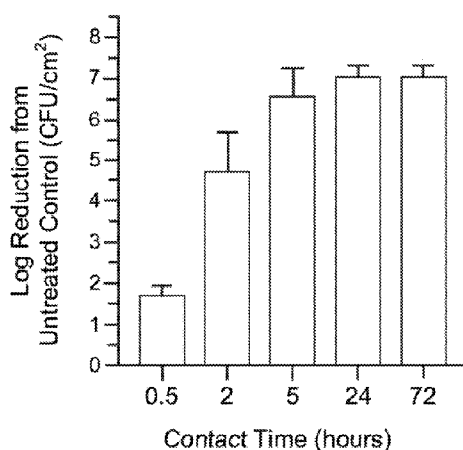




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(54) **Title:** DISINFECTANTS WITH IODINE AND COPPER COMPLEXES FOR BIOFILM ERADICATION AND PREVENTION

FIG. 3



Log reduction *in vitro* against *S. epidermidis* mature biofilms on silicone

(57) **Abstract:** Stable antimicrobial compositions for disinfection are disclosed. Such disinfectant compositions are highly effective in reducing, preventing and/or eradicating biofilm formation and other prevalent pathogens, have extended antimicrobial and antiviral activity for more than 24 hours, have a bio-compatible pH, are suitable for personal, clinical and surgical use and are safe to skin, mucous membranes and wounds, remain stable without precipitation, discoloration or loss of antimicrobial efficacy for at least 12 months, and are beneficial for a range of applications and uses in improving and supporting human and animal health.



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DISINFECTANTS WITH IODINE AND COPPER COMPLEXES FOR BIOFILM ERADICATION AND PREVENTION

CROSS-REFERENCE TO RELATED APPLICATION

[0001] The present application claims priority to U.S. Application No. 18/345,895, filed on June 30, 2023, which is incorporated by reference herein in its entirety.

BACKGROUND

[0002] Iodine is a well-established antiseptic and has been in use for more than 150 years. It is one of the most widely accepted antimicrobials in the world as it is active against bacteria, fungi and viruses and has no known acquired resistance.

[0003] The mechanisms of action for iodine can include: (1) penetration into the cell wall of the microorganism, causing blocking of the hydrogen bond which results in damage to the phospholipid cell membrane; or (2) damage to and denaturing of the proteins, nucleotides and fatty acids, leading to rapid cell death by binding to thiol and sulphhydryl groups.

[0004] One of the first antiseptic preparations of iodine was Lugol's solution. Developed in 1829, it consisted of an aqueous solution of elemental iodine (5%) and potassium iodide (10%) in distilled water or ethanol and was used as an antiseptic to treat wounds among other applications. However, the high levels of iodine caused pain and irritation when applied to open wounds as well as major problems and concerns regarding cytotoxicity, systemic absorption, and delayed wound healing.

[0005] In 1956 povidone iodine (PVP-I) was developed, consisting of a water-soluble combination of molecular iodine and polyvinylpyrrolidone. The 10% solution generally contains 90 percent water, 8.5 percent polyvinylpyrrolidone and 1 percent iodine/iodide. The terms iodine and iodide are often used interchangeably in the literature, despite the fact that there are several different forms and complexes of iodine which may have differing properties. Povidone-iodine as used in practice to disinfect surgical sites is typically as a solution containing 10% povidone-iodine, equivalent to 1% available iodine/iodide or 10,000 ppm. The available free iodine (I_2) depends on the formulation and concentration as it is a dependent equilibrium of povidone-bound iodine to free

iodine. Studies have shown that most of the iodine in PVP-I is complexed in association with polyvinylpyrrolidone and iodide, with a small amount of free iodine (I₂) released.

[0006] The free iodine level is dependent on the concentration of the solution and follows a bell-shaped curve, with levels of 1 ppm in 10% and .001% solutions and approximately 20 ppm in .1% solutions.

[0007] PVP-I, sold under the tradename Betadine®, is currently available as an antiseptic to treat minor cuts, scrapes or burns. It can be used surgically as a pre-scrub on intact skin. Betadine® is typically a 10% solution and has 1% (10,000 ppm) available iodine, but is not allowed for use in open wounds or non-intact skin. *In vitro* studies have suggested that PVP-I has a cytotoxic effect and can interfere with healing. Dilution may help reduce PVP-I's inhibition of the granulation and epithelialization processes involved in wound healing, but toxicity concerns remain. As a result, any off-label use of this product on non-intact skin, in wounds, or on or in surgical sites generally involves a very short application time followed by an immediate flush with saline. Effects of this dilution followed by saline flushing have not been comprehensively tested or proven with respect to the resulting cytotoxicity or antimicrobial profile and such a use is carried out without any recognized approvals for that indication.

[0008] Persistent use of antibiotics (antibacterials) has provoked the emergence of multidrug resistant (MDR) and extensively drug resistant (XDR) bacteria, which render even the most effective drugs ineffectual. One of the advantages of iodine as an antimicrobial is that bacterial resistance does not develop even with extended use as it does for many antibiotics. Hospital acquired infections are a serious and persistent problem, in part because many such pathogens have developed or exhibit multidrug resistance and virulence. Six of the most common and noxious nosocomial (hospital acquired) pathogens are known by the acronym ESKAPE, which includes: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter*.

[0009] Viruses are another form of pathogen addressed with antimicrobials.

[0010] Coronaviruses are positive-stranded RNA viruses with large genome sizes that are known to cause diseases in animals and in humans. In humans, coronaviruses can cause respiratory tract infections that may be mild, such as the common cold.

[0011] But coronaviruses can also cause much more serious infections such coronavirus-induced severe acute respiratory syndrome (SARS). Woo *et al.*, *Microbiol. Immunol.*

49:899-908 (2005). Seven strains of human coronaviruses are known: human coronavirus 229E (HCoV-229E); human coronavirus OC43 (HCoV-OC43); severe acute respiratory syndrome coronavirus (SARS-CoV); human coronavirus NL63 (HCoV-NL63, New Haven coronavirus); human coronavirus HKU1; Middle East respiratory syndrome-related coronavirus (MERS-CoV, also known as novel coronavirus 2012 and HCoV-EMC); and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also known as 2019-nCoV or novel coronavirus 2019.

- [0012] In March 2020, the World Health Organization declared the outbreak of coronavirus disease 2019 (COVID-19) a pandemic. Like the severe acute respiratory syndrome (SARS) outbreak of 2003, and the Middle East respiratory syndrome (MERS) outbreaks of 2012, 2015, and 2018, COVID-19 has caused serious illness and death around the world.
- [0013] Like iodine, various forms of copper are also known to have broad spectrum antimicrobial properties. Ancient civilizations exploited the antimicrobial properties of copper long before the concept of microbes became understood in the nineteenth century, both as copper based medicinal preparations and for transporting water, where water in copper vessels had far less biofouling or slime formation than when transported in other materials. This antimicrobial effect of copper has been further documented with respect to common nosocomial pathogens in aqueous solutions in more recent research. Copper and copper ions are used today as a water purifier, algacide, fungicide and anti-fouling agent. Copper Iodide (CuI) is known to have some antiviral properties. And copper ions in copper sulfate solutions have been shown to reduce the hydrophobicity of biofilm – the water repelling nature of biofilm's outer layer – which can allow greater access to companion antimicrobials to the interior microbes within a biofilm.
- [0014] Copper ions are essential for cellular physiology in the human body, playing a role in respiration, neural transmission, tissue maturation and iron metabolism. Excess copper levels in the body can be toxic, but in general microorganisms are far more susceptible to copper relative to the lower sensitivity of human tissue. The U.S. Environmental Protection Agency lists copper as both a micro-nutrient and a toxin.
- [0015] Copper toxicity to micro-organisms including viruses, is thought to occur as a result of numerous mechanisms. Such mechanisms can include, for example, (1) the displacement of essential metals from their native binding sites; (2) interference with

oxidative phosphorylation and osmotic balance; and (3) inducing alterations in the conformational structure of the nucleic acids, membranes and proteins of microbes.

- [0016] The use of antimicrobials, and especially iodine, typically requires carefully balancing an antimicrobial's effectiveness against the risks of its toxicity to living tissue. Antimicrobial efficacy is not useful, beneficial or acceptable if it comes at the expense of damaging healthy tissue or interfering with or delaying the processes of wound healing. Most iodine-based formulations have difficulty achieving the appropriate balance.
- [0017] Copper, while more differentially damaging to microbes than human tissue at low concentrations, must also maintain this balance. With respect to iodine, formulations such as Betadine®'s PVP-I are not approved for use on wounds or non-intact skin. If medical professionals choose to use them on open wounds or surgical sites by diluting them, applying them and then flushing them out quickly in an attempt to avoid the toxic side effects, they engage in such off-label use at risk, as the procedure is not based on scientific evidence and the side effects are risky and unknown.
- [0018] U.S. Pat. Nos. 8,846,067 and 8,642,057 describe antimicrobial solutions and delivery systems for them using liquid antimicrobial solutions with at least 80% of total weight of a carrier liquid comprising water, alcohol or a mixture of water and alcohol; at least 0.0001% by weight of the solution of iodine; at least 0.0001% by weight of copper sulfate; and sufficient acid in the solution to provide a pH of less than 7.0. A buffering system is also preferable in the solution, and the solution may be provided directly to wounds, burns or other skin damage as a liquid, as a spray or as a gel. US Patent Application No. 20180228837 describes antimicrobial solutions and delivery systems for them using liquid antimicrobial solutions that required at least medically effective amount of a peptide or metallo-peptide to try to impart stability to the solution and without using an acidic agent as described in the '067 and '057 patents.
- [0019] These prior art iodine solutions described above are not physiologically effective against pathogens at the entire low end of the ranges indicated and it was found through extensive testing that when the indicated additional functional additives are added to these types of iodine solutions such as an acidifying agent, buffer, or peptide or metallo-peptide, the resulting solution's efficacy, pH and/or stability (either visual or functional or both) is significantly impaired to a point that renders the solution ineffective and unacceptable for use in wounds, surgical sites and/or burns.

[0020] As iodine is considered one of the most efficacious broad spectrum topical antiseptics, with no known resistance, it would represent a significant breakthrough to find or develop the formulation of a composition that releases sufficiently low levels of iodine so as to not cause any delay in wound healing or result in any toxicity to healthy tissues even under prolonged contact, while still proving highly effective against infection, pathogens and biofilms with sustained antimicrobial activity, all in a solution that demonstrates the long-term stability required of an effective medical product and a bio-compatible pH. Such a formulation, further leveraged by the antimicrobial and anti-biofilm characteristics of appropriate copper complexes would represent a significant advancement in the standard of care and treatment of wounds and infection.

[0021] Such a composition that is non-toxic, safe and non-irritating to human skin and tissue, (including under prolonged contact) that is simultaneously highly effective at destroying or inactivating viruses such as coronaviruses would further provide an extremely useful and effective antimicrobial disinfectant for treating surfaces such as personal protective equipment (PPE), gloves, masks and/or skin and eliminating coronaviruses from such surfaces, one that could be used in situ as often and as frequently as necessary without causing any insult or irritation to surrounding skin and tissues.

SUMMARY

[0022] The present invention is directed to a stable antimicrobial composition for disinfection against biofilm and pathogens, as well as the treatment of wounds and the prevention and/or treatment of infection. The composition is non-toxic, safe, and non-irritating (including at a cellular level and for extended contact) to endogenous tissue and skin, has an effective antimicrobial activity for 24 hours or more with a greater than 4 log kill against pathogens (including but not limited to the ESKAPE pathogens), is effective against biofilm and coronaviruses, has a pH suitable for application on or within a body or wound, and is stable for at least 12 months without precipitation, discoloration or loss of antimicrobial efficacy.

[0023] The inventors of the present application spent many years of exploration, research and testing to develop such a product. After extensive work and experimentation on formulations and detailed stability, pH and efficacy testing, it was unexpectedly found that a mixture of potassium iodide at a first effective concentration by weight and copper

sulfate at a second effective concentration by weight in a carrier liquid such as water or saline, without any other buffers, pH adjusters (e.g., acidifying agents) or surfactants, yielded a unique stable composition with exceptional antimicrobial and wound healing effects while having only an amount of free iodine in the composition of from about 100 ppm to about 330 ppm (e.g. about 250 ppm).

[0024] The present disclosure provides even further experimental evidence demonstrating the unexpected antimicrobial and anti-biofilm efficacy compared to other disinfectants. Enhanced testing protocols disclosed herein with lower levels of detection demonstrated even better results than previously disclosed.

[0025] Such a composition, even without any other additives such as acidifying agents or peptides, has a pH of from about 3.5 to about 7.5 (typically from about 4.5 to about 6.0) which is biologically acceptable and does not cause harm or irritation to surrounding tissues, has an effective antimicrobial activity for 24 hours or more with a greater than 4 log kill rate against common pathogens, and remains stable without precipitation, discoloration or loss of antimicrobial efficacy for at least 12 months (e.g., up to 24 months or longer as required by the FDA for commercial use). The resulting product has a very safe and non-toxic profile, yet achieves the efficacy of its iodine predecessors that incorporate iodine at 1,000 – 100,000 ppm or more.

[0026] The bactericidal properties of this iodine copper complex solution have been assessed against bacterial pathogens commonly associated with wound infections, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE), and *Pseudomonas aeruginosa*. The composition has been shown to result in a greater than 4 log kill against common pathogens (including but not limited to the ESKAPE pathogens) within 24 hours and continues to exhibit safety and sustained activity at 3 days.

[0027] The present disclosure provides additional antimicrobial test results for the composition where after initial treatment of 15 various common pathogens with the composition, the samples were re-infected (with a re-exposure time of 10 minutes each time) at 10 minutes, 4 hours and 24 hours without retreating with the composition. The extremely high log kill results demonstrate more clearly than any previous data the unexpected time extended efficacy of the composition, even after re-infection.

[0028] The composition described herein has also been tested against biofilm using an *in vivo* porcine model and exhibited a 2.0-2.4 log reduction against mature biofilm at 24

hours, more than double the 1 log reduction which is the current standard of care in the medical industry and the log reduction achieved using Povidone Iodine.

[0029] The present disclosure also provides additional anti-biofilm test results using the composition against mature biofilm on both silicone – as found in breast implants, and on titanium alloy – as found in joint implants. The composition showed cumulative and increasing efficacy against biofilm over a 24 hour period with no colonies detected at both 24 and 72 hours. Again, this demonstrates the long term efficacy of the composition as compared to known disinfectants, most of which need to be rinsed out due to their toxicity. In addition, many commercial disinfectants are subject to a biofilm rebound effect, wherein the biofilm is often not completely eliminated and reforms, possibly even stronger. The compositions described herein, with their long term efficacy and ability to remain in contact for an extended period with no need to rinse out, do not have these limitations.

[0030] Some embodiments have further been tested against coronavirus, specifically SARS-CoV-2, and exhibited total eradication below detectable limits within 30 minutes (> 6 log kill) against coronavirus.

[0031] Some embodiments described herein are useful for the treatment of wounds and burns, and to prevent and/or treat infections, including but not limited to bacterial, viral, fungal, and parasitic infections. It can be used as a treatment or prophylactically for all chronic or surgical site infections, including pathological wounds (e.g. diabetic or pressure ulcers), traumatic wounds due to injury, all manner of surgical wounds including oral surgery, and surgical wound cavities such as joint replacements. It can also be used to treat abrasions or skin infections and can also be used prophylactically as an oral rinse.

[0032] The disclosure describes iodine copper complex solutions that are also effective as a hospital-grade disinfectant powerful enough to provide disinfection and an added layer of protection for PPE and other surfaces while being gentle enough to use on skin including face and hands. The embodiments described herein are a safe, non-cytotoxic, tissue friendly solution with long-lasting antimicrobial activity (up to 3 days shown in GLP studies).

[0033] Other disinfectants are either intended for hard surfaces only and cannot be used on skin, or are intended only for skin (e.g., hand sanitizers). Hand sanitizers cannot be used on PPE, are typically active on the skin for only a few seconds up to a few minutes, and it is widely recognized that repeated application results in skin stripping and drying or

cracking due to the high alcohol content. The iodine copper complex solutions described herein do not cause drying and cracking of the skin and can be safely and repeatedly used on PPE and skin without harm. The inventors have confirmed through level 3 lab testing at University of Texas Medical Branch - Galveston that such solution exhibited total eradication below detectable limits within 30 minutes (> 6 log kill) against coronavirus. In other words, the iodine-copper complex eliminates the COVID-19 virus to below detectable levels, classified as complete inactivation.

[0034] In one embodiment of the present invention, a stable liquid antimicrobial composition that is non-toxic, safe and non-irritating (including at a cellular level and under extended contact) to endogenous tissue is disclosed which comprises at least about 95.0% of total weight of a carrier liquid; potassium iodide at a first effective concentration by weight; copper sulfate at a second effective concentration by weight; wherein a mixture of the first concentration of potassium iodide and the second concentration of copper sulfate generates an amount of free iodine in the composition of from about 100 ppm to about 330 ppm; and wherein the composition has a pH of from about 3.5 to about 7.5, demonstrates sustained antimicrobial activity for at least 24 hours with a greater than 4 log kill rate, and remains stable for at least 12 months.

[0035] In another embodiment, the composition may comprise from about 98.0% to about 99.9% by weight of the carrier liquid. The carrier liquid may comprise purified water, or more preferably saline (water plus NaCl) with a concentration of NaCl of from about 0% to about 3%, or most preferably saline with a concentration of NaCl of about 0.9%. The copper sulfate can be in the form of anhydrous copper sulfate or copper sulfate pentahydrate (the most common commercial form) or any other hydrate thereof. The composition preferably has a pH of from about 4.0 to about 7.0, or most preferably has a pH of from about 4.5 to about 6.0. The amount of free iodine generated in the composition can be from about 200 to about 300 ppm, and in one embodiment is about 250 ppm. In one embodiment, the first effective concentration is from about 0.025% to about 0.100% by weight and the second effective concentration is from about 0.016% to about 0.064% by weight if anhydrous copper sulfate is used or from about 0.025% to about 0.100% by weight if copper sulfate pentahydrate is used.

[0036] In one embodiment, the ratio of the first effective concentration to the second effective concentration is such that the ratio of the moles of potassium iodide to the moles of copper sulfate is from about 1.25 to about 1.75.

- [0037] In another embodiment, the first concentration is about 0.0625% by weight and the second concentration is about 0.040% by weight if anhydrous copper sulfate is used or 0.0625% by weight if copper sulfate pentahydrate is used (where the 2 percentages represent equivalent amounts of copper sulfate), and the combination yields about 250 ppm of free iodine in the solution plus other copper complexes.
- [0038] The composition preferably has an effective activity of at least three days and remains stable without precipitation, discoloration or loss of antimicrobial efficacy for at least 24 months. In an embodiment, the composition does not include any other buffers, pH adjusters or surfactants.
- [0039] In another embodiment of the invention, a method of providing a stable antimicrobial composition that is non-toxic, safe and non-irritating (including at a cellular level and under extended contact) to endogenous tissue, has a pH of from about 4.0 to about 7.0, has an effective antimicrobial activity for at least 24 hours with a greater than 4 log kill rate, and remains stable without precipitation, discoloration or loss of antimicrobial efficacy for at least 24 months, is disclosed which comprises providing a carrier liquid comprising water or saline (e.g., water plus dissolved NaCl) wherein the carrier liquid comprises from about 95% to about 99.9% of the total weight of the composition; adding potassium iodide to the carrier liquid at a first effective concentration by weight; adding copper sulfate to the carrier liquid at a second effective concentration by weight, wherein the mixture of the potassium iodide and the copper sulfate in the carrier liquid generates an amount of free iodine in the composition of from about 100 ppm to about 330 ppm plus other copper complexes; and wherein the carrier liquid does not include any buffers, pH adjusters or surfactants.
- [0040] In another embodiment of the invention, a stable liquid antimicrobial composition that is non-toxic, safe and non-irritating (including at a cellular level and under extended contact) to endogenous tissue is disclosed which comprises a carrier liquid of from about 98% to about 99.9% of total weight of the composition and comprising water or saline (water plus dissolved NaCl); potassium iodide at a first effective concentration by weight; copper sulfate at a second effective concentration by weight, wherein the ratio of the first effective concentration to the second effective concentration is such that the ratio of the moles of potassium iodide to the moles of copper sulfate is from about 1.25 to about 1.75; wherein the mixture of the potassium iodide and copper sulfate generates both an amount of free iodine in the composition of from about 100 ppm to about 330 ppm plus other

copper complexes; and wherein the composition has a pH of from about 4.0 to about 7.0, has an effective antimicrobial activity for at least 24 hours with a greater than 4 log kill rate for the ESKAPE and other pathogens, and remains stable for at least 24 months without significant precipitation, discoloration or loss of antimicrobial efficacy.

[0041] In another embodiment of the invention, a stable liquid antimicrobial composition that is non-toxic, safe and non-irritating (including at a cellular level and under extended contact) to endogenous tissue is disclosed which comprises: a carrier liquid of from about 98% to about 99.9% of total weight of the composition and comprising water or saline (water plus dissolved NaCl); potassium iodide at a concentration by weight of about 0.0625%; copper sulfate pentahydrate at a concentration by weight of about 0.0625%, wherein the mixture of the respective concentrations of potassium iodide and copper sulfate pentahydrate is such that the potassium iodide is the reaction limiting component and generates both free iodine in solution at an amount of about 250 ppm plus other copper complexes, without precipitation or discoloration of the composition; and wherein the composition has a pH of from about 4.0 to about 7.0, has an effective antimicrobial activity for at least 24 hours with a greater than 4 log kill rate for the ESKAPE and other pathogens, remains stable without loss of antimicrobial efficacy for at least 24 months, and does not include any buffers, pH adjusters or surfactants.

[0042] In one embodiment of the present invention, a stable liquid antimicrobial disinfectant composition that is non-toxic, safe and non-irritating (including at a cellular level and under extended contact) to skin and endogenous tissue is disclosed which comprises at least 95.0% of total weight of a carrier liquid; potassium iodide at a first effective concentration by weight; copper sulfate at a second effective concentration by weight; wherein a mixture of the first concentration of potassium iodide and the second concentration of copper sulfate generates an amount of free iodine in the composition of from about 100 ppm to about 330 ppm; and wherein the composition has a pH of from about 3.5 to about 7.5, demonstrates sustained antimicrobial and antiviral activity for 24 hours or more with total eradication below detectable limits within 30 minutes (> 6 log kill) against coronavirus, and remains stable for at least 12 months.

[0043] In an embodiment, the composition may comprise from about 98.0% to about 99.9% by weight of the carrier liquid. The carrier liquid may comprise purified water, or more preferably saline (water plus NaCl) with a concentration of NaCl of from about 0% to about 3%, or most preferably saline with a concentration of NaCl of about 0.9%. The

copper sulfate can be in the form of anhydrous copper sulfate or copper sulfate pentahydrate (the most common commercial form) or any other hydrate thereof. The composition preferably has a pH of from about 4.0 to about 7.0, or most preferably a pH of from about 4.5 to about 6.0. The amount of free iodine generated in the composition can be from about 200 to about 300 ppm, and in one embodiment is about 250 ppm. In one embodiment, the first effective concentration is from about 0.025% to about 0.100% by weight and the second effective concentration is from about 0.016% to about 0.064% by weight if anhydrous copper sulfate is used or from about 0.025% to about 0.100% by weight if copper sulfate pentahydrate is used.

[0044] In an embodiment, the ratio of the first effective concentration to the second effective concentration is such that the ratio of the moles of potassium iodide to the moles of copper sulfate is from about 1.25 to about 1.75.

[0045] In an embodiment, the first concentration is about 0.0625% by weight and the second concentration is about 0.040% by weight if anhydrous copper sulfate is used or 0.0625% by weight if copper sulfate pentahydrate is used (where the 2 percentages represent equivalent amounts of copper sulfate), and the combination yields about 250 ppm of free iodine in the solution plus other copper complexes.

[0046] The composition preferably has an effective activity of at least three days and remains stable without precipitation, discoloration or loss of antimicrobial efficacy for at least 24 months. In an embodiment, the composition does not include any other buffers, pH adjusters or surfactants.

[0047] In an embodiment, the coronavirus is a human coronavirus. The human coronavirus can be HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, MERS-CoV, or SARS-CoV-2, or a mutated strain thereof. In an embodiment, the coronavirus is the human coronavirus SARS-CoV-2, or a mutated strain thereof.

[0048] In another embodiment of the invention, a method of providing a stable antimicrobial disinfectant solution that is non-toxic, safe and non-irritating (including at a cellular level and under extended contact) to skin and endogenous tissue, has a pH of from about 4.0 to about 7.0, has an effective antimicrobial and antiviral activity for at least 24 hours with total eradication below detectable limits within 30 minutes (> 6 log kill) against coronavirus, and remains stable without precipitation, discoloration or loss of antimicrobial efficacy for at least 24 months, is disclosed which comprises providing a carrier liquid comprising water or saline (e.g., water plus dissolved NaCl) wherein the

carrier liquid comprises from about 95% to about 99.9% of the total weight of the composition; adding potassium iodide to the carrier liquid at a first effective concentration by weight; adding copper sulfate to the carrier liquid at a second effective concentration by weight, wherein the ratio of the first and second effective concentrations is such that the potassium iodide is the reaction limiting component, and wherein the mixture of the potassium iodide and the copper sulfate in the carrier liquid generates an amount of free iodine in the composition of from about 100 ppm to about 330 ppm plus other copper complexes; and wherein the carrier liquid does not include any buffers, pH adjusters or surfactants. In an embodiment, the coronavirus is a human coronavirus such as HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, MERS-CoV, or SARS-CoV-2, or a mutated strain thereof. In an embodiment, the coronavirus is the human coronavirus SARS-CoV-2, or a mutated strain thereof.

[0049] In another embodiment of the invention, a stable liquid antimicrobial disinfectant composition that is non-toxic, safe and non-irritating (including at a cellular level and under extended contact) to skin and endogenous tissue is disclosed which comprises a carrier liquid of from about 98% to about 99.9% of total weight of the composition and comprising water or saline (water plus dissolved NaCl); potassium iodide at a first effective concentration by weight; copper sulfate pentahydrate at a second effective concentration by weight, wherein the ratio of the first effective concentration to the second effective concentration is such that the ratio of the moles of potassium iodide to the moles of copper sulfate is from about 1.25 to about 1.75; wherein the mixture of the potassium iodide and copper sulfate generates both an amount of free iodine in the composition of from about 100 ppm to about 330 ppm plus other copper complexes; and wherein the composition has a pH of from about 4.0 to about 7.0, has an effective antimicrobial and antiviral activity for at least 24 hours with total eradication below detectable limits within 30 minutes (> 6 log kill) against coronavirus, and remains stable for at least 24 months without significant precipitation, discoloration or loss of antimicrobial efficacy. In an embodiment, the coronavirus is a human coronavirus such as HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, MERS-CoV, or SARS-CoV-2, or a mutated strain thereof. In an embodiment, the coronavirus is the human coronavirus SARS-CoV-2, or a mutated strain thereof.

[0050] In another embodiment of the invention, a stable liquid antimicrobial disinfectant composition that is non-toxic, safe and non-irritating (including at a cellular level and

under extended contact) to skin and endogenous tissue is disclosed which comprises: a carrier liquid of from about 98% to about 99.9% of total weight of the composition and comprising water or saline (water plus dissolved NaCl); potassium iodide at a concentration by weight of about 0.0625%; copper sulfate pentahydrate at a concentration by weight of about 0.0625%, wherein the mixture of the respective concentrations of potassium iodide and copper sulfate pentahydrate is such that the potassium iodide is the reaction limiting component and generates both free iodine in solution at an amount of about 250 ppm plus other copper complexes, without significant precipitation or discoloration of the composition; and wherein the composition has a pH of from about 4.0 to about 7.0, has an effective antimicrobial and antiviral activity for at least 24 hours with total eradication below detectable limits within 30 minutes (> 6 log kill) against coronavirus, remains stable for at least 24 months without loss of antimicrobial efficacy, and does not include any buffers, pH adjusters or surfactants. In an embodiment, the coronavirus is a human coronavirus such as HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, MERS-CoV, or SARS-CoV-2, or a mutated strain thereof. In an embodiment, the coronavirus is the human coronavirus SARS-CoV-2, or a mutated strain thereof.

[0051] In all the described embodiments, the copper sulfate used may be in either of the two commercially available forms - anhydrous copper sulfate (CuSO_4) or copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) - or in any other hydrated form, so long as the concentration is adjusted to keep the net moles of Cu the same (e.g. 0.040% by weight of CuSO_4 can be replaced by 0.0625% by weight of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$). The small increase in the weight due to the hydrated forms of copper sulfate would be offset by an equivalent small reduction in the weight or percentage of water or carrier liquid as would be clear to one skilled in the art.

[0052] These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description and appended claims.

BRIEF DESCRIPTION OF THE FIGURES

[0053] **FIG. 1** shows free iodine levels in povidone-iodine aqueous solutions measured in parts per million.

- [0054] FIG. 2 shows the stability of pH levels of the test product over time under room temperature and accelerated conditions.
- [0055] FIG. 3 shows the log reduction in biofilm from the untreated control in CFU/cm² over 72 hours on smooth silicone material coupons.
- [0056] FIG. 4 shows the log reduction in biofilm from the untreated control in CFU/cm² over 72 hours on hydroxyapatite or titanium alloy substrates of various treatment compositions compared to the Clyra™ Solution.
- [0057] FIG. 5 shows the log reduction in biofilm from the untreated control in CFU/cm² over 24 hours on a titanium alloy substrate in a lavage system.
- [0058] FIG. 6 shows the log reduction in biofilm from the untreated control in CFU/cm² over 2 hours on a titanium alloy substrate in static versus pulsed lavage systems.

DETAILED DESCRIPTION

- [0059] The present invention is directed to a stable antimicrobial composition for the treatment of wounds and the prevention and/or treatment of infection or pathogens, including disinfection against the SARS-CoV-2 virus and other coronaviruses, where the composition is non-toxic, safe and non-irritating (including at a cellular level and for extended contact) to endogenous tissue and skin, has an effective antimicrobial activity for 24 hours or more with a greater than 4 log kill against pathogens (including but not limited to the ESKAPE pathogens), is effective against biofilm and coronaviruses, has a pH suitable for application on or within a body or wound, and is stable for at least 12 months without precipitation, discoloration or loss of antimicrobial efficacy.
- [0060] All publications, patents, and other references mentioned herein are incorporated by reference in their entireties for all purposes as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

Terminology

- [0061] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present application including the definitions

will control. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0062] To the extent that section headings are used, they should not be construed as necessarily limiting.

[0063] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof. The terms "a," "an," "the," "one or more," and "at least one," for example, can be used interchangeably herein.

[0064] As used herein, the term "about," when used to modify an amount related to the invention, refers to variation in the numerical quantity that can occur, for example, through routine testing and handling; through inadvertent error in such testing and handling; through differences in the manufacture, source, or purity of ingredients employed in the invention; and the like. Whether or not modified by the term "about", the claims include equivalents of the recited quantities. In some embodiments, the term "about" means plus or minus 10% of the reported numerical value.

[0065] Throughout this application, various embodiments of this invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range, such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 2, from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 3, from 2 to 4, from 2 to 5, from 2 to 6, from 3 to 4, from 3 to 5, from 3 to 6, etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0066] The terms "comprises," "comprising," "includes," "including," "having," and their conjugates are interchangeable and mean "including but not limited to." It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0067] The term "consisting of" means "including and limited to."

- [0068]** The term "consisting essentially of" means the specified material of a composition, or the specified steps of a method, and those additional materials or steps that do not materially affect the basic characteristics of the material or method.
- [0069]** The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).
- [0070]** The term "antimicrobial" refers to agents that act against and typically kill, or stop or slow the growth or spread of various types of pathogens (e.g., bacteria (antibacterial), viruses (antiviral), fungi (antifungal) and protozoa (antiprotozoal)).
- [0071]** The term "effective amount" as used herein refers to an amount that achieves a desired result.
- [0072]** As used herein, a "therapeutically effective amount" or "medically effective amount" refers to an amount, that achieves a desired therapeutic result, including, e.g., an amount whether used alone or in combination with other components. A therapeutic result can be, e.g., lessening of symptoms, prolonged survival, improved mobility, and the like. A therapeutic result need not be a "cure."
- [0073]** The terms "treat," "treating," and "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological condition, disease, or disorder, or to obtain beneficial or desired physiological results (e.g., clinical, medical, and/or veterinary results). For purposes of this invention, beneficial or desired results include, but are not limited to, alleviation or elimination of the symptoms or signs associated with a condition, disease, or disorder; diminishment of the extent of a condition, disease, or disorder; stabilization of a condition, disease, or disorder, (i.e., where the condition, disease, or disorder is not worsening); delay in onset or progression of the condition, disease, or disorder; amelioration of the condition, disease, or disorder; remission (whether partial or total and whether detectable or undetectable) of the condition, disease, or disorder; or enhancement or improvement of a condition, disease, or disorder. Treatment includes eliciting a

physiologically significant response without excessive side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment.

- [0074]** As used herein, the terms "prevent" and "preventing" refer to partially or completely delaying onset of an infection, disease, disorder, and/or condition; partially or completely delaying onset of one or more signs, symptoms, features, or manifestations (e.g., clinical or physiological signs, symptoms, features, or manifestations) of a particular infection, disease, disorder, and/or condition; partially or completely delaying progression from an infection, a particular disease, disorder, and/or condition; and/or decreasing the risk of developing a pathology associated with the infection, the disease, disorder, and/or condition.
- [0075]** The terms "invention" and "disclosure" can be used interchangeably when describing or used, for example, in the phrases "the present invention" or "the present disclosure."
- [0076]** The terms "animal" or "subject" refer to any organism belonging to the kingdom Animalia and includes, without limitation, aquatic animals and terrestrial animals such as fish; commercial fish; ornamental fish; fish larvae; bivalves; mollusks; crustaceans; shellfish; shrimp; larval shrimp; artemia; rotifers; brine shrimp; filter feeders; amphibians; reptiles; mammals; humans; non-human animals; domestic animals; farm animals; zoo animals; sport animals; breeding stock; racing animals; show animals; heirloom animals; rare or endangered animals; companion animals; pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, or horses; primates such as monkeys (e.g., cebus, rhesus, African green, patas, cynomolgus, and cercopithecus), apes, orangutans, baboons, gibbons, and chimpanzees; canids such as dogs and wolves; felids such as cats, lions, and tigers; equids such as horses, ponies, donkeys, mules, and zebras; food animals such as cows, buffalo, cattle, pigs, poultry, and sheep; ungulates such as deer and giraffes; avians (i.e., birds); poultry such as chickens, geese, ducks, quails, turkeys, pigeons, emus, and ostriches.
- [0077]** The term "infection" refers to invasion and multiplication of microorganisms (e.g., bacteria, viruses, fungi, and protozoa).
- [0078]** The term "non-toxic" or "safe" refers to not being harmful or damaging to cells, tissues, or organisms. The term "non-irritating" refers to not causing or resulting in irritation when applied to or in contact with skin or tissue.
- [0079]** The term "pathogen" refers to an infectious agent and/or a biological agent that causes disease or illness to its host (e.g., bacteria, viruses, fungi, and protozoa).

- [0080] The term "biofilm" refers to a collection of one or more types of microorganisms enveloped or embedded within a polymer matrix, that can grow on many different surfaces.
- [0081] The term "endogenous tissue" refers to the native cells and tissues of the human or animal host that is being treated; tissues that may be healthy or damaged but that are an integral portion of the whole organism.
- [0082] The term "copper complexes" refers to all reaction byproducts comprising copper, including the CuI, the CuSO₄, the Cu⁺⁺ ions resulting from its dissociation, and other forms found in solution such Cu(H₂O)₆²⁺ (Hexaaquacopper ion), which result from the mixture of the components of the composition.
- [0083] The term "iodine copper complex solution" refers to the solution containing the free iodine and the copper complexes which result from the mixture of the components of the composition.
- [0084] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, can also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, can also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.
- [0085] Although methods and materials similar or equivalent to those described herein can be used in practice or testing of the present invention, suitable methods and materials are described below. The materials, methods and examples are illustrative only and are not intended to be limiting. Other features and advantages of the invention will be apparent from the detailed description and from the claims.

Antimicrobial compositions

- [0086] As noted above, iodine solutions have been used for many years as antimicrobial agents, but have a reputation for interfering with wound healing and for being cytotoxic. Indeed, most antimicrobials of all types (not just iodine-based) such as chlorhexidine, iodine tincture, Octenisept, Lugol's solution, silver, PVP-I, etc. can have a cytotoxic effect on cells, cause inflammation, result in tissue necrosis, or interfere with granulation and

epithelialization processes involved in wound healing. Doctors are constantly required to balance the anti-microbial and anti-viral effectiveness and properties of the products and compositions they use against their cytotoxicity and potential to do harm.

[0087] It would therefore be highly desirable to have a clear, odorless and colorless iodine-based antimicrobial and antiviral solution that is non-toxic, safe and non-irritating (including at a cellular level and under extended contact) to skin and endogenous tissue, while simultaneously exhibiting sustained antimicrobial and antiviral activity for 24 hours or more with a greater than 4 log kill rate against common pathogens (including the noxious nosocomial ESKAPE pathogens) and total eradication below detectable limits within 30 minutes (> 6 log kill) against coronavirus (with continued activity for 3 days or more), while having a pH that is bio-compatible (for example from about 4.0 to about 7.0) to insure no pain or stinging on application plus no acidic/alkaline negative clinical outcomes, and while exhibiting long term stability for at least 24 months without precipitation, discoloration or loss of antimicrobial efficacy. Such a solution would permit far greater effective application across a wide range of medical needs without concern for detrimental effects to skin, tissue or wounds than existing products, offerings or compositions provide.

[0088] The inventors of the present invention spent many years of experimentation and development to develop just such a composition. Many formulas of varying concentrations and with numerous different additives associated with some degree of medical benefit were tested. However, much of their work proved to be generally unacceptable as the combinations that were developed and tested often did not exhibit stability for commercially acceptable periods of time, showed discoloration and/or precipitation, had a pH too low (e.g., below 3.5) to allow for medical application, exhibited some cytotoxicity, or were not sufficiently effective as an antimicrobial.

[0089] Unexpectedly, after many previous attempts to find an optimal iodine-based composition for wound healing and the prevention and treatment of infection, the inventors surprisingly discovered that the iodine and copper based formula as described herein remains stable at room temperature (e.g. at least 6 months, more preferably at least 1 year and most preferably 2 years or more) without precipitation, discoloration or loss of antimicrobial efficacy, has an acceptable/optimal pH for wounds (e.g., a pH of from about 3.5 to about 7.5, e.g. from about 4.0 to about 7.0, e.g. from about 4.5 to about 6.0), is non-toxic, safe and non-irritating (including at a cellular level and under extended contact) to

skin and endogenous tissue, has an effective antimicrobial and antiviral activity of at least 24 hours with a greater than 4 log kill rate against common pathogens (including the ESKAPE pathogens) and total eradication below detectable limits within 30 minutes (> 6 log kill) against coronavirus, exhibits sustained activity for more than 3 days, and does not contain any buffers, acidifying agents (such as sulfamic acid or other acids which can lead to an unacceptably low pH) or any other peptide ingredients. The claimed invention described herein is a result of that long effort and provides a unique medical material that is antimicrobial, antiviral, stable and provides exceptional infection control and wound healing benefits not previously available in a solution that can be applied in such a broad variety of ways, with far greater latitude, and without any concerns or need to rinse it out for fear of toxicity.

[0090] As explained below, the invention hinged on the discovery of a careful balance or ratio of the components coupled with concentrations fine-tuned to keep the iodine concentration below solubility levels, while simultaneously enlisting the added efficacy of certain copper complex reaction byproducts.

[0091] All the embodiments of the invention as detailed above comprise potassium iodide and copper sulfate. The copper sulfate used may be in either of the two commercially available forms - anhydrous copper sulfate (CuSO_4) or copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) - or in any other hydrated form. For the purposes of the discussion of chemistry below, we will use copper sulfate pentahydrate as this is the most commonly available commercial form.

[0092] The combination of potassium iodide and copper sulfate pentahydrate (the common commercial form) is governed by the following chemical reaction when the components are in the appropriate stoichiometric ratio of 2:1.



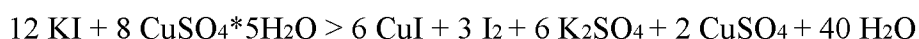
[0093] As an aside, note that if excess potassium iodide (KI) is available in the presence of elemental iodine then the following reaction may occur:



[0094] In the presence of such excess potassium iodide, the molecular or free iodine (I_2) is thus largely converted to triiodide (I_3^-) or other polyiodide ions. High concentrations of triiodide ions are present in Lugol's solution and are responsible for the brown color of the solution.

[0095] One of the contributing insights of the inventors was that by limiting the ratio of potassium iodide to copper sulfate to less than the stoichiometric ratio of the first equation above, then the potassium iodide would become the reaction limiting component. Thus if the molar quantity of potassium iodide is less than twice that of the copper sulfate, the potassium iodide is the reaction limiting component. It is believed that this results in the iodine of the KI being fully converted to free iodine (I₂) and copper iodide (CuI) without any subsequent conversion to triiodide and leaves residual unreacted copper sulfate in solution as a source of copper ions. On the other hand, if the ratio of potassium iodide to copper sulfate is too low, then very little free iodine is generated (and very little copper iodide), which is problematic as it is believed that the free iodine is largely responsible for a significant portion of the antimicrobial efficacy. So a balance needs to be struck, using enough KI to generate a useful amount of I₂ and CuI, while making sure that the KI is the reaction limiting factor. A molar ratio (KI to CuSO₄) of more than 1:1 and less than 2:1 was settled on with a preferred ratio of from about 1.25:1 to about 1.75:1, for example with a ratio of about 1.5:1. Note that the full conversion of the KI to I₂ and CuI insures that a high level (50%) of the total iodine in the solution is present as free iodine (I₂), in contrast to formulations such as PVP-I where, as shown in Figure 1, the amount of free iodine is minimal compared to the total iodine present in the solution and does not exceed around 20 ppm.

[0096] The effective chemical reaction at the indicated molar ratio of KI to CuSO₄(*5H₂O) of about 1.5 to 1 can be modeled as follows:



[0097] Experimentation further determined that if the concentration of potassium iodide was selected so as to yield 200-660 ppm in solution while keeping the molar ratio of potassium iodide to copper sulfate below 2:1 as described above, the resulting solution would contain an antimicrobially effective concentration of I₂ of 100-330 ppm (half the iodine reacts to become I₂ while the other half combines with the copper to become CuI) while remaining below the free iodine solubility level of about 330 ppm. It is believed that these low concentrations also allow the relatively insoluble CuI to remain in solution and support the extended stability exhibited by and required of the composition, as well as to provide additional antimicrobial activity. Copper sulfate also has known antimicrobial properties and the residual copper sulfate may further enhance the

composition's effectiveness while the extremely low concentration present may help avoid any irritation it might otherwise have to healthy tissue in higher doses.

[0098] Additionally, the copper ions of the residual copper sulfate are believed to interact with biofilms so as to reduce their hydrophobicity, thereby allowing the balance of the solution's antimicrobial components access to the bacteria interior to the biofilm and enhancing the antimicrobial/antibiofilm efficacy.

[0099] Together these factors combine to create and support the non-toxicity and safety of the formulation even under long term contact (e.g., 24 hours, or as tested up to 30 days) while simultaneously allowing it to serve as an effective antimicrobial and antiviral. The extended contact (with skin or endogenous tissue) allowed by the lack of toxicity further leverages the sustained antimicrobial action of the solution, which helps the composition exhibit the previously seemingly unachievable balance of a greater than 4 log kill with complete safety and non-toxicity, providing a substantial and highly desirable utility in wound care, the treatment and prevention of infection, the killing of dangerous pathogens, and the disinfection of viruses such as coronaviruses on skin, PPE, and surfaces.

[0100] It was further discovered through testing that the addition of numerous other elements with potentially desirable properties such as buffers, acidifiers, emulsifiers, medical peptides, etc. often disrupted the subtle balance that had been achieved and resulted in unacceptable pH, precipitation, discoloration, lack of long-term stability, reduced antimicrobial efficacy, or a degree of cyto-toxicity, which helped to confirm and validate the uniqueness, novelty and unexpected results of the solution that the inventors had developed. This was further confirmed by the extensive testing required by regulatory agency personnel who remained unconvinced until confronted with incontrovertible test results including extensive live animal studies that it was possible that an iodine copper solution simultaneously be non-toxic, safe and non-irritating to skin, tissue and wounds even with prolonged contact, cause no detrimental effects or delay in wound healing, have a biocompatible pH, demonstrate long term stability without discoloration, precipitation or loss of efficacy, and yet demonstrate sustained antimicrobial and antiviral activity for at least 24 hours with a 4 log kill against the nosocomial ESKAPE and other pathogens and total eradication below detectable limits within 30 minutes (> 6 log kill) against coronavirus.

[0101] An embodiment of the present invention has been approved by the FDA and is intended for use by healthcare professionals for cleansing, irrigating, moistening and debriding (removing wound debris) acute and chronic dermal lesions that are partial or full thickness wounds such as 1st and 2nd degree burns, stage I - IV pressure ulcers, diabetic ulcers, stasis ulcers, abrasions and minor skin irritations, post-surgical wounds, and grafted and donor sites, as well as for use in moistening and lubricating absorbent wound dressings. The compositions described herein can also be used as a preventative and treatment for infection on or in the body, wounds or surgical openings or cavities. An embodiment of the present invention has also been registered by the FDA for use as a disinfectant effective against coronavirus and SARS-CoV-2, the virus responsible for COVID-19.

[0102] The determination of an appropriate pH range for the present invention was made based on the pH of healthy skin and wounds. Healthy, intact skin has a slightly acidic pH ranging from 4.0 to 6.0. The pH is an important aspect of the skin's barrier function, helping to regulate bacterial flora and prevent infection. When a wound occurs, the skin's acidic milieu and pH is disrupted, exposing the more neutral pH of the underlying tissue. With successful healing and re-epithelialization, the skin returns to being acidic. Acute wounds have a more neutral pH and, during acute wound healing, there is a drop in pH caused by various factors, including hypoxia and increased production of lactic acid. An acidic pH environment is considered to be beneficial, by increasing fibroblast proliferation and migration and also regulating bacterial colonization. If, however wound healing is delayed, then the pH will oscillate and become increasingly alkaline over time. At this stage the wound is described as chronic and the synthesis of ECM molecules becomes impaired, thus arresting the healing process. In light of this, the desired pH range of an effective antimicrobial is optimally in the range of 4.0-7.0, for example the pH is in the range 4.5-6.0.

[0103] As described above, the copper sulfate used in the compositions may be in either of the two commercially available forms - anhydrous copper sulfate (CuSO_4) or copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) - or in any other hydrated form, so long as the respective concentration is adjusted to keep the net moles of Cu the same.

[0104] The subtle balance and concentration ranges of the components of the composition discovered by the inventors is easily disturbed by the addition of other elements which might be thought to have a salutary effect, often resulting instead in one

of more of unacceptable pH, lack of stability, precipitation, discoloration, interference with sustained activity, diminishment of antimicrobial and antiviral activity, or some level of cyto-toxicity, as was determined by the inventors during the extensive testing of the composition with various additives and alterations. Nonetheless, it may be possible within carefully tested and constrained limits to augment the composition with additional elements while maintaining the fundamental characteristics and benefits thereof.

- [0105]** In some embodiments, the compositions according to the present invention can be directly applied to skin or wounds, used in all surgical sites before, during or after surgery to prevent or treat infection, used as a lavage, used as an oral rinse or wash, or used as a spray to disinfect skin, surfaces and PPE, as well as being used to impregnate fabric, sheets or layers of other materials such as cotton, gauze, dressings, compresses, bandages and the like. However, in addition to such uses, other materials may be used to apply, disperse or augment the compositions. Examples include emulsions, alginates, natural and synthetic thickeners (e.g., cellulose gum, dextrose, CMC, Thymulen®, etc.), polysiloxanes, polymeric carriers, liquid polyols (such as glycerol and PEG), solvents, superabsorbents, collagen, biological carriers such as tissue or cells, PPP (platelet poor plasma), or PRP (platelet rich plasma), oxygen or nitric oxide donors, and the like.
- [0106]** In such applications, the compositions and solutions may be incorporated within such additional materials or carriers or be combined with them, possibly in greater concentration where the composition or solution may subsequently flow out of the carrier and onto the skin or into the wound at the appropriate concentration levels.
- [0107]** Although a buffering agent is not needed to provide the composition described herein with a medical benefit, a buffering agent such as inorganic cation buffering agents including carbonates, bicarbonates, phosphates and other inorganic salts (such as those of sodium, calcium, potassium and lithium) can be added to the solutions.
- [0108]** The compositions according to the present invention may additionally comprise an antibiotic including without limitation neomycin, vancomycin, bacitracin, gentamicin, polymyxin, or may additionally comprise a sulfa drug (e.g., prontosil, sulfadiazine, sulfamethizole (Thiosulfil Forte™), sulfainethoxazole (Gantanol™), sulfasalazine (Azulfidine™), sulfisoxazole (Gantrisin™), and various high-strength combinations of three sulfonamides).
- [0109]** The compositions according to the present invention may further comprise an agent that promotes degradation of biofilms on open wounds such as surfactants or

amphiphilic compounds like polyhexamethylene biguanide or PHMB (Prontosan®), benzalkonium chloride or Bactisure®, and the like.

[0110] The compositions according to the present invention can optionally further comprise any active pharmaceutical agent for specific treatment regimens, or any acceptable excipient, including without limitation colorants, deodorants, fragrances, flavorings, preservatives and the like.

[0111] The compositions according to the present invention can be used as a treatment for all manner of wounds and burns, for the prevention or treatment of infection on skin, in wounds, or for any surgical site for both intact or non-intact skin, or for use as a disinfectant to eliminate viral contamination and as a treatment against coronavirus on skin, mucous membranes, personal protective equipment (PPE), and equipment or surfaces.

[0112] The compositions described herein are useful in direct medical treatment of wounds, sores, topical conditions, skin infections, including tissue injuries and those characterized by delay or complete failure of healing. Typical but non-limiting examples of such injuries are traumatic injury including burns, injury resulting from surgery, diabetic wounds, pressure ulcers, arterial ulcers, and decubitus ulcers. The compositions may be directly applied to the region of the patient where treatment is desired. They may be used for cleansing, irrigating, and debriding dermal wounds in addition to moistening and lubricating absorbent wound dressings (e.g., gauze). They may be used in plastic surgery operations and in total joint replacements, either as a prophylactic, during surgery or to treat post-surgical infection. As the compositions are non-toxic, safe and non-irritating to tissue even with extended contact, no wash-out of the composition is necessary. It may be applied and left in place, or flushed into or through a wound where any residual solution can be left in place and remains antimicrobially active.

[0113] The treatment of open wounds and burns according to the present invention typically comprises topical administration of the composition or of a combination of the composition and other medicaments, or of medicaments containing the composition, to the open wound or burn. The medicament is preferably applied to the wounds or burns in amounts sufficient to completely cover the entire surface of the wound. There is no need to rinse or remove the treatment, which may be left in place to take advantage of the lack of toxicity and the sustained activity of the compositions. The treatment is continued as

long as necessary to completely heal the wounds or burns it is applied to, or as long as beneficial effects are observed.

[0114] The compositions described herein can also be used in oral surgery applications as a pre-surgical rinse, a wash during surgery, and a post-surgical rinse. They can also be applied through the dental instruments as a spray rinse during oral surgery or dental procedures. They can also be used as a regular mouthwash to reduce the bacteria responsible for plaque buildup on the teeth.

[0115] The compositions described herein can also be used on animals in the same ways described herein for use on humans, including but not limited to the treatment of wounds, the prevention and/or treatment of infection, as a prophylactic or treatment before, during or after surgery of all types, as an oral rinse, as a disinfectant, etc.

[0116] Although the aforementioned methods of treatment generally apply, it is within the skill and within the objective of any professional, trained in the art of wound healing

[0117] or infection prevention or treatment, or surgery, or oral or dental care to adjust the preferred amounts of the composition and/or the frequency with which it is applied, as well as the duration of the treatment, in order to optimize the efficacy for each individual patient or subject. The materials and compositions of the invention may be applied to wounds, burns, cuts, surgical sites, mucosal tissue, mucosal membrane, and/or the skin for any after-event medical condition or pre-event medical condition. In some embodiments, the antimicrobial compositions are effective as a hospital-grade disinfectant powerful enough to provide disinfection and an added layer of protection for PPE and other surfaces against coronavirus, e.g., SARS-CoV-2, while being gentle enough to use on skin including face and hands. They can also be used to sanitize used protective masks and other PPE, especially from coronavirus, e.g., SARS-CoV-2. They provide a safe, non-toxic, non-irritating, tissue friendly solution with long-lasting antimicrobial and antiviral activity (up to 3 days shown in GLP studies) that can be applied directly to the hands, face and skin. By contrast, alternative antimicrobials for skin such as hand sanitizer products based on 60-70% alcohol are active on the skin for only a few seconds up to a few minutes, and are known to cause drying and cracking of the skin with continued use. The compositions according to the present invention do not cause any such drying or cracking of the skin, are not sticky and leave no residue, and can be safely and repeatedly used on PPE and skin without harm. Level 3 lab testing at University of Texas Medical Branch - Galveston has confirmed that the composition

exhibits total eradication below detectable limits at 30 minutes (> 6 log kill) against coronavirus, specifically the COVID-19 virus, thereby resulting in complete inactivation.

[0118] The present disclosure also provides the following numbered embodiments.

[0119] Embodiment 1. A stable liquid antimicrobial disinfectant composition that is non-toxic, safe and non-irritating (including at a cellular level and under extended contact) to skin and endogenous tissue comprising:

[0120] a. at least 95.0% of total weight of a carrier liquid;

[0121] b. potassium iodide at a first effective concentration by weight;

[0122] c. copper sulfate at a second effective concentration by weight;

[0123] d. wherein a mixture of the first concentration of potassium iodide and the second concentration of copper sulfate generates an amount of free iodine in the composition of from about 100 ppm to about 330 ppm; and

[0124] e. wherein the composition has a pH of from about 3.5 to about 7.5, has an effective antimicrobial and antiviral activity for at least 24 hours with total eradication below detectable limits within 30 minutes (> 6 log kill) against coronavirus.

[0125] Embodiment 2. The composition of Embodiment 1, where the composition includes from about 98% to about 99.9% by weight of the carrier liquid.

[0126] Embodiment 3. The composition of Embodiment 1, wherein the carrier liquid comprises purified water.

[0127] Embodiment 4. The composition of Embodiment 1, wherein the carrier liquid comprises water plus NaCl at a concentration of from about 0% to about 3%.

[0128] Embodiment 5. The composition of Embodiment 1, wherein the carrier liquid comprises water plus NaCl at concentration of about 0.9%.

[0129] Embodiment 6. The composition of Embodiment 1, where the copper sulfate is anhydrous copper sulfate or copper sulfate pentahydrate.

[0130] Embodiment 7. The composition of Embodiment 1, wherein the first effective concentration is from about 0.025% to about 0.100% by weight, and either the copper sulfate is anhydrous copper sulfate and the second effective concentration is from about 0.016% to about 0.064% by weight, or the copper sulfate is copper sulfate pentahydrate and the second effective concentration is from about .025% to about 0.100% by weight.

[0131] Embodiment 8. The composition of Embodiment 7, wherein the ratio of the first effective concentration to the second effective concentration is such that the ratio of

the moles of potassium iodide to the moles of copper sulfate is from about 1.25 to about 1.75.

- [0132] Embodiment 9. The composition of Embodiment 1, wherein the first concentration is about 0.0625% by weight, and either the copper sulfate is anhydrous copper sulfate and the second effective concentration is about 0.040% by weight, or the copper sulfate is copper sulfate pentahydrate and the second effective concentration is about .0625% by weight.
- [0133] Embodiment 10. The composition of Embodiment 1, wherein an amount of free iodine generated in the composition is from about 200 to about 300 ppm.
- [0134] Embodiment 11. The composition of Embodiment 1, wherein an amount of free iodine generated in the composition is about 250 ppm.
- [0135] Embodiment 12. The composition of Embodiment 1, wherein the composition has an effective antimicrobial activity of at least three days.
- [0136] Embodiment 13. The composition of Embodiment 1, wherein the composition has a pH of from about 4.5 to about 6.0.
- [0137] Embodiment 14. The composition of Embodiment 1, wherein the composition remains stable without significant precipitation, discoloration or loss of antimicrobial efficacy for at least 24 months.
- [0138] Embodiment 15. The composition of Embodiment 1, wherein the composition does not include any buffers, pH adjusters or surfactants.
- [0139] Embodiment 16. The composition of Embodiment 1, wherein the coronavirus is a human coronavirus.
- [0140] Embodiment 17. The composition of Embodiment 16, wherein the human coronavirus is HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, MERS-CoV, or SARS-CoV-2, or a mutated strain thereof.
- [0141] Embodiment 18. A method of providing a stable antimicrobial disinfectant solution against coronavirus that is non-toxic, safe and non-irritating (including at a cellular level and under extended contact) to skin and tissue, has a pH of from about 4.0 to about 7.0, has effective antimicrobial and antiviral activity for at least 24 hours with total eradication below detectable limits within 30 minutes (> 6 log kill) against coronavirus, and remains stable for at least 24 months without significant precipitation, discoloration or loss of antimicrobial efficacy, comprising:

- [0142] a. providing a carrier liquid comprising water or saline wherein the carrier liquid comprises from about 95% to about 99.9% of the total weight of the composition;
- [0143] b. adding potassium iodide to the carrier liquid at a first effective concentration by weight;
- [0144] c. adding copper sulfate to the carrier liquid at a second effective concentration by weight, wherein the ratio of the first and second effective concentrations is such that the potassium iodide is the reaction limiting component, and wherein the mixture of the potassium iodide and the copper sulfate in the carrier liquid generates an amount of free iodine in the composition of from about 100 ppm to about 330 ppm plus other copper complexes; and
- [0145] d. wherein the carrier liquid does not include any acidifying agents buffers or surfactants.
- [0146] Embodiment 19. The method of Embodiment 18, wherein the coronavirus is a human coronavirus.
- [0147] Embodiment 20. The method of Embodiment 19, wherein the human coronavirus is HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, MERS-CoV, or SARS-CoV-2, or a mutated strain thereof.
- [0148] Embodiment 21. The method of Embodiment 19, wherein the human coronavirus is SARS-CoV-2.
- [0149] Embodiment 22. The method of Embodiment 19, wherein the human coronavirus is a mutated strain of SARS- CoV-2.
- [0150] Embodiment 23. A stable liquid antimicrobial disinfectant composition that is non-toxic, safe and non- irritating (including at a cellular level and under extended contact) to skin and endogenous tissue and can be used as a disinfectant against coronavirus comprising:
- [0151] a. a carrier liquid of from about 98% to about 99.9% of total weight of the composition and comprising water or saline;
- [0152] b. potassium iodide at a first effective concentration by weight;
- [0153] c. copper sulfate at a second effective concentration by weight, wherein the ratio of the first effective concentration to the second effective concentration is such that the ratio of the moles of potassium iodide to the moles of copper sulfate is from about 1.25 to about 1.75;

- [0154] d. wherein the mixture of the potassium iodide and copper sulfate generates both an amount of free iodine in the composition of from about 100 ppm to about 330 ppm without precipitation, discoloration or loss of antimicrobial efficacy of the composition plus other copper complexes;
- [0155] e. wherein the composition has a pH of from about 4.0 to about 7.0, has an effective antimicrobial and antiviral activity for at least 24 hours with total eradication below detectable limits within 30 minutes (> 6 log kill) against coronavirus, and remains stable for at least 24 months without significant precipitation, discoloration or loss of antimicrobial efficacy.
- [0156] Embodiment 24. The composition of Embodiment 23, wherein the copper sulfate is either anhydrous copper sulfate or copper sulfate pentahydrate.
- [0157] Embodiment 25. The composition of Embodiment 23, wherein the composition does not include any acidifying agents, buffers or surfactants.
- [0158] Embodiment 26. The composition of Embodiment 23, wherein the coronavirus is a human coronavirus.
- [0159] Embodiment 27. The composition of Embodiment 26, wherein the human coronavirus is SARS-CoV-2 or a mutated strain of SARS-CoV-2.
- [0160] Embodiment 28. A stable liquid antimicrobial disinfectant composition that is non-toxic, safe and non-irritating (including at a cellular level and under extended contact) to skin and endogenous tissue comprising:
- [0161] a. a carrier liquid of from about 98% to about 99.9% of total weight of the composition and comprising water or saline;
- [0162] b. potassium iodide at a concentration by weight of about 0.0625%;
- [0163] c. copper sulfate pentahydrate at a concentration by weight of about 0.0625%, wherein the mixture of the respective concentrations of potassium iodide and copper sulfate pentahydrate is such that the potassium iodide is the reaction limiting component and generates both free iodine in solution at an amount of about 250 ppm plus other copper complexes without precipitation or discoloration of the composition;
- [0164] d. wherein the composition has a pH of from about 4.0 to about 7.0, has an effective antimicrobial and antiviral activity for at least 24 hours with total eradication below detectable limits within 30 minutes (> 6 log kill) against coronavirus, and remains stable for at least 24 months without loss of antimicrobial efficacy, and does not include any acidifying agents, buffers or surfactants.

[0165] Embodiment 29. The composition of Embodiment 28, wherein the coronavirus is a human coronavirus.

[0166] Embodiment 30. The composition of Embodiment 29, wherein the human coronavirus is SARS-CoV-2 or a mutated strain of SARS-CoV-2.

[0167] All references cited in this disclosure are incorporated by reference in their entirety.

Testing Studies and Results

1. Iodine concentration testing:

[0168] The primary purpose of this study, performed by an independent third party laboratory, was to provide supporting data requested by the U.S. Food and Drug Administration (FDA) related to chemical composition data for the Clyra™ Solution described below. In addition, corresponding data points have been included to show iodine/iodide concentrations over multiple time points to model room temperature storage and stability.

[0169] The Clyra™ Solution used in the studies below is as follows (expressed as concentrations):

Compounds

[0170] $625 \text{ ppm KI} + 625 \text{ ppm CuSO}_4 \cdot 5\text{H}_2\text{O} \Rightarrow 359 \text{ ppm CuI} + 239 \text{ ppm I}_2 + 328 \text{ ppm K}_2\text{SO}_4 (+ 99 \text{ ppm CuSO}_4 \text{ residual}) + 225 \text{ ppm H}_2\text{O}$

Elemental

[0171] $(147 \text{ ppm K} + 478 \text{ ppm I}) + (159 \text{ ppm Cu} + 80 \text{ ppm S} + 25 \text{ ppm H} + 361 \text{ ppm O}) \Rightarrow (120 \text{ ppm Cu} + 239 \text{ ppm I}) + 239 \text{ ppm I}_2 + (147 \text{ ppm K} + 60 \text{ ppm S} + 121 \text{ ppm O}) + (39 \text{ ppm Cu} + 20 \text{ ppm S} + 40 \text{ ppm O}) \text{ Residual} + (25 \text{ ppm H} + 200 \text{ ppm O})$

Test Results and Discussion

[0172] Chemical analysis using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was performed to determine total iodine/iodide concentration on fresh batches and at numerous time points from 0 to 24 months at Room Temperature (25 °C).

[0173] All samples tested were within the specification of 478 ppm ($\pm 20\%$) being consistent with the theoretical value calculated by stoichiometry. Note that the actual free

Iodine concentration is computed as half the total Iodine/Iodide concentration as per the stoichiometric equation, a measurement assumption that was accepted by the FDA, yielding approximate free Iodine concentrations of about 245 ppm. Table 1 provides iodine concentration results for the solution at various time points.

Table 1

Chemical Element	Theoretical concentration range	Measured concentration (ppm)-Batches stored at RT							Mean	Standard Deviation
		0 months	3 months	6 months	12 months	17 months	21 months	24 months		
Total iodine/iodide	373-564 ppm+ [478 ppm (+/- 20%)]	504**	471**	502	493	523	466	475	492	22.5
iodine*	186-282 ppm+ [239 ppm (+/- 20%)]	252	235.5	251	246.5	261.5	233	237.5	245	10.4
iodide*	186-282 ppm+ [239 ppm (+/- 20%)]	252	235.5	251	246.5	261.5	233	237.5	245	10.4

(* Based on 50/50 ratio per the theoretical stoichiometric equation.

(**) Average from batches LN319-015 and LN319-016.

(+) Specification range based on a 98% of mean recovery according to the analytical technique and +/-20% of experimental error applied to the theoretical concentration calculated by stoichiometry.

2. Biocompatibility and Toxicity testing:

[0174] Biocompatibility testing was performed by an independent third party laboratory to confirm that the components and materials contained in the Clyra™ Solution are not adverse to human tissue.

[0175] Wound Irrigation / wound cleansers typically have to be rinsed out with saline. Wound Treatments (as opposed to wound irrigation or wound cleansers which typically have to be rinsed out with saline) are generally left in contact with the human body for from about 24 (twenty-four) hours to about 7 (seven) days depending on the wound management protocol in place and the required dressing changes. This corresponds to biocompatibility protocol categorization of >24 hours to 30 days for exposure to breached or compromised surfaces.

[0176] The Clyra™ Solution was tested for cytotoxicity (ISO 10993-5), sensitization (ISO 10993-10), irritation (ISO 10993-10) and material mediated pyrogenicity (ISO 10993- 11) with the results summarized below. The results of the biocompatibility testing showed that there are no negative impacts from the materials that are used in the Clyra™ Solution.

- [0177] **A. Cytotoxicity Testing:** The Cytotoxicity testing showed no cytotoxicity or cell lysis in any of the test wells. No pH shift was observed at 48 hours. The reagent control, negative control, and the positive control performed as anticipated. The individual reactivity grades were found to be a grade 2 (mild reactivity) and was found by the third party laboratory to meet the requirements of the test.
- [0178] **B. Guinea Pig Maximization Sensitization Testing:** Maximization sensitization testing was conducted to evaluate the allergenic potential of sensitization capacity of the Clyra™ Solution. This test was used as a procedure for the screening of contact allergens in guinea pigs and extrapolating the results to humans, but it does not establish the actual risk of sensitization. The albino guinea pig model was chosen as the appropriate animal model for human allergic contact dermatitis. None of the animals in the study showed abnormal clinical signs during the test period. None of the negative control animals challenged with the control solution were observed with a sensitization response greater than "0." None of the test animals challenged with the test solution were observed with a sensitization response greater than "0." A negative sensitization incidence was interpreted for all test animals. Under the conditions of this protocol, the test solution was found by the third party laboratory to not elicit a sensitization response.
- [0179] **C. Irritation Testing:** Primary skin irritation showed there were no significant dermal reactions observed at the test sites on the rabbits at the 60 minute, 24, 48 and 72 hour observation periods. In addition, none of the animals in the study showed abnormal clinical signs during the 60 minute, 24, 48 and 72 hour observation periods. The test was considered valid as the test solution remained in contact with abraded or compromised skin for a minimum four hour exposure. The irritation response was negligible under the conditions of the protocol, and the test solution was found to be a negligible irritant, meeting the acceptance criteria of ISO 10993-10.
- [0180] **D. Materials Mediated Rabbit Pyrogen Testing:** The baseline temperatures for all the rabbits in this study were within 1 °C at the start of the test and no animals had a baseline temperature above 39.8 °C or less than 38.5 °C. All of the validity criteria were met during the assay; therefore, the test was considered valid.
- [0181] During the 3 hour observation period, none of the rabbits administered with the test solution had a temperature rise > 0.5 °C at the required observation time points. This response did not exceed the USP<151> and ISO 10993-11 limits and meets the

requirements for this test. Therefore these results indicate that the test solution was found by the third party laboratory to be non-pyrogenic.

3. Antimicrobial Effectiveness Testing:

- [0182] Properties of the Clyra™ Solution were evaluated to determine the effectiveness of the product. The product was evaluated for antimicrobial activity in compliance with USP General Chapter 51 (<51>) - Antimicrobial Effectiveness Test and antimicrobial testing (ASTM E2315-03 discussed below). In addition, microbial testing USP General Chapters 61 and 62 (<61> and <62>) for non-sterile products was carried out as part of the overall stability testing (color, odor, appearance, pH) to ensure product remained within its specification and effective for its intended use over the proposed shelf life.
- [0183] Antimicrobial effectiveness testing was performed by an independent third party lab, to demonstrate the Clyra™ Solution meets the criteria set forth by the USP General Chapter 51 on Antimicrobial Effectiveness Testing. The Antimicrobial Preservative Efficacy study tested the antimicrobial preservative efficacy of Clyra™ Solution against the following organisms: *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10231), and *Aspergillus brasiliensis* (ATCC 16404). Both the initial testing of the solution and repeat testing of the solution after it had been stored for 11 months at 40 C show the device meets the USP criteria of acceptance for the Antimicrobial Effectiveness Test for Category 2.
- [0184] The Clyra™ Solution was tested as a Category 2 product, defined in USP<51> as "topically used products made with aqueous bases or vehicles." The bacteria are grown on soy casein digest agar plates (SCDA) and harvested on the day of testing. The yeast is grown on sabouraud dextrose agar (SDEX) and harvested on the day of testing. The mold is grown on SDEX and harvested within a week before testing. All organisms are adjusted to approximately 1.0×10^8 colony-forming units (CFU)/mL by diluting in sterile saline. Individual test samples and a positive control are prepared for each organism.
- [0185] At the 0-hour, the test samples and positive controls are individually inoculated with each organism to reach a concentration of $1.0 \times 10^5 - 1.0 \times 10^6$ CFU/mL. The volume of the inoculum used is from about 0.5% to about 1.0% of the volume of the product. The positive controls and samples are assayed immediately after inoculation to

determine the challenge level and to provide confirmation that the samples were inoculated.

[0186] After the samples have been inoculated and the 0-hour assay has been performed, the samples are stored in a 20 – 25 °C incubator for the remainder of the test. At 48 hours, 7 days, 14 days and 28 days after inoculation, the samples are removed from the incubator and assayed for surviving organisms. At each of these time points, an aliquot is removed from the samples, diluted in an appropriate neutralizer and plated per the standard plate count procedure to determine the remaining concentration of organisms.

[0187] The organisms recovered from each time point are compared to those seen in the 0-hour positive control. Changes in the concentrations of organisms are converted to Log reduction values for assessing the antimicrobial effectiveness of the samples, per the USP<51> criteria.

Table 2A – Previous Reported Data

Test Organism	Initial Inoculum /g		Log ₁₀ Reduction 14 Days		Log ₁₀ Reduction 28 Days	
	T0	11mos 40 C	T0	11mos 40 C	T0	11mos 40 C
<i>Escherichia coli</i>	3.1x10 ⁵	2.0x10 ⁵	4.5	4.3	4.5	4.3
<i>Pseudomonas aeruginosa</i>	3.5x10 ⁵	5.3x10 ⁵	4.5	4.3	4.5	4.3
<i>Staphylococcus aureus</i>	3.0x10 ⁵	5.4x10 ⁵	4.5	4.4	4.5	4.4
<i>Candida albicans</i>	2.3x10 ⁵	5.1x10 ⁵	4.3	4.1	4.3	4.1
<i>Aspergillus brasiliensis</i>	2.5x10 ⁵	5.5x10 ⁵	4.4	4.5	4.4	4.5

Test results should demonstrate at least a 4-log reduction. The above original test results served to show that the solution achieved > log-4 reduction in the tested organisms, and further demonstrated shelf stability with maintained efficacy of the solution since a sample of the solution which had been stored for 11 months at 40 degrees C demonstrated virtually identical performance and effectiveness.

[0188] The present disclosure provides additional testing results and test data which were performed in the ongoing effort to further evaluate and quantify the efficacy and characteristics of the solution. Using more sophisticated and sensitive test procedures, this subsequent testing revealed even greater effectiveness (by roughly an order of magnitude) than previously noted as shown in the comparable results of Table 2B. Since the stability of the solution had already been amply demonstrated, this additional testing was simply directed at determining the log-kill data at 14 days and 28 days after application.

Table 2B – New Data using Higher Sensitivity Test

Test Organism	Log ₁₀ Reduction 14 Days	Log ₁₀ Reductions 28 Days
<i>Escherichia coli</i>	>5.7	>5.7
<i>Pseudomonas aeruginosa</i>	>5.3	>5.3
<i>Staphylococcus aureus</i>	>5.4	>5.4
<i>Candida albicans</i>	>5.3	>5.3
<i>Aspergillus brasiliensis</i>	>5.1	>5.1

4. Kill Time Study:

[0189] An evaluation of products for anti-microbial activity was performed by an independent third party lab, against selected organisms at representative contact times. Products were evaluated in a liquid matrix. The test organisms and contact times were chosen by the sponsor. This is a quantitative test that allows the determination of the amount of organism reduction at pre-determined intervals. All test method acceptance criteria were met.

[0190] A kill time study measures the antimicrobial activity of the tested product over time. It can be used to measure the antimicrobial activity of a wide variety of products such as hand sanitizers, antiseptic wound gels, mouthwashes, and any other products that are expected to have microbiocidal properties. A Kill Time study is a generalized solution-based challenge study where the challenge organisms and time points are determined based on clinical relevance for the product being tested. It provides a quantitative kinetic kill model for the selected organisms and time points. This test complies with the ASTM Standard E2315-03, the Standard Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure. The following organisms were tested at 10 minutes; 24 hours and 3 days. Table 3A below shows the Log reduction of microbial amounts over time, demonstrating effectiveness of the tested product.

Table 3A – Previous Reported Data

Test Organism	Log ₁₀ Reduction 10 minutes	Log ₁₀ Reduction 24 hours	Log ₁₀ Reduction 3 days
<i>Enterococcus faecium</i>	2.49	>5.13	>5.13
<i>Staphylococcus aureus</i>	1.02	>5.53	>5.53
<i>Klebsiella pneumoniae</i>	>5.12	>5.12	>5.12
<i>Acinetobacter baumannii</i>	~3.94	~5.35	~5.35
<i>Pseudomonas aeruginosa</i>	>5.28	>5.28	>5.28
<i>Klebsiella aerogenes</i>	0.85	>6.12	>6.12
<i>Bacteroides fragilis</i>	2.94	>4.87	>4.87
<i>Candida albicans</i>	0.03	>5.31	>5.31
<i>Candida tropicalis</i>	0.18	>5.35	>5.35

The acceptance criteria for Kill test was met as follows. Negative controls negative for growth. Positive controls positive for growth. Neutralization confirmed at $\geq 70\%$. Test results should demonstrate at least a 4-log reduction. Results show Clyra™ Wound Irrigation Solution achieved $> \log_4$ reduction in all tested organisms by 24 hours that is fully maintained at 3 days.

(Note: *Klebsiella aerogenes* is the new designation for *Enterobacter aerogenes* ~ the name used in the original specification)

[0191] The test procedure used to determine the kill time shown in Table 3A above (Table 3 of the originally filed parent application specification) was based on the standard spiral plate technique, which has a statistical limit of detection that is also affected by the sample size of the test material and the sample dilutions used in the case of each organism. The inventors subsequently determined that they were in fact being limited, in terms of the efficacy or log kill data, by the limitations of the testing procedure. That is to say, the inventors determined that efficacy could not be shown against an organism below the limit of detection of the test itself on that organism. In addition to measuring efficacy, the test also revealed that effectiveness at 24 hours proved durable and constant through 3 days and that this result was consistent across the organisms.

[0192] In order to more accurately determine the true efficacy of the Clyra™ solution in treating these organisms, the inventors engaged in additional testing using a more sensitive experimental process based on the pour plate technique. This technique uses larger sample volumes and less sample dilution, and results in dramatically lower limits of detection, i.e. significantly greater sensitivity and accuracy. Given that durability of the solution was previously demonstrated from 24 hours out to 3 days, for reasons of efficiency the new testing was done at just the 10 minute and 24 hour intervals. The results of this enhanced sensitivity testing were even more exceptional and unexpected,

yielding extremely high log kill reductions even at 10 minutes and with those results maintained or further improved at 24 hours, as shown in Table 3B below (to allow for comparison with the prior results shown in Table 3A). The complete list of tested organisms and results is subsequently shown in Table 4 below.

Table 3B – New Data using Higher Sensitivity Test

Test Organism	Log ₁₀ Reduction 10 minutes	Log ₁₀ Reduction 24 hours
<i>Enterococcus faecium</i>	>6.7	>6.7
<i>Staphylococcus aureus</i>	>6.7	>6.7
<i>Klebsiella pneumoniae</i>	~5.8	>6.8
<i>Acinetobacter baumannii</i>	>6.9	>6.9
<i>Pseudomonas aeruginosa</i>	>6.5	>6.5
<i>Klebsiella aerogenes</i>	>7.0	>7.0
<i>Candida albicans</i>	>5.3*	>5.3
<i>Candida tropicalis</i>	>5.1*	>5.1

*The greater mass of yeast (relative to bacteria) significantly limits the density of the sample, which caps the experimental Limit of Detection and hence limits the measurable log reduction. Log reduction was assessed after 30 min of contact time with Clyra Solution for the yeast samples.

5. Stability Testing:

[0193] Stability data is based on the results of the chemical and physical stability, microbiological testing per USP<61>, and USP<62>, and Antimicrobial Effectiveness Testing (AET) per USP<51>. The measured physical/chemical characteristics of color, odor, appearance, and pH remained within specifications at 6 months Accelerated (40 °C) storage conditions. AET testing (USP<51>, Antimicrobial Effectiveness Testing) met USP acceptance for the Antimicrobial Effectiveness Test for Category 2. In addition, AET testing was submitted and met USP acceptance on the accelerated aging sample at 11 months at 40 °C confirming that the product is adequately preserved over its shelf life.

[0194] Microbial testing was also conducted at the initial time point and 11 months and 17 months at 40 °C, and at 24 months at 25 °C. All results were within specification with <10 CFU per gram for Total Aerobic Count, and Total Combined Molds and Yeasts tests. Also, both *Staphylococcus aureus* and *Pseudomonas aeruginosa* were seen to be absent indicating the product is adequately preserved over its expected shelf life, which is being proposed for 24 months.

[0195] Therefore based on the above studies, a shelf life of two (2) years has been assigned to the Clyra™ Solution, when stored unopened at ambient temperature, in dry conditions away from excessive heat, in accordance with the manufacturer's recommendations.

6. Clyra™ Solution pH Study:

[0196] A pH stability study was conducted to provide real time and accelerated stability data for the Clyra™ Solution. The pH was tested using the Bioscreen Test Method M576.R09 at both room temperature and 40 degrees C (accelerated storage). Figure 2 displays the average pH of the 6 batches tested, all of which were similarly within the pH range of about 4.4 to about 5.6 over the time period of the study under both room temperature and accelerated conditions.

7. Clyra™ Solution Efficacy Against Coronavirus SARS-CoV-2:

[0197] Level 3 lab testing at an independent third party laboratory demonstrated and confirmed that the Clyra™ Solution exhibits total eradication below detectable limits at 30 minutes (> 6 log kill) against coronavirus, specifically the COVID-19 virus, thereby resulting in complete inactivation.

8. Clyra™ Solution Efficacy Against Biofilm Formation

[0198] *In vitro* testing of Clyra™ solution for efficacy in biofilm prevention was conducted with a method that was adapted from ASTM E3161-18, "Standard Practice For Preparing A *Pseudomonas aeruginosa* Or *Staphylococcus aureus* Biofilm Using The CDC Biofilm Reactor". Mature biofilms of *S. epidermidis* ATCC 35984 were grown for 48 hours (h) on smooth silicone breast implant shell material coupons (1 cm²) using a CDC Biofilm Reactor.

[0199] All breast implant coupons received initial bacterial load of ~10⁷ CFU/cm² and an untreated silicone coupon acted as a control. Three separate experiments were performed over 72 hour contact time, and data analyzed with one-way Anova and Tukey post-hoc tests with p-value of less than 0.05. The log reduction in biofilm CFU/cm² from the untreated control is presented in FIG. 3. A 1.7 log reduction was observed at 30 minutes (min), 4.7 log reduction at 2 hours, 6.6 log reduction at 5 hours, and 7.0 log reduction at 24 hours and 72 hours. No colonies were observed at the 24 hour and 72 hour time points

pointing to the efficacy of the Clyra™ solution to inhibit and prevent biofilm growth over an extended period of time.

- [0200] For purposes of comparison, efficacy data for other commercially available wound cleansers on *S. epidermidis* was drawn from published information on the products. Biofilms were grown on hydroxyapatite or titanium alloy substrates. Contact times of Betadine, Irrisept, Vashe, XPerience and Bactisure are determined by the manufacturer's instructions including any requirements to rinse out. Efficacy of Clyra™ solution over 72 hours was tested as is shown in FIG. 4. The efficacy of the Clyra™ solution surpassed the other wound cleansers at the 30 min time period, continued to further rise through the 5 hour period, and remained effective up to 72 hours, whereas other cleansers only were effective up to a maximum of 5 hours.
- [0201] Efficacy of Clyra™ solution against *S. aureus* mature biofilms on titanium alloy substrate was tested in a method adapted from ASTM E2647-20, "Standard Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown Using Drip Flow Biofilm Reactor with Low Shear and Continuous Flow". Mature biofilms of *Staphylococcus aureus* (MRSA) MBL strain 10943, a clinical chronic wound isolate, were grown for 72 h on Grade 5 (6AL-4V) titanium coupons (18.75 cm²) using a Drip Flow Biofilm Reactor® (DFR). Three independent experiments were conducted. Data was analyzed with one-way Anova and Tukey post-hoc tests with a p-value of less than 0.05. FIG. 5 shows log reduction in CFU/cm² from the untreated control. A 0.6 log reduction was observed at 5 min, 1.8 log reduction at 0.5 hours, 4.7 log reduction at 2 hours and 7.5 log reduction at 24 hours. No colonies were observed at 24 hours.
- [0202] A variation of this study was performed using a pulsed lavage system (InterPulse® by Stryker) for 1.5 min (time required to dispense an entire 32 ounce Clyra™ bottle) simulating an irrigation procedure. The coupons were irrigated at a distance of 3±1 inches with the InterPulse® pulsed lavage system guided over the coupon back and forth approximately 1 sweep per second. After irrigating, coupons were returned to the DFR and maintained under static immersion for 30 min and 2 hours. As shown in FIG. 6, the application of Clyra™ solution using this pulsed lavage technique resulted in a significant incremental increase in its efficacy against biofilm on a titanium alloy coupon, on the order of 3 orders of magnitude at both 30 minutes and 2 hours.

9. Clyra™ Solution Antimicrobial Efficacy Against Planktonic Pathogens

[0203] Clyra™ solution antimicrobial efficacy against planktonic pathogens was tested in time kill and antimicrobial persistence tests. For the time kill study, Clyra™ Solution, was challenged with a bacterial/yeast suspension (density of $\sim 10^6$ CFU/mL) for 10 min and 24 hours with 15 of the most prevalent pathogens in hospital acquired infections and wound and surgical infections, including both bacterial and yeast strains as enumerated in Table 4 below. To then test the sustained or persistent antimicrobial activity of Clyra™ Solution under potential re-contamination events, Clyra™ solution was re-challenged via re-inoculation with the same initial bacterial/yeast density of $\sim 10^6$ CFU/mL of the same microorganisms at 10 min, 4 hours and 24 hours for an exposure time of 10 min after each re-inoculation time point. No additional Clyra™ solution was added to the systems. Data demonstrating the antimicrobial persistency of Clyra™ solution is presented in Table 5 below. Some of the values indicated in Tables 4 and 5 are considered approximate. The approximate symbol (\sim) is used when plate counts were outside the statistically accurate range of 20-200 colony forming units (CFU). More than symbols ($>$) are used for samples where no CFUs were observed on the plates. Saline was also tested at the same time points as Clyra™ Solution and no significant pathogen reduction was exhibited for any of the tested pathogens by saline.

[0204] The log reduction values were calculated using the following formula:

$$\text{Log reduction} = \log_{10} U - \log_{10} C$$

Where: U = Average titer control, C = Average recovered counts

Table 4

Microorganism	Exposure Time	Log ₁₀ Reduction
<i>Staphylococcus epidermidis</i> ATCC 35984	10 min	>6.7
	24 hours	>6.7
<i>Staphylococcus aureus</i> ATCC 6538	10 min	>6.7
	24 hours	>6.7
<i>Streptococcus salivarius</i> FUA3532	10 min	>6.1
	24 hours	>6.1
<i>Streptococcus pyogenes</i> ATCC 19615	10 min	>7.0
	24 hours	>7.0
<i>Enterococcus faecalis</i> ATCC 29212	10 min	\sim 5.7
	24 hours	>6.7
<i>Cutibacterium acnes</i> ATCC 11827	10 min	>6.2
	24 hours	>6.2

<i>Escherichia coli</i> ATCC 25922	10 min	>6.9
	24 hours	>6.9
<i>Proteus mirabilis</i> ATCC 29906	10 min	>6.9
	24 hours	>6.9
<i>Candida tropicalis</i> ATCC 750	30 min	>5.1
	24 hours	>5.1
<i>Candida albicans</i> ATCC 10231	30 min	>5.3
	24 hours	>5.3
<i>Enterococcus faecium</i> ATCC 19434	10 min	>6.7
	24 hours	>6.7
<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> ATCC 4352	10 min	~5.8
	24 hours	>6.8
<i>Pseudomonas aeruginosa</i> ATCC 10145	10 min	>6.5
	24 hours	>6.5
<i>Klebsiella aerogenes</i> ATCC 13048	10 min	>7.0
	24 hours	>7.0
<i>Acinetobacter baumannii</i> ATCC 19606	10 min	>6.9
	24 hours	>6.9

Table 5

Microorganism	Re-challenge interval	Log ₁₀ Reduction
<i>Staphylococcus epidermidis</i> ATCC 35984	10 min	>6.7
	4 hours	>6.7
	24 hours	>6.7
<i>Staphylococcus aureus</i> ATCC 6538	10 min	>6.7
	4 hours	~5.7
	24 hours	~5.7
<i>Streptococcus salivarius</i> FUA3532	10 min	>6.1
	4 hours	>6.1
	24 hours	>6.1
<i>Streptococcus pyogenes</i> ATCC 19615	10 min	>7.0
	4 hours	>7.0
	24 hours	>7.0
<i>Enterococcus faecalis</i> ATCC 29212	10 min	>6.7
	4 hours	>6.7
	24 hours	>6.7
<i>Cutibacterium acnes</i> ATCC 11827	10 min	~5.2
	4 hours	>6.2
	24 hours	>6.2
<i>Escherichia coli</i> ATCC 25922	10 min	>6.9
	4 hours	>6.9
	24 hours	>6.9
<i>Proteus mirabilis</i> ATCC 29906	10 min	>6.9
	4 hours	>6.9
	24 hours	>6.9
<i>Candida tropicalis</i> ATCC 750	30 min	>5.1
	4 hours	>5.1
	24 hours	>5.1

<i>Candida albicans</i> ATCC 10231	30 min	>5.3
	4 hours	>5.3
	24 hours	>5.3
<i>Enterococcus faecium</i> ATCC 19434	10 min	>6.7
	4 hours	>6.7
	24 hours	>6.7
<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i> ATCC 4352	10 min	~5.8
	4 hours	>6.8
	24 hours	>6.8
<i>Pseudomonas aeruginosa</i> ATCC 10145	10 min	>6.5
	4 hours	>6.5
	24 hours	~5.5
<i>Klebsiella aerogenes</i> ATCC 13048	10 min	>7.0
	4 hours	>7.0
	24 hours	>7.0
<i>Acinetobacter baumannii</i> ATCC 19606	10 min	>6.9
	4 hours	>6.9
	24 hours	>6.9

CLAIMS:

1. A method for reducing, preventing and/or eradicating biofilm growth comprising providing a stable liquid antimicrobial disinfectant composition comprising:
 - a. at least 95.0% of total weight of a carrier liquid;
 - b. potassium iodide at a first effective concentration by weight;
 - c. copper sulfate at a second effective concentration by weight;
 - d. wherein a mixture of the first concentration of potassium iodide and the second concentration of copper sulfate generates an amount of free iodine in the composition of from about 100 ppm to about 330 ppm;
 - e. wherein the carrier liquid does not include any metallo-peptides, acidifying agents, buffers or surfactants; and
 - f. wherein the composition maintains a pH of from about 4.0 to about 7.0 without discoloration or solid iodine precipitation for at least 6 months; to a surface.
2. The method of claim 1, where the composition includes from about 98% to about 99.9% by weight of the carrier liquid.
3. The method of claim 1 or 2, wherein the carrier liquid comprises purified water.
4. The method of any one of claims 1-3, wherein the carrier liquid comprises water plus NaCl at a concentration of from about 0% to about 3%.
5. The method of any one of claims 1-4, wherein the carrier liquid comprises water plus NaCl at a concentration of about 0.9%.
6. The method of any one of claims 1-5, where the copper sulfate is anhydrous copper sulfate or copper sulfate pentahydrate.
7. The method of any one of claims 1-6, wherein the first effective concentration is from about 0.025% to about 0.100% by weight, and either the copper sulfate is anhydrous copper sulfate and the second effective concentration is from about 0.016% to about

0.064% by weight, or the copper sulfate is copper sulfate pentahydrate and the second effective concentration is from about 0.025% to about 0.100% by weight.

8. The method of any one of claims 1-7, wherein the ratio of the first effective concentration to the second effective concentration is such that the ratio of the moles of potassium iodide to the moles of copper sulfate is from about 1.25 to about 1.75.
9. The method of any one of claims 1-8, wherein the first concentration is about 0.0625% by weight, and either the copper sulfate is anhydrous copper sulfate and the second effective concentration is about 0.040% by weight, or the copper sulfate is copper sulfate pentahydrate and the second effective concentration is about 0.0625% by weight.
10. The method of any one of claims 1-9, wherein an amount of free iodine generated in the composition is from about 200 to about 300 ppm.
11. The method of any one of claims 1-10, wherein an amount of free iodine generated in the composition is about 250 ppm.
12. The method of any one of claims 1-11, wherein the composition has an effective antimicrobial activity of at least three days.
13. The method of any one of claims 1-12, wherein the composition has a pH of from about 4.5 to about 6.0.
14. The method of any one of claims 1-13, wherein the composition remains stable without significant precipitation, discoloration or loss of antimicrobial efficacy for at least 24 months.
15. The method of any one of claims 1-14, wherein the biofilm is eliminated within 24 hours following providing the composition to the surface.
16. The method of any one of claims 1-15, wherein the biofilm is reduced within the first 30 minutes following providing the composition to the surface.

17. The method of any one of claims 1-16, wherein no biofilm is observed from at least 24 hours following providing the composition to the surface to at least 48 hours following providing the composition to the surface.
18. The method of any one of claims 1-17, wherein no biofilm is observed from at least 24 hours following providing the composition to the surface to at least 72 hours following providing the composition to the surface.
19. The method of any one of claims 1-18, wherein the surface is silicone.
20. The method of any one of claims 1-18, wherein the surface is titanium.
21. The method of any one of claims 1-18, wherein the surface is titanium alloy.
22. The method of any one of claims 1-21, wherein the composition is provided via lavage.
23. The method of any one of claims 1-22, wherein the composition is provided via pulsed lavage.
24. The method of any one of claims 1-22, wherein the composition is provided via static lavage.
25. The method of any one of claims 1-24, wherein the biofilm comprises microorganisms from the genus *Staphylococcus*.

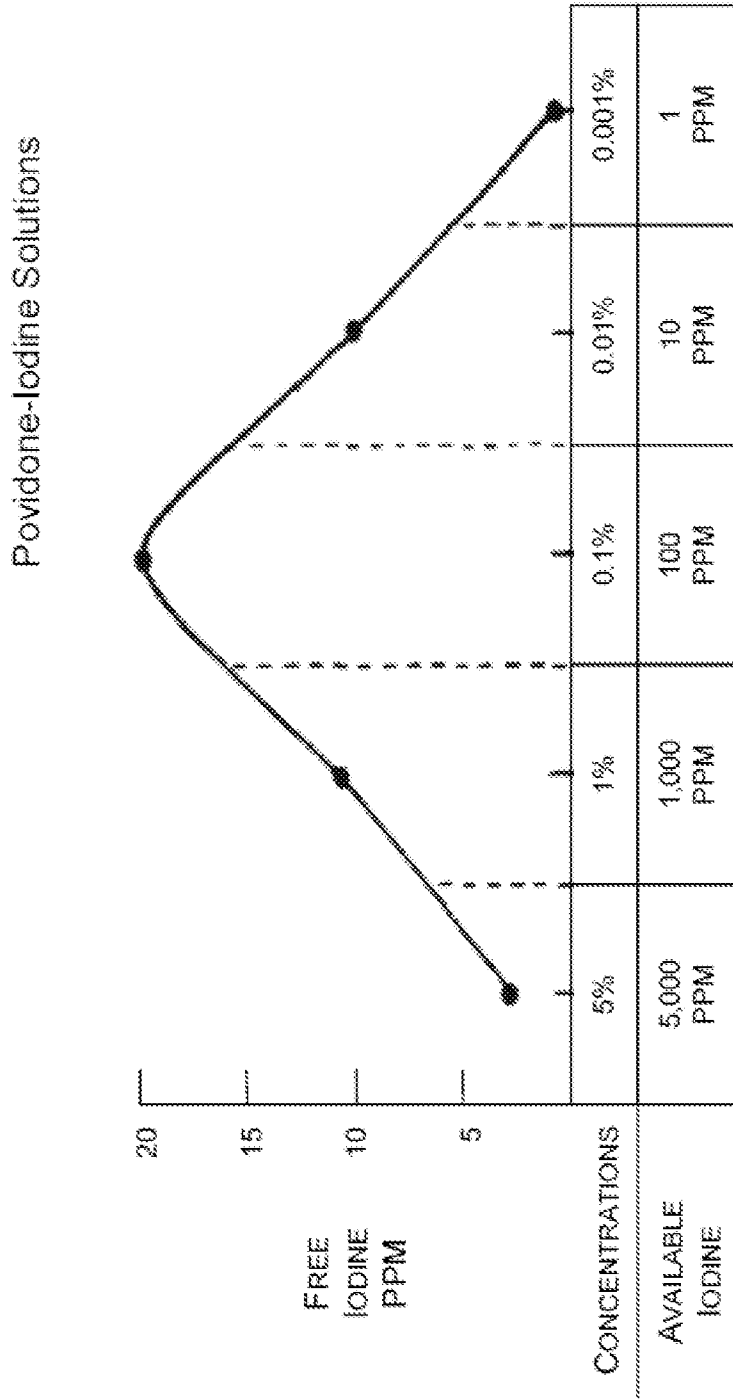


FIG. 1

Average of Batches #: LN273-003, LN273-101, LN291-061, LN319-015, LN319-016

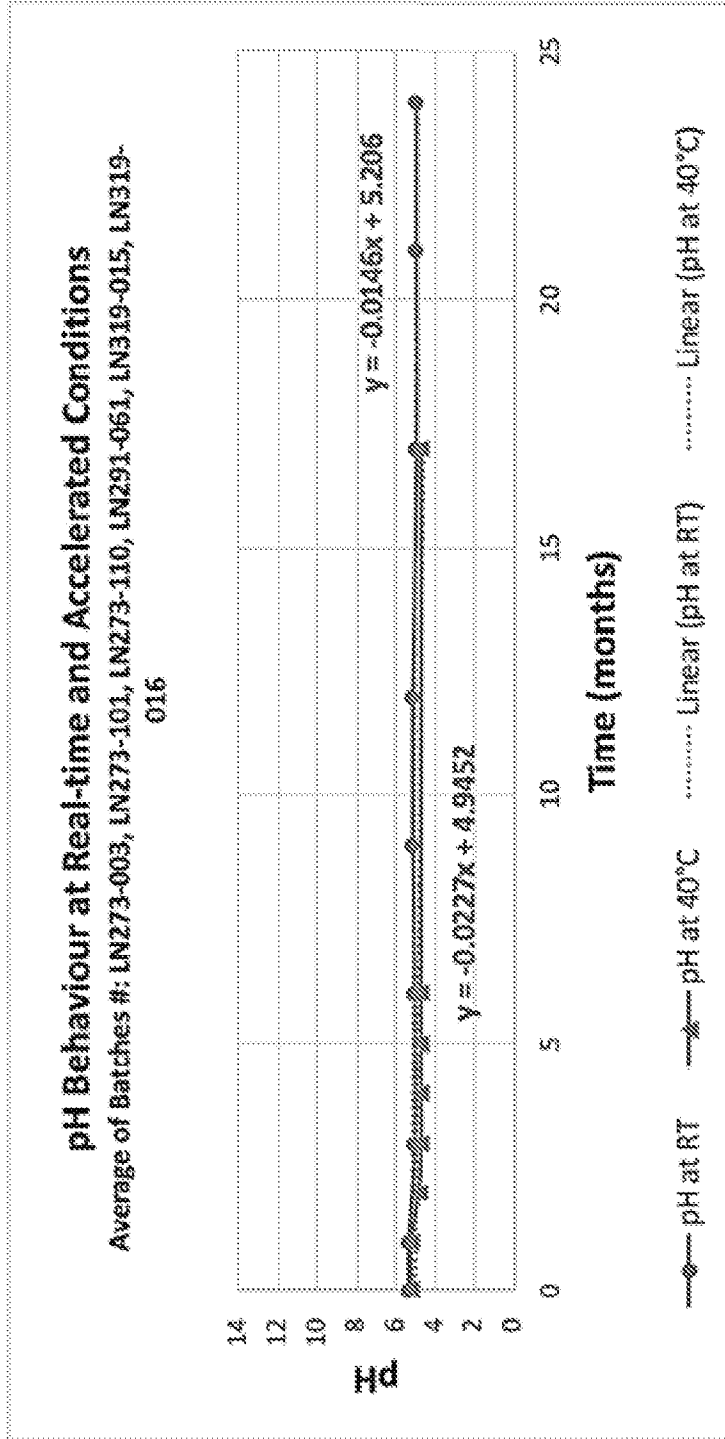
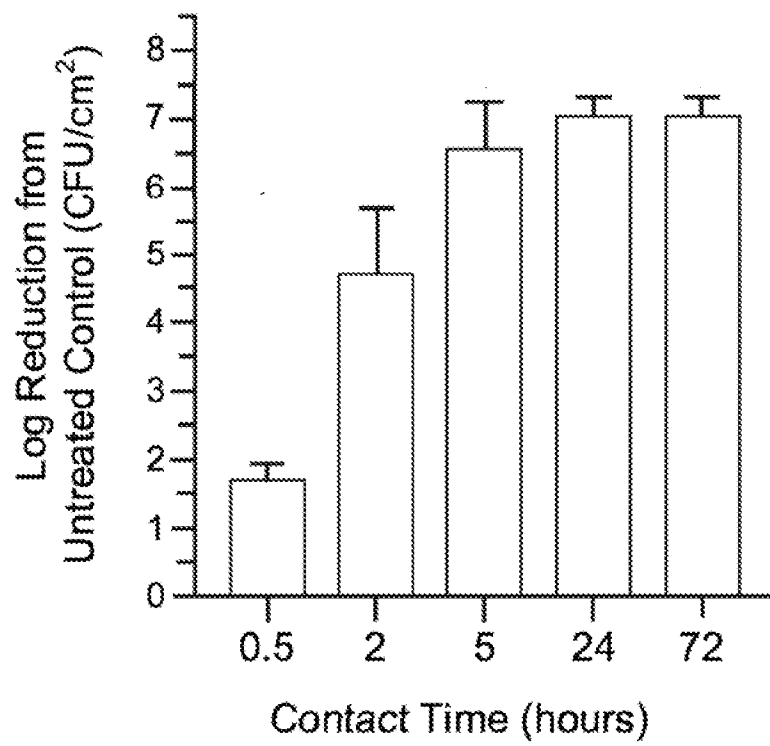


FIG. 2

FIG. 3

Log reduction *in vitro* against *S. epidermidis* mature biofilms on silicone

FIG. 4

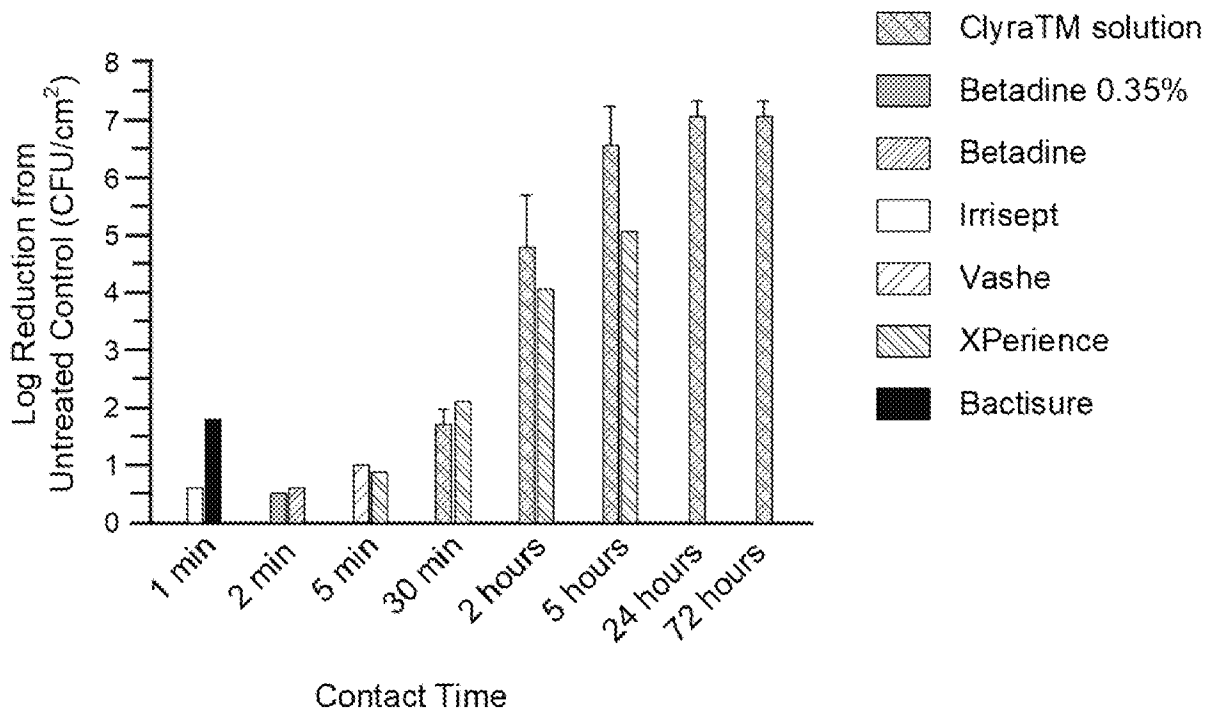
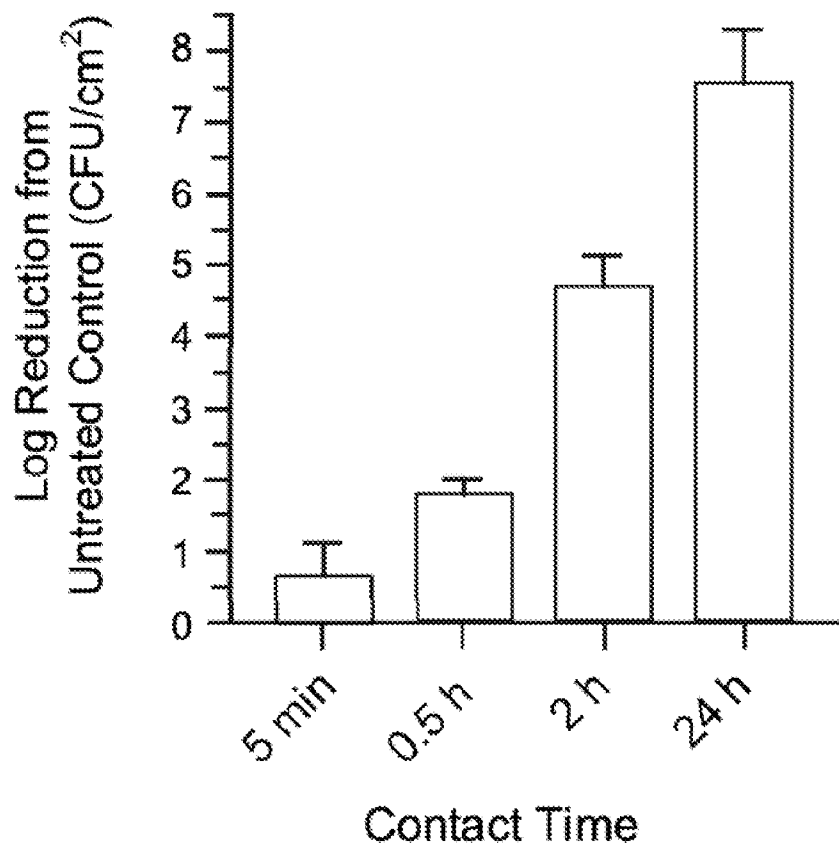


FIG. 5

Log reduction *in vitro* against *S. aureus* mature biofilms on titanium alloy

FIG. 6

