) administering the apparatus to the wound; (c) instilling the wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprises about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent; (d) soaking the wound in the wound instillation fluid composition for a first temporal interval; (e) applying negative pressure on the wound for a second temporal interval; and (f) repeating steps (c)-(e) at least once. In another aspect, the present disclosure provides a method for treating a wound in a subject in need thereof,

comprising (a) providing an apparatus comprising a wound dressing, an instillation pump configured to instill a wound instillation fluid composition to the wound dressing, and a vacuum source for applying negative pressure to the wound, wherein the vacuum source is fluidly connected to the wound dressing through a first tube connection and the instillation pump is fluidly connected to the wound dressing through a second tube connection; (b) administering the apparatus to the wound; (c) instilling the wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprises about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent; (d) soaking the wound in the wound instillation fluid composition for a first temporal interval; (e) applying negative pressure on the wound for a second temporal interval; and (f) repeating steps (c)-(e) at least once. In some embodiments of the methods disclosed herein, steps (c)-(e) are repeated for about 2 to about 1000 cycles. Additionally or alternatively, in some embodiments of the methods disclosed herein, the first tube connection and/or the second connection is composed of polyvinyl chloride, polyethylene, polypropylene, or any combination thereof.

[0017] Additionally or alternatively, in some embodiments of the methods disclosed herein, the wound is a chronic wound, an acute wound, a traumatic wound, a subacute wound, a dehiscent wound, a partial-thickness burn, an ulcer, a flap, or a graft. The chronic wound may comprise infectious wounds, venous ulcers, arterial ulcers, decubitus ulcers and diabetic ulcers.

[0018] In any and all embodiments of the methods described herein, the wound instillation fluid composition further comprises about 0.1% to about 20% or about 0.25% to about 1% acetic acid by weight. In certain embodiments, the wound instillation fluid composition comprises 25 mM to 800 mM citric acid, or about 50 mM to 400 mM citric acid. Additionally or alternatively, in some embodiments, the wound instillation fluid composition further comprises about 0.1% to about 20% of one or more organic acids by weight, wherein the one or more organic acids are selected from the group consisting of malic acid, gallic acid, ascorbic acid, boric acid, alginic acid, tartaric acid, and formic acid. The solvent may comprise water or phosphate-buffered saline (PBS).

[0019] Additionally or alternatively, in some embodiments of the methods disclosed herein, the wound dressing comprises a collagen, an oxidized cellulose, and optionally silver. In some embodiments, the oxidized cellulose comprises oxidized regenerated cellulose (ORC).

[0020] In any of the preceding embodiments of the methods described herein, exposure of a wound to the wound instillation fluid compositions of the present technology results in the killing of at least 90%-99.9% of microbes in the wound within 24 hours. Additionally, or alternatively, in some embodiments, exposure of a wound to the wound instillation fluid compositions disclosed herein results in partial or complete removal of a microbial biofilm present in the wound and/or prevents the formation of a microbial biofilm in the wound. In some embodiments, the microbial biofilm

comprises Gram-positive bacteria (e.g., *Staphylococcus aureus* and *Streptococcus mutans*), Gram-negative bacteria (e.g., *Pseudomonas aeruginosa*), a fungus (e.g., yeast), or any combination thereof.

[0021] Additionally or alternatively, in some embodiments of the methods disclosed herein, the first temporal interval is about 10 seconds to about 30 minutes and/or the second temporal interval is about 10 seconds to about 100 minutes. In certain embodiments, the negative pressure applied to the wound dressing is about -5 mm Hg to about -500 mm Hg, or about -75 mm Hg to about -300 mm Hg. Additionally or alternatively, in some embodiments of the methods disclosed herein, the volume of the wound instillation fluid composition instilled to the wound dressing is about 1 ml to about 20 ml per cycle.

[0022] A kit comprising a wound instillation fluid composition comprising about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent, and instructions for using the wound instillation fluid composition in negative pressure and instillation wound therapy (NPIWT).

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] FIGS. 1A-1H demonstrate the antimicrobial activity of citric acid in a Lab-Tek™ chamber slide™ system (ThermoFisher Scientific, Ottawa, Canada). *Pseudomonas aeruginosa* and *Staphylococcus aureus* were grown on Lab-Tek chamber slides for 24 hrs. The biofilms formed on the slides were washed and were either left untreated or were treated for 24 hours with 25% or 50% (vol/vol) dilution of Suprox® multi-purpose cleaner (Hillyard, St Joseph MO) containing stabilized hydrogen peroxide (used as a positive control for antimicrobial activity), or solutions comprising 100 mM or 200 mM citric acid. The slides were stained using the LIVE/DEAD™ BacLight™ Biofilm Viability Kit (ThermoFisher Scientific, Ottawa, Canada) and imaged via confocal microscopy. The biofilm viability kit includes SYTO 9 (green)/propidium iodide (red) fluorescent stain. FIG. 1A shows the untreated *Pseudomonas aeruginosa* biofilm. FIG. 1B shows the untreated *Staphylococcus aureus* biofilm. FIG. 1C shows the *Pseudomonas aeruginosa* biofilm treated with 25% dilution of Suprox® multi-purpose cleaner. FIG. 1D shows the *Staphylococcus aureus* biofilm treated with 50% dilution of Suprox® multi-purpose cleaner. FIG. 1E shows the *Pseudomonas aeruginosa* biofilm treated with 200 mM citric acid. FIG. 1F shows the *Staphylococcus aureus* biofilm treated with 200 mM citric acid. FIG. 1G shows the *Pseudomonas aeruginosa* biofilm treated with 100 mM citric acid. FIG. 1H shows the *Staphylococcus aureus* biofilm treated with 100 mM citric acid.

[0024] FIG. 2 shows the antimicrobial activity of citric acid against *Pseudomonas aeruginosa* in a Minimum Biofilm Eradication Concentration-High Throughput Plates (MBEC™-HTP) assay (Innovotech Inc., Edmonton Canada). Shown is a bar graph plotting log₁₀ bacterial density (CFU/ml) of a 24-hour *P. aeruginosa* ATCC 15442 biofilm following a 24-hour treatment with 50 mM (0.96 g/100 ml), 100 mM (1.92 g/100 ml), 200 mM (3.84 g/100 ml), or 400 mM (7.68 g/100 ml) citric acid solutions (diluted with PBS), or the indicated concentrations (% vol/vol) of Suprox® multi-purpose

cleaner containing stabilized hydrogen peroxide. An untreated biofilm sample was used as a negative control for antimicrobial activity. Five replicates were performed for each concentration of citric acid or Suprox® multi-purpose cleaner, and eight replicates for the untreated control. Error bars represent standard error of the mean. A significant log reduction is indicated on the graph * = p 0.0001.

[0025] FIG. 3 shows the antimicrobial activity of citric acid against *Staphylococcus aureus* in the Minimum Biofilm Eradication Concentration-High Throughput Plates (MBEC™-HTP) assay (Innovotech Inc., Edmonton Canada). Shown are bar graphs plotting $\log_{10}$ bacterial density (CFU/ml) of a 24-hour *S. aureus* ATCC 29213 biofilm following a 24-hour treatment with the 50 mM (0.96 g/100 ml), 100 mM (1.92 g/100 ml), 200 mM (3.84 g/100 ml), 400 mM (7.68 g/100 ml) citric acid solutions (diluted with PBS), or the indicated concentrations (% vol/vol) of Suprox® multi-purpose cleaner containing stabilized hydrogen peroxide. An untreated sample was used as a negative control for antimicrobial activity. Five replicates were performed for each concentration of citric acid or Suprox® multi-purpose cleaner, and eight replicates for the untreated control. Error bars represent standard error of the mean. A significant log reduction is indicated on the graph * = p 0.0001.

[0026] FIG. 4 shows the antimicrobial activity of citric acid against *Pseudomonas aeruginosa* in a CDC Biofilm Reactor® model (Biosurface Technologies Corp., Bozeman MT). Shown is a bar graph plotting $\log_{10}$ bacterial density of a 24-hour *P. aeruginosa* ATCC 15442 biofilm following a 24-hour treatment with the 100 mM (1.92 g/100 ml), 200 mM (3.84 g/100 ml) citric acid, or Suprox® multi-purpose cleaner containing stabilized hydrogen peroxide (100% vol/vol). An untreated sample was used as a negative control for antimicrobial activity. Three replicates were performed for each concentration of citric acid or Suprox® multi-purpose cleaner. Error bars represent standard error of the mean. A significant log reduction is indicated on the graph * = p 0.0158.

[0027] FIG. 5 shows the antimicrobial activity of citric acid against *Staphylococcus aureus* in a CDC Biofilm Reactor® model (Biosurface Technologies Corp., Bozeman MT). Shown is a bar graph plotting $\log_{10}$ bacterial density of a 24-hour *S. aureus* ATCC 29213 biofilm following a 24-hour treatment with the 100 mM (1.92 g/100 ml), 200 mM (3.84 g/100 ml) citric acid, or Suprox® multi-purpose cleaner containing stabilized hydrogen peroxide (100% vol/vol). An untreated sample was used as a negative control for antimicrobial activity. Three replicates were performed for each concentration of citric acid or Suprox® multi-purpose cleaner. Error bars represent standard error of the mean. A significant log reduction is indicated on the graph * = p 0.0097.

[0028] FIG. 6 shows the antimicrobial activity of citric acid against *Pseudomonas aeruginosa* in a drip flow bioreactor model (Dressing testing). Shown is a bar graph plotting $\log_{10}$ bacterial density of a 24-hour *P. aeruginosa* ATCC 700888 biofilm grown in a Drip Flow Bioreactor® (Biosurface Technologies Corp., Bozeman MT) using tryptic soy broth following a 24-hour treatment with PROMOGRAN® dressing (Acelity, San Antonio TX), wound dressing A (PROMOGRAN® dressing (Acelity, San Antonio TX) comprising 100 mM citric acid and 0.05M acetic acid), or wound dressing

B (PROMOGRAN® dressing (Acelity, San Antonio TX) comprising 200 mM citric acid and 0.05M acetic acid). Dressing A and dressing B were tested in triplicates in two channels (n = 6).

PROMOGRAN® (Acelity, San Antonio TX) dressing was tested in triplicate (n = 3). Error bars represent standard error of the mean. The differences between the test dressings and the control Promogran® dressing were statistically significant.

[0029] FIG. 7 is a perspective view of an exemplary negative pressure and instillation wound therapy system.

[0030] FIG. 8 is a block diagram of the negative pressure and instillation wound therapy system of FIG. 7.

[0031] FIG. 9 is a flowchart of a process for negative pressure and instillation wound therapy.

DETAILED DESCRIPTION

[0032] It is to be appreciated that certain aspects, modes, embodiments, variations and features of the present methods are described below in various levels of detail in order to provide a substantial understanding of the present technology.

[0033] Wounds infected by bacteria or other microorganisms cause deterioration and delay in the wound healing process. Thus, a reduction in bacterial colonization is vital in wound therapy. There is a long-held need for NPWT-compatible fluid instillation solutions that can reduce biofilm colonization with deep wounds, including those with implanted metals.

[0034] Antiseptic solutions such as povidone-iodine, chlorhexidine and hydrogen oxide, however, are toxic to the total tissue and can prevent wound healing. Back *et al.*, *Int Wound J.* 2013;10 Suppl 1:32-42; see also Daeschlein *et al.*, *Skin Pharmacol Physiol* 2007; 20:292-6 (showing that irrigation with silver nitrate is not as effective in treating soft-tissue wounds without bone participation). While saline can be an effective instillation fluid for the purpose of reducing infection, it is still necessary to open the wound, and in some instances remove and replace the metal implants within the deep wounds.

[0035] The present disclosure is directed to non-silver wound instillation fluid compositions for use in negative pressure wound therapy systems involving instillation therapy. The non-silver instillation fluid compositions of the present technology are suitable for deep tissue delivery and effectively reduce bio-film formation without the need to open a closed wound. The wound instillation fluid compositions of the present technology exhibit both biofilm reduction and anti-microbial properties. Specifically, the wound instillation fluid compositions of the present technology kill pathogens present in the wound bed, and can dislodge pathogens from the wound bed during instillation therapy. Accordingly, the wound instillation fluid compositions disclosed herein are useful in the methods for treating infected complex wounds.

Definitions

[0036] The definitions of certain terms as used in this specification are provided below. Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this present technology belongs.

5 [0037] As used herein and in the appended claims, singular articles such as “a”, “an”, and “the” and similar referents in the context of describing the elements (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within
10 the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.*, “such as”) provided herein, is intended merely to better illuminate the embodiments and does not pose a limitation on the scope of the claims
15 unless otherwise stated. No language in the specification should be construed as indicating any non-claimed element as essential.

[0038] As used herein, “about” will be understood by persons of ordinary skill in the art and will vary to some extent depending upon the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art, given the context in which it is used, “about”
20 will mean up to plus or minus 10% of the particular term.

[0039] As used herein, the “administration” of a wound dressing composition to a subject includes any route of introducing or delivering to a subject a wound dressing composition to perform its intended function. Administration can be carried out by any suitable route, including but not limited to, topical administration. Administration includes self-administration and the administration by
25 another.

[0040] As used herein, the term “biofilm” refers to an association of microorganisms, *e.g.*, single or multiple species that can be encased or embedded in a matrix material, which may be self-produced by resident microorganisms. The biofilm may be present or adhere to living and/or non-living surfaces, *e.g.*, tissue, a wound, medical implants, such as but not limited to orthopedic implants, dental
30 implants, catheters, stents, *etc.* Exemplary microorganisms include, but are not limited to bacteria, *e.g.*, Gram-negative bacteria, such as *Pseudomonas aeruginosa*, Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus mutans*, and fungi, such as yeasts, *e.g.*, *Candida albicans*. The term “matrix material” is intended to encompass extracellular polymeric substances. Exemplary matrix materials include, but are not limited to polysaccharides, glycoproteins and/or nucleic acids.
35 The term “biofilm” is further intended to include biological films that develop and persist at interfaces in aqueous environments. The language “biofilm development” or “biofilm formation” is intended to

include the formation, growth, and modification of the bacterial colonies contained with biofilm structures, as well as the synthesis and maintenance of the exopolysaccharide of the biofilm structures. “Reducing” or “disrupting” a biofilm includes reducing the number of total viable microorganisms making up at least part of the biofilm, for example, as measured by total viable counts (TVC) of microorganisms (*e.g.*, bacteria, yeast).

[0041] As used herein, the term “effective amount” refers to a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, *e.g.*, an amount which results in wound healing or a reduction of one or more signs or symptoms associated with a wound described herein. In the context of therapeutic and/or prophylactic applications, the wound dressing administered to the subject will vary depending on the composition, the degree, type, and severity of the wound and on the characteristics of the individual.

[0042] As used herein, the terms “individual”, “patient”, or “subject” can be an individual organism, a vertebrate, a mammal, or a human. In some embodiments, the individual, patient or subject is a human.

[0043] As understood by one of ordinary skill in the art, “molecular weight” (also known as “relative molar mass”) is a dimensionless quantity that can be converted to molar mass by multiplying by 1 gram/mole – for example, collagen with a weight-average molecular weight of 5,000 has a weight-average molar mass of 5,000 g/mol.

[0044] As used herein, “prevention” or “preventing” of biofilm formation refers to a compound that, in a statistical sample, reduces the occurrence of biofilm formation in the treated sample relative to an untreated control sample, or delays the onset of one or more of biofilm formation relative to the untreated control sample.

[0045] “Tissue site” as used herein refers to a wound, defect, or other treatment target located on or within tissue, including but not limited to, bone tissue, adipose tissue, muscle tissue, neural tissue, dermal tissue, vascular tissue, connective tissue, cartilage, tendons, or ligaments. A wound may include chronic, acute, traumatic, subacute, and dehiscent wounds, partial-thickness burns, ulcers (such as diabetic, pressure, or venous insufficiency ulcers), flaps, grafts, or a combination of any two or more thereof. The term “tissue site” may also refer to areas of any tissue that are not necessarily wounded or defective, but are instead areas in which it may be desirable to add or promote the growth of additional tissue.

[0046] As used herein, the terms “tube”, “tubing” or “tube connection” are interchangeable and broadly refer to a tube, pipe, hose, conduit, or other structure with one or more lumina adapted to convey a fluid between two ends. Typically, a tube is an elongated, cylindrical structure with some flexibility, but the geometry and rigidity may vary. Typically, a tube is an elongated, cylindrical structure with some flexibility, but the geometry and rigidity may vary.

[0047] “Treating” or “treatment” as used herein covers the treatment of a wound described herein, in a subject, such as a human, and includes: (i) inhibiting a wound, *i.e.*, arresting its development; (ii) relieving a wound, *i.e.*, causing regression of the wound; (iii) slowing progression of the wound; and/or (iv) inhibiting, relieving, or slowing progression of one or more symptoms of the wound. In some embodiments, treatment means that the symptoms associated with the wound are, *e.g.*, alleviated, reduced, cured, or placed in a state of remission.

[0048] It is also to be appreciated that the various modes of treatment of wounds as described herein are intended to mean “substantial,” which includes total but also less than total treatment, and wherein some biologically or medically relevant result is achieved. The treatment may be a continuous prolonged treatment for a chronic wound or a single, or several administrations for the treatment of an acute wound.

NPWT Systems with Instillation and Dwell Time

[0049] “NPWT” refers to negative pressure wound therapy, which is a type of wound therapy that involves applying negative pressure (relative to atmospheric pressure) to a wound bed to promote wound healing. Typically, a dressing is sealed over a wound site and air is pumped out of the dressing to create negative pressure at the wound site. In some NPWT systems, wound exudate and other fluid is pumped out of the dressing and collected by a canister. In negative pressure wound therapy with instillation and dwell time, a second tube is introduced (in addition to the one for drainage) for the purpose of intermittently instilling solutions into the wound (*e.g.*, V.A.C. Instill® Wound Therapy, KCI, an Acelyty company, San Antonio, TX). Briefly, fluid was instilled via gravity into a foam interface from an intravenous bag or bottle. The solution is held at the wound site for a short period of time (*a.k.a.*, dwell time) followed by removal of wound fluid under negative pressure; this sequence of events was repeated in cycles. FIGS. 7-9 show exemplary negative pressure and instillation wound therapy (NPIWT) system and its functioning.

[0050] Referring to FIGS. 7 and 8, exemplary embodiments of a negative pressure and instillation wound therapy (NPIWT) system 100 is shown. FIG. 7 shows a perspective view of the NPIWT system 100, according to an exemplary embodiment. FIG. 8 shows a block diagram of the NPIWT system 100, according to an exemplary embodiment. The NPIWT system 100 may be used to provide instillation therapy by providing instillation fluid to the dressing 104. The NPIWT system 100 is shown to include a therapy unit 102 fluidly coupled to a dressing 104 via a vacuum tube 106 and an instillation tube 108. The dressing 104 may be detachable, disposable, reusable, or recyclable. A dressing 104 may include a wound-facing side, an environmental-facing side, or both. The NPIWT system 100 is also shown to include an instillation fluid 110, containing one of the wound instillation fluid compositions disclosed herein, fluidly coupled to the instillation tube 108. The NPIWT system 100 is configured to provide negative pressure wound therapy at a wound bed by reducing the

pressure at the dressing 104 relative to atmospheric pressure. The NPIWT system 100 may be, for example, a V.A.C. Ultra™ System available from Kinetic Concepts, Inc. (San Antonio, TX).

[0051] The dressing 104 is coupled to a wound bed, *i.e.*, a location of a wound (*e.g.*, sore, laceration, burn, etc.) on a patient. The dressing 104 may be substantially sealed over the wound model such that a pressure differential may be maintained between the atmosphere and the wound bed (*i.e.*, across the dressing 104). The dressing 104 may be coupled to the vacuum tube 106 and the instillation tube 108, for example to place the vacuum tube 106 and/or the instillation tube 108 in fluid communication with the wound bed. Any wound dressing known in the art may be implemented as the dressing 104 in the NPIWT systems disclosed herein. For example, the dressing comprises a collagen and an oxidized cellulose (*e.g.*, oxidized regenerated cellulose (ORC)), and optionally silver, including but not limited to PROMOGRAN® (Acelity, San Antonio, TX), PROMOGRAN PRISMA® (Acelity, San Antonio, TX), and the like. In some embodiments, the dressing 104 may be a V.A.C. VERAFLOR™ dressing (Acelity, San Antonio, TX) or a V.A.C. VERAFLOR CLEANSE CHOICE™ dressing (Acelity, San Antonio, TX).

[0052] The therapy unit 102 includes a negative pressure pump 112 (shown in FIG. 8 and obscured within the therapy unit 102 in the perspective view of FIG. 7) configured to pump air, wound exudate, and/or other debris (*e.g.*, necrotic tissue) and/or fluids (*e.g.*, instillation fluid) out of the dressing 104 via the vacuum tube 106, thereby creating a negative pressure at the dressing 104. The negative pressure pump 112 is fluidly communicable with the vacuum tube 106 and the dressing 104. Wound exudate and/or other debris and/or fluids removed from the wound bed by the negative pressure pump 112 may be collected in a canister 114 located on the therapy unit 102.

[0053] Operating the negative pressure pump 112 may therefore both create a negative pressure at the wound bed and remove undesirable fluid and debris from the wound bed. In some cases, operating the negative pressure pump 112 may cause deformation of the wound bed and/or provide other energy to the wound bed to facilitate debridement and healing of the wound bed. The negative pressure pump 112 may be operated in accordance with one or more dynamic pressure control approaches that may facilitate wound healing.

[0054] The therapy unit 102 also includes an instillation pump 116. The instillation pump 116 is configured to selectively provide instillation fluid from the instillation fluid source 110 to the dressing 104. The instillation pump 116 is operable to control the timing and amount (volume) of instillation fluid provided to the dressing 104. As described in detail below, the instillation pump 116 may be controlled in coordination with the negative pressure pump 112 to provide one or more wound treatment cycles that may facilitate wound healing.

[0055] The therapy unit 102 also includes an input/output device 118. The input/output device 118 is configured to provide information relating to the operation of the NPIWT system 100 to a user and to receive user input from the user. The input/output device 118 may allow a user to input various

preferences, settings, commands, *etc.* that may be used in controlling the negative pressure pump 112 and the instillation pump 116 as described in detail below. The input/output device 118 may include a display (*e.g.*, a touchscreen), one or more buttons, one or more speakers, and/or various other devices configured to provide information to a user and/or receive input from a user.

5 **[0056]** As shown in FIG. 7, the therapy unit 102 may also include one or more sensors 200 and a control circuit 202. The sensor(s) 200 may be configured to monitor one or more of various physical parameters relating to the operation of the NPIWT system 100. For example, the sensor(s) 200 may measure pressure at the vacuum tube 106, which may be substantially equivalent and/or otherwise indicative of the pressure at the dressing 104. As another example, the sensor(s) 200 may measure an
10 amount (*e.g.*, volume) of instillation fluid provided to the dressing 104 by the instillation pump 116. The sensor(s) 200 may provide such measurements to the control circuit 202. The sensor 200 may be coupled or configured to be coupled to the dressing 104 and to the negative-pressure pump 112.

[0057] The control circuit 202 is configured to control the operation of the therapy unit 102, including by controlling the negative pressure pump 112, the instillation pump 116, and the
15 input/output device 118. The control circuit 202 may receive measurements from the sensor(s) 200 and/or user input from the input/output device 118 and use the measurements and/or the user input to generate control signals for the instillation pump 116 and/or the negative pressure pump 112. The control circuit 202 may control the negative pressure pump 112 and the instillation pump 116 to provide various combinations of instillation phase, soak phase (corresponding to dwell time), and
20 negative pressure phase to support and encourage wound healing.

[0058] Referring to FIG. 9, a flowchart depicting a process for treating a wound using the NPIWT system 100 of FIGs. 7-8 is shown, according to an exemplary embodiment. The NPIWT process is shown as a cycle through three phases, namely an instillation phase, a soak phase, and a negative pressure phase. The control circuit 202 may be configured to control the instillation pump 116 and
25 the negative pressure pump 112 to execute the process.

[0059] At the instillation phase, the control circuit 202 controls the instillation pump 116 to provide instillation fluid from the instillation fluid source 110 to the dressing 104 via the instillation tube 108. In one illustrative embodiment, the instillation fluid source 110 may include a storage component for the solution and a separate cassette for holding the storage component and delivering
30 the solution to a tissue site, such as a V.A.C. VeraLink™ Cassette available from Kinetic Concepts, Inc. (San Antonio, TX). At the instillation phase, the control circuit 202 may control the instillation pump 116 to provide a particular amount (*e.g.*, volume) of instillation fluid and/or to provide instillation fluid for a particular duration of time. Instillation fluid may thereby be placed in contact with the wound bed. The amount of instillation fluid provided at the instillation phase and/or the
35 duration of time of the instillation phase may be user-selectable (*e.g.*, by a doctor, nurse, caregiver,

patient) via the input/output device 118 and/or otherwise customizable (*e.g.*, for various wound types, for various types of instillation fluid).

[0060] At the soak phase, the control circuit 202 provides a dwelling time between the instillation phase and the negative pressure phase. During the soak phase, the control circuit 202 controls the instillation pump 116 to prevent additional fluid from being added to the dressing 104 and prevents the negative pressure pump 112 from operating. The soak phase thereby provides a dwelling time during which the instillation fluid added at the instillation phase can soak into the wound model, for example to soften, loosen, dissolve, *etc.* the biofilm. The duration of the soak period may be user-selectable via the input/output device 118 and/or otherwise customizable (*e.g.*, for various wound types, for various types of instillation fluid). For example, the soak period may have a duration of between ten seconds and 20 minutes.

[0061] At the negative pressure phase, the control circuit 202 controls the negative pressure pump 112 to create a negative pressure at the dressing 104. In some embodiments, the instillation pump 116 is also controlled to provide instillation fluid to the dressing 104 during the negative pressure phase.

[0062] During the negative pressure phase, the negative pressure pump 112 is controlled to remove air, tryptic soy broth medium, biofilm and/or debris from the wound bed and the dressing 104. In some cases, the negative pressure pump 112 may remove the instillation fluid 110 added at the instillation phase. The instillation phase, the soak phase, and the negative pressure phase thereby work together to provide improved wound therapy.

[0063] As illustrated by FIG. 9, the control circuit 202 may control the NPIWT system 100 to repeatedly cycle through the sequence of the instillation phase, the soak phase, and the negative pressure phase. Various parameters (*e.g.*, amount of instillation fluid 110 provide, the length of the soak phase, the low pressure value, the high pressure value) of the phases may remain constant between cycles, may vary between cycles, or some combination thereof. Accordingly, the NPIWT process is highly configurable for various wound types, wound sizes, patients, instillation fluids, dressings 104, *etc.* Other NPIWT systems are described in US Publication Nos. 20170182230, and 20180214315, the contents of which are herein incorporated by reference in their entirety.

The Wound Instillation Fluid Compositions of the Present Technology

[0064] The present disclosure is directed to wound instillation fluid compositions that include at least one organic acid selected from the group consisting of citric acid, acetic acid, malic acid, gallic acid, ascorbic acid, boric acid, alginic acid, tartaric acid, and formic acid, in an amount that is sufficient (*e.g.*, an effective amount) to prevent, reduce, inhibit, or disrupt biofilm levels in a wound after application. The effects of the wound instillation fluid compositions described herein were unexpected given that previous studies have shown that the use of diluted acetic acid as an instillation fluid in NPIWT is not as effective as polyhexanide, and is comparable to saline solution. Kim *et al.*, *Plastic and Reconstructive Surgery* 132: 1569 (2013); Kim *et al.*, *Wounds*. 27(12):S2-S19 (2015)

(“Normal saline (0.9%) is the preferred solution for NPWTi, except in special situations”). Moreover, the present disclosure demonstrates that NPIWT using citric acid as an instillation fluid at concentrations as low as 12.5 mM (0.24 g/100 ml) led to inhibition of *P. aeruginosa* growth.

Compare with Nagoba *et al.*, *Wounds* 27(1):5-11 (2015) (reporting the direct application of citric acid to a wound site at a 10-15 × higher concentration (*i.e.*, 2%-3%) for the treatment of pseudomonal wound infections).

[0065] The wound instillation fluid solutions of the present technology may comprise pharmaceutically acceptable carriers, optional active materials, and excipients. As used herein, such a “pharmaceutically acceptable” component is one that is suitable for use with humans and/or animals without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio. In certain embodiments, the wound instillation fluid solutions comprise a pharmaceutically acceptable carrier, such as water or phosphate-buffered saline (PBS). The wound instillation fluid composition may have a pH in the range of 1-5 or 2-3, and/or may be isotonic.

[0066] In some embodiments, the wound instillation fluid compositions disclosed herein may comprise about 0.01%, about 0.025%, about 0.05%, about 0.1%, about 0.25%, about 0.5%, about 0.75%, about 1%, about 1.5%, about 2%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 5.5%, about 6%, about 6.5%, about 8%, about 10%, about 12%, about 14%, about 16%, about 18%, about 20%, or any range including and/or in between any two of the preceding values, of at least one organic acid selected from the group consisting of citric acid, acetic acid, malic acid, gallic acid, ascorbic acid, boric acid, alginic acid, tartaric acid, and formic acid.

[0067] Additionally or alternatively, in some embodiments, the wound instillation fluid compositions disclosed herein may comprise about 0.05 mM, about 0.1 mM, about 0.5 mM, about 1 mM, about 5 mM, about 7.5 mM, about 10 mM, about 12.5 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 40 mM, about 50 mM, about 60 mM, about 70 mM, about 80 mM, about 90 mM, about 100 mM, about 110 mM, about 120 mM, about 130 mM, about 140 mM, about 150 mM, about 160 mM, about 170 mM, about 180 mM, about 190 mM, about 200 mM, about 210 mM, about 220 mM, about 230 mM, about 240 mM, about 250 mM, about 260 mM, about 270 mM, about 280 mM, about 290 mM, about 300 mM, about 310 mM, about 320 mM, about 330 mM, about 340 mM, about 350 mM, about 360 mM, about 370 mM, about 380 mM, about 390 mM, about 400 mM, about 410 mM, about 420 mM, about 430 mM, about 440 mM, about 450 mM, about 460 mM, about 470 mM, about 480 mM, about 490 mM, about 500 mM or any range including and/or in between any two of the preceding values, of at least one organic acid selected from the group consisting of citric acid, acetic acid, malic acid, gallic acid, ascorbic acid, boric acid, alginic acid, tartaric acid, and formic acid.

[0068] Additionally or alternatively, in certain embodiments, the wound instillation fluid compositions disclosed herein comprise about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition; and a solvent (*e.g.*, water or PBS). In certain embodiments, the wound instillation fluid compositions disclosed herein comprise about 0.1%, about 0.25%, about 0.5%, about 0.75%, about 1%, about 1.25%, about 1.5%, about 1.75%, about 2%, about 2.25%, about 2.5%, about 3%, about 3.5%, about 4%, about 4.5%, about 5%, about 5.5%, about 6%, about 6.5%, about 7%, about 7.5%, about 8%, about 8.5%, about 9%, about 9.5%, about 10%, about 10.5%, about 11%, about 11.5%, about 12%, about 12.5%, about 13%, about 13.5%, about 14%, about 14.5%, about 15%, about 15.5%, about 16%, about 16.5%, about 17%, about 17.5%, about 18%, about 18.5%, about 19%, about 19.5%, about 20%, or any range including and/or in between any two of the preceding values, of citric acid. Additionally or alternatively, in some embodiments, the wound instillation fluid compositions disclosed herein further comprise about 0.1% to about 2.5% or about 0.1% to about 20% of acetic acid by weight of the wound instillation composition. In certain embodiments, the wound instillation fluid compositions disclosed herein comprise about 0.1%, about 0.25%, about 0.5%, about 0.75%, about 1%, about 1.25%, about 1.5%, about 1.75%, about 2%, about 2.25%, about 2.5%, or any range including and/or in between any two of the preceding values, of acetic acid.

[0069] Additionally or alternatively, in some embodiments, the wound instillation fluid compositions further comprise about 0.1% to about 2.5% or about 0.1% to about 20% of any one or more of malic acid, gallic acid, ascorbic acid, boric acid, alginic acid, tartaric acid, or formic acid by weight of the wound instillation composition.

[0070] Additionally, or alternatively, in some embodiments, exposure of a wound to the wound instillation fluid compositions of the present technology results in the killing of at least 90%-99.9% of microbes in the wound within 24 hours. Additionally, or alternatively, in some embodiments, exposure of a wound to the wound instillation fluid compositions disclosed herein results in partial or complete removal of a microbial biofilm present in the wound and/or prevents the formation of a microbial biofilm in the wound.

Therapeutic Methods of the Present Technology

[0071] To combat the growing threat of infections, a wound instillation fluid composition of the present technology may be used in conjunction with the automated systems and methods described herein including, for example, instilling the wound instillation fluid composition to a tissue interface in a continuous or intermittent mode followed by negative pressure therapy for treating the wound at the tissue site.

[0072] In one aspect, the present disclosure provides a method for treating a wound in a subject in need thereof, comprising (a) providing a device to the wound, wherein the device comprises: a wound dressing, optionally a retainer layer, a drape, an instillation pump configured to instill a wound

instillation fluid composition to the wound dressing, and a vacuum source for applying negative pressure to the wound, wherein each of the vacuum source and the instillation pump are fluidly connected to the drape through tubing; (b) administering to the wound the wound dressing; (c) optionally applying the retainer layer over the wound dressing; (d) applying the drape over the wound dressing and/or the retainer layer, wherein the drape is configured to seal the wound dressing and/or the retainer layer and the wound site; (e) instilling the wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprises about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent; (f) soaking the wound in the wound instillation fluid composition for a first temporal interval; (g) applying negative pressure on the wound for a second temporal interval; and (h) repeating steps (e)-(g) at least once.

[0073] In another aspect, the present disclosure provides a method for treating or preventing biofilm formation in a wound in a subject in need thereof, comprising (a) providing a device to the wound, wherein the device comprises: a wound dressing, optionally a retainer layer, a drape, an instillation pump configured to instill a wound instillation fluid composition to the wound dressing, and a vacuum source for applying negative pressure to the wound, wherein each of the vacuum source and the instillation pump are fluidly connected to the drape through tubing; (b) administering to the wound the wound dressing; (c) optionally applying the retainer layer over the wound dressing; (d) applying the drape over the wound dressing and/or the retainer layer, wherein the drape is configured to seal the wound dressing and/or the retainer layer and the wound site; (e) instilling the wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprises about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent; (f) soaking the wound in the wound instillation fluid composition for a first temporal interval; (g) applying negative pressure on the wound for a second temporal interval; and (h) repeating steps (e)-(g) at least once.

[0074] Additionally or alternatively, in some embodiments of the methods of the present technology, steps (e)-(g) are repeated for about 2 to about 1000 cycles. In certain embodiments of the methods disclosed herein, steps (e)-(g) are repeated for about 2 to about 6 cycles, about 5 to about 15 cycles, about 10 to about 30 cycles, about 20 to about 60 cycles, about 50 to about 150 cycles, about 100 to about 300 cycles, about 200 to about 600 cycles, about 300 to about 1000 cycles, or any range including and/or in between any two of these values.

[0075] In one aspect, the present disclosure provides a method for treating or preventing biofilm formation in a wound in a subject in need thereof, comprising (a) providing an apparatus comprising a wound dressing, an instillation pump configured to instill a wound instillation fluid composition to the wound dressing, and a vacuum source for applying negative pressure to the wound, wherein the vacuum source is fluidly connected to the wound dressing through a first tube connection and the instillation pump is fluidly connected to the wound dressing through a second tube connection; (b)

administering the apparatus to the wound; (c) instilling the wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprises about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent; (d) soaking the wound in the wound instillation fluid composition for a first temporal interval; (e) applying negative pressure on the wound for a second temporal interval; and (f) repeating steps (c)-(e) at least once. In another aspect, the present disclosure provides a method for treating a wound in a subject in need thereof, comprising (a) providing an apparatus comprising a wound dressing, an instillation pump configured to instill a wound instillation fluid composition to the wound dressing, and a vacuum source for applying negative pressure to the wound, wherein the vacuum source is fluidly connected to the wound dressing through a first tube connection and the instillation pump is fluidly connected to the wound dressing through a second tube connection; (b) administering the apparatus to the wound; (c) instilling the wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprises about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent; (d) soaking the wound in the wound instillation fluid composition for a first temporal interval; (e) applying negative pressure on the wound for a second temporal interval; and (f) repeating steps (c)-(e) at least once. In some embodiments of the methods disclosed herein, steps (c)-(e) are repeated for about 2 to about 1000 cycles. In certain embodiments of the methods disclosed herein, steps (c)-(e) are repeated for about 2 to about 6 cycles, about 5 to about 15 cycles, about 10 to about 30 cycles, about 20 to about 60 cycles, about 50 to about 150 cycles, about 100 to about 300 cycles, about 200 to about 600 cycles, about 300 to about 1000 cycles, or any range including and/or in between any two of these values.

[0076] In any embodiment disclosed herein, the dressing 104 comprises an environmental-facing side and a wound-facing side, and may be mated to a retainer layer while in use for NPWT. The retainer layer may be configured to be adjoined to the environmental-facing side of the dressing. The retainer layer may include, but is not limited to, a cellular foam, an open-cell foam, a reticulated foam, porous tissue collections, and/or other porous material (*e.g.*, gauze). The retainer layer may have pores that range in diameter from about 60 μm to about 2000 μm . Thus, the retainer layer may have pores that range in diameter from about 60 μm , about 100 μm , about 250 μm , about 500 μm , about 750 μm , about 1000 μm , about 1250 μm , about 1500 μm , about 1750 μm , about 2000 μm , or any range including and/or in between any two of these values. In some embodiments, the retainer layer may include an open-cell, reticulated polyurethane foam such as a GRANUFOAM™ dressing available from Kinetic Concepts, Inc. of San Antonio, Texas. In some embodiments, the retainer layer may include an open-cell, reticulated polyurethane foam such as a V.A.C. VERAFLOR™ dressing (Acelity, San Antonio TX) or a V.A.C. VERAFLOR CLEANSE CHOICE™ dressing (Acelity, San Antonio TX).

[0077] In any embodiment disclosed herein, the drape may be composed of a polyurethane film or an elastomeric film. The drape may be applied over the wound dressing of the present technology

and/or the retainer layer during NPWT. The drape may be configured to seal the wound dressing and/or the retainer layer, and the wound site during NPWT. Examples of an elastomeric film include, but are not limited to, natural rubber, polyisoprene, styrene butadiene rubber, chloroprene rubber, polybutadiene, nitrile rubber, butyl rubber, ethylene propylene rubber, ethylene propylene diene monomer, chlorosulfonated polyethylene, polysulfide rubber, ethylene vinyl acetate (EVA) film, co-polyester, or silicone. Suitable drape materials and methods of use are described in U.S. Pat. Nos. 7,534,240, 7,611,500, 9,918,733, and U.S. Pat. App. No. 14/708,078, of which the entire contents are incorporated herein by reference.

[0078] In any embodiment disclosed herein, the wound dressing may be connected to tubing or tube connections while in use for NPWT. The tubing or tube connections may include, but is not limited to, a tube, pipe, hose, conduit, or any other structure with one or more lumina adapted to convey liquid between two ends. Additionally or alternatively, in some embodiments, the tubing or tube connections may be composed of polyvinyl chloride, polyethylene, polypropylene, or any combination thereof. The tubing or tube connections may be configured to connect the drape to an instillation pump or a vacuum source for applying negative pressure, such as a V.A.C.[®] Therapy system, while in use for NPWT. Suitable tubing or tube connection materials and methods of use are described in U.S. Pat. Nos. 7,534,240, 7,611,500, 9,918,733, and U.S. Pat. App. No. 14/708,078, of which the entire contents are incorporated herein by reference.

[0079] Additionally or alternatively, in some embodiments, the vacuum source for applying negative pressure and/or the instillation pump may be a vacuum pump, a suction pump, a micro-pump, or a wall vacuum port available in many healthcare facilities.

[0080] In any embodiment disclosed herein, the wound dressing may be fluidly coupled to a vacuum source *via* the tubing to apply negative pressure to a wound in need thereof. Additionally or alternatively, in some embodiments, negative pressure refers to a pressure less than local ambient pressure, such as the pressure in a local environment external to a sealed wound site. Additionally or alternatively, in some embodiments, the vacuum source is used to apply negative pressure to a wound. Additionally or alternatively, in some embodiments, the negative pressure applied to a wound may be about -5 mm Hg to about -500 mm Hg, or about -75 mm Hg to about -300 mm Hg. Thus, the negative pressure applied to a wound may be about -5 mm Hg, about -25 mm Hg, about -50 mm Hg, about -75 mm Hg, about -100 mm Hg, about -125 mm Hg, about -150 mm Hg, about -175 mm Hg, about -200 mm Hg, about -225 mm Hg, about -250 mm Hg, about -275 mm Hg, about -300 mm Hg, about -325 mm Hg, about -350 mm Hg, about -375 mm Hg, about -400 mm Hg, about -425 mm Hg, about -450 mm Hg, about -475 mm Hg, about -500 mm Hg, or any range including and/or in between any two of these values. Methods of use of negative pressure therapy devices are described in U.S. Pat. Nos. 7,534,240, 7,611,500, 9,918,733, and U.S. Pat. App. No. 14/708,078, of which the entire contents are incorporated herein by reference.

[0081] Additionally or alternatively, in some embodiments of the methods disclosed herein, negative pressure may be applied to the wound for about 1 second to about 100 minutes.

Accordingly, in some embodiments, the second temporal interval is about 1 second, about 5 seconds, about 10 seconds, about 15 seconds, about 30 seconds, about 45 seconds, about 1 minute, about 1.25 minutes, about 2 minutes, about 4 minutes, about 6 minutes, about 8 minutes, about 10 minutes, about 12 minutes, about 14 minutes, about 16 minutes, about 18 minutes, about 20 minutes, about 25 minutes, about 30 minutes, about 35 minutes, about 40 minutes, about 45 minutes, about 50 minutes, about 60 minutes, about 70 minutes, about 80 minutes, about 90 minutes, about 100 minutes, or any range including and/or in between any two of these values.

[0082] Additionally or alternatively, in some embodiments of the methods disclosed herein, about 1 ml to about 20 ml of any and all embodiments of the wound instillation fluid compositions disclosed herein may be instilled per cycle. Additionally or alternatively, in some embodiments, about 1 ml, about 2 ml, about 4 ml, about 6 ml, about 8 ml, about 10 ml, about 12 ml, about 14 ml, about 16 ml, about 20 ml, or any range including and/or in between any two of these values of any and all embodiments of the wound instillation fluid compositions disclosed herein may be instilled per cycle.

[0083] Additionally or alternatively, in some embodiments of the methods disclosed herein, the first temporal interval (dwell time) may be about 1 second to about 30 minutes. Additionally or alternatively, in some embodiments, the first temporal interval (dwell time) may be about 1 second, about 5 seconds, about 10 seconds, about 15 seconds, about 30 seconds, about 45 seconds, about 1 minute, about 1.25 minutes, about 1.5 minutes, about 1.75 minutes, about 2 minutes, about 2.25 minutes, about 2.5 minutes, about 2.75 minutes, about 3 minutes, about 3.5 minutes, about 4 minutes, about 5 minutes, about 6 minutes, about 8 minutes, about 10 minutes, about 12 minutes, about 15 minutes, about 18 minutes, about 20 minutes, about 25 minutes, about 30 minutes, or any range including and/or in between any two of these values.

[0084] Additionally or alternatively, in some embodiments of the methods of the present technology, steps (e)-(g) are repeated for about 2 to about 1000 cycles. In certain embodiments of the methods disclosed herein, steps (e)-(g) are repeated for about 2 to about 6 cycles, about 5 to about 15 cycles, about 10 to about 30 cycles, about 20 to about 60 cycles, about 50 to about 150 cycles, about 100 to about 300 cycles, about 200 to about 600 cycles, about 300 to about 1000 cycles, or any range including and/or in between any two of these values.

[0085] Any method known to those in the art for administering the wound instillation fluid composition to an acute wound or a chronic wound disclosed herein may be employed. Suitable methods include *in vitro* or *in vivo* methods. *In vivo* methods typically include the administration of one or more wound dressings to a subject in need thereof, suitably a human. In any embodiment disclosed herein, the wound instillation fluid composition may be applied directly to the wound. In any embodiment disclosed herein, the wound instillation fluid composition may be applied directly to

a dressing contacting a wound. When used *in vivo* for therapy, the wound instillation fluid composition described herein are administered to the subject in effective amounts (*i.e.*, amounts that have desired therapeutic effect). The dose and dosage regimen will depend upon the state of the wound of the subject, and the characteristics of the particular wound dressing used. The effective amount may be determined during pre-clinical trials and clinical trials by methods familiar to physicians and clinicians. Additionally or alternatively, in some embodiments, the subject is human.

[0086] In any embodiment disclosed herein, the wound instillation fluid compositions may be administered as disclosed herein daily for 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 8 hours, 12 hours, 24 hours or more. Additionally or alternatively, in some embodiments, the wound instillation fluid compositions may be administered one, two, three, four, five, or more than five times per day. Additionally or alternatively, in some embodiments, the wound instillation fluid compositions may be administered daily for one, two, three, four or five weeks. Additionally or alternatively, in some embodiments, the wound instillation fluid compositions may be administered daily for less than 6 weeks. Additionally or alternatively, in some embodiments, the wound instillation fluid compositions may be administered daily for 6 weeks or more. Additionally or alternatively, in some embodiments, the wound instillation fluid compositions may be administered daily for 12 weeks or more.

[0087] Additionally or alternatively, in some embodiments, the wound instillation fluid compositions may be administered every day, every other day, every third day, every fourth day, every fifth day, or every sixth day. Additionally or alternatively, in some embodiments, the wound instillation fluid compositions may be administered weekly, bi-weekly, tri-weekly, or monthly. Additionally or alternatively, in some embodiments, the wound instillation fluid compositions may be administered for a period of one, two, three, four, or five weeks. Additionally or alternatively, in some embodiments, the wound instillation fluid compositions may be administered for six weeks or more. Additionally or alternatively, in some embodiments, the wound instillation fluid compositions may be administered for twelve weeks or more. Additionally or alternatively, in some embodiments, the wound instillation fluid compositions may be administered for a period of less than one year. Additionally or alternatively, in some embodiments, the wound instillation fluid compositions may be administered for a period of more than one year.

[0088] The wound may be chronic, acute, traumatic, subacute, and dehiscent wounds, partial-thickness burns, ulcers (such as diabetic, pressure, or venous insufficiency ulcers), flaps, grafts, or a combination of any two or more thereof. Additionally or alternatively, in some embodiments, the wound is a chronic wound selected from the group consisting of infectious wounds, venous ulcers, arterial ulcers, decubitus ulcers and diabetic ulcers. Additionally or alternatively, in some embodiments, the wound is an acute wound selected from the group consisting of burns, skin grafts, and dehiscent surgical wounds.

Kits Comprising the Wound Dressings of the Present Technology

[0089] In a further related aspect, the present disclosure provides kits that include a wound instillation fluid composition of any embodiment described herein and instructions for use with NPWT systems with instillation therapy. The kits of the present technology may also include instructions for treating a wound in a subject in need thereof (recommended concentrations of the wound instillation fluid composition, recommended dwell times, number of cycles *etc.*). The kit may optionally comprise components such as a wound dressing 104, antiseptic wipes, ointment, adhesive tape, tweezers, scissors, an NPIWT system 100 *etc.*

EXAMPLES

[0090] The present technology is further illustrated by the following Example, which should not be construed as limiting in any way. The examples herein are provided to illustrate advantages of the present technology and to further assist a person of ordinary skill in the art with preparing or using the compositions and systems of the present technology. The examples should in no way be construed as limiting the scope of the present technology, as defined by the appended claims. The examples can include or incorporate any of the variations, aspects, or embodiments of the present technology described above. The variations, aspects, or embodiments described above may also further each include or incorporate the variations of any or all other variations, aspects or embodiments of the present technology.

Example 1: Minimum Inhibitory Concentrations (MIC) of the Fluid Instillation Solutions of the Present Technology

[0091] *Experimental Procedure:* A solution of 400 mM citric acid was made in water. The solution was subjected to two-fold serial dilution in tryptic soy broth (TSB). *Pseudomonas aeruginosa* and *Staphylococcus aureus* were separately grown in the diluted citric acid/TSB solution overnight at 37 °C. MIC was determined as the lowest concentration that showed no visually observable growth.

[0092] *Results:* Bacterial growth was inhibited by as little as 12.5 mM (0.24 g/100 ml) and 25 mM (0.48 g/100 ml) citric acid against *P. aeruginosa* and *S. aureus*, respectively, as evidenced by a decrease in turbidity of the cultures (data not shown). Both 100 mM and 200 mM citric acid showed antimicrobial activity, as indicated by a lack of detectable growth.

[0093] These results demonstrate that the wound instillation fluid compositions of the present technology exhibit antibacterial effects, and are thus useful in NPWT systems with instillation therapy.

Example 2: Effects of Wound Instillation Fluid Compositions of the Present Technology in Lab-Tek Chamber Slide Biofilm Model

[0094] *Experimental Procedure:* *P. aeruginosa* and *S. aureus* were added to separate 8 well Lab-Tek chamber slides (ThermoFisher Scientific, Ottawa, Canada) and grown for 24 hrs to establish

biofilms. The biofilms were washed with saline, and 100 mM and 200 mM citric acid solutions (n=3) were subsequently added into the wells. Suprox® multi-purpose cleaner (Hillyard, St Joseph MO) containing stabilized hydrogen peroxide was used as a positive control for antimicrobial activity.

Untreated slides were used as negative controls for biofilm removal and/or antimicrobial activity. The slides were washed and were either left untreated in saline or treated for 24 hours with 25% or 50% (vol/vol) dilution of Suprox® multi-purpose cleaner (Hillyard, St Joseph MO) containing stabilized hydrogen peroxide, or solutions comprising 100 mM or 200 mM citric acid. The slides were stained using the LIVE/DEAD™ Biofilm Viability Kit (ThermoFisher Scientific, Ottawa, Canada) and imaged via confocal microscopy.

[0095] *Results:* As shown in FIGs. 1A and 1B, the untreated slides containing *P. aeruginosa* (FIG. 1A) and *S. aureus* (FIG. 1B) had uniform biofilms, which stained green thus indicating the presence of live cells. As shown in FIG. 1C, the slides containing *P. aeruginosa* biofilms treated with 25% dilution of Suprox® (Hillyard, St Joseph MO) for 24 hrs had a mixture of live and dead cells, as indicated by red and green staining. Similarly, the slides containing *S. aureus* biofilms treated with 50% dilution of Suprox® (Hillyard, St Joseph MO) for 24 hrs showed red and green staining, indicating the presence of both live and dead cells (FIG. 1D). In contrast, 24 hour treatment with 200 mM citric acid led to the removal of >90% and ~80% of the *P. aeruginosa* biofilm and *S. aureus* biofilm, respectively. See FIGs. 1E and 1F. Likewise, as shown in FIGs. 1G and 1H, 24 hour treatment with 100 mM citric acid led to the removal of ~80% and ~60% of the *P. aeruginosa* (FIG. 1G) biofilm and *S. aureus* (FIG. 1H) biofilm, respectively. Small areas of *P. aeruginosa* biofilm could be observed, but these appeared to be dislodged from the surface and showed folding (FIG. 1G). Similarly, small areas of *S. aureus* biofilm could also be observed (see FIG. 1F and 1H), with a large proportion of the biofilm staining red (indicating the presence of dead cells). Thus, the wound instillation fluid compositions disclosed herein are more effective in inhibiting the formation of microbial biofilms compared to Suprox®.

[0096] These results demonstrate that the wound instillation fluid compositions of the present technology are effective in inhibiting microbial biofilms. It is anticipated that the biofilm reducing properties of the wound instillation fluid compositions disclosed herein will remove the bacteria from the wound bed during instillation therapy. Accordingly, the wound instillation fluid compositions of the present technology are useful in NPWT systems with instillation therapy.

Example 3: Effects of Wound Instillation Fluid Compositions of the Present Technology in a Minimum Biofilm Eradication Concentration (MBEC) Model

[0097] *Experimental Procedure:* The MBEC assay evaluates biofilm grown under batch conditions (no flow of nutrients into or out of an individual well). The model can be used to determine the efficacy of multiple antimicrobial products simultaneously at multiple concentrations. *Pseudomonas aeruginosa* ATCC 15442 and *Staphylococcus aureus* ATCC 29213 bacteria were

separately grown using MBEC™-HTP. Pegs were placed into the growing bacterial cultures and incubated at 37°C for 24 hrs to allow the formation of biofilms. The pegs containing biofilms were then transferred to a plate with different concentrations of citric acid (400 mM, 200 mM, 100 mM, or 50 mM), Suprox® (12.5%, 25%, 50% or 100% vol/ vol dilutions, which were used as positive controls for antimicrobial activity), or left untreated (negative controls for antimicrobial activity). The pegs were incubated for 24 hrs with the solutions. Bacterial viability in the biofilms was measured based on plate counts.

[0098] *Results:* As shown in FIG. 2, no *P. aeruginosa* colonies were detectable on the Trypticase soy agar (TSA) plates following a 24-hour treatment with any of the tested concentrations of citric acid or Suprox®. Given that untreated biofilms contained $>10^5$ live bacterial cells, citric acid caused a 5-log reduction in bacterial cell density ($p = 0.0001$). Therefore, the effect of the 24-hr treatment with all tested concentrations of citric acid (400 mM, 200 mM, 100 mM and 50 mM) on *Pseudomonas* biofilm was as robust as Suprox®.

[0099] Similarly, as shown in FIG. 3, no *S. aureus* colonies were detectable on the TSA plates following a 24-hour treatment with all tested concentrations of citric acid or Suprox®. Given that untreated biofilms contained $>10^5$ live bacterial cells as indicated by the plate counts (FIG. 3), citric acid caused a 5-log reduction in bacterial cell density ($p = 0.0005$). Therefore, the effect of the 24-hr treatment with all tested concentrations of citric acid (400 mM, 200 mM, 100 mM and 50 mM) on *Pseudomonas* biofilm was as robust as Suprox®.

[00100] These results demonstrate that the Minimum Biofilm Eradication Concentration of citric acid is lower than the 50 mM, the lowest concentration tested herein. Therefore, the wound instillation fluid compositions of the present technology are effective. Accordingly, the wound instillation fluid compositions of the present technology are useful in NPWT systems with instillation therapy.

Example 4: Effects of Wound Instillation Fluid Compositions of the Present Technology in a CDC Bioreactor Model

[00101] *Experimental Procedure:* The CDC Bioreactor, also known as a CDC Biofilm Reactor® model (Biosurface Technologies Corp., Bozeman MT), allows formation of robust biofilms in the presence of shear generated by fluid movement and mixing. *Pseudomonas aeruginosa* ATCC 15442 and *Staphylococcus aureus* ATCC 29213 were separately grown for 24 hr in the CDC Bioreactors. Coupons were exposed to bacteria for 24 hrs to allow formation of biofilms. The coupons containing biofilms were then taken out and exposed to saline, Suprox® or citric acid (100 mM and 200 mM concentrations) for 24 hours. Bacterial cell density in the biofilms was measured based on plate counts.

[00102] *Results:* Untreated *P. aeruginosa* biofilms contained $>10^7$ bacterial cells. As shown in FIG. 4, no colonies were seen following treatment with all tested concentrations of citric acid (100 mM and

200 mM) as well as Suprox®, indicating that the *P. aeruginosa* biofilms were completely destroyed. The biofilm-killing effect of citric acid and Suprox® against *Pseudomonas* biofilm was statistically significant compared to the untreated controls ($p<0.0158$). These data demonstrate that the biofilm-killing effects of 100 mM and 200 mM citric acid on a 24 hr old *Pseudomonas* biofilm was as robust as Suprox®.

[00103] Untreated *S. aureus* biofilms contained $>10^6$ bacterial cells. As shown in FIG. 5, there was a decrease in colony formation following treatment with all tested concentrations of citric acid (100 mM and 200 mM) as well as Suprox®. 100 mM citric acid caused a 2 log reduction in viable bacteria counts, and 200 mM citric acid showed a 4 log reduction in viable bacteria counts (FIG. 5). The biofilm-killing effect of citric acid and Suprox® against the *S. aureus* biofilms was statistically significant compared to the untreated controls ($p<0.0097$).

[00104] These results demonstrate that the wound instillation fluid compositions of the present technology are effective in the eradication of microbial biofilms. Accordingly, the wound instillation fluid compositions of the present technology are useful in NPWT systems with instillation therapy.

Example 5: Effects of Wound Instillation Fluid Compositions of the Present Technology in a Drip Flow Bioreactor Model (Dressing testing)

[00105] *Experimental Procedure:* *Pseudomonas aeruginosa* ATCC 70888 biofilm was generated in a Drip Flow Bioreactor® (Biosurface Technologies Corp., Bozeman MT) using TSB (tryptic soy broth). After growing the biofilm for 24 hr, the biofilms were either left untreated or treated with three dressings for 24 hrs: PROMOGRAN® (Acelity, San Antonio TX) dressing (containing 45% oxidized regenerated cellulose (ORC) and 55% collagen), Dressing A (PROMOGRAN® containing 100 mM citric acid), or Dressing B (PROMOGRAN® containing 200 mM citric acid). Dressings A and B also contained 0.05M acetic acid. The number of viable bacteria in the biofilms were enumerated.

[00106] *Results:* The untreated biofilm contained $>10^7$ viable bacterial cells (FIG. 6). PROMOGRAN® (Acelity, San Antonio TX) alone showed a 3 log reduction in viable count. As shown in FIG. 6, dressings containing 100 mM or 200 mM citric acid caused a 7 log reduction of the same *Pseudomonas* biofilm. These data demonstrate that the wound instillation fluid compositions of the present technology are more effective in eradicating microbial biofilms compared to dressings containing ORC and cellulose.

[00107] These results demonstrate that the wound instillation fluid compositions of the present technology are effective in the eradication of microbial biofilms. Accordingly, the wound instillation fluid compositions of the present technology are useful in NPWT systems with instillation therapy.

Example 6: Effects of Wound Instillation Fluid Compositions of the Present Technology in a Advanced/Aged Biofilm (72 hr)

[00108] *Experimental Procedure:* Various strains of bacteria, including MRSA strains, multidrug resistant strains and clinical isolates, will be separately grown using the biofilm models described herein. The biofilms will be grown for 24 hrs, 48 hrs, or 72 hrs. The resultant biofilms will either be left untreated or treated for 24 hrs with the following compositions: Suprox® (the positive control), 50 mM citric acid, 100 mM citric acid, 200 mM citric acid, 50 mM citric acid + 0.5 % acetic acid, 100 mM citric acid + 0.5 % acetic acid, 200 mM citric acid + 0.5 % acetic acid, or 0.5 % acetic acid. The number of viable bacteria in the biofilms will be enumerated. Bacterial biofilms will also be stained and visualized using confocal microscopy.

[00109] *Results:* It is anticipated that each of 50 mM citric acid, 100 mM citric acid, 200 mM citric acid, 50 mM citric acid + 0.5 % acetic acid, 100 mM citric acid + 0.5 % acetic acid, 200 mM citric acid + 0.5 % acetic acid, and 0.5 % acetic acid will show notable antimicrobial activity against at least some of the different tested strains. It is also anticipated that the combination of citric acid and acetic acid will show a synergistic effect with respect to reducing bacterial viability and/or biofilm stability. It is further anticipated that the combination of citric acid and acetic acid will show a synergistic effect with respect to the eradication of microbial biofilms.

[00110] These results demonstrate that the wound instillation fluid compositions of the present technology are effective in inhibiting the formation of microbial biofilms and/or eradicating microbial biofilms. Accordingly, the wound instillation fluid compositions of the present technology are useful in NPWT systems with instillation therapy.

Example 7: Effects of Wound Instillation Fluid Compositions of the Present Technology in a Complex Biofilm (Mixed Species)

[00111] *Experimental Procedure:* Various strains of bacteria, including MRSA strains, multidrug resistant strains and clinical isolates, will be grown as various combinations of mixed cultures using the biofilm models described herein. The biofilms will be grown for 24 hrs, 48 hrs, or 72 hrs. The resultant biofilms will either be left untreated or treated for 24 hrs with the following compositions: Suprox® (the positive control), 50 mM citric acid, 100 mM citric acid, 200 mM citric acid, 50 mM citric acid + 0.5 % acetic acid, 100 mM citric acid + 0.5 % acetic acid, 200 mM citric acid + 0.5 % acetic acid, or 0.5 % acetic acid. The number of viable bacteria in the biofilms will be enumerated. Bacterial biofilms will also be stained and visualized using confocal microscopy.

[00112] *Results:* It is anticipated that each of 50 mM citric acid, 100 mM citric acid, 200 mM citric acid, 50 mM citric acid + 0.5 % acetic acid, 100 mM citric acid + 0.5 % acetic acid, 200 mM citric acid + 0.5 % acetic acid, and 0.5 % acetic acid will show notable antimicrobial activity against at least some of the different tested strains. It is also anticipated that the combination of citric acid and acetic acid will show a synergistic effect with respect to reducing bacterial viability and/or biofilm stability.

It is further anticipated that the combination of citric acid and acetic acid will show a synergistic effect with respect to the eradication of microbial biofilms.

[00113] The results will demonstrate that the wound instillation fluid composition of the present technology are effective in killing a variety of pathogenic bacteria in complex biofilms. Accordingly, the wound instillation fluid compositions of the present technology are useful in NPWT systems with instillation therapy.

Example 8: NPIWT Using the Wound Instillation Fluid Compositions of the Present Technology in a Wound Model

[00114] *Experimental Procedure:* Various combinations of strains of bacteria, including MRSA strains, multidrug resistant strains and clinical isolates, will be grown using the biofilm models described herein. The biofilms will be transplanted on wound models and a dressing will be applied. The biofilms in the wound models will be simultaneously fed with TSB or wound fluid, and treated with negative pressure and instillation wound therapy. The following wound instillation fluid compositions will be used as instillation fluid: normal saline, Dakin's solution, PHMB solution, Mycrocyn (diluted bleach) solution, silver nitrate solution (positive control for antimicrobial effect), 50 mM citric acid, 100 mM citric acid, 200 mM citric acid, 50 mM citric acid + 0.5 % acetic acid, 100 mM citric acid + 0.5 % acetic acid, 200 mM citric acid + 0.5 % acetic acid, or 0.5 % acetic acid.

[00115] An exemplary negative pressure and instillation wound therapy (NPIWT) system is shown in FIGs. 7 and 8 and described herein. NPIWT process will be carried out as described in FIG. 9.

Briefly, the NPIWT process will cycle through three phases, namely an instillation phase, a soak phase, and a negative pressure phase. At the instillation phase, about 8 ml of the wound instillation fluid compositions will be instilled into the dressing. At the soak phase, the wound instillation fluid compositions will soak into the wound model for a dwell time of 2 minutes. The negative pressure phase will last for about 10 min. NPIWT will be carried out for multiple cycles for 8 hrs or 24 hrs on the wound model. At end of NPIWT, the viable counts of bacteria in the wound model will be measured.

[00116] *Results:* It is anticipated that each of 50 mM citric acid, 100 mM citric acid, 200 mM citric acid, 50 mM citric acid + 0.5 % acetic acid, 100 mM citric acid + 0.5 % acetic acid, 200 mM citric acid + 0.5 % acetic acid, and 0.5 % acetic acid will show notable antimicrobial activity against at least some of the different tested strains. It is also anticipated that the combination of citric acid and acetic acid will show a synergistic effect with respect to reducing bacterial viability and/or biofilm stability. It is further anticipated that the combination of citric acid and acetic acid will show a synergistic effect with respect to the eradication of microbial biofilms. It is further anticipated that 50 mM citric acid, 100 mM citric acid, 200 mM citric acid, 50 mM citric acid + 0.5 % acetic acid, 100 mM citric acid + 0.5 % acetic acid, 200 mM citric acid + 0.5 % acetic acid will exhibit equivalent or better

antimicrobial activity and/or biofilm removal compared to at least one of Dakin's solution, PHMB solution, Myrocyn (diluted bleach) solution, or silver nitrate solution.

[00117] These results demonstrate that the wound instillation fluid compositions of the present technology are effective in inhibiting the formation of microbial biofilms and/or eradicating microbial biofilms. Accordingly, the wound instillation fluid compositions of the present technology are useful in NPWT systems with instillation therapy.

EQUIVALENTS

[001] The present technology is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the present technology. Many modifications and variations of this present technology can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the present technology, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the present technology. It is to be understood that this present technology is not limited to particular methods, reagents, compounds, compositions, or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[002] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[003] As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third, and upper third, *etc.* As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like, include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 cells refers to groups having 1, 2, or 3 cells. Similarly, a group having 1-5 cells refers to groups having 1, 2, 3, 4, or 5 cells, and so forth.

[004] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

CLAIMS

1. An apparatus comprising:
 - a. a wound dressing configured to be applied on to a wound surface,
 - b. an instillation fluid source comprising a wound instillation fluid composition that includes about 0.1% to about 20% citric acid by weight and a solvent, wherein the instillation fluid source is configured to be coupled to the wound dressing through a first tube connection,
 - c. an instillation pump configured to selectively deliver the wound instillation fluid composition from the instillation fluid source to the wound dressing, and
 - d. a vacuum source for applying negative pressure to the wound dressing, wherein the vacuum source is configured to be coupled to the wound dressing through a second tube connection.
2. The apparatus of claim 1, wherein the wound instillation fluid composition further comprises about 0.1% to about 20% or about 0.25% to about 1% acetic acid by weight.
3. The apparatus of claim 1 or 2, wherein the wound instillation fluid composition further comprises about 0.1% to about 20% of one or more organic acids by weight, wherein the one or more organic acids are selected from the group consisting of malic acid, gallic acid, ascorbic acid, boric acid, alginic acid, tartaric acid, and formic acid.
4. The apparatus of any one of claims 1-3, wherein the solvent comprises water or phosphate-buffered saline (PBS).
5. The apparatus of any one of claims 1-4, wherein the wound instillation fluid composition comprises 25 mM to 800 mM citric acid, or about 50 mM to 400 mM citric acid.
6. The apparatus of any one of claims 1-5, wherein the wound instillation fluid composition has a pH in the range of 1-5 or 2-3.
7. The apparatus of any one of claims 1-6, wherein the wound instillation fluid composition is isotonic.
8. The apparatus of any one of claims 1-7, wherein exposure of a wound to the wound instillation fluid composition results in the killing of at least 90%-99.9% of microbes in the wound within 24 hours.
9. The apparatus of any one of claims 1-8, wherein exposure of a wound to the wound instillation fluid composition results in partial or complete removal of a microbial biofilm present in the wound.
10. The apparatus of any one of claims 1-9, wherein exposure of a wound to the wound instillation fluid composition prevents the formation of a microbial biofilm in the wound.
11. The apparatus of claim 9 or 10, wherein the microbial biofilm comprises Gram-positive bacteria, Gram-negative bacteria, a fungus, or any combination thereof.

12. The apparatus of claim 8, wherein the microbes are Gram-positive bacteria, Gram-negative bacteria, a fungus, or any combination thereof.
13. The apparatus of claim 11 or 12, wherein the Gram-negative bacteria is *Pseudomonas aeruginosa*.
14. The apparatus of any one of claims 11-13, wherein the Gram-positive bacteria are *Staphylococcus aureus* and *Streptococcus mutans*.
15. The apparatus of any one of claims 11-14, wherein the fungus is a yeast.
16. The apparatus of any one of claims 1-15, wherein the wound dressing comprises a collagen, an oxidized cellulose, and optionally silver.
17. The apparatus of claim 16, wherein the oxidized cellulose comprises oxidized regenerated cellulose (ORC).
18. The apparatus of any one of claims 1-17, wherein the first tube connection and/or the second connection is composed of polyvinyl chloride, polyethylene, polypropylene, or any combination thereof.
19. The apparatus of any one of claims 1-18, wherein the vacuum source for applying negative pressure and/or the instillation pump is a vacuum pump, a suction pump, a micro-pump, or a wall vacuum port.
20. A negative pressure and instillation wound therapy (NPIWT) system, comprising:
 - (a) a wound dressing configured to be applied on to a wound surface,
 - (b) an instillation pump configured to (i) be fluidly coupled to the wound dressing through a first connection and (ii) provide a wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprising about 0.1% to about 20% citric acid by weight and a solvent,
 - (c) a negative pressure pump for applying negative pressure to the wound dressing, wherein the negative pressure pump is configured to be coupled to the wound dressing through a second connection, and
 - (d) a control circuit that is communicably coupled to the instillation pump and the negative pressure pump and is configured to:
 - control the instillation pump to provide an amount of the wound instillation fluid composition to the dressing;
 - provide a dwell time; and
 - control the negative pressure pump to provide a cyclic variation of negative pressure at the wound dressing.
21. The NPIWT system of claim 20, wherein the wound instillation fluid composition further comprises about 0.1% to about 20% or about 0.25% to about 1% acetic acid by weight.
22. The NPIWT system of claim 20 or 21, wherein the wound instillation fluid composition further comprises about 0.1% to about 20% of one or more organic acids by weight, wherein

- the one or more organic acids are selected from the group consisting of malic acid, gallic acid, ascorbic acid, boric acid, alginic acid, tartaric acid, and formic acid.
23. The NPIWT system of any one of claims 20-22, wherein the solvent comprises water or phosphate-buffered saline (PBS).
24. The NPIWT system of any one of claims 20-23, wherein the wound instillation fluid composition comprises 25 mM to 800 mM citric acid, or about 50 mM to 400 mM citric acid.
25. The NPIWT system of any one of claims 20-24, wherein exposure of a wound to the wound instillation fluid composition results in the killing of at least 90%-99.9% of microbes in the wound within 24 hours.
26. The NPIWT system of any one of claims 20-25, wherein exposure of a wound to the wound instillation fluid composition results in partial or complete removal of a microbial biofilm present in the wound and/or prevents the formation of a microbial biofilm in the wound.
27. The NPIWT system of claim 26, wherein the microbial biofilm comprises Gram-positive bacteria, Gram-negative bacteria, a fungus, or any combination thereof.
28. The NPIWT system of any one of claims 20-27, wherein the wound dressing comprises a collagen, an oxidized cellulose, and optionally silver.
29. The NPIWT system of any one of claims 20-28, wherein the negative pressure pump and/or the instillation pump is a vacuum pump, a suction pump, a micro-pump, or a wall vacuum port.
30. The NPIWT system of any one of claims 20-29, wherein the amount of the wound instillation fluid composition provided to the dressing is about 1 ml to about 20 ml.
31. The NPIWT system of any one of claims 20-30, wherein the dwell time is about 10 seconds to about 30 minutes.
32. The NPIWT system of any one of claims 20-31, wherein the negative pressure applied to the wound dressing is about -5 mm Hg to about -500 mm Hg, or about -75 mm Hg to about -300 mm Hg.
33. A method for treating a wound in a subject in need thereof, comprising
- (a) providing a device to the wound, wherein the device comprises: a wound dressing, optionally a retainer layer, a drape, an instillation pump configured to instill a wound instillation fluid composition to the wound dressing, and a vacuum source for applying negative pressure to the wound, wherein each of the vacuum source and the instillation pump are fluidly connected to the drape through tubing;
 - (b) administering to the wound the wound dressing;
 - (c) optionally applying the retainer layer over the wound dressing;
 - (d) applying the drape over the wound dressing and/or the retainer layer, wherein the drape is configured to seal the wound dressing and/or the retainer layer and the wound site;

- (e) instilling the wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprises about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent;
 - (f) soaking the wound in the wound instillation fluid composition for a first temporal interval;
 - (g) applying negative pressure on the wound for a second temporal interval; and
 - (h) repeating steps (e)-(g) at least once.
34. A method for treating or preventing biofilm formation in a wound in a subject in need thereof, comprising
- (a) providing a device to the wound, wherein the device comprises: a wound dressing, optionally a retainer layer, a drape, an instillation pump configured to instill a wound instillation fluid composition to the wound dressing, and a vacuum source for applying negative pressure to the wound, wherein each of the vacuum source and the instillation pump are fluidly connected to the drape through tubing;
 - (b) administering to the wound the wound dressing;
 - (c) optionally applying the retainer layer over the wound dressing;
 - (d) applying the drape over the wound dressing and/or the retainer layer, wherein the drape is configured to seal the wound dressing and/or the retainer layer and the wound site;
 - (e) instilling the wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprises about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent;
 - (f) soaking the wound in the wound instillation fluid composition for a first temporal interval;
 - (g) applying negative pressure on the wound for a second temporal interval; and
 - (h) repeating steps (e)-(g) at least once.
35. The method of claim 33 or 34, wherein steps (e)-(g) are repeated for about 2 to about 1000 cycles.
36. The method of any one of claims 33-35, wherein the tubing comprises polyvinyl chloride, polyethylene, polypropylene, or any combination thereof.
37. A method for treating or preventing biofilm formation in a wound in a subject in need thereof, comprising
- (a) providing an apparatus comprising a wound dressing, an instillation pump configured to instill a wound instillation fluid composition to the wound dressing, and a vacuum source for applying negative pressure to the wound, wherein the vacuum source is fluidly connected to the wound dressing through a first tube connection and the instillation pump is fluidly connected to the wound dressing through a second tube connection;
 - (b) administering the apparatus to the wound;

- (c) instilling the wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprises about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent;
 - (d) soaking the wound in the wound instillation fluid composition for a first temporal interval;
 - (e) applying negative pressure on the wound for a second temporal interval; and
 - (f) repeating steps (c)-(e) at least once.
38. A method for treating a wound in a subject in need thereof, comprising
- (a) providing an apparatus comprising a wound dressing, an instillation pump configured to instill a wound instillation fluid composition to the wound dressing, and a vacuum source for applying negative pressure to the wound, wherein the vacuum source is fluidly connected to the wound dressing through a first tube connection and the instillation pump is fluidly connected to the wound dressing through a second tube connection;
 - (b) administering the apparatus to the wound;
 - (c) instilling the wound instillation fluid composition to the wound dressing, wherein the wound instillation fluid composition comprises about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent;
 - (d) soaking the wound in the wound instillation fluid composition for a first temporal interval;
 - (e) applying negative pressure on the wound for a second temporal interval; and (f) repeating steps (c)-(e) at least once.
39. The method of claim 37 or 38, wherein the first tube connection and/or the second connection is composed of polyvinyl chloride, polyethylene, polypropylene, or any combination thereof.
40. The method of any one of claims 37-39, wherein steps (c)-(e) are repeated for about 2 to about 1000 cycles.
41. The method of any one of claims 33-40, wherein the wound is a chronic wound, an acute wound, a traumatic wound, a subacute wound, a dehiscent wound, a partial-thickness burn, an ulcer, a flap, or a graft.
42. The method of claim 41, wherein the chronic wound is selected from the group consisting of infectious wounds, venous ulcers, arterial ulcers, decubitus ulcers and diabetic ulcers.
43. The method of any one of claims 33-42, wherein the wound dressing comprises a collagen, an oxidized cellulose, and optionally silver.
44. The method of claim 43, wherein the oxidized cellulose comprises oxidized regenerated cellulose (ORC).
45. The method of any one of claims 33-44, wherein the wound instillation fluid composition further comprises about 0.1% to about 20% or about 0.25% to about 1% acetic acid by weight.

46. The method of any one of claims 33-45, wherein the wound instillation fluid composition further comprises about 0.1% to about 20% of one or more organic acids by weight, wherein the one or more organic acids are selected from the group consisting of malic acid, gallic acid, ascorbic acid, boric acid, alginic acid, tartaric acid, and formic acid.
47. The method of any one of claims 33-46, wherein the solvent comprises water or phosphate-buffered saline (PBS).
48. The method of any one of claims 33-47, wherein the wound instillation fluid composition comprises 25 mM to 800 mM citric acid, or about 50 mM to 400 mM citric acid.
49. The method of any one of claims 33-48, wherein exposure of the wound to the wound instillation fluid composition results in the killing of at least 90%-99.9% of microbes in the wound within 24 hours.
50. The method of any one of claims 33-49, wherein exposure of a wound to the wound instillation fluid composition results in partial or complete removal of a microbial biofilm present in the wound and/or prevents the formation of a microbial biofilm in the wound.
51. The method of any one of claims 33-50, wherein the first temporal interval is about 10 seconds to about 30 minutes.
52. The method of any one of claims 33-51, wherein the second temporal interval is about 10 seconds to about 100 minutes.
53. The method of any one of claims 33-52, wherein the negative pressure applied to the wound dressing is about -5 mm Hg to about -500 mm Hg, or about -75 mm Hg to about -300 mm Hg.
54. The method of any one of claims 33-53, wherein the volume of the wound instillation fluid composition instilled to the wound dressing is about 1 ml to about 20 ml per cycle.
55. A kit comprising a wound instillation fluid composition comprising about 0.1% to about 20% citric acid by weight of the wound instillation fluid composition and a solvent, and instructions for using the wound instillation fluid composition in negative pressure and instillation wound therapy (NPIWT).

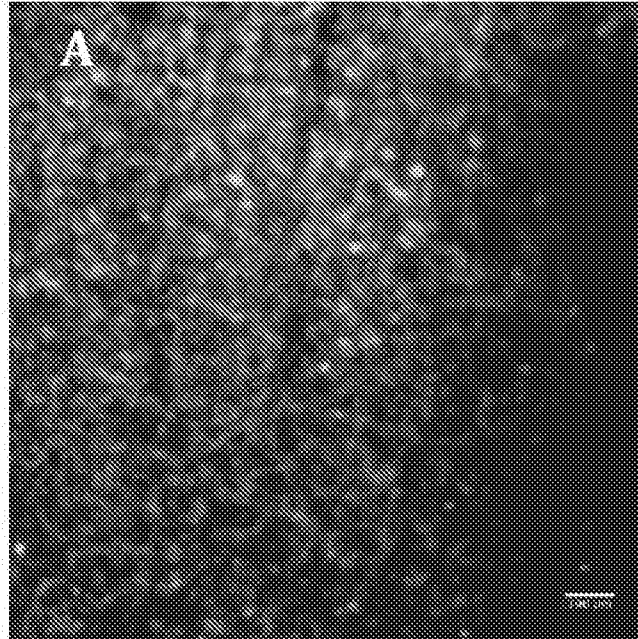


FIG. 1A

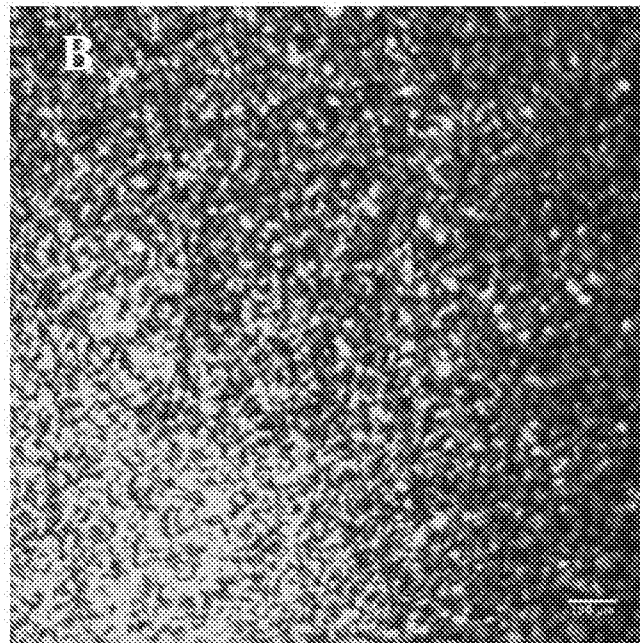


FIG. 1B

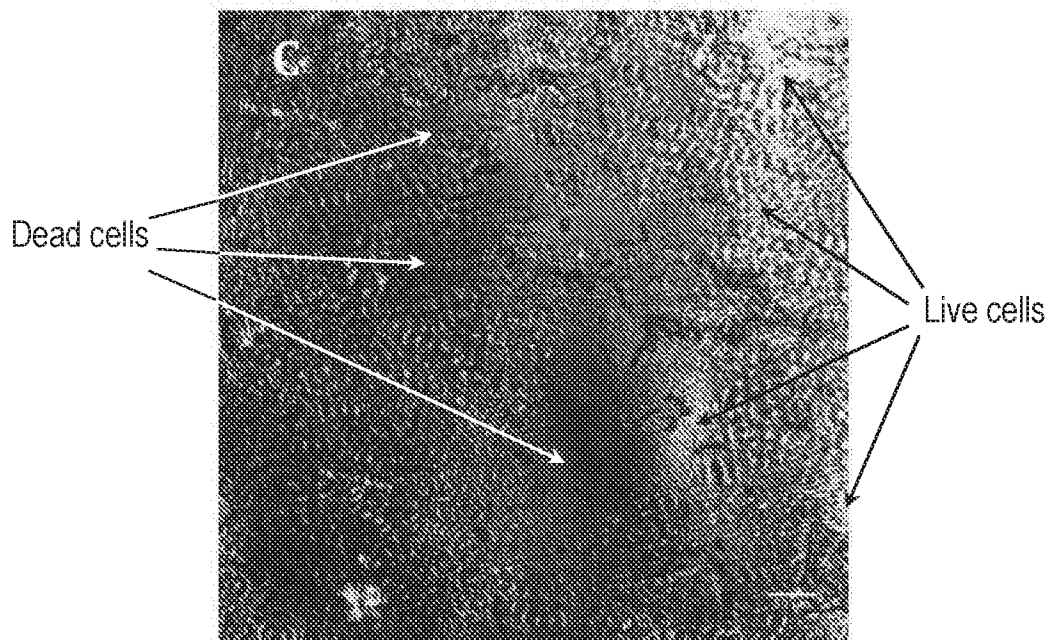


FIG. 1C

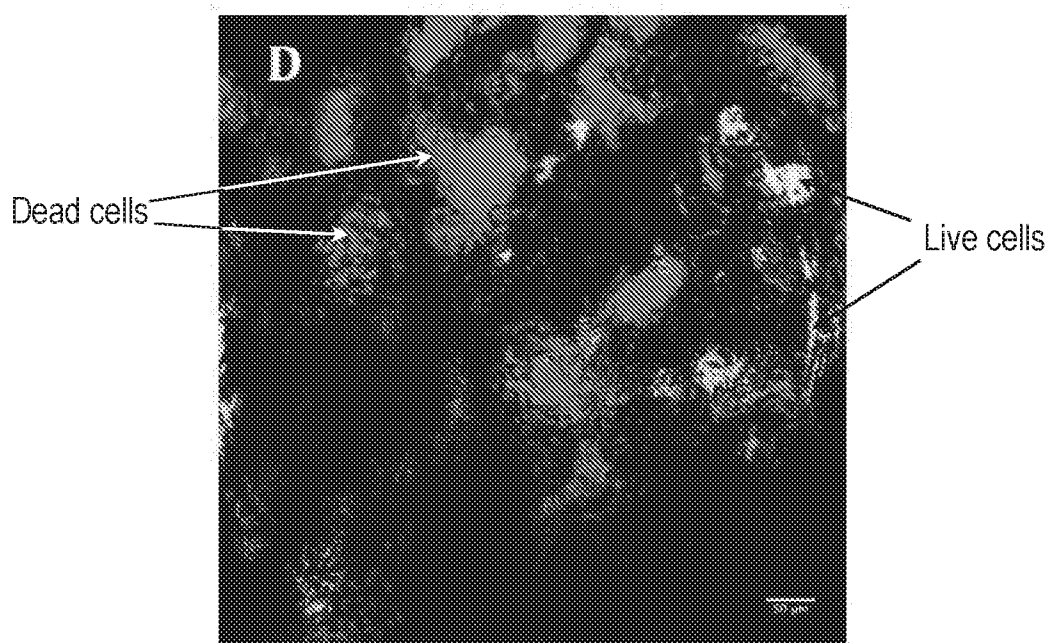
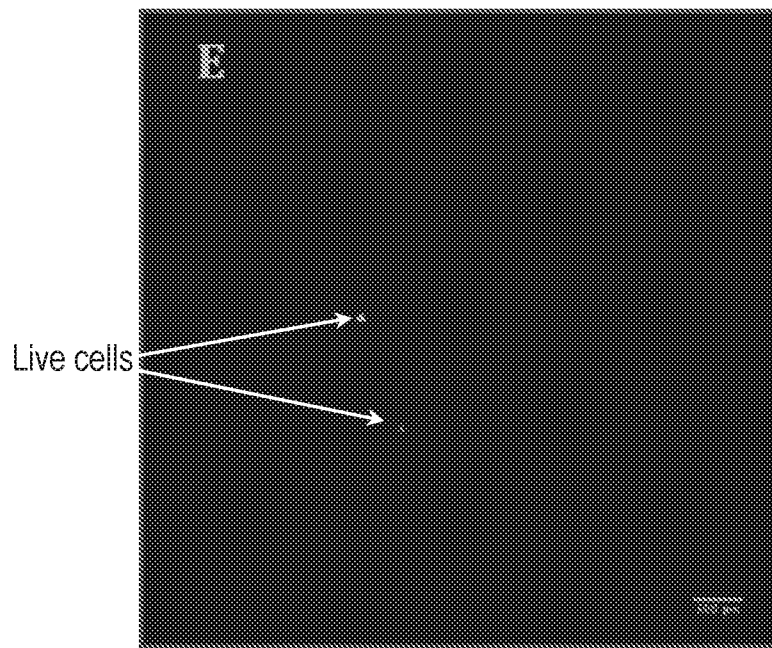
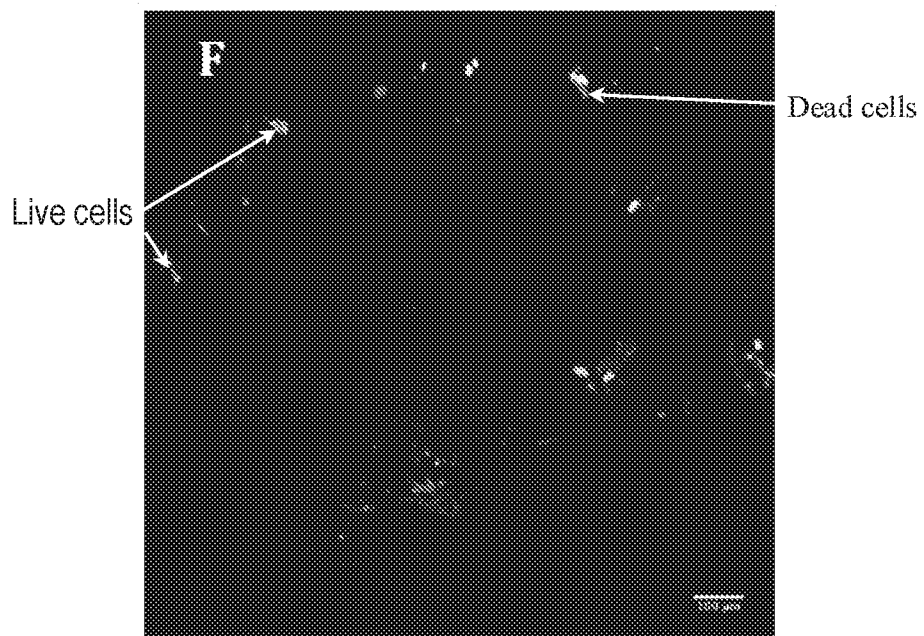
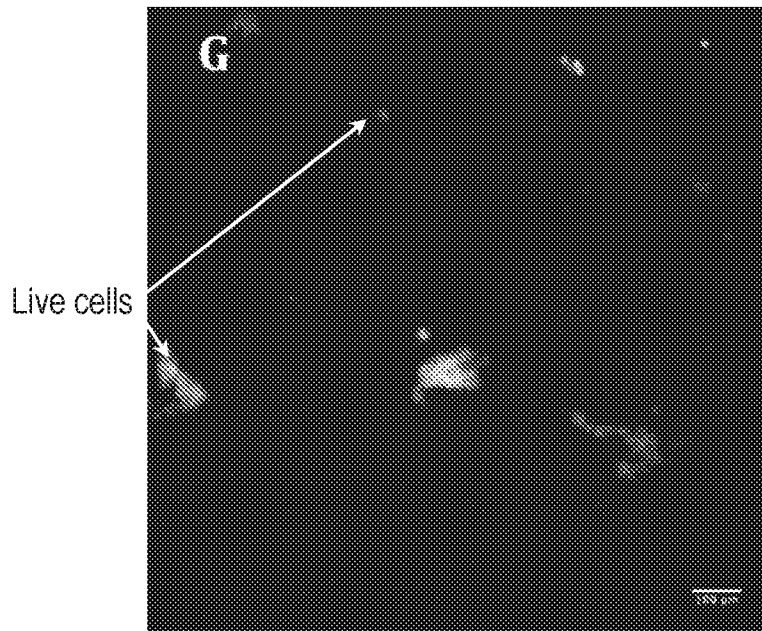
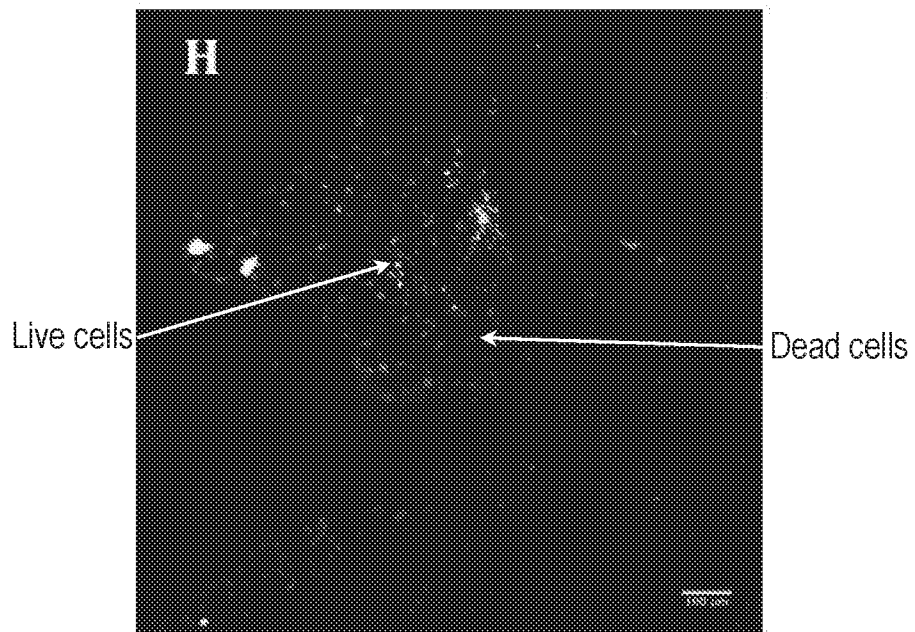


FIG. 1D

*FIG. 1E**FIG. 1F*

*FIG. 1G**FIG. 1H*

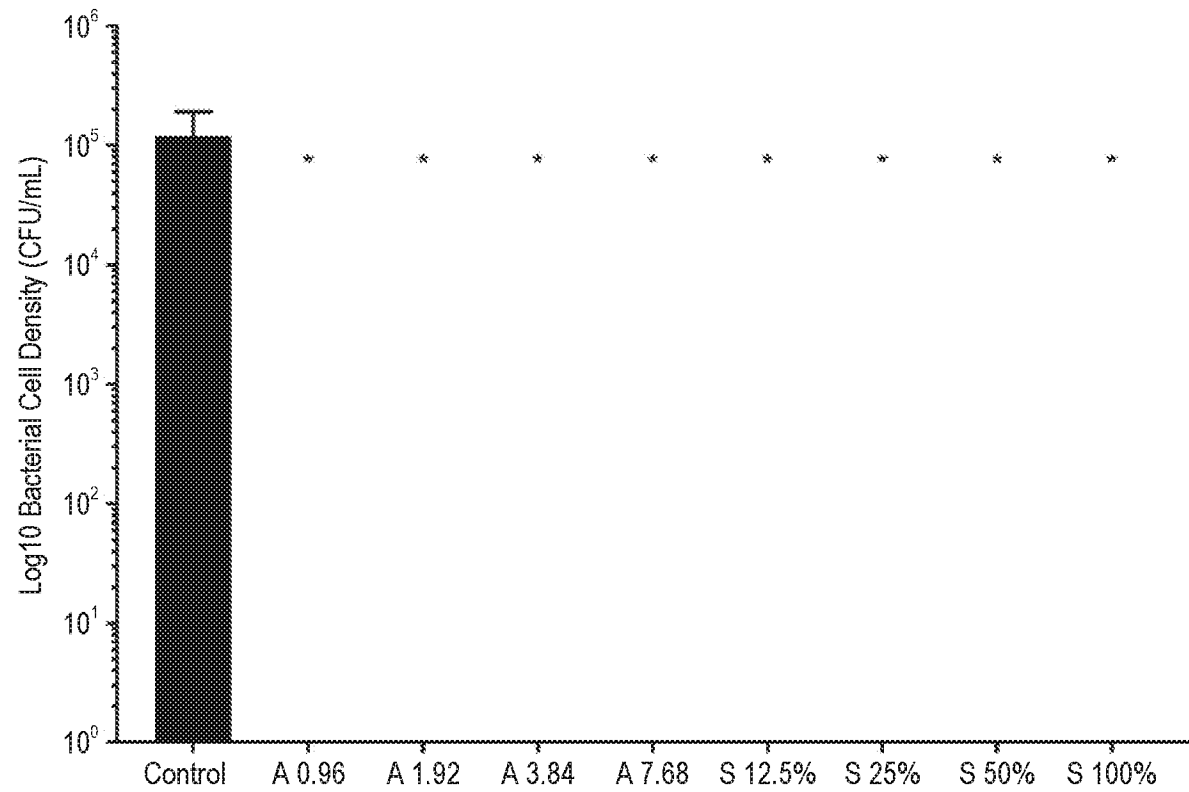


FIG. 2

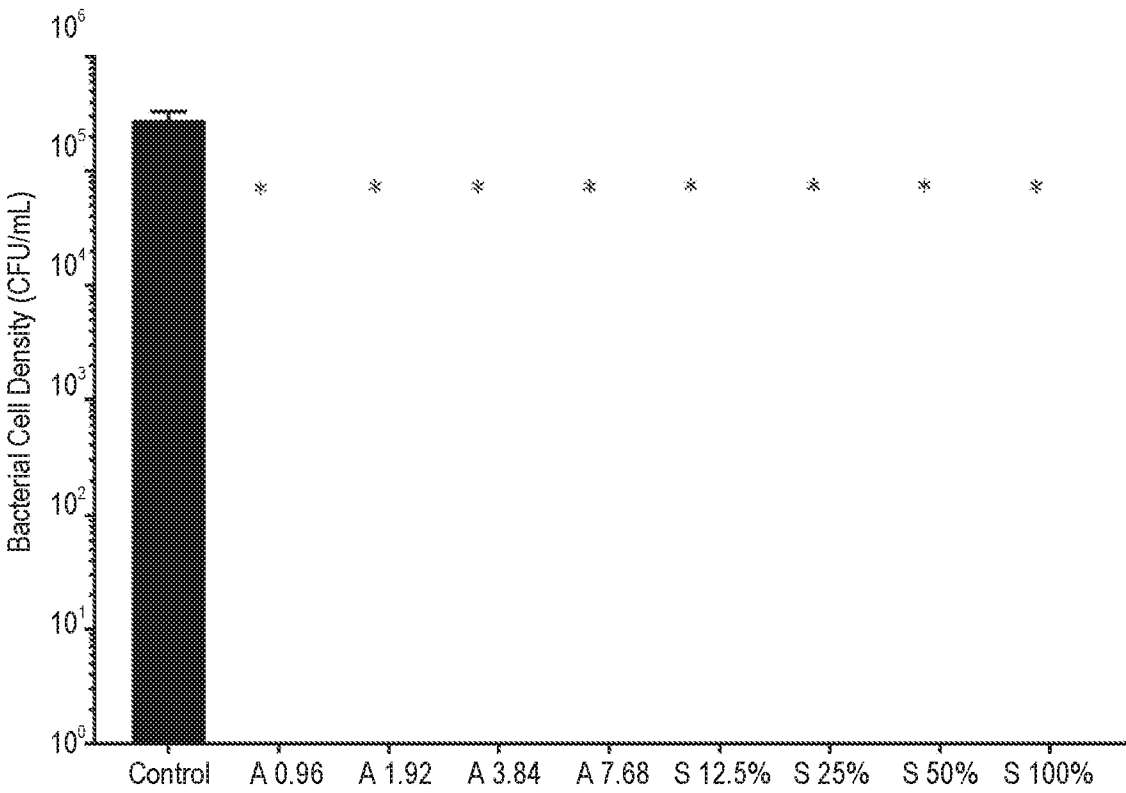
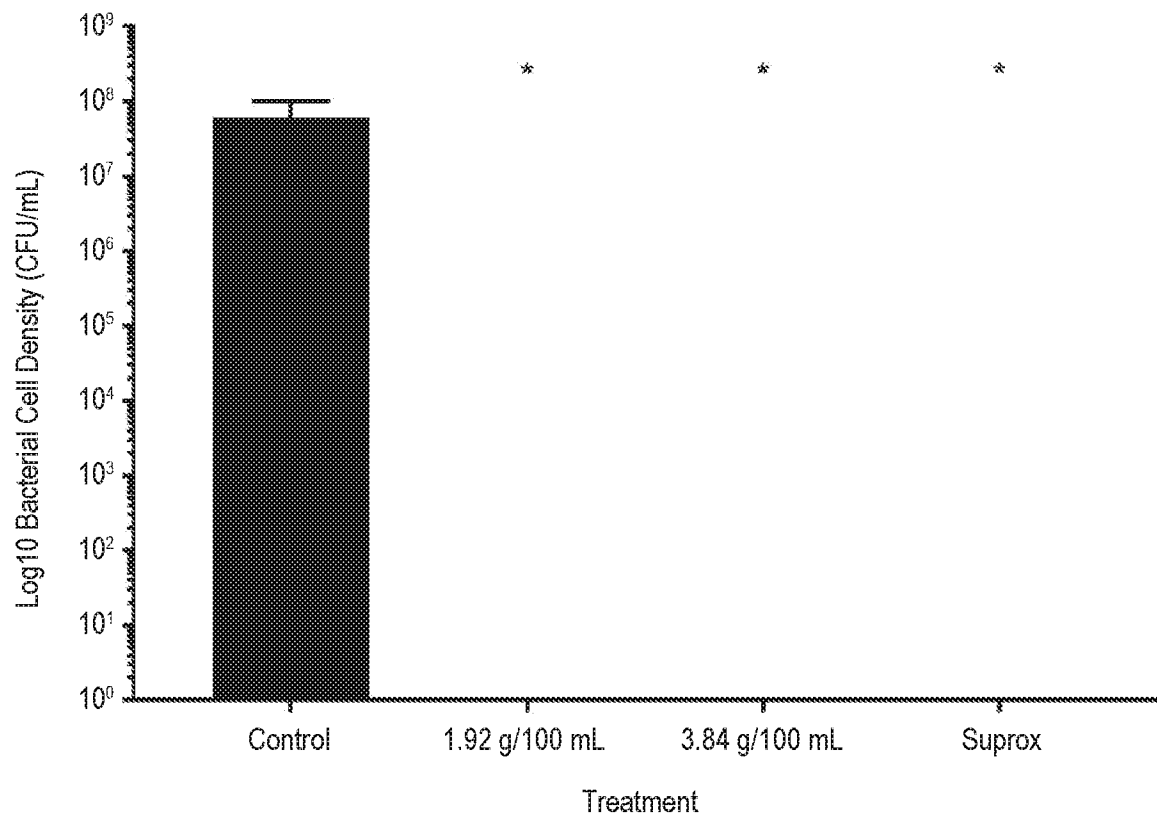
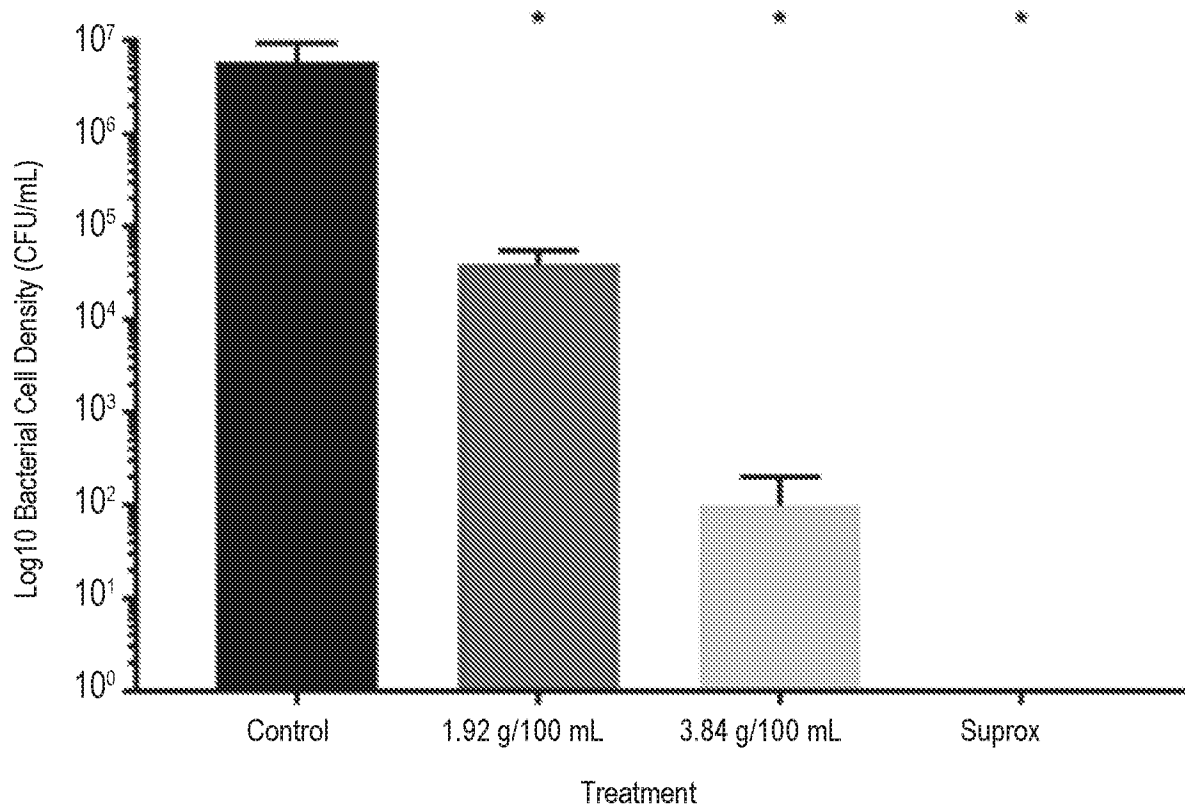
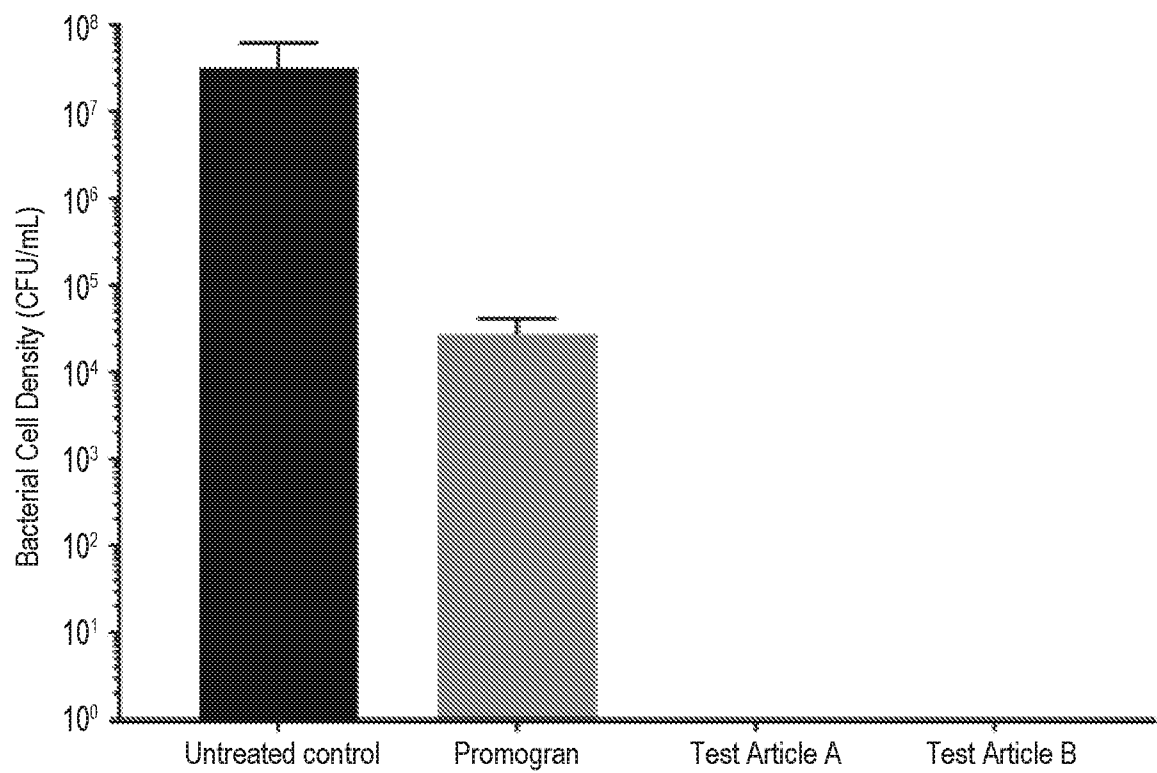
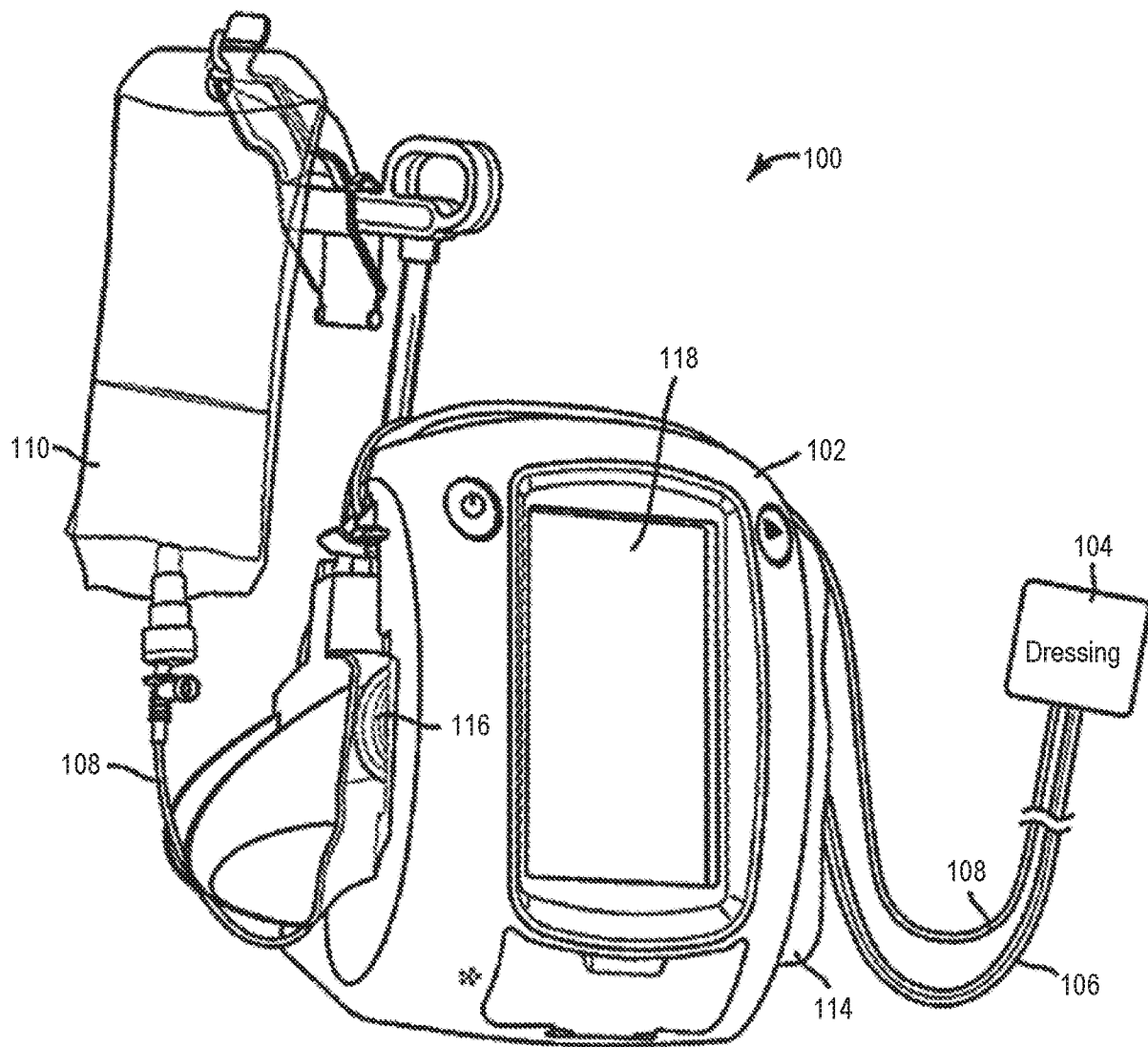


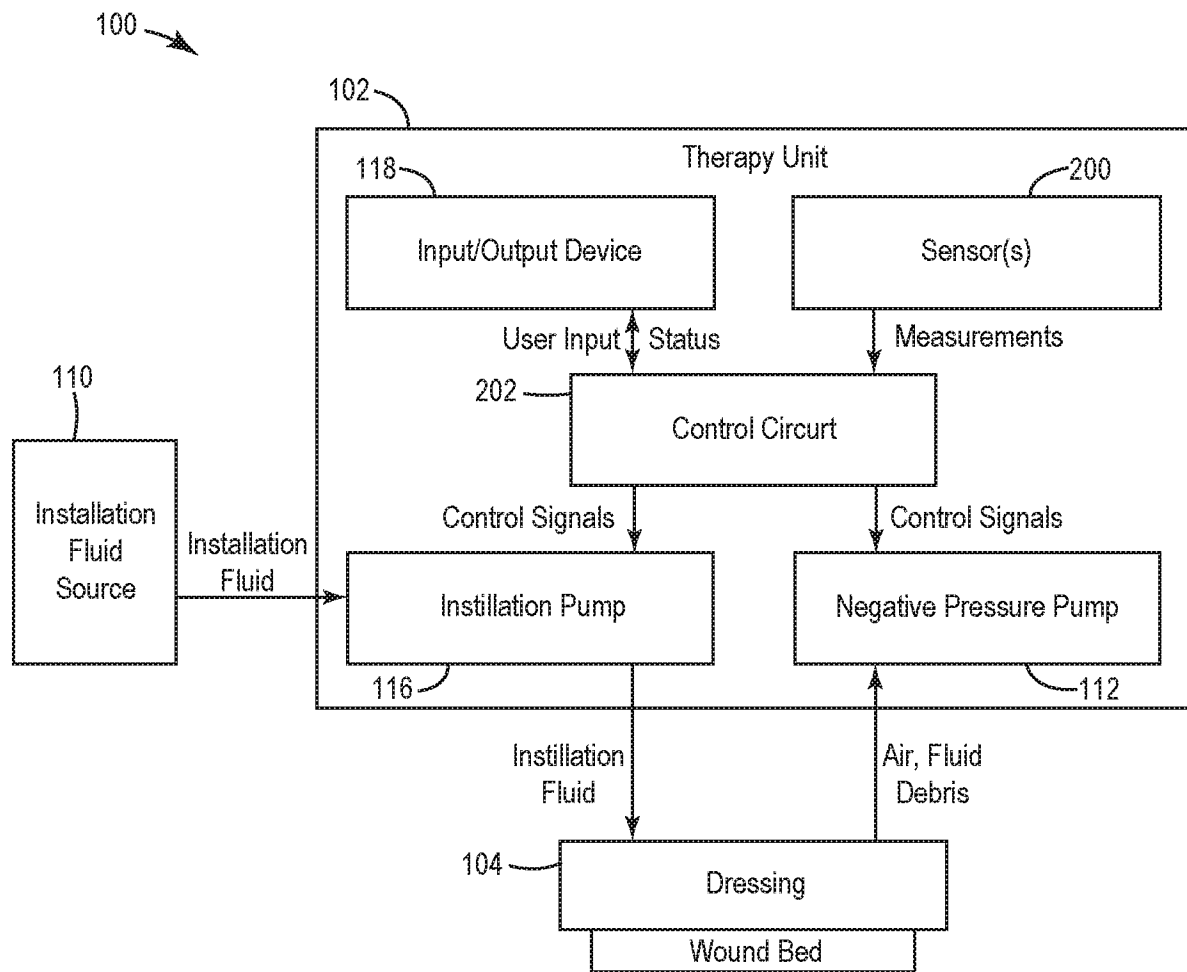
FIG. 3

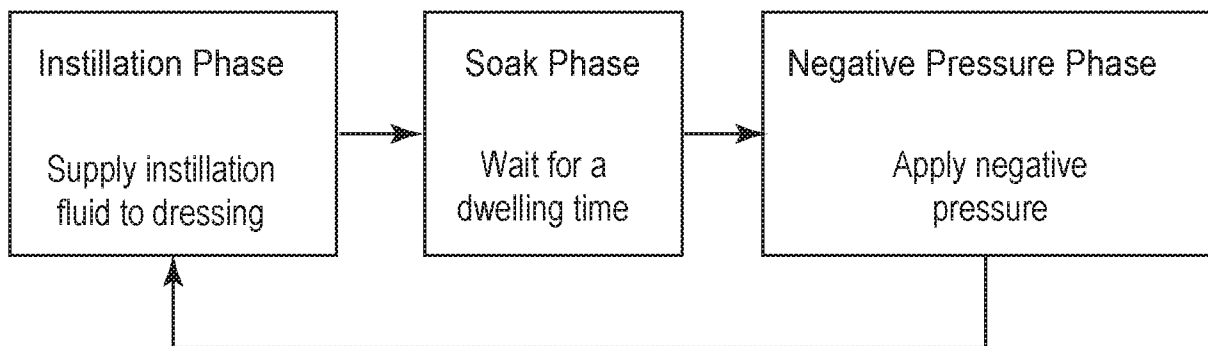
*FIG. 4*

*FIG. 5*

*FIG. 6*

*FIG. 7*

*FIG. 8*

*FIG. 9*

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2021/055225

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61M1/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EP0-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 9 974 693 B2 (ROBINSON TIMOTHY MARK [GB]; PERKINS JOANNA [US] ET AL.) 22 May 2018 (2018-05-22) claims 1,7,11,17 -----	1-32,55
A	US 2020/078224 A1 (CARROLL CHRISTOPHER A [US] ET AL) 12 March 2020 (2020-03-12) claim 1; figure 2 -----	1-32

☐

Further documents are listed in the continuation of Box C.

☒

See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 August 2021

Date of mailing of the international search report

01/09/2021

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Mata Vicente, Teresa

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2021/055225

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **33-54**
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 33-54 are directed to a method of treatment of the human body by therapy. Consequently said claims were not searched (Article 17(2)(a)(i) /(ii) and Rule 39.1 (iv) PCT) and no opinion will be formulated on the subject matter thereof (Article 34(4)(a)(i) and Rule 67.1(iv) PCT).
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2021/055225

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