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(54) Title: PYROGEN BINDING COMPOSITION AND USE OF SAME IN TREATMENT AND PREVENTION OF SEPSIS

(57) Abstract: The subject invention provides materials and methods for reducing the amount of bioavailable pyrogens in a subject's body. Advantageously, by applying a composition comprising chlorhexidine gluconate to a site in a subject's body, for example, a site that is afflicted with a bacterial infection, the methods can be used to treat and/or prevent sepsis and/or pyrogenic fever in the subject.



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PYROGEN BINDING COMPOSITION AND USE OF SAME IN TREATMENT AND PREVENTION OF SEPSIS

CROSS-REFERNCE TO A RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application Serial No. 62/690,019, filed June 26, 2018, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

The management and treatment of a wound, a surgical site, a surgical incision, or otherwise infection-prone tissues in the body, has three primary objectives: (1) prevention of
5 infection, (2) preservation and/or restoration of function, and (3) preservation and/or restoration of cosmetic appearance. The most important of these objectives is the prevention of infection. Success in the prevention of infection directly affects the healing process and the degree to which function and cosmetic appearance can be preserved and/or restored.

The number and virulence of bacteria present at a site are critical determinants of
10 whether the site becomes infected. Experimental evidence suggests that a critical level of bacteria is approximately 10^5 organisms per gram of tissue. Below this level, a site or a tissue typically heals; at levels greater than 10^5 bacteria per gram of tissue, infections often develop. Dirty wounds, or wounds that have not been treated within six hours, are likely to be contaminated with bacteria at levels that are higher than the critical level. Reducing the number
15 of bacteria in and around the wound is critical for avoiding infection and expediting wound healing.

If a severe bacterial infection is present in, for example, a wound, the bloodstream, the pulmonary tree or the urinary tract, one potentially serious result could be the development of a condition known as sepsis, where the body initiates an extreme immune response to the infection
20 that can cause damage to tissues and organs. Sepsis can present in the form of fever, increased heart rate, decreased blood pressure, and confusion, among other serious symptoms.

Fever, in particular, may develop in critically ill patients as a result of the production of fever-inducing agents, or pyrogens, by the infecting bacteria. One important pyrogen is an endotoxin compound, a lipopolysaccharide (LPS), which is a potent toxin present in the cell
25 walls of Gram-negative, and some Gram-positive bacteria. Typically, the endotoxin will be released once a cell dies and disintegrates.

A fever is an inflammatory response that affects the entire body, resulting in an increase in body temperature above a subject's baseline temperature. Pyrogenic fever is a common response to sepsis, and occurs through several mechanisms. Exogenous pyrogens such as, e.g., those produced by microorganisms, and endogenous pyrogens, such as, e.g., the cytokines IL-1, IL-6 and TNF- α , interact with the hypothalamus, the section of the brain that maintains homeostasis in the body. The part of the hypothalamus affected by this interaction lacks a blood-brain barrier, allowing it to be stimulated directly by pyrogenic substances. Stimulation of the hypothalamus leads to increased synthesis of, for example, PGE₂ and ceramide, which act to slow the firing rate of the warm sensitive neurons in the hypothalamus, resulting in an increase in body temperature.

Initially, a low-level fever can assist the body in immune defense by, for example, stimulating the leukocytes to kill pathogens, as well as by inhibiting the growth of certain pathogens that do not thrive at elevated temperatures; however, when such an immune response becomes too strong, the results can include tissue and organ damage and, in severe cases, death. In extreme cases, for example, if a bacterial infection reaches the bloodstream, endotoxemia can result, where large quantities of endotoxins, or pyrogens, are released into the blood. This can eventually lead to a condition known as septic shock. The strong immune and inflammatory responses that occur with septic shock can cause a severe drop in blood pressure, intravascular blood clotting, development of thrombi and emboli that block blood vessels and lead to tissue death, multiple organ failure, and even death of the patient.

Sepsis is a widespread problem that affects hundreds of thousands of people each year. While many cases of sepsis are linked to hospital acquired infections (HAI), doctors' offices, nursing homes and/or other healthcare facilities, many other cases are acquired outside of these facilities, for example, at home, school, or work. Any infection can lead to sepsis, from, for example, a simple cut, to food poisoning, to a urinary tract infection.

Currently, methods of treating bacterial infections and sepsis primarily consist of applying broad spectrum and/or target-specific antibiotics; however, with the rapid evolution of bacterial strains, many of these drugs become ineffective over time as the bacteria develop resistance to them. Furthermore, once a patient has developed sepsis, the presence of bacterial endotoxins and pyrogenic substances in the body pose the risk of a patient developing more serious conditions, such as septic shock.

Thus, there is a need for improved and/or complimentary methods of treating and/or preventing sepsis and symptoms thereof. In particular, there is a need for compositions and methods for combatting bacterial infections in, for example, wound patients, while inhibiting the activity of pyrogenic substances that are present in the body as a result of these infections.

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BRIEF SUMMARY OF THE INVENTION

The subject invention provides materials and methods for preventing and/or treating sepsis by, for example, decreasing the amount of bioavailable pyrogens in a subject's body.

More specifically, the subject invention provides compositions and methods of their use for reducing the activity of pyrogenic substances, or pyrogens, in a subject diagnosed with an infection and/or with sepsis, or in a subject at risk of developing sepsis. Advantageously, the compositions of the subject invention are capable of binding to pyrogens and rendering them incapable of inducing the immune response that leads to sepsis.

In preferred embodiments, the subject invention provides pyrogen-binding compositions comprising chlorhexidine. It has been found that the subject compositions, which, in preferred embodiments, comprise a low concentration chlorhexidine without other excipients, can be administered to a subject according to the procedures of the subject invention without causing hemolysis. In a preferred embodiment, the active agent of the subject compositions is a salt of chlorhexidine, such as, for example, chlorhexidine gluconate.

Advantageously, when administered according to the procedures of the subject invention, the compositions of the subject invention do not cause deleterious absorption of chlorhexidine, systemic toxicity, or fibrosis. Furthermore, the compositions can be applied to tissue of the nervous system, including tissue of the central nervous system (CNS), without causing deleterious effects.

In certain embodiments, the pyrogen-binding compositions of the subject invention comprise chlorhexidine at a concentration of about 1.0% or less, more preferably at about 0.1% or less, and even more preferably at about 0.05% or less, and for some uses at 0.02% or less. Typically, the compositions would have at least, 0.005%, 0.01%, or 0.02% or more of chlorhexidine. In one embodiment, chlorhexidine dissolved in water can be used according to the subject invention.

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In certain embodiments, wherein a pyrogen is present in a subject's body as a result of, for example, the presence of bacteria and/or a bacterial infection, the subject invention can be useful for inhibiting the bioavailability of the pyrogen in the subject's body. In other words, the subject invention can be useful for reducing or removing the ability of a pyrogen to induce the symptoms of sepsis, such as, for example, fever, in a subject's body.

In one embodiment, a method is provided for inhibiting the bioavailability of a pyrogen in a subject's body, wherein a composition comprising chlorhexidine gluconate is contacted with the pyrogen. The composition can be contacted with the pyrogen by way of administration to a site in the subject's body. Preferably, the site is afflicted with, or susceptible to, a bacterial infection.

In one embodiment, inhibiting the bioavailability of a pyrogen can comprise rendering the pyrogen inactive or otherwise incapable of inducing the immune response that leads to sepsis. This can be achieved by any number of mechanisms, for example, by changing the configuration of the pyrogen molecule or blocking a binding site on the pyrogen molecule.

In one embodiment, the method can be used to decrease the amount of bioavailable pyrogens, reduce the number of bioavailable pyrogen molecules, and/or reduce the potency of a pyrogen in a subject's body.

The pyrogen can be an exogenous or endogenous pyrogen. In specific embodiments, the pyrogen is an exogenous pyrogen produced by bacteria. Even more specifically, the pyrogen can be an endotoxin produced by bacteria, such as, for example, a lipopolysaccharide (LPS) endotoxin. In one embodiment, the endotoxin-producing bacteria is a Gram-negative bacteria. In another embodiment, the endotoxin-producing bacteria is a Gram-positive bacteria.

In some embodiments, the chlorhexidine composition can also be used to control the endotoxin-producing bacteria itself while inhibiting the bioavailability of a pyrogen.

In one embodiment, the method may be used to treat and/or prevent a pyrogenic fever, or to treat and/or prevent any other symptom of sepsis in a subject that has, preferably, been diagnosed with a bacterial infection and/or with sepsis.

In one embodiment, the subject methods comprise contacting the composition, under turbulent conditions, with a site in the subject's body. For example, in one embodiment, the composition can be administered under pressure to the site using a device fitted with a nozzle or head capable of spraying multiple, simultaneous, pressurized streams of liquid. In one

embodiment, the device is a pressurized wound irrigation device.

In certain embodiments, the administration of the chlorhexidine-containing composition is followed by a rinse with, for example, saline. In other embodiments, no such rinse is applied. Suction may also be applied.

5 The pyrogen-binding composition may have other components including, for example, pH modifiers, buffers, local anesthetic agents, agents that promote wound healing (such as agents that help degrade biofilm), agents that stop bleeding and/or promote clot formation, fever-reducing agents, and other therapeutic and non-therapeutic components. In one embodiment, the composition “consists essentially” of an aqueous solution of CHG, which means that the solution
10 contains no other active agent that materially changes the ability of the solution to bind to pyrogens and/or to control microbial growth.

 The composition of the subject invention can be used at a variety of applications in a variety of bodily sites, such as, for example, a postoperative surgical site, an incision on the skin, the urogenital tract, an implant, a joint, the respiratory tract, an intraperitoneal site, an ocular site,
15 the colon, the sinuses, an intra-articular site, a mediastinal site, a healing tissue site, an intracranial site, a cerebrospinal site, or other nervous system tissue.

 In certain specific examples, the site can be an anastomosis or an anastomotic leak (e.g., after colorectal surgery); a perforated intestinal viscus; a spinal and/or cranial surgical site; a prosthetic joint infection; a cardiac surgery postoperative infection; and/or a urinary tract
20 infection.

 In preferred embodiments, the composition of the subject invention is packaged to facilitate use in a sterile field.

DETAILED DESCRIPTION OF THE INVENTION

25 The subject invention provides materials and methods for preventing and/or treating sepsis by, for example, decreasing the amount of bioavailable pyrogens in a subject’s body.

 More specifically, the subject invention provides compositions and methods of their use for reducing the activity of pyrogenic substances, or pyrogens, in a subject diagnosed with an infection and/or with sepsis, or in a subject at risk of developing sepsis due to, for example, a
30 wound or infection. Advantageously, the compositions of the subject invention are capable of

binding to pyrogens and rendering them incapable of inducing the immune response that leads to sepsis and symptoms thereof, e.g., fever.

Selected Definitions

5 As used herein, the term “bioavailability” of a molecule refers to the ability of the molecule to have an effect at a site of action in a subject’s body. In a specific embodiment, the bioavailability of a pyrogen refers to the ability of the pyrogen molecule to induce an immune and/or inflammatory response in the subject’s body. Bioavailability of a pyrogen may also include the ability of the pyrogen molecule to interact with the hypothalamus and induce fever in
10 the subject’s body, and/or the ability of the pyrogen molecule to bind with certain immune response receptors present in or on immune cells to induce an immune response by those cells.

 As used herein, the term “subject” refers to an animal, especially a mammal. Non-limiting examples of mammals include humans, non-human primates, dogs, cats, equines, bovines, and pigs. The preferred subject in the context of this invention, however, is a human of any gender
15 and of any age or stage of development, including infant, toddler, child, adolescent, teenager, adult and senior.

 As used herein, the term “treatment” refers to eradicating, reducing, ameliorating, or reversing, a sign or symptom of a condition or disorder to any extent, and includes, but does not require, a complete cure of the condition or disorder. Treating can be curing, improving, or
20 partially ameliorating a disorder.

 As used herein, “preventing” a condition or disorder refers to avoiding, delaying, forestalling, or reducing the onset of a particular sign or symptom of the condition or disorder. Prevention can, but is not required to be, absolute or complete, meaning the sign or symptom may still develop at a later time. Prevention can include reducing the severity of the onset of such a
25 condition or disorder, and/or inhibiting the progression of the condition or disorder to a more severe condition or disorder.

 The transitional term “comprising,” which is synonymous with “including,” or “containing,” is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. By contrast, the transitional phrase “consisting of” excludes any element, step, or
30 ingredient not specified in the claim. The transitional phrase “consisting essentially of” limits the scope of a claim to the specified materials or steps “and those that do not materially affect the

basic and novel characteristic(s)” of the claimed invention. Use of the term “comprising” contemplates other embodiments that “consist” or “consist essentially” of the recited component(s).

Unless specifically stated or obvious from context, as used herein, the term “or” is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms “a,” “an” and “the” are understood to be singular or plural.

The terms “about,” “approximately,” “approximate,” and “around” are used in this patent application to describe some quantitative aspects of the invention, for example, the concentration of the active agent. It should be understood that absolute accuracy is not required with respect to those aspects for the invention to operate. When these terms are used to describe a quantitative aspect of the invention the relevant aspect may be varied by up to $\pm 10\%$. Thus, the terms “about,” “approximately,” “approximate,” and “around” allow for variation of the various disclosed quantitative aspects of the invention by $\pm 1\%$, $\pm 2\%$, $\pm 3\%$, $\pm 4\%$, $\pm 5\%$, $\pm 6\%$, $\pm 7\%$, $\pm 8\%$, $\pm 9\%$, or up to $\pm 10\%$. For example, a composition comprising about 1% active agent can contain 0.9% to 1.1% active agent.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims. All references cited herein are hereby incorporated by reference.

Compositions and Methods

In certain embodiments, wherein a pyrogen is present in a subject’s body as a result of bacteria and/or a bacterial infection therein, the subject invention is useful for inhibiting the activity of the pyrogen in the subject’s body, and/or for decreasing the amount of bioavailable pyrogens in the subject’s body.

In one embodiment, a pyrogen-binding composition is provided, wherein the composition contains an active agent that preferably comprises chlorhexidine. In one embodiment, the composition is sterile.

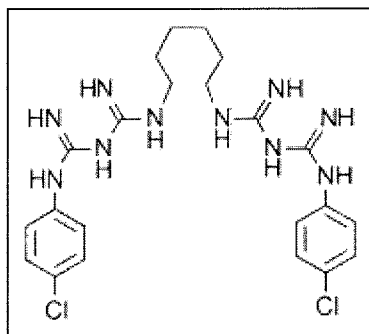
In one embodiment of the subject invention, a low concentration solution of chlorhexidine can be used to effectively decrease the amount of bioavailable pyrogen molecules in a subject's body. Chlorhexidine-containing solutions can be administered to a subject according to the current invention without causing hemolysis or other deleterious effects on the blood, blood cells, or vascular system. Furthermore, when administered according to the procedures of the subject invention, the chlorhexidine-containing solutions of the subject invention do not result in deleterious absorption of chlorhexidine, systemic toxicity, or fibrosis. Additionally, the compositions of the subject invention can be applied to tissue of the nervous system, including tissue of the central nervous system (CNS), without causing deleterious effects.

Based on these findings, it is possible to utilize chlorhexidine-containing solutions in novel and advantageous ways, as described herein, to effectively decrease the amount of bioavailable pyrogenic substances in a subject's body, as well as to treat and/or prevent infections and the complications associated therewith (e.g., sepsis and/or pyrogenic fever) in a wide range of tissues and locations in or on a subject.

In one embodiment, the sterile pyrogen-binding composition contains an active agent that preferably comprises chlorhexidine at a concentration of about 2% w/v or less, about 1% w/v or less, about 0.1% w/v or less, about 0.05% w/v or less, about 0.025% w/v or less, or about 0.02% w/v or less. Typically, the composition would have at least, 0.005%, 0.01%, or 0.02% or more of chlorhexidine. In one embodiment, the chlorhexidine concentration is from 0.02% w/v to 0.05% w/v.

The chlorhexidine can be, for example, chlorhexidine gluconate (CHG), chlorhexidine acetate, chlorhexidine hydrochloride, or a combination thereof. The chlorhexidine may also be modified with, for example, a phosphate group to enhance efficacy, further reducing the likelihood of the development of resistant microbes. The pyrogen-binding composition can further contain one or more additional active agents. In certain embodiments, the composition contains no alcohol, or less than 1%, 4%, 5%, 10%, 25%, or 50% alcohol.

Specifically exemplified herein is the use of CHG. In a specific embodiment, the CHG used according to the subject invention has the following chemical structure:



CHG	
Systematic (IUPAC) Name	1-[amino-[6-[amino-[amino-(4-chlorophenyl)amino-methylidene]amino-methylidene]amino-hexylimino]methyl]imino-N-(4-chlorophenyl)-methanediamine
Chemical Data	
Formula	C ₂₂ H ₃₀ Cl ₂ N ₁₀
Mol. weight	505.446 g/mol

The pH of the pyrogen-binding composition is preferably neutral or slightly acidic. Preferably, the pH is 5.0 to 7.5. More preferably the pH is 5.5 to 7.0.

In certain embodiments, wherein a pyrogen is present in a subject's body as a result of, for example, the presence of bacteria and/or a bacterial infection, the subject compositions can be useful for inhibiting the bioavailability of the pyrogen in the subject's body. In other words, the subject invention can be useful for reducing or removing the ability of a pyrogen to induce the symptoms of sepsis, such as, for example, fever, in a subject's body.

In one embodiment, a method is provided for inhibiting the bioavailability of a pyrogen in a subject's body, wherein a composition comprising chlorhexidine gluconate is contacted with the pyrogen. The composition can be contacted with the pyrogen by way of administration to a site in the subject's body where the pyrogen is located or produced. Preferably, the site has a bacterial infection.

In one embodiment, the method can comprise the steps of: (a) providing a sterile pyrogen-binding composition comprising an active agent comprising chlorhexidine at a concentration of about 2% or less; and (b) administering the sterile pyrogen-binding composition, directly or indirectly, to a site in the subject, wherein the site is afflicted with a bacterial infection.

In one embodiment, the method can be used to decrease the amount of bioavailable pyrogen producing agents, reduce the number of bioavailable pyrogen molecules, and/or reduce the potency of a pyrogen in a subject's body.

5 In one embodiment, the method can be used to reduce a pyrogenic fever in a subject diagnosed with pyrogenic fever. In another embodiment, the method can be used to treat and/or prevent sepsis, septic shock or septicemia in a subject that has, preferably, been diagnosed with an infection and/or with sepsis, septic shock or septicemia.

10 In one embodiment, inhibiting the bioavailability of a pyrogen can comprise rendering the pyrogen inactive or otherwise incapable of inducing the immune response that leads to sepsis. This can be achieved by any number of mechanisms, for example, by changing the configuration of the pyrogen molecule or blocking a binding site on the pyrogen molecule. In a preferred embodiment, a chlorhexidine in the composition of the subject invention binds with a pyrogen to inhibit the pyrogen molecule's bioavailability.

15 The pyrogen can be an exogenous or endogenous pyrogen. In certain embodiments, the pyrogen is an exogenous pyrogen produced by bacteria. In specific embodiments, the pyrogen is an endotoxin produced by bacteria, such as, for example, a lipopolysaccharide (LPS) endotoxin. In one embodiment, the endotoxin-producing bacteria is a Gram-negative bacteria. In another embodiment, the endotoxin-producing bacteria is Gram-positive.

20 In some embodiments, the chlorhexidine composition can also be used to control the endotoxin-producing bacteria itself while inhibiting the bioavailability of a pyrogen.

25 The pyrogen-binding composition and methods of the current invention can be used in a variety of applications in a variety of bodily sites, such as, for example, a postoperative surgical site, an incision on the skin, the urogenital tract, an implant, a joint, the respiratory tract, an intraperitoneal site, an ocular site, the colon, the sinuses, an intra-articular site, a mediastinal site, a healing tissue site, an intracranial or cerebrospinal site, or other nervous system tissue.

In certain specific examples, the site can be an anastomosis; a colorectal surgery postoperative anastomotic leak; a perforated intestinal viscus; a spinal and/or cranial surgical site infection; a prosthetic joint infection; a cardiac surgery postoperative infection; and/or a urinary tract infection.

30 In a preferred embodiment, the administration of the pyrogen-binding composition of the present invention to a site in a subject's body results in a reduction in the amount of bioavailable

pyrogens in the body while also reducing the number of bacteria or other microbes at the site, when compared to either an untreated site or a site administered with saline or water that does not contain chlorhexidine.

Advantageously, administration of the pyrogen-binding composition according to the
5 subject invention can result in effective reduction of pyrogen activity, effective control of an infection, and in some embodiments, effective treatment and/or prevention of sepsis and/or pyrogenic fever, without causing tissue damage.

Examples of additional active agents that can be administered to a subject in accordance with the subject invention include, but are not limited to, anti-bacterial agents, anti-viral agents,
10 fungicidal agents, chemotherapeutic agents, topical antiseptics, anesthetic agents, fever-reducers, oxygenated fluids and/or agents, antibiotics, diagnostic agents, homeopathic agents, agents that stop bleeding, and over-the-counter medications/agents. The subject may receive, for example, vancomycin, ceftriaxone, ceftin, cefuroxime, or tobramycin. In one embodiment, the additional agent can be an anti-microbial peptide (AMP). AMPs are well known in the art.

15 The subject may also receive an intravenous fluid and/or a vasopressor.

In certain embodiments, the additional agent is a diagnostic agent. The diagnostic agent may be, for example, an antibody, protein, or polynucleotide that binds to a target biomolecule. Any such binding may then be visualized utilizing technologies known to those skilled in the art.

For the purpose of this invention, a plain aqueous solution of the active agent comprises
20 the active agent and/or a second agent in a solution of water that is essentially devoid of solutes that provide osmolarity to the solution, for example, a salt or a sugar. For the purpose of this invention, an isotonic solution refers to a solution having the same osmotic pressure as blood. Typically, isotonic solutions contain about 0.85% of NaCl in water. Accordingly, an isotonic solution containing the active agent according to the current invention refers to a solution of the
25 active agent and/or a second agent in about 0.85% NaCl in water.

Spectrum of Activity

Chlorhexidine is capable of binding to pyrogens in a subject's body and rendering them bio-unavailable, or inactive, within the body. In some embodiments, the pyrogen is an
30 endogenous pyrogen, released by a monocyte, neutrophil, lymphocyte, endothelium glial cells, mesangial and/or mesenchymal cell in the subject's body. Exemplary endogenous pyrogens

include cytokines, such as, for example, interleukin-6 (IL-6), IL-1, tumor necrosis factor (TNF), interferon-alpha and/or gp130 receptor ligands.

In preferred embodiments, the pyrogen is an exogenous pyrogen produced by, for example, a microorganism. In particular, the exogenous pyrogen can be an endotoxin, such as a lipopolysaccharide (LPS) complex associated with the outer membrane of Gram-negative bacterial cells of, for example, *Bordetella pertussis*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Salmonella enterica*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Shigella*, *Neisseria*, and *Vibrio cholerae*. In some embodiments, the endotoxin is associated with a Gram-positive bacteria.

LPS molecules comprise three covalently joined regions: a lipid, a core oligosaccharide, and O-antigen. The molecule is anchored into the bacterial membrane by the "lipid A" domain, which comprises hydrophobic fatty acid chains, while the remainder of the molecule extends from the cell surface. When bacterial cells are lysed by, for example, the immune system or an antibiotic, fragments of membrane containing lipid A are released into a subject's blood circulation, a condition known as endotoxemia. The endotoxin fragments bind to receptors and proteins in the cell membranes of macrophages, e.g., CD14, mannose binding protein and toll-like receptors, to activate the release of pro-inflammatory mediators, e.g., IL-1 and TNF- α , against the invading pathogens; however, this immune response can become so strong that it can result in, for example, fever, changes in blood pressure, diarrhea, tissue and organ failure, and/or death, i.e., septic shock or endotoxic shock.

In one embodiment, the chlorhexidine in the compositions of the subject invention is capable of binding the lipid A portion of LPS and rendering it incapable of inducing the immune response that leads to sepsis.

Advantageously, in addition to its pyrogen-binding capabilities, chlorhexidine is also capable of controlling (e.g., killing and/or eliminating) aerobic and anaerobic Gram-positive and Gram-negative bacteria. Chlorhexidine also has activity against *Chlamydia trachomatis*, certain fungi, and certain viruses.

Other bacteria against which chlorhexidine is effective include a variety of Gram-positive aerobic bacteria, such as, e.g., *Streptococcus* mutants, *S. pyogenes* (group A β -hemolytic streptococci), *S. salivarius*, and *S. sanguis*; *Staphylococcus aureus* (e.g., *S. epidermidis*, *S. haemolyticus*, *S. hominis*, and *S. simulans*); both oxacillin-resistant (ORSA) and oxacillin-

susceptible staphylococci (also known as methicillin-resistant [MRSA] or methicillin-susceptible staphylococci); *Enterococcus* (e.g., *E. faecalis* and *E. faecium*); and both vancomycin-susceptible and vancomycin-resistant strains of bacteria.

Chlorhexidine is also capable of controlling some anaerobic bacteria, such as, for example, some strains of *Bacteroides*, *Propionibacterium*, *Clostridium difficile*, and *Selenomonas*, but is less active against *Veillonella*.

Chlorhexidine can control *Candida* (e.g., *C. albicans*, *C. dubliniensis*, *C. glabrata* (formerly *Torulopsis glabrata*), *C. guilliermondii*, *C. kefyr* (formerly *C. pseudotropicalis*), *C. krusei*, *C. lusitaniae*, and *C. tropicalis* (formerly *C. parapsilosis*)); and some dermatophytes, including *Epidermophyton floccosum*, *Microsporum gypseum*, *M. canis*, and *Trichophyton mentagrophytes*.

In addition to controlling bacteria, the sterile pyrogen binding composition of the subject invention can also “depathogenize” certain bacteria including, for example, *Escherichia coli* and *Klebsiella aerogenes*, making these bacteria less potent to cause infection.

Chlorhexidine also has antiviral activity against viruses that have a lipid component in their outer coat or have an outer envelope such as cytomegalovirus (CMV), human immunodeficiency virus (HIV), herpes simplex virus types 1 (HSV-1) and 2 (HSV-2), influenza virus, parainfluenza virus, and variola virus (smallpox virus).

Modes of Administration

The methods of the subject invention can be used in conjunction with the delivery of a chlorhexidine-containing solution by many routes. Of particular interest are: cutaneous, intra-abdominal, intracranial, intralesional, intrathoracic (during surgery), nasal, otic (in the ear canal), as a gastric lavage, ocular (as an eye wash), periodontal, rectal, soft tissue, oral, subcutaneous, and vaginal routes.

Chlorhexidine solutions of the subject invention can be administered using any of a wide range of currently-available delivery devices, systems, and methods.

In preferred embodiments, the composition is contacted with a site in the subject's body under turbulent rather than laminar conditions. For example, in one embodiment, the composition can be administered under pressure to the site using a device fitted with a nozzle or head capable of spraying single or multiple, simultaneous pressurized streams of liquid. In one

embodiment, the device is a wound irrigator fitted with such a nozzle or head. Advantageously, the active flow of fluid under pressure can remove bacteria and particulate matter. Furthermore, the turbulence and increased mixing of the solution with body fluids can lead to greater binding of pyrogens with chlorhexidine, i.e., greater reduction in the number of bioavailable pyrogen molecules.

The composition can also be delivered via an intravenous fluid line, a catheter, a syringe, or as a spray, shower or mist, and/or orally (e.g., as a capsule or tablet).

The subject invention also provides a sepsis treatment kit comprising a composition comprising chlorhexidine and also having in the kit at least one antibiotic. The subject invention further provides an intravenous tube or fluid bag containing a fluid comprising chlorhexidine and an antibiotic. In one embodiment, the invention provides an aqueous composition consisting of, consisting essentially of, or comprising chlorhexidine and an antibiotic.

In one embodiment, the sterile pyrogen-binding composition can be administered to an internal surgical site (or other site of infection or potential infection) via depositing a porous material containing the active agent (e.g., chlorhexidine) that releases the active agent over a period of time to the site. The presence of the active agent in and around the site can prevent and/or treat an infection; reduce the amount of bioavailable pyrogens at the site; and/or prevent or treat sepsis and/or pyrogenic fever resulting from an infection. The porous material containing the active agent can be administered to a surgical site when the surgery is performed. In certain embodiments of the invention, the porous material is a disc, a sphere, or a shape designed to fit at the site.

The porous material containing the active agent can release the active agent over a period of about 1 hour to about 6 months, about 2 months to about 5 months, about 3 months to about 4 months, about 1 week to about 4 weeks, about 2 weeks to about 3 weeks, or any other permutation of these time periods.

Non-limiting examples of materials that can be used to produce the porous implants include silicate feldspar matrix, hydroxyapatite, porous titanium, or sponge. Additional examples of materials appropriate to produce sustained release implants are well known to a person of ordinary skill in the art and such materials are within the purview of the current invention. For example, hydrogels or other such coatings that incorporate therein chlorhexidine can also be used.

In preferred embodiments of the invention, the pyrogen-binding composition is administered to a site of healing tissue. For the purpose of this invention, a healing tissue site is an area of the tissue that suffered an injury or a disease and is recovering after the treatment for the injury or the disease (e.g., after surgery). A healing tissue site can be at the surface of the skin or internal.

In certain embodiments of the current invention, the pyrogen-binding composition is administered to a healing tissue site via a patch, biodegradable gel, bandage, or dressing containing the chlorhexidine; a thick viscous solution containing the chlorhexidine; or a suture containing chlorhexidine.

Advantageously, chlorhexidine binds to healing tissues, for example, to sub-cutaneous layers of skin, to provide antimicrobial, anti-pyrogen, and healing effects. Accordingly, the sterile composition provides an active agent that can bind to a healing tissue to enhance healing tissue recovery, prevent infection, treat an existing infection, and/or reduce the bioavailability of pyrogenic substances produced by an infection.

In additional embodiments of the invention, the sterile pyrogen-binding composition can be administered to a site as a tablet taken orally, microcapsule delivery spheres, nanoparticles, targeted nanoparticles (for example, receptor mediated targeted nanoparticles), a time controlled delivery system, a frozen block of the sterile pyrogen binding composition, a plain aqueous solution of the active agent, an isotonic solution of the active agent, or an implantable time release delivery system.

In certain embodiments, the pyrogen-binding composition is left at the site after administration thereto.

Alternatively, after administration of the pyrogen-binding composition to a site or a tissue, the site or tissue is optionally rinsed with, for example, a sterile solution free of the active agent. Suction (negative pressure) may also be applied at the time of administering the chlorhexidine or within, for example, 1, 2, 3, 5, 10, or 20 minutes thereafter. Examples of solutions free of the active agent include, but are not limited to, plain water, saline, and isotonic solutions free of the active agent. The rinsing can be performed by administering the solution free of the active agent to the site and removing the resultant solution from the site or the tissue by, for example, suction. In certain embodiments, the rinsing is performed within about 1 minute to about 10 minutes, about 2 minutes to about 5 minutes, or about 3 minutes from the time of

administering the sterile pyrogen-binding composition to the site in the subject. In other embodiments, suction is performed, with or without rinsing.

Under optimal circumstances, the methods of the subject invention are utilized by trained medical technicians; however, because of the simplicity and convenience of the subject invention, they can be used to greatly enhance the effectiveness of the administration of the pyrogen binding composition regardless of the training level of the operator performing the irrigation.

CLAIMS

We claim:

1. A method for reducing the bioavailability of a pyrogen, wherein the method comprises contacting chlorhexidine with the pyrogen.
2. The method, according to claim 1, wherein the pyrogen is in a subject's body and the method comprises administering to the subject a composition comprising chlorhexidine.
3. The method of claim 2, wherein the composition is administered to a site in the subject's body having an infection.
4. The method of claim 3, wherein the pyrogen is an endotoxin produced by a Gram-negative or Gram-positive bacteria.
5. The method of claim 3, wherein the site is a colorectal surgery postoperative anastomosis or anastomotic leak.
6. The method of claim 3, wherein the site is a perforated intestinal viscus.
7. The method of claim 3, wherein the site is a prosthetic joint infection.
8. The method of claim 3, used to treat a cardiac surgery postoperative infection.
9. The method of claim 1, wherein the chlorhexidine is chlorhexidine gluconate and the composition has a concentration of chlorhexidine gluconate of 2.0% w/v or lower.
10. The method of claim 9, wherein the concentration of chlorhexidine gluconate is from 0.02% w/v to 0.05% w/v.

11. The method of claim 3, wherein the chlorhexidine is contacted with the site under turbulent conditions.
12. The method of claim 11, wherein the turbulent conditions are achieved using a pressurized wound irrigation device.
13. The method of claim 1, wherein the method is used to treat and/or prevent sepsis, septic shock and/or septicemia in a subject who has been diagnosed with sepsis, septic shock and/or septicemia, or who has been diagnosed as being at risk for sepsis, septic shock and/or septicemia.
14. The method of claim 1, wherein the method is used to treat and/or prevent a pyrogenic fever in a subject who has been diagnosed as having a pyrogenic fever or who has been diagnosed as being at risk for developing a pyrogenic fever.
15. The method, according to claim 2, further comprising administering an antibiotic to the subject.
16. The method, according to claim 13, further comprising administering an intravenous fluid and/or a vasopressor to the subject.
17. A sepsis treatment kit comprising a composition comprising chlorhexidine and at least one antibiotic.
18. An intravenous tube or fluid bag containing a fluid comprising chlorhexidine and an antibiotic.

INTERNATIONAL SEARCH REPORT	International application No. PCT/US 2019/038618																	
A. CLASSIFICATION OF SUBJECT MATTER <i>A61K 31/135 (2006.01)</i> <i>A61K 31/155 (2006.01)</i> <i>A61P 31/04 (2006.01)</i> <i>A61P 41/00 (2006.01)</i> According to International Patent Classification (IPC) or to both national classification and IPC																		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) A61K 31/135, 31/155, A61P 31/04, C07G 11/00, A61P 41/00, A61K 45/06 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PatSearch (RUPTO Internal), USPTO, PAJ, Espacenet, Information Retrieval System of FIPS, Google																		
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%;">Category*</th> <th style="width: 70%;">Citation of document, with indication, where appropriate, of the relevant passages</th> <th style="width: 20%;">Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td style="text-align: center;">X</td> <td rowspan="2">US 2015/0164829 A1 (INNOVATION TECHNOLOGIES, INC.) 18.06.2015, abstract, paragraphs [0009], [0015], [0020], [0024] - [0027], [0029], [0039], [0048], [0051] - [0057], [0060], [0076], [0080] - [0081], [0104], [0114], [0116], claims 1-3, 7, 18</td> <td style="text-align: center;">1-4, 7, 9-18</td> </tr> <tr> <td style="text-align: center;">Y</td> <td style="text-align: center;">5, 6, 8</td> </tr> <tr> <td style="text-align: center;">Y</td> <td>ЛИТВИЦКИЙ П.Ф. Нарушения теплового баланса организма. Лихорадка. Вопросы современной педиатрии, 2009, Т.8, №6, pp. 55-63, p. 56, non-official translation (LITVITSKIY P.F. Violations of the heat balance. Fever. Questions of modern pediatrics, 2009, vol.8, №6, pp. 55-63)</td> <td style="text-align: center;">8</td> </tr> <tr> <td style="text-align: center;">Y</td> <td>BICALHO Paulo Roberto Rodrigues et al. Treatment with chlorhexidine modifies the healing of colon anastomosis in rats. Journal of Investigative Surgery, 2011, 24(1): pp.8-12, doi: 10.3109/08941939.2010.519595, abstract</td> <td style="text-align: center;">5</td> </tr> <tr> <td style="text-align: center;">Y</td> <td>BONDAR Victor M. et al. Chlorhexidine lavage in the treatment of experimental intra-abdominal infection. Arch Surg., 2000, 135(3): pp. 309-314, abstract</td> <td style="text-align: center;">5</td> </tr> </tbody> </table>		Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X	US 2015/0164829 A1 (INNOVATION TECHNOLOGIES, INC.) 18.06.2015, abstract, paragraphs [0009], [0015], [0020], [0024] - [0027], [0029], [0039], [0048], [0051] - [0057], [0060], [0076], [0080] - [0081], [0104], [0114], [0116], claims 1-3, 7, 18	1-4, 7, 9-18	Y	5, 6, 8	Y	ЛИТВИЦКИЙ П.Ф. Нарушения теплового баланса организма. Лихорадка. Вопросы современной педиатрии, 2009, Т.8, №6, pp. 55-63, p. 56, non-official translation (LITVITSKIY P.F. Violations of the heat balance. Fever. Questions of modern pediatrics, 2009, vol.8, №6, pp. 55-63)	8	Y	BICALHO Paulo Roberto Rodrigues et al. Treatment with chlorhexidine modifies the healing of colon anastomosis in rats. Journal of Investigative Surgery, 2011, 24(1): pp.8-12, doi: 10.3109/08941939.2010.519595, abstract	5	Y	BONDAR Victor M. et al. Chlorhexidine lavage in the treatment of experimental intra-abdominal infection. Arch Surg., 2000, 135(3): pp. 309-314, abstract	5
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☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.																		
<table style="width: 100%;"> <tr> <td style="width: 50%; vertical-align: top;"> * Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed </td> <td style="width: 50%; vertical-align: top;"> "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family </td> </tr> </table>		* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family															
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<table style="width: 100%;"> <tr> <td style="width: 50%;"> Name and mailing address of the ISA/RU: Federal Institute of Industrial Property, Berezhevskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37 </td> <td style="width: 50%;"> Authorized officer E. Redo Telephone No. 8(495) 531-64-81 </td> </tr> </table>		Name and mailing address of the ISA/RU: Federal Institute of Industrial Property, Berezhevskaya nab., 30-1, Moscow, G-59, GSP-3, Russia, 125993 Facsimile No: (8-495) 531-63-18, (8-499) 243-33-37	Authorized officer E. Redo Telephone No. 8(495) 531-64-81															
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2019/038618

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	SULTONOV Sh.R. et al. Complex diagnosis and treatment of typhoid peritonitis among children. <i>Российский вестник</i> , 2013, т.3, №1, pp. 40-43, p. 41, last paragraph - p. 42, left col. line 1	6
Y	SEGRS Patrique et al. Prevention of nosocomial infection in cardiac surgery by decontamination of the nasopharynx and oropharynx with chlorhexidine gluconate: a randomized controlled trial. <i>JAMA</i> , 2006, 296(20): pp. 2460-2466, abstract	8