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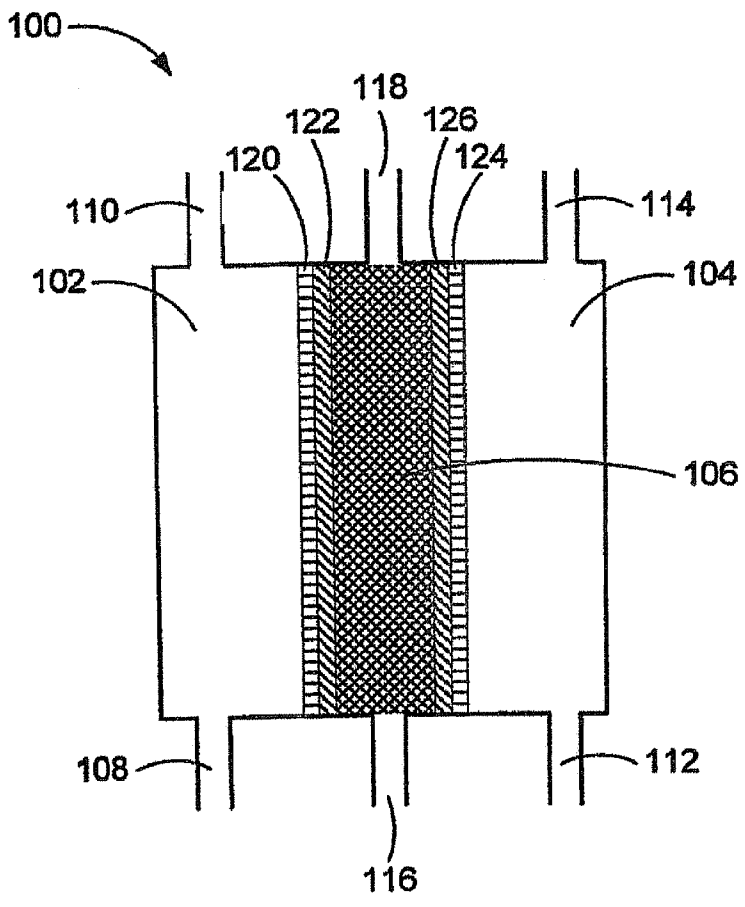
(54) Title: METHODS OF TREATING OR PREVENTING SINUSITIS WITH OXIDATIVE REDUCTIVE POTENTIAL WATER SOLUTION



(57) Abstract: Provided is a method for preventing or treating sinusitis by administering a therapeutically effective amount of an oxidative reduction potential (ORP) water solution that is stable for at least about twenty-four hours. The ORP water solution administered in accordance with the invention can be combined with one or more suitable carriers. The ORP water solution can be administered alone or, e.g., in combination with one or more additional therapeutic agents.

WO 2007/085019 A2

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METHODS OF PREVENTING OR TREATING SINUSITIS WITH OXIDATIVE REDUCTIVE POTENTIAL WATER SOLUTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Patent Application Nos. 60/760,635 filed January 20, 2006; 60/760,567 filed January 20, 2006; 60/760,645 filed January 20, 2006; and 60/760,557 filed January 20, 2006; all of which are hereby incorporated by reference in their entireties.

BACKGROUND OF THE INVENTION

[0002] The cranial sinuses are air chambers within the bone of the cheeks, eyebrows and jaw. These chambers include the frontal sinuses in the eyebrow area, maxillary sinuses inside each cheekbone, ethmoid sinuses just behind the bridge of the nose and between the eyes, and sphenoid sinuses located behind the ethmoids in the upper region of the nose and behind the eyes. The sinuses are lined by a respiratory type epithelium with an underlining subepithelial layer rich in mucus glands and small blood vessels.

[0003] Sinusitis is a condition in which the lining of the sinuses becomes inflamed. Sinusitis can be acute or chronic. Viruses are a frequent cause of acute sinusitis, which produces significant inflammation. This inflammation results in increased mucus production and congestion of the nasal passages. When there is swelling of the mucous membranes of the sinuses, air and mucus are trapped behind the narrowed openings of the sinuses. This congestion predisposes the individual to bacterial sinusitis. Chronic inflammation of the nasal passages, such as allergic rhinitis (hay fever) also predisposes the individual to episodes of acute sinusitis. Vasomotor rhinitis, which can be caused by, e.g., humidity, cold air, alcohol, perfumes, and other environmental conditions, also can predispose the individual to sinus infection.

[0004] Most healthy people harbor bacteria, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*, in their upper respiratory tissues. The mucus trapped behind the narrowed openings of the sinuses allows the resident bacteria to multiply and invade the sinuses' lining, causing an acute bacterial infection. Similarly, fungal infections can cause acute sinusitis. Although fungi are abundant in the environment, they usually are harmless to healthy people. However, fungi, such as *Aspergillus*, can cause serious illness in people whose immune systems are hypersensitive to *Aspergillus*.

[0005] The etiology of chronic sinusitis is often unclear. Chronic sinusitis is an inflammatory disease that often occurs in patients with asthma. It can be caused by infectious agents, although airborne allergens, such as dust, mold, and pollen, which trigger allergic rhinitis, may contribute to or cause chronic sinusitis. An immune response to antigens in fungi also can be responsible for at least some cases of chronic sinusitis.

[0006] Sinusitis is typically treated with drugs including decongestants, anti-histamines, non-steroidal anti-inflammatory agents, steroids, antibiotics, and antivirals. Each of these

drugs has side effects and other drawbacks. For example, nonsteroidal anti-inflammatory agents can produce adverse gastronintestinal and cardiovasular side effects. In addition, the use of anti-infective agents such as antibiotics can produce allergic reactions and also can create an environment that can give rise to the emergence of antibiotic-resistant bacteria. Steroids have systemic side effects, must be tapered to prevent withdrawal, and, because of their immunosuppressive effects, must be used carefully to avoid infection from emerging as a result of immunosuppression. When drug therapy fails, surgery is the only alterative to treat sinusitis but, surgery can result in significant morbidity, pain, and can prolong recovery. Accordingly, there is a need for new safe and effective methods for treating or preventing sinusitis.

[0007] The present invention provides such methods. These and other advantages of the invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

BRIEF SUMMARY OF THE INVENTION

[0008] The present invention provides a method of treating or preventing sinusitis in a patient, which method includes administering to the patient a therapeutically effective amount of an oxidative reductive potential (ORP) water solution, wherein the solution is stable for at least about twenty-four hours. The ORP water solution administered in accordance with the present invention is stable for at least about twenty-four hours, and preferably is stable for at least about two months, more preferably is stable for at least about six months, and most preferably is stable for at least about one year (e.g., one year or longer).

[0009] In accordance with the present invention, the ORP water solution can be administered to the upper respiratory airway (e.g., the upper respiratory tract) and/or one or more cranial sinuses in the patient, e.g., so as to contact one or more tissues in the upper respiratory airway and/or cranial sinuses with the ORP water solution. The cranial sinuses can include, e.g., the frontal sinuses, maxillary sinuses, ethmoid sinuses, and sphenoid sinuses. In one embodiment, the ORP water solution is administered to one or more of the patient's ethmoid sinuses, e.g., so as to contact one or more tissues of the ethmoid sinuses with the ORP water solution.

[0010] In accordance with the present invention, the ORP water solution can be administered by any suitable route including, for example, intranasally, through the mouth or both. In addition, the ORP water solution can be administered in any suitable form such as, e.g., a liquid, spray, mist or aerosol, and can be delivered by any suitable method, e.g., aerosolization, nebulization and atomization. In one embodiment, the ORP water solution is administered in the form of droplets having a diameter in the range of from about 0.1 micron to about 100 microns, preferably 1 micron to about 10 microns.

[0011] The method of the present invention can be effective for treating or preventing acute sinusitis and chronic sinusitis, and can be effective for treating or preventing sinusitis that results from, e.g., an allergic reaction, asthma, or inflammation affecting one or more tissues in the one or more of the cranial sinuses or in the upper respiratory airway. The method of the present invention also can be effective for treating or preventing sinusitis that results from an infection, e.g., by one or more microorganisms, which can include viruses, bacteria, and fungi, which are preferably susceptible to the ORP water solution. Susceptible viruses can include, e.g., coxsackie viruses, adenoviruses, rhinoviruses and influenza viruses. Susceptible bacteria can include, e.g., *Streptococcus pneumoniae, Haemophilus influenzae,* staphylococci, non-pneumococcal streptococci, corynebacterium, and anaerobes. Susceptible fungi can include, e.g., zygomycetes, aspergillus, and candida.

[0012] In accordance with the present invention, the ORP water solution can be administered alone or in combination (e.g., diluted) with one or more suitable carriers. For example, the ORP water solution can be combined with up to about 25% (wt./wt. or vol./vol.) of one or more suitable carriers, up to about 50% (wt./wt. or vol./vol.) of one or more suitable carriers, up to about 75% (wt./wt. or vol./vol.) of one or more suitable carriers, up to about 90% (wt./wt. or vol./vol.) of one or more suitable carriers, or up to about 95% (wt./wt. or vol./vol.) or more of one or more suitable carriers. Suitable carriers can include, e.g., water (e.g., distilled water, sterile water, e.g., sterile water for injection, sterile saline and the like). Suitable carriers also can include one or more carriers described in U.S. Patent Application No. 10/916,278 (hereby incorporated by reference).

[0013] In accordance with the present invention, the OPR water solution can be administered alone or in combination with (or in conjunction with) at least one additional therapeutic agent (i.e., one or more therapeutic agents other than the ORP water solution administered in accordance with the invention). For example, the ORP water solution can be administered in combination with or in conjunction with one or more therapeutic agents selected from the group consisting of antihistamines, decongestants, anti-infective agents (e.g., antibiotics, anti-viral agents, or anti-fungal agents), anti-inflammatory agents, and combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1 illustrates a three-chambered electrolysis cell for producing an exemplary ORP water solution.

[0015] FIG. 2 illustrates a three-chambered electrolysis cell and depicts ionic species that are believed to be generated during the production process.

[0016] FIG. 3 is a schematic flow diagram of a process for producing an exemplary ORP water solution.

[0017] FIG.s 4A-4C depict a graphical comparison of cell viability, apoptosis and necrosis in human diploid fibroblasts (HDFs) treated with an exemplary ORP water solution (MCN) versus hydrogen peroxide (HP).

[0018] FIG. 5 is a graphical comparison of the levels of 8-hydroxy-2'-deoxiguanosine (8-OHdG) adducts in HDFs treated with an exemplary ORP water solution (MCN) versus 500 μM hydrogen peroxide (HP).

[0019] FIG. 6 illustrates cellular senescence demonstrated by β-galactosidase expression in HDFs after chronic exposure to low concentrations of an exemplary ORP water solution (MCN) versus hydrogen peroxide (HP).

[0020] FIG. 7 illustrates the effect on degranulation of antigen-activated mast cells treated with various concentrations of an exemplary ORP water solution (MCN).

[0021] FIG. 8 comparatively illustrates the effect on degranulation of antigen-activated mast cells treated with cromoglycate.

[0022] FIG. 9 illustrates the effect on degranulation of antigen-activated and calcium ionophore (A23187)-activated mast cells treated with various concentrations of an exemplary ORP water solution (MCN).

[0023] FIG. 10A-10B are RNAse protection assay illustrating cytokine mRNA levels after antigen challenge in control versus ORP water solution-treated mast cells.

[0024] FIG. 11 is a graphical comparison of TNF-α secretion by antigen-activated mast cells treated with various concentrations of an exemplary ORP water solution (MCN).

[0025] FIG. 12 is a graphical comparison of MIP 1-α secretion by antigen-activated mast cells treated with various concentrations of an exemplary ORP water solution (MCN).

[0026] FIG. 13 is a graphical comparison of IL-6 secretion by antigen-activated mast cells treated with various concentrations of an exemplary ORP water solution (MCN).

[0027] FIG. 14 is a graphical comparison of IL-13 secretion by antigen-activated mast cells treated with various concentrations of an exemplary ORP water solution (MCN).

DETAILED DESCRIPTION OF THE INVENTION

[0028] The present invention provides a method of preventing or treating sinusitis (e.g., rhinosinusitis, acute sinusitis, chronic sinusitis, and the like) in a patient, which method comprises administering to the patient a therapeutically effective amount of an oxidative reductive potential (ORP) water solution (also known as superoxidized water (SOW)), wherein the solution is stable for at least about twenty-four hours. In accordance with the present invention, the ORP water solution can be administered to the upper respiratory airway (e.g., the upper respiratory tract) and/or one or more cranial sinuses in the patient, e.g., so as to contact one or more tissues in the upper respiratory airway or cranial sinuses with the ORP water solution. The cranial sinuses can include, e.g., the frontal sinuses, maxillary sinuses,

ethmoid sinuses, and sphenoid sinuses. In one embodiment, the ORP water solution is administered to one or more of the patient's ethmoid sinuses, e.g., so as to contact one or more tissues residing in the ethmoid sinuses with the ORP water solution.

[0029] In accordance with the present invention, the ORP water solution can be administered in an amount effective for treating or preventing (e.g., inhibiting the onset of, inhibiting the escalation of, decreasing the likelihood of) sinusitis, including acute sinusitis and chronic sinusitis. The sinusitis treatable or preventable in accordance with the present invention can include sinusitis that results from, e.g., contact with a noxious stimulus, injury, infection, inflammation, autoimmune reaction, hypersensitivity, asthma, and allergic reaction, including allergic reactions associated with cellular histamine release.

[0030] Chronic sinusitis typically refers to inflammation of the sinuses that continues for at least 3 weeks, but the inflammation can (and often does) continue for months or even years. Allergies are frequently associated with chronic sinusitis. In addition, patients with asthma have a particularly high frequency of chronic sinusitis. Inhalation of airborne allergens (substances that provoke an allergic reaction), such as dust, mold, and pollen, often set off allergic reactions (e.g., allergic rhinitis) that, in turn, may contribute to sinusitis (particularly rhinosinusitis or rhinitis). People who are allergic to fungi can develop a condition called "allergic fungal sinusitis." Damp weather or pollutants in the air and in buildings also can affect people subject to chronic sinusitis.

[0031] Like acute sinusitis, chronic sinusitis is more common in patients with immune deficiency or abnormalities of mucus secretion or movement (e.g., immune deficiency, HIV infection, cystic fibrosis, Kartagener's syndrome). In addition, some patients have severe asthma, nasal polyps, and severe asthmatic responses to aspirin and aspirin-like medications (so-called non-steroidal anti-inflammatory drugs, or NSAIDs). These latter patients have a high frequency of chronic sinusitis.

[0032] A doctor can diagnose sinusitis by medical history, physical examination, X-rays, and if necessary, MRIs or CT scans (magnetic resonance imaging and computed tomography). After diagnosing sinusitis and identifying a possible cause, a doctor can prescribe a course of treatment that will reduce the inflammation and relieve the symptoms. Treating acute sinusitis typically requires re-establishing drainage of the nasal passages, controlling or eliminating the source of the inflammation, and relieving the pain. Doctors generally recommend decongestants to reduce the congestion, antibiotics to control a bacterial infection, if present, and pain relievers to reduce the pain. When treatment with drugs fails, surgery may be the only alternative for treating chronic sinusitis, e.g., removal of adenoids, removal of nasal polyps, repair of a deviated septum, endoscopic sinus surgery, and the like. It is believed that the ORP water solution administered in accordance with the present invention can be used for treating chronic sinusitis as an alternative to potentially avoid more aggressive therapies, such as antibiotics and surgery.

[0033] Surprisingly, it has been found that the ORP water solution administered in accordance with the invention is a highly effective inhibitor of mast cell degranulation, one of the primary inflammation-causing biological cascades. The ORP water solution administered in accordance with the invention inhibits degranulation of mast cells regardless of whether they are activated with an antigen or a calcium ionophore. Also surprisingly, it has been found that the ORP water solution administered in accordance with the present invention non-selectively inhibits the secretion of pro-inflammatory cytokines in mast cells. For example, the ORP water solution of the present invention can inhibit the secretion of, e.g., TNF-α and MIP 1-α in mast cells. It is believed that the ORP water solution administered in accordance with the invention also can inhibit the secretion of pro-inflammatory cytokines in other cytokine-secreting cells. These findings demonstrate that the ORP water administered in accordance with the present invention should exhibit broad anti-inflammatory efficacy.

[0034] The ORP water solution administered in accordance with the invention preferably inhibits mast cell degranulation by more than about 50% relative to untreated mast cells, more preferably by more than about 80% relative to untreated mast cells, still more preferably by more than about 90% relative to untreated mast cells, and even more preferably by more than about 95% relative to untreated mast cells, when contacted with the ORP water solution for up to about 30 minutes, more preferably for up to about 15 minutes, and still more preferably for up to about 5 minutes. In accordance with the method of the invention, histamine secretion (e.g., from degranulation) can be therapeutically inhibited by the administration of the ORP water solution alone or in combination with a diluent (e.g., water or saline solution). For instance, histamine secretion can be therapeutically inhibited by administering compositions in which the ORP water solution is diluted, e.g., by a ratio of up to about 50% (vol./vol.) ORP water solution/diluent, by a ratio of up to about 25% (vol./vol.) ORP water solution/diluent, by a ratio of up to about 10% (vol./vol.) ORP water solution/diluent, by a ratio of up to about 5 % (vol./vol.) ORP water solution/diluent, or even by a ratio of up to about 1% (vol./vol.) ORP water solution/diluent.

[0035] The ORP water solution administered in accordance with the invention also preferably inhibits the secretion of TNF-α by more than about 50%, more preferably by more than about 60%, still more preferably by more than about 70%, and even more preferably by more than about 85%. In addition, the ORP water solution administered in accordance with the invention also preferably inhibits the secretion of MIP1-α by more than 25%, more preferably by more than about 50%, and still more preferably by more than about 60%. Further, the ORP water solution administered in accordance with the invention also preferably inhibits the secretion of IL-6 and/or IL-13 by more than 25%, more preferably by more than about 50%, and still more preferably by more than about 60%. In accordance with the method of the invention, cytokine secretion can be therapeutically inhibited by the administration of the ORP water solution alone or in combination with a diluent (e.g., water

or saline solution). For instance, cytokine secretion can be therapeutically inhibited by administering compositions in which the ORP water solution is diluted, e.g., up to about 50% (vol./vol.) ORP water solution/diluent, up to about 25% (vol./vol.) ORP water solution/diluent, up to about 10% (vol./vol.) ORP water solution/diluent, up to about 5 % (vol./vol.) ORP water solution/diluent, or even up to about 1% (vol./vol.) ORP water solution/diluent.

[0036] The sinusitis treatable or preventable in accordance with the present invention also can include sinusitis that results from an infection. In one embodiment, the present invention provides a method of treating or preventing sinusitis, wherein the sinusitis results from infection caused by, e.g., one or more microorganisms selected from the group consisting of viruses, bacteria, and fungi. Accordingly, the present invention provides a method of treating or preventing a viral sinusitis, wherein the sinusitis is associated with infection by one or more viruses, which are preferably susceptible to the ORP water solution administered to the patient. Susceptible viruses can include, e.g., one or more viruses selected from the group consisting of HIV, coxsackie viruses, adenoviruses, rhinoviruses, herpes viruses, influenza viruses, and combinations thereof.

[0037] The present invention also provides a method of treating or preventing a bacterial sinusitis, wherein the sinusitis is associated with infection by one or more bacteria, which are preferably susceptible to the ORP water solution administered to the patient. Susceptible bacteria can include, e.g., one or more bacteria selected from the group consisting staphylococci, streptococci, corynebacterium, anaerobes, and, particularily, *Streptococcus pneumoniae* and *Haemophilus influenzae*. The present invention further provides a method of treating or preventing a fungal sinusitis, wherein the sinusitis is associated with infection by one or more fungi, which are preferably susceptible to the ORP water solution administered to the patient. Susceptible fungi can include, e.g., one or more fungi selected from the group consisting of zygomycetes, aspergillosis, and candida.

[0038] The invention also provides methods for killing bacteria in biofilms, e.g., *Pseudomonas aeruginosa* in biofilms. The invention further provides methods for killing of *Moraexlla catarrhalis* and antibotic resistant bacteria, e.g., penicillin resistant Streptococcus. The methods disclosed herein can be used in accordance with the invention for killing bacteria using ORP water solutions faster than with using Bacitracin.

[0039] The present invention can further include administering with the ORP water solution with (e.g., by co-administering the ORP water solution with, by administering the ORP water solution in conjunction with, or by combining the ORP water solution with) a therapeutically effective amount of at least one additional therapeutic agent (i.e., one or more therapeutic agents other than the ORP water solution administered in accordance with the invention). The additional therapeutic agent can include, e.g., one or more drugs selected from the group consisting of anti-histamines, decongestants, anti-infective agents (e.g.,

antibacterial agents (e.g., antibiotics), anti-viral agents, and anti-fungal agents), anti-inflammatory agents, and combinations thereof.

[0040] Suitable antihistamines can include, e.g., diphenhydramine, chlorpheniramine, brompheniramine, loratadine, clemastine, fexofenadine, derivatives thereof, and combinations thereof. Suitable decongestants can include, e.g., phenylephrine, pseudoephedrine, other α- and β-adrenergic agonists, derivatives thereof, and combinations thereof. Suitable antibacterial agents can include, e.g., penicillins, cephalosporins or other β-lactams, macrolides (e.g., erythromycin, 6-0-methylerythromycin, and azithromycin), fluoroquinolones, sulfonamides, tetracyclines, aminoglycosides, clindamycin, quinolones, metronidazole, vancomycin, chloramphenicol, antibacterially effective derivatives thereof, and combinations thereof. Suitable antifungal agents can include, e.g., amphotericin B, fluconazole, flucytosine, ketoconazole, miconazole, derivatives thereof, and combinations thereof. Suitable antiviral agents can include, e.g., acyclovir, amantadine, didanosine, famciclovir, fortovase, gancicolvir, valacyclovir, zanamivir, interferons, derivatives thereof, and combinations thereof. Suitable anti-inflammatory agents can include, e.g., one or more anti-inflammatory drugs, e.g., one or more anti-inflammatory steroids or one or more non-steroidal anti-inflammatory drugs (NSAIDs). Exemplary anti-inflammatory drugs can include, e.g., leukotriene receptor antagonists, cyclophilins, FK binding proteins, steroids, and NSAIDs.

[0041] In accordance with the present invention, the ORP water solution can be administered topically, e.g., as a liquid, spray, mist, aerosol or steam by any suitable process, e.g., by spraying, aerosolization, nebulization, atomization, and the like. In one embodiment, the ORP water solution is administered to the upper respiratory airway and/or one or more cranial sinuses as a spray, mist, or aerosol. When the ORP water solution is administered by aerosolization, nebulization or atomization. In one embodiment, the method of the present invention includes administering the ORP water solution in the form of droplets having a diameter in the range of from about 1 micron to about 10 microns so as to contact one or more mucosal tissues in the upper respiratory airway or one or more cranial sinuses with the ORO water solution.

[0042] In accordance with the present invention, the ORP water solution can be administered by delivering the ORP water solution alone, or by combining (e.g., mixing) the ORP water solution with one or more suitable carriers (e.g., a diluent). For example, the ORP water solution can be mixed with one or more suitable carriers in the chamber of a device (e.g., a nebulizer or a device that can dispense the mixture as a spray), and the resulting mixture can be delivered from the chamber of the device, e.g., directly to the upper respiratory airway and/or one or more cranial sinuses (e.g., intranasallay, through the mouth, or both). Alternatively, the ORP water solution can be mixed with one or more suitable carriers (e.g., a diluent) using a multiple-chamber device, e.g., a dual-chamber device, in

which the ORP water solution and carrier(s) reside in separate chambers and are combined and/or mixed when they exit the chambers so that the ORP water solution and carrier(s) are combined upon (e.g., immediately prior to or simultaneously upon) delivery to the patient.

[0043] Methods and devices, which are useful for aerosolization, nebulization and atomization, are well known in the art. Medical nebulizers, for example, have been used to deliver a metered dose of a physiologically active liquid into an inspiration gas stream for inhalation by a recipient. See, e.g., U.S. Patent No. 6,598,602 (hereby incorporated by reference). Medical nebulizers can operate to generate liquid droplets, which form an aerosol with the inspiration gas. In other circumstances medical nebulizers may be used to inject water droplets into an inspiration gas stream to provide gas with a suitable moisture content to a recipient, which is particularly useful where the inspiration gas stream is provided by a mechanical breathing aid such as a respirator, ventilator or anaesthetic delivery system.

[0044] An exemplary nebulizer is described, for example, in WO 95/01137, which describes a hand held device that operates to eject droplets of a medical liquid into a passing air stream (inspiration gas stream), which is generated by a recipient's inhalation through a mouthpiece. Another example can be found in U.S. Patent No. 5,388,571 (hereby incorporated by reference), which describes a positive-pressure ventilator system which provides control and augmentation of breathing for a patient with respiratory insufficiency and which includes a nebulizer for delivering particles of liquid medication into the airways and alveoli of the lungs of a patient. U.S. Patent No. 5,312,281 (hereby incorporated by reference) describes an ultrasonic wave nebulizer, which atomizes water or liquid at low temperature and reportedly can adjust the size of mist. In addition, U.S. Patent No. 5,287,847 (hereby incorporated by reference) describes a pneumatic nebulizing apparatus with scalable flow rates and output volumes for delivering a medicinal aerosol to neonates, children and adults. Further, U.S. Patent No. 5,063,922 (hereby incorporated by reference) describes an ultrasonic atomizer. The ORP water solution also may be dispensed in aerosol form as part of an inhaler system for treatment of infections in the lungs and/or air passages or for the healing of wounds in such parts of the body.

[0045] For larger scale applications, a suitable device may be used to disperse the ORP water solution into the air including, but not limited to, humidifiers, misters, foggers, vaporizers, atomizers, water sprays, and other spray devices. Such devices can permit the dispensing of the ORP water solution on a continuous basis. An ejector which directly mixes air and water in a nozzle may be employed. The ORP water solution may be converted to steam, such as low pressure steam, and released into the air stream. Various types of humidifiers may be used such as ultrasonic humidifiers, stream humidifiers or vaporizers, and evaporative humidifiers. The particular device used to disperse the ORP water solution may be incorporated into a ventilation system to provide for widespread application of the ORP water solution throughout an entire house or healthcare facility (e.g., hospital, nursing home,

etc.). The ORP water solution also can be administered to a patient in a chamber or tent, or can be administered through a mask or endoscopically.

[0046] In accordance with the present invention, as indicated herein, the ORP water solution can be administered alone or in combination with one or more pharmaceutically acceptable carriers, which can include, e.g., vehicles, adjuvants, excipients, diluents, combinations thereof, and the like. Such carriers are preferably compatible with one or more of the chemical species that exist in the ORP water solution. One skilled in the art can easily determine the appropriate formulation and method for administering the ORP water solution administered in accordance with the present invention. Any necessary adjustments in dose can be readily made by a skilled practitioner to address the nature and/or severity of the condition being treated in view of one or more clinically relevant factors, such as, e.g., side effects, changes in the patient's overall condition, and the like.

[0047] For example, the ORP water solution can be formulated by combining or diluting the ORP water solution with up to about 25% (wt./wt. or vol./vol.) of a suitable carrier, up to about 50% (wt./wt. or vol./vol.) of a suitable carrier, up to about 75% (wt./wt. or vol./vol.) of a suitable carrier, up to about 90% (wt./wt. or vol./vol.) of a suitable carrier, up to about 95% (wt./wt. or vol./vol.) of a suitable carrier, or even up to about 99% (wt./wt. or vol./vol.) or more of a suitable carrier. Suitable carriers can include, e.g., water (e.g., distilled water, sterile water, e.g., sterile water for injection, sterile saline and the like). Suitable carriers also can include one or more carriers described in U.S. Patent Application No. 10/916,278 (hereby incorporated by reference). Exemplary formulations can include solutions in which the ORP water solution is diluted with sterile water, sterile saline, or a combination thereof. For example, the ORP water solution can be diluted by up to about 25% (vol./vol.), by up to about 50% (vol./vol.), by up to about 75% (vol./vol.), by up to about 90% (vol./vol.), by up to about 95% (vol./vol.), or by up to 99% (vol./vol.) or more with sterile water, sterile saline, or a combination thereof.

[0048] It has been found that the ORP water solution administered in accordance with the invention is virtually free of toxicity to normal tissues and normal mammalian cells. The ORP water solution administered in accordance with the invention causes no significant decrease in the viability of eukaryotic cells, no significant increase in apoptosis, no significant acceleration of cell aging and/or no significant oxidative DNA damage in mammalian cells. The non-toxicity is particularly advantageous, and perhaps even surprising, given that the disinfecting power of the ORP water solution administered in accordance with the invention is roughly equivalent to that of hydrogen peroxide, yet, unlike hydrogen peroxide, is virtually non-toxic to normal tissues and normal mammalian cells. These findings demonstrate that the ORP water solution administered in accordance with the present invention is safe for use, e.g., in mammals, including humans.

[0049] For the ORP water solution administered in accordance with the invention, the cell viability rate is preferably at least about 65%, more preferably at least about 70%, and still more preferably at least about 75% after from about 5 to about 30 minute exposure to the ORP water solution. Additionally, the ORP water solution administered in accordance with the invention preferably causes only up to about 10% of cells, more preferably only up to about 5% of cells, and still more preferably only up to about 3% of cells to expose Annexin-V on their cellular surfaces when contacted with the ORP water solution for up to about thirty minutes or less (e.g., after about thirty minutes or after about five minutes of contact with the ORP water solution).

[0050] Further, the ORP water solution administered in accordance with the invention preferably causes less than about 15% of cells, more preferably less than about 10% of cells, and still more preferably less than about 5% of cells to express the SA-β-galactosidase enzyme after chronic exposure to the OPR water solution. The ORP water solution administered in accordance with the invention preferably causes only a fraction of the oxidative DNA adduct formation caused by hydrogen peroxide in cells treated under equivalent conditions, e.g., less than about 20% of the oxidative DNA adduct formation, less than about 10% of the oxidative DNA adduct formation, or about 5% or less of the oxidative DNA adduct formation normally caused by hydrogen peroxide in cells treated under equivalent conditions.

[0051] The ORP water solution administered in accordance with the invention produces no significant RNA degradation. Accordingly, RNA extracted from human cell cultures after about 30 minutes of exposure to the ORP water solution or after about 3 hours of exposure, and analyzed by denaturing gel electrophoresis, will typically show no significant RNA degradation and will typically exhibit two discreet bands corresponding to the eukaryotic ribosomal RNAs (i.e. 28S and 18S) indicating that the ORP water solution administered in accordance with the invention leaves the RNA substantially intact. Similarly, RNA extracted from human cell cultures after about 30 minutes of exposure to the ORP water solution or after about 3 hours of exposure, can be subjected reverse transcription and amplification (RT-PCR) of the constitutive human GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) gene and result in a strong GAPDH band on gel electrophoresis of the RT-PCR products. By contrast, cells treated with HP for a similar period show significant RNA degradation and little if any GAPDH RT-PCR product.

[0052] Generally, the ORP water solution administered in accordance with the present invention can be administered by a variety of different routes, e.g., parenterally, endoscopically or directly to the surface of a biological tissue, e.g., to the skin and/or one or more mucosal surfaces. Parenteral administration can include using, for example, administering the ORP water solution intramuscularly, subcutaneously, intravenously, intra-arterially, intrathecally, intravesically or into a synovial space. Endoscopic administration of

the ORP water solution can include using, e.g., nasoscopy, bronchoscopy, colonoscopy, sigmoidoscopy, hysterscopy, laproscopy, athroscopy, gastroscopy or a transurethral approach. Administering the ORP water solution to a mucosal surface can include, e.g., administration to a sinus, nasal, oral, tracheal, bronchial, esophageal, gastric, intestinal, peritoneal, urethral, vesicular, urethral, vaginal, uterine, fallopian, and synovial mucosal surface. Parenteral administration also can include administering the ORP water solution intravenously, subcutaneously, intramuscularly, or intraperitoneally. For example, the ORP water solution can be administered intravenously, e.g., as described in U.S. Patent Nos. 5,334,383 and 5,622,848 (hereby incorporated by reference), which describe methods of treating viral myocarditis, multiple sclerosis, and AIDS via intravenous administration of ORP water solutions.

[0053] The therapeutically effective amount administered to the patient, e.g., a mammal, particularly a human, in the context of the present invention should be sufficient to affect a therapeutic or prophylactic response in the patient over a reasonable time frame. The dose can be readily determined using methods that are well known in the art. One skilled in the art will recognize that the specific dosage level for any particular patient will depend upon a variety of potentially therapeutically relevant factors. For example, the dose can be determined based on the strength of the particular ORP water solution employed, the severity of the condition, the body weight of the patient, the age of the patient, the physical and mental condition of the patient, general health, sex, diet, and the like. The size of the dose also can be determined based on the existence, nature, and extent of any adverse side effects that might accompany the administration of a particular ORP water solution. It is desirable, whenever possible, to keep adverse side effects to a minimum.

[0054] Factors, which can be taken into account for a specific dosage can include, for example, bioavailability, metabolic profile, time of administration, route of administration, rate of excretion, the pharmacodynamics associated with a particular ORP water solution in a particular patient, and the like. Other factors can include, e.g., the potency or effectiveness of the ORP water solution with respect to the particular condition to be treated, the severity of the symptoms presented prior to or during the course of therapy, and the like. In some instances, what constitutes a therapeutically effective amount also can be determined, in part, by the use of one or more of the assays, e.g., bioassays, which are reasonably clinically predictive of the efficacy of a particular ORP water solution for the treatment or prevention of a particular condition.

[0055] In accordance with the present invention, the ORP water solution can be administered alone or in combination with one or more additional therapeutic agents to a patient, e.g., a human, to treat an existing condition, including sinusitis. The ORP water solution also can be administered prophylactically, alone or in combination with one or more additional therapeutic agents, to a patient, e.g., a human, that has been exposed to one or more causative

agents associated with the condition. For example, the ORP water solution administered in accordance with the invention can be suitably administered to a patient that has been exposed to one or more infectious microorganisms (e.g., viruses, bacteria and/or fungi) prophylactically to inhibit or decrease the likelihood of sinusitis (and/or infection) associated with the microorganism in a patient, or decrease the severity of the sinusitis (and/or infection) that may develop as a result of such exposure.

[0056] One skilled in the art will appreciate that suitable methods of administering the ORP water solution are available, and, although more than one route of administration can be used, it is possible that one particular route can provide a more immediate and more effective reaction than another route. The therapeutically effective amount can be the dose necessary to achieve an "effective level" of the ORP water solution in an individual patient. The therapeutically effective amount can be defined, for example, as the amount required to be administered to an individual patient to achieve a blood level, tissue level (e.g., level in one or more tissues of the upper respiratory airway and/or cranial sinus(es)), and/or intracellular level of the ORP water solution (or of one or more active species contained therein) to prevent or treat the condition in the patient.

[0057] When the effective level is used as a preferred endpoint for dosing, the actual dose and schedule can vary depending, for example, upon interindividual differences in pharmacokinetics, distribution, metabolism, and the like. The effective level also can vary when the ORP water solution is used in combination with one or more additional therapeutic agents, e.g., one or more anti-infective agents, one or more "moderating," "modulating" or "neutralizing agents," e.g., as described in U.S. Patent Nos. 5,334,383 and 5,622,848 (hereby incorporated by reference), one or more anti-inflammatory agents, and the like.

[0058] An appropriate indicator can be used for determining and/or monitoring the effective level. For example, the effective level can be determined by direct analysis (e.g., analytical chemistry) or by indirect analysis (e.g., with clinical chemistry indicators) of appropriate patient samples (e.g., blood and/or tissues). The effective level also can be determined, for example, by direct or indirect observations such as, e.g., the concentration of urinary metabolites, changes in markers associated with the condition (e.g., viral count in the case of a viral infection), histopathology and immunochemistry analysis, decrease in the symptoms associated with the condition, and the like.

[0059] Conventional ORP water solutions have an extremely limited shelf-life, usually only a few hours. As a result of this short lifespan, using conventional ORP water solutions requires the production to take place in close proximity to the point of use. From a practical standpoint, this means that the facility, e.g., a healthcare facility such as a hospital, must purchase, house and maintain the equipment necessary to produce such conventional ORP water solutions. Additionally, conventional manufacturing techniques have not been able to

produce sufficient commercial-scale quantities to permit widespread use, e.g., as a general disinfecting agent for healthcare facilities.

[0060] Unlike conventional ORP water solutions, the ORP water solution administered in accordance with the invention is stable for at least about twenty-hours after its preparation. In addition, the ORP water solution administered in accordance with the invention is generally environmentally safe and, thus, avoids the need for costly disposal procedures. Preferably, the ORP water solution administered in accordance with the invention is stable for at least about one week (e.g., one week, two weeks, three weeks, four weeks, etc.), and more preferably is stable for at least about two months. Still more preferably, the ORP water solution administered in accordance with the invention is stable for at least about six months. Even more preferably, the ORP water solution administered in accordance with the invention is stable for at least about one year, and most preferably is stable for more than about one year, e.g., at least about two years or at least about three years.

[0061] Stability can be measured based on the ability of the ORP water solution to remain suitable for one or more uses, for example, inhibiting mast cell degranulation, inhibiting cytokine secretion, decontamination, disinfection, sterilization, anti-microbial cleansing, and wound cleansing, for a specified period of time after its preparation under normal storage conditions (e.g., room temperature). The stability of the ORP water solution administered in accordance with the invention also can be measured by storage under accelerated conditions, e.g., from about 30 °C to about 60 °C, in which the ORP water solution preferably is stable for up to about 90 days, and more preferably for up to about 180 days.

[0062] Stability also can be measured based on the concentration over time of one or more species (or precursors thereof) present in solution during the shelf-life of the ORP water solution. Preferably, the concentrations of one or more species, e.g., free chlorine and are maintained at about 70% or greater of their initial concentration for at least about two months after preparation of the ORP water solution. More preferably, the concentration of one of more of these species is maintained at about 80% or greater of their initial concentration for at least about two months after preparation of the ORP water solution. Still more preferably, the concentration of one or more of such species is maintained at about 90% or greater, and most preferably is maintained at about 95% or greater of their initial concentration for at least about two months after preparation of the ORP water solution.

[0063] Stability also can be determined based on the reduction in the amount of organisms present in a sample following exposure to the ORP water solution. Measuring the reduction of organism concentration can be made on the basis of any suitable organism including, e.g., bacteria, fungi, yeasts, or viruses. Exemplary organisms that can be used for determining stability can include, e.g., *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Bacillus athrophaeus* (formerly *B. subtilis*).

[0064] Stability also can be determined based on the reduction in the amount of endotoxins (e.g. lipopolysacharides), growth factors, cytokines and other proteins and lipids present in a sample following exposure to the ORP water solution.

[0065] The ORP water solution administered in accordance with the invention can function as a low-level disinfectant capable of a four log (10^4) reduction in the concentration of live microorganisms, and also can function as a high-level disinfectant capable of a six log (10^6) reduction in concentration of live microorganisms. Preferably, the ORP water solution administered in accordance with the invention is capable of yielding at least about a four log (10^4) reduction in total organism concentration, following exposure for one minute when measured at least about two months after preparation of the solution. More preferably, the ORP water solution is capable of a 10^4-10^6 reduction of organism concentration when measured at least about six months after preparation of the solution. Still more preferably, the ORP water solution is capable of a 10^4-10^6 reduction of organism concentration when measured at least about one year after preparation of the ORP water solution, and most preferably when measured more than about one year, e.g., at least about two years or at least about three years, after preparation of the ORP water solution.

[0066] For instance, the ORP water solution is capable of at least about five log (10^5) reduction in the concentration of a sample of live microorganism selected from the group consisting of *Pseudomonas aeruginosa, Escherichia coli, Enterococcus hirae, Acinetobacter baumannii, Acinetobacter species, Bacteroides fragilis, Enterobacter aerogenes, Enterococcus faecalis, Vancomycin Resistant-Enterococcus faecium (VRE, MDR), Haemophilus influenzae, Klebsiella oxytoca, Klebsiella pneumoniae, Micrococcus luteus, Proteus mirabilis, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus saprophyticus, Streptococcus pneumoniae, Streptococcus pyogenes, Candida albicans and Candida tropicalis,* within 30 seconds of exposure, when measured at least two months after preparation of the ORP water solution.

[0067] In one embodiment, the ORP water solution administered in accordance with the invention can reduce a sample of live microorganisms including, but not limited to, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*, from an initial concentration of from about 1 x 10^6 to about 1 x 10^8 organisms/ml to a final concentration of about zero organisms/ml within about one minute of exposure when measured at least about two months after preparation of the ORP water solution. This corresponds to from about a six log (10^6) to about an eight log (10^8) reduction in organism concentration. Preferably, the ORP water solution is capable of achieving a 10^6-10^8 reduction of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* or *Candida albicans* organisms when measured at least about six months after preparation, and more preferably when measured at least about one year after preparation.

[0068] Alternatively, the ORP water solution administered in accordance with the present invention can produce about a six log (10^6) reduction in the concentration of a spore suspension of *Bacillus athrophaeus* spores within about five minutes of exposure when measured at least about two months after preparation of the ORP water solution. Preferably, the ORP water solution administered in accordance with the invention can achieve about a 10^6 reduction in the concentration of *Bacillus athrophaeus* spores when measured at least about six months after preparation, and more preferably when measured at least about one year after preparation.

[0069] The ORP water solution administered in accordance with the invention also can produce about a four log (10^4) reduction in the concentration of a spore suspension of *Bacillus athrophaeus* spores within about thirty (30) seconds of exposure when measured at least about two months after preparation of the ORP water solution. Preferably, the ORP water solution can achieve this reduction in the concentration of *Bacillus athrophaeus* spores when measured at least about six months after preparation, and more preferably when measured at least about one year after preparation.

[0070] The ORP water solution administered in accordance with the invention further can produce about a six log (10^6) reduction in the concentration of fungal spores, such as *Aspergillis niger* spores, within about five to about ten minutes of exposure when measured at least about two months after preparation of the ORP water solution. Preferably, the ORP water solution can achieve a 10^6 reduction in the concentration of fungal spores when measured at least about six months after preparation, and more preferably when measured at least about one year after preparation.

[0071] The ORP water solution administered in accordance with the invention further can produce more than 3 log (10^3) reduction in the concentration of viruses, such as Human Immunedeficiency Virus (HIV) and adenovirus, within about five to about ten minutes of exposure when measured at least about two months after preparation of the ORP water solution. Preferably, the ORP water solution can achieve a $> 10^3$ reduction in the concentration of viruses when measured at least about six months after preparation, and more preferably when measured at least about one year after preparation.

[0072] The ORP water solution administered in accordance with the invention further can completely inhibit the growth of *Mycobacterium bovis* within about five minutes of exposure when measured at least about two months after preparation of the ORP water solution. Preferably, the ORP water solution can achieve the total inhibition in the concentration of *Mycobacteria* when measured at least about six months after preparation, and more preferably when measured at least about one year after preparation.

[0073] The ORP water solution administered in accordance with the invention can be acidic, neutral or basic, and generally can have a pH of from about 1 to about 14. Within this pH range, the ORP water solution can be safely applied in suitable quantities, e.g., to surfaces

without damaging the surfaces or harming objects, such as human skin, that comes into contact with the ORP water solution. Preferably, the pH of the ORP water solution administered in accordance with the invention is from about 3 to about 8. More preferably, the pH of the ORP water solution is from about 6.4 to about 7.8, and still more preferably, the pH is from about 7.4 to about 7.6.

[0074] The ORP water solution administered in accordance with the invention can have an oxidation-reduction potential of from about –1000 millivolts (mV) to about +1150 millivolts (mV). This potential is a measure of the tendency (i.e., the potential) of a solution to either accept or transfer electrons that are sensed by a metal electrode and compared with a reference electrode in the same solution. This potential may be measured by standard techniques including, for example, measuring the electrical potential in millivolts of the ORP water solution relative to a standard reference such as, e.g., a silver/silver chloride electrode.

[0075] The ORP water solution administered in accordance with the invention preferably has a potential of from about –400 mV to about +1300 mV. More preferably, the ORP water solution has a potential of from about 0 mV to about +1250 mV, and still more preferably from about +500 mV to about +1250 mV. Even more preferably, the ORP water solution administered in accordance with the present invention has a potential of from about +800 mV to about +1100 mV, and most preferably from about +800 mV to about +1000 mV.

[0076] Various ionic and other species may be present in the ORP water solution administered in accordance with the invention. For example, the ORP water solution may contain chlorine (e.g., free chlorine). The presence of one or more of these species is believed to contribute to at least the disinfectant ability of the ORP water solution to kill a variety of microorganisms, such as bacteria and fungi, as well as viruses. Although not wishing to be bound by any particular theory, it is believed that or more of such species also may contribute the efficacy of the ORP water solution for treating or preventing sinusitis.

[0077] Free chlorine typically includes, but is not limited to, hypochlorous acid (HClO), hypochlorite ions (ClO^-), sodium hypochlorite (NaOCl), chloride ion (Cl^-), dissolved chlorine gas (Cl_2), and precursors thereof. The ratio of hypochlorous acid to hypochlorite ion is dependent upon pH. At a pH of 7.4, hypochlorous acid levels are typically from about 25 ppm to about 75 ppm. Temperature also can impact the ratio of the free chlorine component.

[0078] Chlorine can be present in the ORP water solution administered in accordance with the invention in any suitable amount. The levels of these components may be measured by any suitable method, including methods known in the art.

[0079] Preferably, the total chlorine content, which includes both free chlorine and bound chlorine, is from about 50 parts per million (ppm) to about 400 ppm. More preferably, the total chlorine content is about 80 ppm to about 150 ppm.

[0080] The chlorine content may be measured by methods known in the art, such as the DPD colorimeter method (Lamotte Company, Chestertown, Maryland) or other known

methods such as, e.g., methods established by the Environmental Protection Agency. In the DPD colorimeter method, a yellow color is formed by the reaction of free chlorine with N,N-diethyl-p-phenylenediamine (DPD) and the intensity is measured with a calibrated calorimeter that provides the output in parts per million. Further addition of potassium iodide turns the solution a pink color to provide the total chlorine value. The amount of bound chlorine present is then determined by subtracting free chlorine from the total chlorine.

[0081] The total amount of oxidizing chemical species present in the ORP water solution is preferably in the range of about 2 millimolar (mM), which includes the aforementioned chlorine species, superoxidized water species, and additional species, including those, which can be difficult to measure such as, e.g., Cl^-, ClO_3, Cl_2^-, and ClO_x.

[0082] In one embodiment, the ORP water solution administered in accordance with the invention comprises one or more chlorine species and/or one or more additional superoxidized water species. Preferably, the chlorine species present is a free chlorine species. The free chlorine species can include one or more species selected from the group consisting of hypochlorous acid (HOCl), hypochlorite ions (OCl^-), sodium hypochlorite (NaOCl), chloride ion (Cl^-), dissolved chlorine gas (Cl_2), precursors thereof and mixtures thereof.

[0083] The total amount of free chlorine species is preferably from about 10 ppm to about 400 ppm, more preferably from about 50 ppm to about 200 ppm, and most preferably from about 50 ppm to about 80 ppm. The amount of hypochlorous acid is preferably from about 15 ppm to about 35 ppm. The amount of sodium hypochlorite is preferably in the range of from about 25 ppm to about 50 ppm.

[0084] In one embodiment, the ORP water solution includes one or more chlorine species or one and, optionally, or more precursors thereof, and is stable for at least about 24 hours, preferably for at least about one week, more preferably for at about least two months, and still more preferably for at least about six months after its preparation. Even more preferably, such ORP water solution is stable for at least about one year, and most preferably for more than about one year, e.g., at least about two years or at least about three years.

[0085] It is also preferred that the ORP water solution, which includes one or more chlorine species, one or more additional superoxidized water species (e.g., one or more oxygen species, dissolved oxygen) or one or more precursors thereof and has a pH of from about 6 to about 8. More preferably, the pH of such ORP water solution is from about 6.2 to about 7.8, and most preferably from about 7.4 to about 7.6. An exemplary ORP water solution administered in accordance with the present invention can comprise, e.g., from about 15 ppm to about 35 ppm hypochlorous acid, from about 25 ppm to about 50 ppm sodium hypochlorite, with these components at a pH of from about 6.2 to about 7.8, and can be stable for at least about one week, e.g., at least about two months, at least about six months, at least

about one year, or more than about one year, e.g., at least about two years or at least about three years.

[0086] While in no way limiting the present invention, it is believed that the control of pH and other variables (e.g., salinity) can provide stable ORP water solutions, which contain one or more chlorine species and, optionally, hydrogen peroxide or precursors thereof, such as, e.g., hypochlorous acid and hypochlorite ions.

[0087] The ORP water solutions administer in accordance with the invention preferably comprises one or more oxidized water species which can yield free radicals (such as, e.g., hydroxyl radicals) on exposure to iron. The ORP water can optionally include one or more chemical compounds generated during the production thereof such as, e.g., sodium hydroxide (NaOH), chlorine dioxide (ClO_2), peroxides (e.g., hydrogen peroxide (H_2O_2), and ozone (O_3) although, it has been reported that sodium hydroxide, chlorine dioxide, hydrogen peroxide, and ozone may react with hypocholrite resulting in their consumption and the production of other chemical species.

[0088] The ORP water solution administered in accordance with the present invention can be produced by an oxidation-reduction process, e.g., by an electrolytic process or redox reaction, in which electrical energy is used to produce one or more chemical changes in an aqueous solution. Exemplary processes for preparing suitable ORP water solutions are described, e.g., in U.S. Patent Application Publication Nos. US 2005/0139808 and US 2005/0142157 (hereby incorporated by reference).

[0089] In the electrolytic process, electrical energy is introduced into and transported through water by the conduction of electrical charge from one point to another in the form of an electrical current. In order for the electrical current to arise and subsist there should be charge carriers in the water, and there should be a force that makes the carriers move. The charge carriers can be electrons, as in the case of metal and semiconductors, or they can be positive and negative ions in the case of solutions. A reduction reaction occurs at the cathode while an oxidation reaction occurs at the anode. At least some of the reductive and oxidative reactions that are believed to occur are described in International Application WO 03/048421 A1.

[0090] As used herein, water produced at an anode is referred to as anode water and water produced at a cathode is referred to as cathode water. Anode water typically contains oxidized species produced from the electrolytic reaction while cathode water typically contains reduced species from the reaction. Anode water generally has a low pH, typically of from about 1 to about 6.8. The anode water preferably contains chlorine in various forms including, for example, chlorine gas, chloride ions, hydrochloric acid and/or hypochlorous acid, or one or more precursors thereof. Oxygen in various forms is also preferably present including, for example, oxygen gas and, optionally, peroxides, and/or ozone, or one or more

precursors thereof. Cathode water generally has a high pH, typically from about 7.2 to about 11. Cathode water can contain hydrogen gas, hydroxyl radicals, and/or sodium ions.

[0091] The ORP water solution administered in accordance with the invention can include a mixture of anode water (e.g., water produced in the anode chamber of an electrolytic cell) and cathode water (e.g., water produced in the cathode chamber of an electrolysis cell). Preferably, the ORP water solution administered in accordance with the present invention contains cathode water, e.g., in an amount of from about 10% by volume to about 90% by volume of the solution. More preferably, cathode water is present in the ORP water solution in an amount of from about 10% by volume to about 50% by volume, and still more preferably in an amount of from about 20% by volume to about 40% by volume of the solution, e.g., from about 20% by volume to about 30% by volume of the solution. Additionally, anode water can be present in the ORP water solution, e.g., in an amount of from about 50% by volume to about 90% by volume of the solution. Exemplary ORP water solutions can contain from about 10% by volume to about 50% by volume of cathode water and from about 50% by volume to about 90% by volume of anode water. The anode and cathode water can be produced using the three-chambered electrolysis cell shown in FIG. 1.

[0092] The ORP water solution administered in accordance with the invention is preferably produced using at least one electrolysis cell comprising an anode chamber, a cathode chamber and a salt solution chamber located between the anode and cathode chambers, wherein at least some of the anode and cathode water are combined such that the ORP water solution comprises anode water and cathode water. A diagram of an exemplary three chamber electrolysis cell that can be used in preparing an exemplary ORP water solution is shown in FIG. 2.

[0093] The electrolysis cell 100 has an anode chamber 102, cathode chamber 104 and salt solution chamber 106. The salt solution chamber is located between the anode chamber 102 and cathode chamber 104. The anode chamber 102 has an inlet 108 and outlet 110 to permit the flow of water through the anode chamber 100. The cathode chamber 104 similarly has an inlet 112 and outlet 114 to permit the flow of water through the cathode chamber 104. The salt solution chamber 106 has an inlet 116 and outlet 118. The electrolysis cell 100 preferably includes a housing to hold all of the components together.

[0094] The anode chamber 102 is separated from the salt solution chamber by an anode electrode 120 and an anion ion exchange membrane 122. The anode electrode 120 may be positioned adjacent to the anode chamber 102 with the membrane 122 located between the anode electrode 120 and the salt solution chamber 106. Alternatively, the membrane 122 may be positioned adjacent to the anode chamber 102 with the anode electrode 120 located between the membrane 122 and the salt solution chamber 106.

[0095] The cathode chamber 104 is separated from the salt solution chamber by a cathode electrode 124 and a cathode ion exchange membrane 126. The cathode electrode 124 may be

positioned adjacent to the cathode chamber 104 with the membrane 126 located between the cathode electrode 124 and the salt solution chamber 106. Alternatively, the membrane 126 may be positioned adjacent to the cathode chamber 104 with the cathode electrode 124 located between the membrane 126 and the salt solution chamber 106.

[0096] The electrodes preferably are constructed of metal to permit a voltage potential to be applied between the anode chamber and cathode chamber. The metal electrodes are generally planar and have similar dimensions and cross-sectional surface area to that of the ion exchange membranes. The electrodes are configured to expose a substantial portion of the surface of the ion exchange members to the water in their respective anode chamber and cathode chamber. This permits the migration of ionic species between the salt solution chamber, anode chamber and cathode chamber. Preferably, the electrodes have a plurality of passages or apertures evenly spaced across the surface of the electrodes.

[0097] A source of electrical potential is connected to the anode electrode 120 and cathode electrode 124 so as to induce an oxidation reaction in the anode chamber 102 and a reduction reaction in the cathode chamber 104.

[0098] The ion exchange membranes 122 and 126 used in the electrolysis cell 100 may be constructed of any suitable material to permit the exchange of ions between the salt solution chamber 106 and the anode chamber 102 such as, e.g., chloride ions (Cl^-) and between the salt solution salt solution chamber 106 and the cathode chamber 104 such as, e.g., sodium ions (Na^+). The anode ion exchange membrane 122 and cathode ion exchange membrane 126 may be made of the same or different material of construction. Preferably, the anode ion exchange membrane comprises a fluorinated polymer. Suitable fluorinated polymers include, for example, perfluorosulfonic acid polymers and copolymers such as perfluorosulfonic acid/PTFE copolymers and perfluorosulfonic acid/TFE copolymers. The ion exchange membrane may be constructed of a single layer of material or multiple layers. Suitable ion exchange membrane polymers can include one or more ion exchange membrane polymers marketed under the trademark Nafion®.

[0099] The source of the water for the anode chamber 102 and cathode chamber 104 of the electrolysis cell 100 may be any suitable water supply. The water may be from a municipal water supply or alternatively pretreated prior to use in the electrolysis cell. Preferably, the water is pretreated and is selected from the group consisting of softened water, purified water, distilled water, and deionized water. More preferably, the pretreated water source is ultrapure water obtained using reverse osmosis purification equipment.

[0100] The salt water solution for use in the salt water chamber 106 can include any aqueous salt solution that contains suitable ionic species to produce the ORP water solution. Preferably, the salt water solution is an aqueous sodium chloride (NaCl) salt solution, also commonly referred to as a saline solution. Other suitable salt solutions can include other chloride salts such as potassium chloride, ammonium chloride and magnesium chloride as

well as other halogen salts such as potassium and bromine salts. The salt solution can contain a mixture of salts.

[0101] The salt solution can have any suitable concentration. For example, the salt solution can be saturated or concentrated. Preferably, the salt solution is a saturated sodium chloride solution.

[0102] FIG. 2 illustrates what are believed to be various ionic species produced in the three chambered electrolysis cell useful in connection with the invention. The three chambered electrolysis cell 200 includes an anode chamber 202, cathode chamber 204, and a salt solution chamber 206. Upon application of a suitable electrical current to the anode 208 and cathode 210, the ions present in the salt solution flowing through the salt solution chamber 206 migrate through the anode ion exchange membrane 212 and cathode ion exchange membrane 214 into the water flowing through the anode chamber 202 and cathode chamber 204, respectively.

[0103] Positive ions migrate from the salt solution 216 flowing through the salt solution chamber 206 to the cathode water 218 flowing through the cathode chamber 204. Negative ions migrate from the salt solution 216 flowing through the salt solution chamber 206 to the anode water 220 flowing through the anode chamber 202.

[0104] Preferably, the salt solution 216 is aqueous sodium chloride (NaCl), which contains both sodium ions (Na^+) and chloride ions (Cl^-) ions. Positive Na^+ ions migrate from the salt solution 216 to the cathode water 218. Negative Cl^- ions migrate from the salt solution 216 to the anode water 220.

[0105] The sodium ions and chloride ions may undergo further reaction in the anode chamber 202 and cathode chamber 204. For example, chloride ions can react with various oxygen ions and other species (e.g., oxygen containing free radicals, O_2, O_3) present in the anode water 220 to produce ClOn- and ClO^-. Other reactions may also take place in the anode chamber 202 including the formation of oxygen free radicals, hydrogen ions (H^+), oxygen (e.g., as O_2)and, optionally, ozone (O_3) and peroxides. In the cathode chamber 204, hydrogen gas (H_2), hydroxide ions (OH^-), and other radicals, and, optionally, sodium hydroxide (NaOH) may be formed.

[0106] The apparatus for producing the ORP water solution also can be constructed to include at least two three chambered electrolysis cells. Each of the electrolytic cells includes an anode chamber, cathode chamber, and salt solution chamber separating the anode and cathode chambers. The apparatus includes a mixing tank for collecting the anode water produced by the electrolytic cells and a portion of the cathode water produced by one or more of the electrolytic cells. Preferably, the apparatus further includes a salt recirculation system to permit recycling of the salt solution supplied to the salt solution chambers of the electrolytic cells. A diagram of an exemplary process for producing an ORP water solution using two electrolysis cells is shown in FIG. 3.

[0107] The process 300 includes two three-chambered electrolytic cells, specifically a first electrolytic cell 302 and second electrolytic cell 304. Water is transferred, pumped or otherwise dispensed from the water source 305 to anode chamber 306 and cathode chamber 308 of the first electrolytic cell 302 and to anode chamber 310 and cathode chamber 312 of the second electrolytic cell 304. Advantageously, this process can produce from about 1 liter/minute to about 50 liters/minute of ORP water solution. The production capacity may be increased by using additional electrolytic cells. For example, three, four, five, six, seven, eight, nine, ten or more three-chambered electrolytic cells may be used to increase the output of the ORP water solution administered in accordance with the invention.

[0108] The anode water produced in the anode chamber 306 and anode chamber 310 are collected in the mixing tank 314. A portion of the cathode water produced in the cathode chamber 308 and cathode chamber 312 is collected in mixing tank 314 and combined with the anode water. The remaining portion of cathode water produced in the process is discarded. The cathode water may optionally be subjected to gas separator 316 and/or gas separator 318 prior to addition to the mixing tank 314. The gas separators remove gases such as hydrogen gas that are formed in cathode water during the production process.

[0109] The mixing tank 314 may optionally be connected to a recirculation pump 315 to permit homogenous mixing of the anode water and portion of cathode water from electrolysis cells 302 and 304. Further, the mixing tank 314 may optionally include suitable devices for monitoring the level and pH of the ORP water solution. The ORP water solution may be transferred from the mixing tank 314 via pump 317 for application in disinfection or sterilization at or near the location of the mixing tank. Alternatively, the ORP water solution may be dispensed into one or more suitable containers for shipment to a remote site (e.g., warehouse, hospital, etc.).

[0110] The process 300 further includes a salt solution recirculation system to provide the salt solution to salt solution chamber 322 of the first electrolytic cell 302 and the salt solution chamber 324 of the second electrolytic cell 304. The salt solution is prepared in the salt tank 320. The salt is transferred via pump 321 to the salt solution chambers 322 and 324. Preferably, the salt solution flows in series through salt solution chamber 322 first followed by salt solution chamber 324. Alternatively, the salt solution may be pumped to both salt solution chambers simultaneously.

[0111] Before returning to the salt tank 320, the salt solution may flow through a heat exchanger 326 in the mixing tank 314 to control the temperature of the ORP water solution as needed.

[0112] The ions present in the salt solution are depleted over time in the first electrolytic cell 302 and second electrolytic cell 304. An additional source of ions periodically can be added to the mixing tank 320 to replace the ions that are transferred to the anode water and cathode water. The additional source of ions may be used, e.g., to maintain a constant pH of

the salt solution, which can to drop (i.e., become acidic) over time. The source of additional ions may be any suitable compound including, for example, salts such as, e.g., sodium chloride. Preferably, sodium hydroxide is added to the mixing tank 320 to replace the sodium ions (Na^+) that are transferred to the anode water and cathode water.

[0113] Following its preparation, the ORP water solution can be transferred to one or more suitable containers, e.g., a sealed container for distribution and sale to end users such as, e.g., health care facilities including, e.g., hospitals, nursing homes, doctor offices, outpatient surgical centers, dental offices, and the like. Suitable containers can include, e.g., a sealed container that maintains the sterility and stability of the ORP water solution held by the container. The container can be constructed of any material that is compatible with the ORP water solution. Preferably, the container is generally non-reactive with one or more ions or other species present in the ORP water solution.

[0114] Preferably, the container is constructed of plastic or glass. The plastic can be rigid so that the container is capable of being stored on a shelf. Alternatively, the container can be flexible, e.g., a container made of flexible plastic such as, e.g., a flexible bag.

[0115] Suitable plastics can include, e.g., polypropylene, polyester terephthalate (PET), polyolefin, cycloolefin, polycarbonate, ABS resin, polyethylene, polyvinyl chloride, and mixtures thereof. Preferably, the container comprises one or more polyethylenes selected from the group consisting of high-density polyethylene (HDPE), low-density polyethylene (LDPE), and linear low-density polyethylene (LLDPE). Most preferably, the container is constructed of high density polyethylene.

[0116] The container preferably has an opening to permit dispensing of the ORP water solution. The container opening can be sealed in any suitable manner. For example, the container can be sealed with a twist-off cap or stopper. Optionally, the opening can be further sealed with a foil layer.

[0117] The headspace gas of the sealed container can be air or any other suitable gas, which preferably does not react with one or more species in the ORP water solution. Suitable headspace gases can include, e.g., nitrogen, oxygen, and mixtures thereof.

[0118] The ORP water solution administered in accordance with the present invention also can be used for treating or preventing cell-mediated inflammation and inflammation, which results from an autoimmune reaction, including, but not limited to, SLE, autoimmune thyroiditis, sarcoidosis, inflammatory bowel disease, rheumatoid arthritis, and rheumatic fever. The ORP water solution administered in accordance with the present invention can be used for treating or preventing inflammation, which results from infection, e.g., from an infection by one or more microorganisms selected from the group consisting of viruses, bacteria, and fungi, including hypersensitivity and autoimmune-mediated inflammation resulting from infection.

[0119] The ORP water solution administered in accordance with the present invention also can be used for treating or preventing inflammation associated with an upper respiratory condition. When the inflammation is associated with an upper respiratory condition, the ORP water solution is preferably administered to the upper airway, e.g., as a spray, mist, aerosol or steam, so as to contact one or more upper airway tissues affected by the condition. Any suitable method can be employed for delivering the ORP water solution to the upper airway so as to treat or prevent one or more upper respiratory conditions in accordance with the present invention, including one or more routes of administration described herein.

[0120] The ORP water solution administered in accordance with the present invention also can be used for preventing or treating inflammation affecting one or more upper respiratory airway tissues (e.g., nasal tissue as described herein) or lung tissues. Such conditions can include, for example, pharyngitis, asthma, and the like, which are preventable or treatable with the ORP solution administered in accordance with the invention.

[0121] With regard to pharyngitis, it is estimated that worldwide, 1 to 2% of all visits to doctors' offices, clinics and emergency rooms are because of pharyngitis. In the United States and Mexico, pharyngitis and tonsillitis is believed to account for about 15 and 12 million consultations per year, respectively. These cases are typically caused by various bacteria and viruses. Also, pharyngitis and tonsillitis caused by group A β-hemolytic *Streptococcus* can significantly raise the risk of rheumatic fever in poor populations; however it is believed that only 5 to 15% of pharyngitis cases are caused by this bacterium, and that the rest of the acute cases are due to bacteria and viruses of little epidemiological relevance. The latter cases tend to be self-limiting in a few days and do not leave sequelae.

[0122] It has been verified that a great number of doctors worldwide prescribe antibiotics indiscriminately for acute pharyngitis. This occurs in a daily practice, often because patients tend to request powerful antibiotics. Unfortunately, it is difficult to establish an accurate diagnosis of streptococcal pharyngitis/tonsillitis clinically and the cost/benefit ratio of treating acute pharyngitis/tonsillitis with antibiotics is questionable. In some countries, such as Mexico, there is significant expenditure of government resources to cover the cost of antibiotics, and to cover losses associated with working days missed as a result of illness, all of which represent a significant loss with respect to the national budget.

[0123] It is believed that the ORP water solution administered in accordance with the present invention can provide a safe, efficacious and cost-effective adjuvant therapy for the treatment or prevention of acute pharyngitis and/or tonsillitis. The empirical treatment of acute pharyngitis/tonsillitis may begin with administering an ORP water solution in accordance with the present invention, and, depending on evolution or the result of the rapid test for *Streptococcus*, antibiotics may be initiated from 48-72 hours thereafter only if needed. The ORP water solution administered in accordance with the present invention may thus allow the use of antibiotics to be deferred, and, at the same time, reduce the symptomatology

of the patient and accelerate the patient's recovery if the pharyngitis/tonsillitis is not from group A *Streptococcus*. The adjuvant use of the ORP water solution administered in accordance with the present invention with antibiotics for the treatment of streptococcal pharyngitis/tonsillitis also may shorten the period of clinical response and decrease the incidence of recurrences.

[0124] The ORP water solution administered in accordance with the present invention also can be used for treating or preventing inflammation associated with hypersensitivity. Historically, hypersensitivity reactions have been classified as one of four types, from which significant disease can result. The ORP water solution administered in accordance with the invention can be used to treat and/or prevent (e.g., inhibit the onset of, inhibit the escalation of or decrease the likelihood of) one or more of such reactions. Type I hypersensitivity typically results from the combination of an antigen with an antibody bound to a mast cell or basophil (see Kumar *et al.*, Robbins & Cotran Pathologic Basis of Disease, 2004, pp. 193-268, which is hereby incorporated by reference). Type I reactions occur within minutes of exposure to the antigen in individuals who have been previously sensitized to the antigen. In humans, Type I reactions are mediated by IgE which has high affinity Fc receptors on mast cells and basophils.

[0125] Mast cells' role in Type I hypersensitivity is especially important because they reside in tissues under the epithelial surface near blood vessels and nerves. Multiple clinical symptoms observed in atopic dermatitis, allergic rhinitis and atopic asthma are produced by IgE-antigen stimulation of mast cells located in distinct affected tissues. The currently accepted view of the pathogenesis of atopic asthma is that allergens initiate the process by triggering IgE-bearing pulmonary mast cells (MCs) to release mediators such as histamine, leukotrienes, prostaglandins, kininis, platelet activating factor (PAF), etc. in the so-called early phase of the reaction (Kumar *et al.*, pp. 193-268). In turn, these mediators induce bronchoconstriction and enhance vascular permeability and mucus production. According to this model, following mast cell activation, those cells secrete various cytokines, including tumor necrosis factor alpha (TNF-α), IL-4, IL-5 and IL-6, which participate in the local recruitment and activation of other inflammatory cells such as eosinophils, basophils, T lymphocytes, platelets and mononuclear phagocytes. These recruited cells, in turn, contribute to the development of an inflammatory response that may then become autonomous and aggravate the asthmatic symptoms. This late phase response constitutes a long term inflammatory process which will induce changes in surrounding tissues (see Kumar *et al.*, pp. 193-268). Clinically, Type I reactions can have local effects such as allergic rhinitis, or systemic effects as is found in anaphylaxis which manifests with itching, hives, respiratory distress, and circulatory collapse.

[0126] Type II hypersensitivity is mediated by antibodies directed to antigens on the surfaces of cells and in the extracellular space. These antibodies can direct cell lysis or result

in opsonization of the target molecules (preparation for phagocytosis by other cells). Alternatively, the antibodies can be directed to and activate cell surface receptors. Conditions resulting from Type II reactions include transfusion reactions, Graves disease (thyrotoxicosis), drug reactions, pernicious anemia, and acute rheumatic fever. In rheumatic fever the antibodies are formed against Streptococcal antigens but, cross-react with human tissues such as heat valves.

[0127] Type III hypersensitivity is caused by immune complexes, which are combinations of antibodies and other host immune system proteins, most typically complement proteins. It is the normal function of antibodies to bind and active complement. However, when the resulting macromolecular immune complexes are not adequately processed, they can lead to persistent tissue damage. Macrophages and PMNLs can be activated by immune complexes and lead to the release of toxic chemicals by these cells. Immune complex reactions can be local and may result in conditions such as, e.g., the arthus reaction or cause systemic disease such as serum sickness or some of the aspects of systemic lupus erythematous (SLE).

[0128] Type IV hypersensitivity is cell mediated and is sometimes called delayed-type hypersensitivity. Type IV hypersensitivity is mediated by T lymphocytes and often results in the formation of a granulomatous reaction. In a granulomatous reaction, a form of macrophage called an epitheloid cell attempts to, but fails, to digest an antigen. The antigen's persistence leads to the release of cytokines that attract additional lymphocytes resulting in chronic foci of inflammation. The foci have high concentrations of cyotoxic T-lymphocytes which release granzymes and perforins which are toxic to adjacent cells. Type IV hypersensitivity is a prominent component of autoimmune diseases such as, e.g., Sjogrren's Syndrome, Sarcoidosis, and contact dermatitis.

[0129] Pathologic states can combine different types of hypersensitivity reactions. In autoimmune diseases host antigens stimulate hypersensitivity with serious consequences for the host. For example, in SLE host antigens induce Type II reactions against blood cells while Type III reactions lead to blood vessel and renal glomerular damage. In addition, hypersensitivity reactions are also seen in iatragenic conditions such as drug reactions and transplant rejection. Transplant rejection includes components of Type II and Type IV hypersensivity.

[0130] The ORP water solution administered in accordance with the invention also can be used for the prevention or treatment of an infection, e.g., by one or more infectious pathogens such as, for example, infectious microorganisms. Such microorganisms can include, for example, viruses, bacteria, and fungi. The viruses can include, e.g., one or more viruses selected from the group consisting of adenoviruses, herpes viruses, coxsackie_viruses, HIV, rhinoviruses, coronaviruses, and flu viruses. The bacteria can include, e.g., one or more bacteria selected from the group consisting of *Escherichia coli*, *Pseudomonas aeruginosa*,

Staphylococcus aureus, and *Mycobaterium tuberculosis*. The fungi can include, e.g., one or more fungi selected from the group consisting of *Candida albicans*, *Bacillus subtilis* and *Bacillus athrophaeus*.

[0131] The ORP water solution administered in accordance with the invention also can be effective against adenovirus. The ORP water solution administered in accordance with the invention preferably achieves a log-10 reduction in the adenoviral load of greater than about 2, more preferably greater than about 2.5, and still more preferably greater than about 3, after exposure to the ORP water solution for about 20 minutes, more preferably after exposure for about 15 minutes, and still more preferably after exposure for about 10 minutes. The ORP water solution administered in accordance with the invention also can be effective for reducing the viral load of HIV-1, preferably by a log reduction factor greater than about 2, more preferably by a log reduction factor of greater than about 2.5, and still more preferably by a log reduction factor of greater than about 3 after exposure to the ORP water solution for about 15 minutes, more preferably after exposure for about ten minutes, still more preferably after exposure for about five minutes.

[0132] In accordance with the method of the present invention, administering the ORP water solution for the prevention or treatment of infection also can serve to prevent or treat sinusitis associated with the infection (or the affected tissues) as described herein.

[0133] The ORP water solution administered in accordance with the invention also can be used for treating impaired or damaged tissue, e.g., by contacting one or more impaired or damaged tissues with a therapeutically effective amount of the ORP water solution. Any suitable method can be used for contacting the impaired or damaged tissue, so as to treat the impaired or damaged tissue. For example, the impaired or damaged tissue can be treated by irrigating the tissue with the ORP water solution, so as to contact the impaired or damaged tissue with a therapeutically effective amount of the ORP water solution. The ORP water solution can be administered as a steam or a spray, or by aerosolization, nebulization or atomization as described herein, so as to contact the impaired or damaged tissue with a therapeutically effective amount of the ORP water solution.

[0134] The ORP water solution administered in accordance with the invention can be used for treating tissues, which have been impaired or damaged, e.g., by surgery. For instance, the ORP water solution can be used for treating tissues, which have been impaired or damaged by an incision. In addition, the ORP water solution can be used for treating tissues, which have been impaired or damaged by oral surgery, graft surgery, implant surgery, transplant surgery, cauterization, amputation, radiation, chemotherapy, and combinations thereof. The oral surgery can include, for example, dental surgery such as, e.g., root canal surgery, tooth extraction, gum surgery, and the like.

[0135] The ORP water solution administered in accordance with the invention can be used for treating tissues, which have been impaired or damaged by one or more burns, cuts,

abrasions, scrapes, rashes, ulcers, puncture wounds, combinations thereof, and the like, which are not necessarily caused by surgery. The ORP water solution administered in accordance with the invention can be used for treating impaired or damaged tissue, which is infected, or tissue impaired or damaged due to infection. Such infection can be caused by one or more infectious pathogens, such as, e.g., one or more microorganisms selected from the group consisting of viruses, bacteria, and fungi, as described herein.

[0136] In accordance with the present invention, administering the ORP water solution for treating impaired or damaged tissue also can serve to prevent or treat sinusitis associated with the impairment or damage (or with the impaired or damaged tissue) .

[0137] The ORP water solution administered in accordance with the invention also can be used as a disinfectant to eradicate microorganisms, including bacteria, viruses and spores, in a variety of settings, e.g., in the healthcare and medical device fields, to disinfect surfaces and medical equipment, and also can be applied in wound care, medical device sterilization, food sterilization, hand disinfection in medical personnel, hospitals, consumer households and anti-bioterrorism. The ORP water solution can be used for disinfecting a surface, e.g., by contacting the surface with an anti-infective amount of the ORP water solution. The surface can be contacted using any suitable method. For example, the surface can be contacted by irrigating the surface with the ORP water solution, so as to disinfect the surface. Additionally, the surface can be contacted by applying the ORP water solution to the surface as a steam or a spray, or by aerosolization, nebulization or atomization, as described herein, so as to disinfect the surface. Further, the ORP water solution can be applied to the surface with a cleaning wipe, as described herein. By disinfecting a surface, the surface may be cleansed of infectious microorganisms. Alternatively (or additionally), the ORP water solution administered in accordance with the present invention can be applied to the surface to provide a barrier to infection, to thereby disinfect the surface.

[0138] The surface(s) can include one or more biological surfaces, one or more inanimate surfaces, and combinations thereof. Biological surfaces can include, for example, tissues within one or more body cavities such as, for example, the oral cavity, the sinus cavity, the cranial cavity, the abdominal cavity, and the thoracic cavity. Tissues within the oral cavity include, e.g., mouth tissue, gum tissue, tongue tissue, and throat tissue. The biological tissue also can include muscle tissue, bone tissue, organ tissue, mucosal tissue, vascular tissue, neurological tissue, and combinations thereof. Inanimate surfaces include, for example, surgically implantable devices, prosthetic devices, and medical devices. In accordance with the method of the present invention, the surfaces of internal organs, viscera, muscle, and the like, which may be exposed during surgery, can be disinfected, e.g., to maintain sterility of the surgical environment.

[0139] The ORP water solution may also be applied to humans and/or animals to treat various conditions, including inflammation, associated with one or more of the following:

surgical/open wound cleansing agent; skin pathogen disinfection (e.g., for bacteria, mycoplasmas, virus, fungi, prions); battle wound disinfection; wound healing promotion; burn healing promotion; treatment of stomach ulcers; wound irrigation; skin fungi; psoriasis; athlete's foot; pinkeye and other eye infections; ear infections (e.g., swimmer's ear); lung/nasal/sinus infections; and other medical applications on or in the human or animal body. The use of ORP water solutions as a tissue cell growth promoter is further described in U.S. Patent Application Publication 2002/0160053 (hereby incorporated by reference).

[0140] Organisms that can be controlled, reduced, killed or eradicated by treatment with the ORP water solution used in accordance with the invention include, e.g., *Pseudomonas aeruginosa*, *Escherichia coli, Enterococcus hirae*, *Acinetobacter baumannii*, *Acinetobacter* species, *Bacteroides fragilis*, *Enterobacter aerogenes*, *Enterococcus faecalis*, Vancomycin resistant-*Enterococcus faecium* (VRE, MDR), Vancomycin resistant-*Escherichia coli, Haemophilus influenzae*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Proteus mirabilis*, *Serratia marcescens*, *Staphylococcus aureus, Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis, Staphylococcus saprophyticus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Salmonella choleraesuis*, *Shigella dysenteriae*, and other susceptible bacteria, as well as yeasts, e.g., *Trichophyton mentagrophytes, Candida albicans* and *Candida tropicalis*. The ORP water solution can also be used in accordance with the invention to controll, reduce, kill or eradicate viruses including, for example, adenovirus, human immunodeficiency virus (HIV), rhinovirus, influenza (e.g., influenza A), hepatitis (e.g., hepatitis A), coronavirus (responsible for Severe Acute Respiratory Syndrome (SARS)), rotavirus, respiratory syncytial virus, herpes simplex virus, varicella zoster virus, rubella virus, and other susceptible viruses.

[0141] The ORP water solution used in accordance with the invention also can be used in controlling the activity of allergens present in the environment. In this context, allergens typically include any substance other than bacteria, fungi, yeasts, or viruses that can trigger an adverse immune response, or allergy, in susceptible people or animals. Asthma is a common physiological response following exposure to one or more of such allergens. Allergens can be either viable (i.e., from living or dead organisms) or non-viable (e.g., non-living such as textiles), and may be present in the environment, for example, in households and/or workplaces.

[0142] Protein-based household allergens that may be treated with the ORP water solution can include, for example, animal fur, skin, and feces, household dust, weeds, grasses, trees, mites, and pollens. Animal allergens can include, for example, cat epithelium, dog epithelium, horse dander, cow dander, dog dander, guinea pig epithelium, goose feathers, mouse epithelium, mouse urine, rat epithelium and rat urine.

[0143] Occupational allergens can include, for example, high-molecular-weight agents, such as natural proteins generally derived from plant or animal proteins, and low-molecular-

weight chemicals, such as diisocyanates, and other material found in some textiles. Other chemical allergens that may be present in the workplace can include, for example, anhydrides, antibiotics, wood dust and dyes. Numerous proteins may be occupational allergens including vegetable gums, enzymes, animal proteins, insects, plant proteins, and legumes.

[0144] Additional allergens that can be treated by the ORP water solution are described in Korenblat and Wedner, Allergy Theory and Practice (1992) and Middleton, Jr., Allergy Principles and Practice (1993).

[0145] The ORP water solution may be applied to disinfect and sterilize in any suitable manner. For example, to disinfect and sterilize medical or dental equipment, the equipment can be maintained in contact with the ORP water solution for a sufficient period of time to reduce the level of organisms present on the equipment to a desired level.

[0146] For disinfection and sterilization of hard surfaces, the ORP water solution can be applied to the hard surface directly from a container in which the ORP water solution is stored. For example, the ORP water solution can be poured, sprayed or otherwise directly applied to the hard surface. The ORP water solution can then be distributed over the hard surface using a suitable substrate such as, for example, cloth, fabric or paper towel. In hospital applications, the substrate is preferably sterile. Alternatively, the ORP water solution can first be applied to a substrate such as cloth, fabric or paper towel. The wetted substrate can then be contacted with the hard surface. Alternatively, the ORP water solution can be applied to hard surfaces by dispersing the solution into the air as described herein. The ORP water solution can be applied in a similar manner to humans and animals.

[0147] The ORP water solution also can be applied with a cleaning wipe comprising a water insoluble substrate and the ORP water solution as described herein, wherein the ORP water solution is dispensed onto the substrate. The ORP water solution can be impregnated, coated, covered or otherwise applied to the substrate. Preferably, the substrate is pretreated with the ORP water solution before distribution of the cleaning wipes to end users.

[0148] The substrate for the cleaning wipe can be any suitable water-insoluble absorbent or adsorbent material. A wide variety of materials can be used as the substrate. It should have sufficient wet strength, abrasivity, loft and porosity. Further, the substrate should not adversely impact the stability of the ORP water solution. Examples include non woven substrates, woven substrates, hydroentangled substrates and sponges.

[0149] The substrate can have one or more layers. Each layer can have the same or different textures and abrasiveness. Differing textures can result from the use of different combinations of materials or from the use of different manufacturing processes or a combination thereof. The substrate should not dissolve or break apart in water. The substrate can thereby provide a vehicle for delivering the ORP water solution to the surface to be treated.

[0150] The substrate can be a single nonwoven sheet or multiple nonwoven sheets. The nonwoven sheet can be made of wood pulp, synthetic fibers, natural fibers, and blends thereof. Suitable synthetic fibers for use in the substrate can include, without limitation, polyester, rayon, nylon, polypropylene, polyethylene, other cellulose polymers, and mixtures of such fibers. The nonwovens can include nonwoven fibrous sheet materials which include meltblown, coform, air-laid, spun bond, wet laid, bonded-carded web materials, hydroentangled (also known as spunlaced) materials, and combinations thereof. These materials can comprise synthetic or natural fibers or combinations thereof. A binder can optionally be present in the substrate.

[0151] Examples of suitable nonwoven, water insoluble substrates include 100% cellulose Wadding Grade 1804 from Little Rapids Corporation, 100% polypropylene needlepunch material NB 701-2.8-W/R from American Non-wovens Corporation, a blend of cellulosic and synthetic fibres-Hydraspun 8579 from Ahlstrom Fibre Composites, and 70% Viscose/30% PES Code 9881 from PGI Nonwovens Polymer Corp. Additional examples of nonwoven substrates suitable for use in the cleaning wipes are described in U.S. Pat. Nos. 4,781,974, 4,615,937, 4,666,621, and 5,908,707, and International Patent Application Publications WO 98/03713, WO 97/40814, and WO 96/14835 (which are hereby incorporated by reference).

[0152] The substrate also can be made of woven materials, such as cotton fibers, cotton/nylon blends, or other textiles. Regenerated cellulose, polyurethane foams, and the like, which are used in making sponges, also can be suitable for use.

[0153] The liquid loading capacity of the substrate should be at least about 50%-1000% of the dry weight thereof, most preferably at least about 200%-800%. This is expressed as loading 1/2 to 10 times the weight of the substrate. The weight of the substrate varies without limitation from about 0.01 to about 1,000 grams per square meter, most preferably 25 to 120 grams/m^2 (referred to as "basis weight") and typically is produced as a sheet or web which is cut, die-cut, or otherwise sized into the appropriate shape and size. The cleaning wipes will preferably have a certain wet tensile strength which is without limitation about 25 to about 250 Newtons/m, more preferably about 75-170 Newtons/m.

[0154] The ORP water solution can be dispensed, impregnated, coated, covered or otherwise applied to the substrate by any suitable method. For example, individual portions of substrate can be treated with a discrete amount of the ORP water solution. Preferably, a mass treatment of a continuous web of substrate material with the ORP water solution is carried out. The entire web of substrate material can be soaked in the ORP water solution. Alternatively, as the substrate web is spooled, or even during creation of a nonwoven substrate, the ORP water solution can be sprayed or metered onto the web. A stack of individually cut and sized portions of substrate can be impregnated or coated with the ORP water solution in its container by the manufacturer.

[0155] The cleaning wipes optionally can contain additional components to improve the properties of the wipes. For example, the cleaning wipes can further comprise polymers, surfactants, polysaccharides, polycarboxylates, polyvinyl alcohols, solvents, chelating agents, buffers, thickeners, dyes, colorants, fragrances, and mixtures thereof to improve the properties of the wipes. These optional components should not adversely impact the stability of the ORP water solution. Examples of various components that may optionally be included in the cleaning wipes are described in U.S. Patents 6,340,663, 6,649,584 and 6,624,135 (which are hereby incorporated by reference).

[0156] The cleaning wipes can be individually sealed with a heat-sealable or glueable thermoplastic overwrap (such as polyethylene, Mylar, and the like). The wipes can also be packaged as numerous, individual sheets for more economical dispensing. The cleaning wipes can be prepared by first placing multiple sheets of the substrate in a dispenser and then contacting the substrate sheets with the ORP water solution administered in accordance with the invention. Alternatively, the cleaning wipes can be formed as a continuous web by applying the ORP water solution to the substrate during the manufacturing process and then loading the wetted substrate into a dispenser.

[0157] The dispenser includes, but is not limited to, a canister with a closure, or a tub with closure. The closure on the dispenser can be employed to seal the moist wipes from the external environment and to prevent premature volatilization of the liquid ingredients.

[0158] The dispenser can be made of any suitable material that is compatible with both the substrate and the ORP water solution. For example, the dispenser can be made of plastic, such as high density polyethylene, polypropylene, polycarbonate, polyethylene terephthalate (PET), polyvinyl chloride (PVC), or other rigid plastics.

[0159] The continuous web of wipes can be threaded through a thin opening in the top of the dispenser, most preferably, through the closure. A means of sizing the desired length or size of the wipe from the web can then be desirable. A knife blade, serrated edge, or other means of cutting the web to desired size can be provided on the top of the dispenser, for non-limiting example, with the thin opening actually doubling in duty as a cutting edge. Alternatively, the continuous web of wipes can be scored, folded, segmented, perforated or partially cut into uniform or non-uniform sizes or lengths, which would then obviate the need for a sharp cutting edge. Further, the wipes can be interleaved, so that the removal of one wipe advances the next.

[0160] The ORP water solution administered in accordance with the invention alternatively can be dispersed into the environment through a gaseous medium, such as air. The ORP water solution can be dispersed into the air by any suitable means. For example, the ORP water solution can be formed into droplets of any suitable size and dispersed into a room.

[0161] For small scale applications, the ORP water solution can be dispensed through a spray bottle that includes a standpipe and pump. Alternatively, the ORP water solution can be packaged in aerosol containers. Aerosol containers can include the product to be dispensed, propellant, container, and valve. The valve can include both an actuator and dip tube. The contents of the container can be dispensed by pressing down on the actuator. The various components of the aerosol container should be compatible with the ORP water solution. Suitable propellants can include a liquefied halocarbon, hydrocarbon, or halocarbon-hydrocarbon blend, or a compressed gas such as carbon dioxide, nitrogen, or nitrous oxide. Aerosol systems preferably yield droplets that range in size from about 0.15 μm to about 5 μm.

[0162] For some applications, the ORP water solution optionally can contain a bleaching agent. The bleaching agent can include, e.g., any suitable compound that lightens or whitens a substrate. The ORP water solution containing a bleaching agent can be used in home laundering to disinfect and sterilize bacteria and germs as well as brighten clothing. Suitable bleaching agents include, but are not limited to, chlorine-containing bleaching agents and, optionally, peroxide-containing bleaching agents. Mixtures of bleaching agents also can be added to the ORP water solution. Preferably, the bleaching agent is added in the form of an aqueous solution to the ORP water solution.

[0163] Suitable chlorine-containing bleaching agents can include, e.g., chlorine, hypochlorites, N-chloro compounds, and, optionally, chlorine dioxide. Preferably, the chlorine-containing bleaching agent added to the ORP water solution is sodium hypochlorite or hypochlorous acid. Other suitable chlorine-containing bleaching agents include, e.g., chlorine, calcium hypochlorite, bleach liquor (e.g., aqueous solution of calcium hypochlorite and calcium chloride), bleaching powder (e.g., mixture of calcium hypochlorite, calcium hydroxide, calcium chloride, and hydrates thereof), dibasic magnesium hypochlorite, lithium hypochlorite, chlorinated trisodium phosphate and mixtures thereof.

[0164] The addition of a bleaching agent to the ORP water solution can be carried out in any suitable manner. Preferably, an aqueous solution containing the bleaching agent is first prepared. The aqueous solution containing the bleaching agent can be prepared using household bleach (e.g., Clorox® bleach) or other suitable source of chlorine-containing bleaching agent or other bleaching agent. The bleaching agent solution can then be combined with the ORP water solution.

[0165] The bleaching agent can be added to the ORP water solution in any suitable amount. Preferably, the ORP water solution containing a bleaching agent is non-irritating to human or animal skin. Preferably, the total chloride ion content of the ORP water solution containing a chlorine-containing bleaching agent is from about 1000 ppm to about 5000 ppm, and preferably from about 1000 ppm to about 3000 ppm. The pH of the ORP water solution

containing a chlorine-containing bleaching agent is preferably from about 8 to about 10, and the oxidative-reductive potential is preferably from about +700 mV to about +800 mV.

[0166] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting in its scope.

EXAMPLES 1-3

[0167] These examples demonstrate the unique features of the ORP water solution used in accordance with the invention. The samples of the ORP water solution in Examples 1-3 were analyzed in accordance with the methods described herein to determine the physical properties and levels of ionic and other chemical species present in each sample. Results obtained for chlorine dioxide, ozone and hydrogen peroxide are based on standard tests used to measure such species but may be indicative of different species, which can also generate positive test results. Further, it has been reported that chlorine dioxide, ozone and hydrogen peroxide react with hypocholrite resulting in their consumption and the production of other compounds (e.g., HCl and O_2.) The pH, oxidative-reductive potential (ORP) and ionic species present are set forth in Table 1 for each sample of the ORP water solution.

Table 1: Physical characteristics and ion species present for the ORP water solution samples

	EXAMPLE 1	EXAMPLE 2	EXAMPLE 3
pH	7.45	7.44	7.45
ORP (mV)	+879	+881	+874
Total Cl^- (ppm)	110	110	120
Bound Cl^- (ppm)	5	6	6

[0168] The ORP water solution has suitable physical characteristics for use in, e.g., disinfection, sterilization, cleaning, and/or the prevention and/or treatment of inflammation, sinusitis, peritonitis, or infection.

EXAMPLES 4-10

[0169] These examples demonstrate the addition of a bleaching agent to the ORP water solution according to the invention in various amounts. In particular, these examples demonstrate the antimicrobial activity and fabric bleaching ability of the compositions.

[0170] [0185] A 10% Clorox® bleach solution was prepared using distilled water. The following solutions were then prepared using the 10% bleach solution: 80% ORP water solution/20% bleach (Example 4); 60% ORP water solution/40% bleach (Example 5); 40% ORP water solution/60% bleach (Example 6); 20% ORP water solution/80% bleach (Example 7); and 0% ORP water solution/100% bleach (Example 8). Two control solutions were also used for comparison including 100% ORP water solution/0% bleach (Example 9)

and an ORP water solution with 0.01% Tween 20 detergent (Example 10). The physical characteristics of these samples were determined, specifically pH, oxidative-reductive potential (ORP), total chlorine (Cl-) content, and hypochlorous acid (HClO) content, and were tested for chlorine dioxide content and peroxide content, the results of which are set forth in Table 2.

Table 2: Physical characteristics of ORP water solution/bleach compositions

	pH	ORP	Total Cl^- (ppm)	$HClO^-$ (ppm)
Ex. 4	8.92	+789	1248	62
Ex. 5	9.20	+782	2610	104
Ex. 6	9.69	+743	4006	80
Ex. 7	9.86	+730	4800	48
Ex. 8	9.80	+737	5000	50
Ex. 9	7.06	+901	64	32
Ex. 10	6.86	+914	51	26

[0171] The large bolus of chlorine ions added as part of the bleaching agent prevented the accurate measurement of the chlorine dioxide and peroxide levels as indicated with the n.d. designations. Also, results obtained for chlorine dioxide and peroxide are based on standard tests used to measure such species but may be indicative of different species, which can also generate positive test results. Further, it has been reported that chlorine dioxide, ozone and hydrogen peroxide react with hypocholrite resulting in their consumption and the production of other compounds (e.g., HCl and O_2.) As these examples demonstrate, the hypochlorous acid levels of the ORP water solution with and without the addition of a bleaching agent are similar.

[0172] The samples of Examples 4-10 were subjected to a high spore count test using *Bacillus subtilis* var. *niger* spores (ATCC #9372 obtained from SPS Medical of Rush, New York). Spore suspensions were concentrated (by evaporation in a sterile hood) to 4 x 10^6 spores per 100 microliters. A 100 microliter sample of the spore suspension were mixed with 900 microliters of each of the samples in Examples 4-10. The samples were incubated at room temperature for periods of 1 to 5 minutes as set forth in Table 3. At the indicated times, 100 microliters of the incubated samples were plated onto individual TSA plates and incubated for 24 hours at 35 °C ± 2 °C, after which the number of resulting colonies on each plate was determined. The control plates demonstrated that the starting spore concentrations were > 1 x 10^6 spores/100 microliters. The concentration of Bacillus spores for the various samples at the various incubation times (as the average of two determinations) is set forth in Table 3.

Table 3: Bacillus spore concentrations

	1 minute	2 minutes	3 minutes	4 minutes	5 minutes
Ex. 4	>> 1000	411	1	0	2
Ex. 5	>> 1000	1000	1	0	0
Ex. 6	>> 1000	>> 1000	> 1000	22	0
Ex. 7	>> 1000	>> 1000	> 1000	15	0
Ex. 8	>> 1000	>> 1000	> 1000	3	1
Ex. 9	>> 1000	74	0	0	0
Ex 10	>> 1000	239	3	0	0

[0173] As these results demonstrate, as the concentration of bleach (as 10% aqueous bleach solution) increases, the amount of *Bacillus* spores killed is reduced for the samples incubated for 2-3 minutes. However, for samples incubated for 5 minutes, the bleach concentration does not impact *Bacillus* spore kill. Further, the results demonstrate that the addition of 0.01% detergent to the ORP water solution does not reduce spore kill.

[0174] The samples of Examples 4-10 were subjected to a fabric bleaching test. The fabric upon which the samples were tested was a 100% rayon children's t-shirt with dark blue dye patches. Two inch square pieces of dyed fabric were placed into 50 mL plastic tubes. Each fabric piece was covered by a sample of the solution in Examples 4-10. The elapsed time until complete bleaching was obtained, as determined by the whitening of the fabric, is set forth in Table 4.

Table 4: Time until complete bleaching of fabric sample

Example	Time
Ex. 4	39 minutes
Ex. 5	23 minutes
Ex. 6	18 minutes
Ex. 7	19 minutes
Ex. 8	10 minutes
Ex. 9	> 6 hours
Ex. 10	> 6 hours

[0175] As demonstrated by these examples, as the concentration of the ORP water solution increases in the composition, the time until complete bleaching is achieved increases.

EXAMPLE 11

[0176] The purpose of this study was to assess the safety of the test an exemplary ORP water solution, Microcyn, when administered as drops into the nasal cavity of rabbits. Thirty-three rabbits were randomly assigned to two groups, Groups I and II. Group I (18 animals) served as the control group and Group II (15 animals) was dosed with the test article. On Day -1 or Day 0, body weights were recorded and blood samples were, collected for analysis of selected parameters. On Day 0, 500 μL of sterile saline was administered to the Group I animals and 500 μL of the test article (at a 50% concentration) was administered to Group n annuals. Both the control and the test articles were administered twice daily as drops into the right nostril. The animals were dosed in the same manner on Days 1-6. Animals were observed daily for signs of pharmacologic and/or toxicologic effects with special attention paid to the nose. Body weights were recorded weekly through study termination. On Day 7, one-third of the animals from each group were selected for blood collection, sacrifice and necropsy. The remaining animals continued to be dosed through Day 14, when half of the animals from each group were selected for blood collection, sacrifice and necropsy. On Day 21, after a 7-day recovery period), the remaining animals had blood collected and were sacrificed and necropsied. Samples of the nasal mucosa from both nostrils were collected from each animal for histopathological analysis.

[0177] The necropsy consisted of gross observations of the respiratory tract. The entire nasal passage and associated bone were taken and fixed in buffered formalin. Samples of any visible abnormalities in the respiratory tract were also collected for histopathology. Three biopsy samples (anterior, middle and posterior nasal cavity) per nostril (treated right and untreated left) were examined. The microscopic histopathology of the nasal mucosa included: integrity of epithelium, presence or loss of epithelial cilia, inflammatory cell infiltration, edema, presence of goblet cells, hyperplasia of glands, changes in number or characteristics of blood vessels and any other changes or observations.

[0178] The results (in-life observations including nasal observations, body weights, blood analysis, gross necropsy and histopathology results) from the test group were compared to the control group. The test group was not significantly different from animals treated with saline in terms of mild irritation.

EXAMPLE 12

[0179] This example illustrates a clinical study, which can be used to determine the effectiveness of an exemplary ORP water solution for treating pharyngitis.

[0180] One such ORP water solution for use in this study is known as "Microcyn 60," recently introduced on the Mexican market as an antiseptic. Microcyn 60 is a superoxidized solution of neutral pH with germicidal, sterilizing and wound antiseptic activity in accordance with certifications obtained from the Secretariat of Health of Mexico. Microcyn 60 is prepared from pure water and salt (NaCl), has a small concentration of sodium (<55 ppm) and chlorine (<80 ppm), a pH in the range of 7.2 to 7.8, and oxidation-reduction

potential in the range of 840 mV to 960 mV. Microcyn 60 is produced in one concentration only, and need not be activated or diluted.

[0181] This solution is produced from water obtained by reverse osmosis, which is then subjected to an electrochemical gradient generated by high voltage and sodium chloride. In this way, the reactive species that form in the multiple chambers where the electrochemical gradient is generated are selected in a controlled way to create Microcyn 60. The result is a solution with a controlled content of free radicals that confer a high oxidation-reduction potential (+840 mV to +960 mV) and consequently high antimicrobial activity.

[0182] Hypochlorous acid and sodium hypochlorite are believed to be among the most abundant elements contained in Microcyn 60, with others in minor concentration, such as, e.g., chloride ions among others. Although applicants do not wish to be bound by a particular theory, it is believed that the disinfectant effect does not necessarily depend exclusively on the quantity of chlorine, but also may depend on reactive species of oxygen and/or oxygen or one or more precursors thereof. Also, and in contrast to other superoxidized solutions that have been reported in the literature, Microcyn 60 has a neutral pH (6.4-7.8), is not corrosive and is stable in storage up to 2 years. All these characteristics have made it possible to produce a superoxidized solution that is effective as a high-level disinfectant and compatible for use both on inanimate and biological surfaces (e.g., tissues).

[0183] Accelerated stability tests have demonstrated that Microcyn 60 can be stored in widely varying temperature conditions, from 4 to 65°C, without losing its disinfectant activity for a period of 2 years. Microcyn 60 can be stored and distributed even under relatively harsh conditions without losing its antimicrobial. By contrast, due to lack of stability, conventional solutions have had to be produced by specialized and costly equipment at or near the point of use e.g., the hospital, in order to use the solutions for the intended purposes.

[0184] Because Microcyn 60 is produced in only one concentration, the dose of Microcyn 60 can be changed only by changes in the volume applied per unit area of the skin. In the toxicological studies, the doses of Microcyn 60 applied topically to the intact skin varied from about 0.05 to about 0.07 mL/cm^2; in the study of acute dermatological toxicity and in the investigation of skin irritation, Microcyn 60 can be applied in doses of up to 8.0 mL/cm^2, and in those that investigated its application in deep wounds applied Microcyn 60 in a dose of about 0.09 mL/cm^2.

[0185] Toxicological studies were carried in which Microcyn 60 was applied topically to the intact skin, using a single application with exposure of 4 to 24 h. Multiple applications of Microcyn 60, one or two times a day, during a period of 7 days were assessed for deep wounds in rats.

[0186] Two studies were carried out on the intact skin of rabbits to evaluate the effect of Microcyn 60 as to acute irritation and dermal toxicity. No clinical signs, dermal irritation, or

abnormalities in the skin at autopsy were found in any of the animals exposed to Microcyn 60.

[0187] The characterization of local and systemic toxicity from topically applied Microcyn 60 to a deep wound was evaluated in rats. No abnormalities, significant differences in the parameters of the blood chemistry or hematic cytology were observed, nor anomalies in the autopsies. The skin irritation gradings and the histopathology of the wounds and the tissues around the place of application did not reveal any difference between the wounds treated with Microcyn 60 and those of the control group treated with saline solution.

[0188] The systemic toxicity of Microcyn 60 was also evaluated by means of an intraperitoneal injection in mice. For this, five mice were injected with a single dose (50 mL/kg) of Microcyn 60 by the intraperitoneal route. In the same way, five control mice were injected with a single dose (50 mL/kg) of saline solution (sodium chloride at 0.9%). In this investigation, neither mortality nor any evidence of systemic toxicity was observed in any of the animals that received the single intraperitoneal dose of Microcyn 60, indicating that the LD_{50} is above 50 mL/kg.

[0189] Microcyn 60 was administered by the oral route to rats to allow its absorption and to characterize any inherent toxic effect of the product. In this study, a single dose (4.98 mL/kg) was administered by esophageal tube to three albino rats of the Sprague-Dawley strain. There was no mortality, nor were there clinical signs or abnormalities in the autopsies of any of the animals exposed to the single oral dose of Microcyn 60.

[0190] The potential of topically applied Microcyn 60 for ocular irritation was also evaluated in rabbits. Ocular irritation was not observed nor any other clinical sign in any animal exposed to Microcyn 60 by topical administration through the ocular route.

[0191] Microcyn 60 was applied by the inhalatory route to rats to determine potential acute toxicity by inhalation. All the animals showed a very slight or slight reduction in activity and piloerection after the exposure, but they were all asymptomatic on the following day. Mortality or abnormalities were not observed at autopsy of the animals exposed to Microcyn 60 by inhalation.

[0192] Evaluation of the potential for sensitization of the skin with Microcyn 60 was carried out in guinea pigs using a modified occlusion patch method (Buehler). Irritation was not observed in the animals of the control group after a simple treatment challenge, nor in the animals evaluated (treated by induction) after challenge with the treatment. These studies demonstrate that Microcyn 60 does not provoke a sensitizing reaction.

[0193] Thus, when it has been applied to the intact skin, deep open dermal wounds, in the conjunctival sac, by oral and inhalation routes or by means of intraperitoneal injection, Microcyn 60 has not shown adverse effects related to the product. There is also experience in having treated more than 500 patients with wounds of very diverse nature in the skin and

mucosae, with excellent antiseptic and cosmetic results. Accordingly, topically applied Microcyn 60 should be effective and well-tolerated in this clinical trial.

[0194] Microcyn 60 is packaged in transparent 240 mL PET sealed bottles. This product is stored at ambient temperature and remains stable for up to 2 years in such bottles. From its profile of high biological safety, Microcyn 60 can be safely disposed of, e.g., emptied into the sink without risk of contamination or corrosion.

[0195] Multiple microbial trials have been run with Microcyn 60, both in the United States and in Mexico. Eradication of more than 90% of the bacteria occurs in the first few seconds of exposure. The antibacterial and antimycotic activity that Microcyn 60 exhibits in accordance with this standard is summarized in Table 5.

Table 5. Kill Times.

Bacterium	Catalog	Time of action (reduction below 99.999%)
Ps. aeruginosa	ATCC 25619	1 min
St. aureus	ATCC 6538	1 min
E. coli	ATCC 11229	1 min
S. typhi	CDC 99	1 min
C. albicans	ATCC	1 min
B. subtilis	9372	
Low spore (10^4)		10 min
High spore (10^6)		15 min

[0196] The sporicidal activity trial was carried out in accordance with the PAHO [Pan-American Health Organization]/WHO protocol.

[0197] The virucidal activity of Microcyn 60 has recently been confirmed in studies carried out in the United States against HIV and polio virus, and its activity against *Listeria monocytogenes*, MRSA and *Mycobacterium tuberculosis* has also been documented. Thus, it has been demonstrated that Microcyn 60, when it is administered as recommended, can eradicate bacteria, fungi, viruses and spores from one to fifteen minutes of exposure.

[0198] In this clinical study, 40 patients with acute pharyngitis/tonsillitis caused by group A β-hemolytic *Streptococcus* and who have not received treatment are recruited. The inclusion criteria are as follows: age 12 to 40 years and two or more of the following symptoms: oropharyngeal burning; pain on swallowing; pharyngeal erythema or of the tonsils (with or without exudate); cervical lymphadenopathy; and positive immunoassay for group A *Streptococcus* antigen (StrepA Test-Abbott Labs). The exclusion criteria are as follows: fever >38°C; bronchospasm (excluded by the clinic); severe cough; sinusitis-rhinitis (excluded by the clinic); esophageal reflux (excluded by the clinic); use of antibiotics in the

two weeks prior to the study; patients who have taken part in another clinical study in the last 8 weeks; rheumatic fever; poststreptococcal glomerulonephritis; severe chronic cardiopathy; severe renal, hepatic or pulmonary insufficiencies; and pregnancy or lactation.

[0199] At the beginning of the study, patients may use such concomitant medicines as antipyretics and analgesics, including paracetamol and acetylsalicylics but not anti-inflammatories such as ibuprofen, Mesulid, COX-2 inhibitors, or steroids. Written informed consent must be obtained before the patient submits to any specific procedure of the study.

[0200] The patients are evaluated in three visits. In the first visit, the patient clinically presents acute pharyngitis/tonsillitis, and the clinical history is taken, and a medical examination, rapid immunoassay for *Streptococcus*, and taking of a pharyngeal exudate is carried out. After being declared eligible and after having signed the letter of informed consent, the patient is prescribed two oropharyngeal cleansings of 30 sec and 5 mL Microcyn 60 each. These rinsings are done every 3 h for a total of four times a day for 3 days.

[0201] The second is made 72 h after having been treated with Microcyn 60. In the second visit, the clinical evolution and side effects of Microcyn 60 are evaluated. A new pharyngeal exudate is taken, and it will be decided, in accordance with the clinical evolution, if the continuing treatment will be with antibiotics or a palliative. A third visit is done after 10 days to discharge the patient.

[0202] To be eligible and clinically evaluated in this study, each patient must present A β-hemolytic *Streptococcus* pharyngitis/tonsillitis confirmed by culture. All the patients must comply with 18 rinsings of 30 sec and 5 mL of Microcyn 60 each, or a maximum of 24 rinsings in the space of 72 h.

[0203] The primary parameter of efficacy is a reduction by 3 orders of magnitude in the bacterial load of the initial culture compared to the culture taken after the administration of Microcyn 60. This bacteriological evaluation is realized 72 h after treatment with Microcyn 60. Secondary parameters of efficacy are the improvement reported clinically, with particular emphasis on the reduction of pharyngeal pain and dysphagia. Clinical symptoms are reported in visits 1, 2 and 3.

[0204] Tolerance is evaluated by reports of adverse events. An adverse event is defined as any symptomatic declaration of the patient who submits to the treatment with Microcyn 60, related or not to the antiseptic, that appears in the course of the treatment.

[0205] The results of bacteriological efficacy (the principal criterion of efficacy) are issued by a bacteriologist independently of the clinical symptoms. The tests for the group A *Streptococcus* antigen and the initial pharyngeal exudate culture are done in the first visit (Visit 1), in accordance with the Schedule of Evaluations and before the administration of Microcyn 60. The second taking and culture of pharyngeal exudate is carried out 72 h after the administration of Microcyn 60 (Visit 2). An antibiogram is done on all the cultures to

determine the bacterial resistance to penicillin, erythromycin, clarithromycin and lincomycin by means of the standard diffusion disc test. Bacteriological efficacy is defined as the reduction by three orders of magnitude of the bacterial count between the initial culture and the culture taken 72 h after administering Microcyn 60.

[0206] Bacteriological failure is indicated by a reduction of less than three orders of magnitude of the bacterial count in the culture at 72 h posttreatment. Indeterminate responses are documented in those cases in which the transport of the sample has been delayed for more than 48 h, in those cases in which the swab has not been immersed in the transport medium, or in those cases in which the sample has been lost. These cases are outside the analysis of the study and are replaced by new cases until those of forty eligible patients have been completed.

[0207] The follow-up and reporting phase begins when the patient finishes the administration of Microcyn 60, and from the second visit. In this evaluation, according to the clinical evolution and the presence of possible adverse effects, the patients are categorized as follows:

[0208] Therapeutic failures if their initial signs and symptoms have not been eliminated or if there is worsening of their general condition with systemic symptoms. In these cases an oral antibiotic is prescribed, such as procaine penicillin, clarithromycin or azithromycin at the dose and for the time that the treating doctor indicates, and they are evaluated in one week.

[0209] Clinically cured if the symptoms and signs that were present in Visit 1 have been eliminated. In these cases in which the acute process is resolved, the patient is discharged and reported as clinically cured. In any case, the patient is asked to return for a third check-up visit in one week.

[0210] Indeterminate evolution. The evolution of any patient who could not have been evaluated clinically for any good reason; for example, a coinfection, or if the evaluation was done very late, later than 72 h. In these cases, the patients is still able to be included in the analysis of the study provided it is possible to document the result of the pharyngeal exudate and culture at 72 h.

[0211] The statistical analysis used in this clinical study takes into account all the patients who have received at least 18 rinsings of Microcyn 60 of 30 sec each in a period of 72 h. This same criterion is considered to include any patient in the analysis of tolerance. The principal criterion for analysis of efficacy is the reduction of the bacterial count of β-hemolytic *Streptococcus* by three orders of magnitude in the culture carried out at 72 h posttreatment with Microcyn 60. The statistical analysis is realized by means of a Wilcoxon paired samples test. Statistical analysis of the clinical variables is realized using the ANOVA test for quantitative variables. The minimal evaluable number of patients is 30 patients.

[0212] An adverse event is any contrary medical occurrence in a patient or subject of clinical investigation to whom a pharmaceutical product is administered and that does not

necessarily have a causal relationship with that medicine. An adverse event can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or illness temporarily associated with the use of a medical product, whether it is considered to be related to this use or not. Preexisting conditions that deteriorate during a study are reported as adverse events.

[0213] The treatment is suspended at any time during the 72 h of duration in case of adverse events that are moderate to severe in intensity. Subsequent treatment is determined by the treating doctor. In accordance with this example, the effectiveness of an ORP water solution of the present invention for treating sinusitis is thus demonstrated.

EXAMPLE 13

[0214] This example demonstrates the virucidal activity of an exemplary ORP water solution against Adenovirus-serotype 5. For this example Adenoviral (Ad) vectors based on human adenovirus type 5 which are E1a-, partially E1-b, and partially E3-deleted were used. A shuttle plasmid containing the Green Fluorescent Protein (GFP) reporter gene under the transcriptional control of pCMV was prepared (pAd-Track). Homologous recombination of this pShuttle plasmid with AdEasy 1 plasmid was carried out in electrocompetent bacteria. Clones that had inserts were tested by restriction endonuclease digestions. Once confirmed, supercoiled plasmid DMA was transformed into DH10B cells for large scale amplification. Subsequently, 293 cells (ATCC 1573) were cultured in serum-free medium (OptiMEM®-GIBCO) and transfected with recombinant plasmid digested with *Pad*. Infected cells were monitored for cytopathic effect, collected and lysed with three cycles of freezing and thawing. The resultant viruses (AdGFP) were purified with AdenoPure columns (BD Clontech) according to the manufacturer's instructions. Viruses were quantitated by OD 260/280. Final yield was 1.52×10^{11} pfu/mL.

[0215] The efficacy of the ORP water solution for inactivating adenovirus encoding the green fluorescence protein gene (AdGFP), was evaluated using a test based on the detection of fluorescence emission from HeLa cells infected with either, control AdGFP viruses or ORP water solution-treated AdGFP, using fluorescence-activated flow cytometry. Infection of HeLa cells was always carried out with 7.5×10^7 pfu/mL (i.e. 150 m.o.i.). Under test conditions, cells appeared normal under light microscopy. The background fluorescence measured in control HeLa cells was 0.06%. After infection with control AdGFP, 88.51% of HeLa cells expressed GFP. Following exposure to the ORP water solution, adenovirus infectivity decreased inversely proportionally to the exposure period. Accordingly, ORP water solution-treated virus for 1, 5, and 10 min could only express GFP in 2.8%, 0.13%, and 0.09% of HeLa cell cultures, respectively. Considering the autofluorescence and the initial viral load for all tested conditions (i.e. 7.5×10^7 pfu), the infectious titer was 6.6×10^7 pfu in the control AdGFP-HeLa group. In the groups where the virus had been treated with the

ORP water solution, the infectious titers were 2.0 X 10^6, 5.2 X 10^4 and 2.2 X 10^4 at one, five and ten minutes of virus exposure to the ORP water solution, respectively. Therefore, the log-10 reduction factor was 1.5, 3.1, and 3.5 at one, five and ten minutes of viral exposure to the ORP water solution. Taken together, these results demonstrate that the virus exposure to the ORP water solution for 5 minutes achieves a log-10 reduction in the viral load of > 3.

EXAMPLE 14

[0216] This example demonstrates the viricidal effectiveness of an exemplary ORP water solution against HIV using the United States Environmental Protection Agency protocol for disinfection of inanimate environmental surfaces.

[0217] The SF33 strain of HIV-1 used for this study. Peripheral blood mononuclear cells from healthy donors were activated with PHA (3 μg/mL, Sigma) and human IL-2 (20 U/mL, Roche) in HUT media for three days. Cells were washed and infected with SF33 strain. Supernatant was collected on days 4 and 6, and tested for the p24 HIV-1 antigen by ELISA (Beckman Coulter). Supernatant was centrifuged to remove cell and debris at 3000 RPM for 20 min at room temperature. Supernatant was removed, aliquoted, and the virus was stored at -80 °C until the day of use.

[0218] Frozen aliquots were thawed at 37°C for two minutes immediately prior to its use. Serial logarithmic dilutions (-1 to -5) in HUT medium were used. Films of virus were prepared by spreading 0.2 ml of virus inoculum uniformly over the bottoms of 55 cm^2 sterile polystyrene Petri dishes. The virus films were air-dried at room temperature (21 °C) in a biological safety cabinet until they looked visibly dry (20 minutes). (To assure that the virus strain (SF33) was capable of replicating and causing cytopathic effects, the procedure was repeated with a viral suspension that had remained in HUT medium without being dried.)

[0219] The control film was exposed to 2 ml HUT media for five minutes. The virus was then scraped and diluted. Separate dried films were exposed to 2 ml each of the ORP water solution for five minutes at room temperature. Following the exposure time, the plates were scraped and their contents were resuspended. The virus-ORP water solution mixture was immediately diluted (10:1) in HUT medium. Serial log dilutions of this resulting suspension were assayed for infectivity. (To control for a possible direct cytotoxic effect of ORP water solution on MT-2 cells, a 2 ml aliquot of ORP water solution was diluted serially (10:1 to 10:5) in medium and inoculated into MT-2 cell cultures.)

[0220] The MT-2 cell line was used as the indicator cell line in the infectivity assays. This line shows a cytopathic effect consisting of sincitia formation when infected with HIV-1. Four microwells were inoculated with 0.2 ml of each dilution of the reconstituted virus suspension from test (reconstituted in ORP water) and control (reconsituted with control medium) groups. Uninfected cell controls were inoculated with test medium only. Cultures were incubated at 37 °C and 5% CO_2.

[0221] The cultures were scored periodically every two days for the presence or absence of cytopathic effect as well as presence of p24-HIV-1 antigen by ELISA. Experimental infection with control HIV-1 exerted a cytopathic effect and Ag p24 protein release into the supernatant in infected MT-2 cultures. In contrast, treatment of HIV-1 with the ORP water solution for five minutes, achieved a log reduction factor > 3 in the viral load as measured in MT-2 cultures by both assays. These results thus demonstrate the level of efficacy that is in conformity with the EPA requirements for HIV-1 virucidal activity on inanimate surfaces.

EXAMPLE 15

[0222] This example demonstrates the effect of an exemplary ORP water solution versus hydrogen peroxide (HP) on the viability of human diploid fibroblasts (HDFs). To study this potential toxicity, HDFs were exposed in vitro to ORP water solution and hydrogen peroxide (HP). HP is known to be toxic to eukaryotic cells, increasing apoptosis and necrosis and reducing cellular viability. In this example, cell viability, apoptosis and necrosis were measured in HDFs exposed to pure ORP water solution and 880 mM HP (a concentration employed for antiseptic uses of HP) for 5 and 30 minutes.

[0223] HDF cultures were obtained from three different foreskins, which were pooled and cryopreserved together for the purpose of this study. Only diploid cells were used for all experiments. On cell cycle analysis, DNA diploidy was defined as the presence of a single G0-G1 peak with a CV </= 7% and a corresponding G2/M peak collected from at least 20,000 total events. FIG. 4A-4C discloses the results where exposure times of 5 and 30 minutes are depicted in white and black bars, respectively. Simultaneous analyses of these parameters were performed in the same cell populations by flow cytometry using: A) 7-aminoactinomycin D (7AAD); B) Annexin V-FITC; and C) Propidium iodide. FIG. 4A-4C disclose percentage values expressed as mean ± SD (n=3).

[0224] Cell viability was 75% and 55% after a 5 minute exposure to ORP water solution and HP, respectively (FIG. 4A). If the exposure was prolonged to 30 min, cell viability further decreased to 60% and 5%, respectively. Apparently, the ORP water solution induced cell death through necrosis because 15% of the cells incorporated propidium iodide in the flow cytometry analysis at both times (FIG. 4C). Apoptosis does not seem to be the mechanism by which the ORP water solution induces cell death because only 3 % of ORP water solution-treated cells exposed Annexin -V in the cellular surface (a marker of apoptosis) (FIG. 4B). This percentage was actually similar to the one measured in the control group. On the contrary, HP induced necrosis in 20% and 75% of treated cells and apoptosis in 15% and 20% after 5 and 30 min of exposure, respectively. Altogether these results show that the (undiluted) ORP water solution is far less toxic for HDFs than an antiseptic concentration of HP.

EXAMPLE 16

[0225] This example demonstrates the effect of an exemplary ORP water solution relative to hydrogen peroxide (HP) on oxidative DNA damage and formation of the DNA adduct 8-hydroxy-2'-deoxiguanosine (8-OHdG) in HDFs. It is known that the production of 8-OHdG adducts in a cell is a marker of oxidative damage at specific residues of DNA. In addition, high cellular levels of this adduct correlate with mutagenesis, carcinogenesis and cellular aging.

[0226] FIG. 5 shows the levels of 8-OHdG adducts present in DNA samples from HDFs after control treatments, ORP water solution treatments and HP-treatments for 30 minutes. DNA was extracted right after the exposure (T0, white bars) or three hours after the challenge period (T3, black bars). DNA was digested and the 8-OHdG adducts were measured by ELISA kit as per the manufacturer's instructions. Values are shown (ng/mL) as mean ±SD (n=3). The exposure to ORP water solution for 30 minutes did not increase the formation of adducts in the treated cells in comparison to control cells after incubation for 30 minutes. In contrast, the treatment with 500 μM HP for 30 minutes increased the number of 8-OHdG adducts by about 25 fold relative to the control-treated or ORP water solution-treated cells.

[0227] The ORP water solution-treated cells were able to decrease the levels of 8-OHdG adducts if left in supplemented DMEM for 3 hours after exposure to the ORP water solution. Despite being allowed the same 3 hour recovery period, HP-treated cells still presented about 5 times more adducts than control-treated or ORP water solution treated cells. Altogether, these results demonstrate that acute exposure to the ORP water solution does not induce significant DNA oxidative damage. These results also indicate that the ORP water solution will not likely induce mutagenesis or carcinogenesis *in vitro* or *in vivo*.

EXAMPLE 17

[0228] This example demonstrates the effects on HDFs of chronic exposure to low concentrations of an exemplary ORP water solution versus HP. It is known that chronic oxidative stress induces premature aging of cells. In order to mimic a prolonged oxidative stress, primary HDF cultures were chronically exposed to low concentrations of the ORP water solution (10%) or HP (5μM) during 20 population doublings. The expression and activity of the SA- β-galactosidase enzyme has previously been associated with the senescence process *in vivo* and *in vitro*. In this example the expression of the SA-β-galactosidase enzyme was analyzed after one month of continuous exposure of HDF to the ORP water solution or HP. The results are depicted in FIG. 6. The expression of the enzyme SA-β-galactosidase was analyzed by counting the number of blue cells in 20 microscopic fields. (For an example staining pattern, see Panel A.) Panel B shows that only HP treatment accelerated the aging of cells as indicated by the number of cells over-expressing SA-β-galactosidase (n= 3). Chronic treatment with a low dose of HP increased the SA- β-Gal

expression in 86% of cells while the treatment with the ORP water solution did not induce the overexpression of this protein. It can be concluded from this example that ORP water solution is not an inducer of premature cellular aging.

EXAMPLE 18

[0229] This example demonstrates the effectiveness of an exemplary ORP water solution (Mycrocyn) in inhibiting mast cell degranulation. Mast cells have been recognized as principal players in type I hypersensitivity disorders. Multiple clinical symptoms observed in atopic dermatitis, allergic rhinitis, and atopic asthma are produced by IgE-antigen stimulation of mast cells located in distinct affected tissues. The currently accepted view of the pathogenesis of atopic asthma is that allergens initiate the process by triggering IgE-bearing pulmonary mast cells (MCs) to release mediators such as histamine, leukotrienes, prostaglandins, kininis, platelet activating factor (PAF), etc. in the so-called early phase of the reaction. In turn, these mediators induce bronchoconstriction and enhance vascular permeability and mucus production. According to this model, following mast cell activation, those cells secrete various cytokines, including tumor necrosis factor alpha (TNF-α), IL-4, IL-5 and IL-6, which participate in the local recruitment and activation of other inflammatory cells such as eosinophils, basophils, T lymphocytes, platelets and mononuclear phagocytes. These recruited cells, in turn, contribute to the development of an inflammatory response that may then become autonomous and aggravate the asthmatic symptoms. This late phase response constitutes a long term inflammation process which can induce plastic changes in surrounding tissues (see Kumar *et al.*, pp. 193-268).

[0230] Antigenic stimulation of mast cells occurs via the activation of the high affinity receptor for IgE (the FcεRI receptor), which is a multimeric protein that binds IgE and subsequently can be aggregated by the interaction of the receptor-bound IgE with a specific antigen. Its structure comprises four polypeptides, an IgE binding α chain, a β chain that serves to amplify its signaling capacity, and two disulfide-linked γ chains, which are the principal signal transducers via the encoded immunoreceptor tyrosine-based (ITAM) activation motif. Signaling pathways activated by the cross-linking of this receptor have been characterized using bone marrow-derived mast cells (BMMC), the rat leukemia cell line RBL 2H3, mouse and rat peritoneal mast cells, and other mast cell lines, such as MC-9, In all of them, the presence of antigen bound to IgE causes mast cell degranulation, calcium mobilization, cytoskeletal re-arrangements and activation of different transcription factors (NFAT, NFκB, AP-1, PU.1, SP1, Ets, etc.) which activate cytokine gene transcription that culminate with cytokine production.

[0231] Mature murine bone marrow-derived mast cells (BMMC) were loaded with a monoclonal anti-Dinitrophenol IgE (300 ng/million cell) during 4 hours at 37 °C. Culture media was removed and cells were resuspended in physiological buffer (Tyrode's Buffer/BSA). Cells were then treated 15 minutes at 37 °C with distinct concentrations of the

ORP water solution (in its Microcyn embodiment). Buffer was removed and cells resuspended in fresh Tyrode's/BSA and stimulated with different concentrations of antigen (Human Albumin coupled to Dinitrophenol) during a 30 minute incubation at 37 °C. Degranulation was measured by β-hexosaminidase activity determination in supernatants and pellets of the stimulated cells, using a colorimetric reaction based on the capacity of this enzyme to hydrolize distinct carbohydrates. (β-hexosaminidase has been shown to be located in the same granules that contain histamine in mast cells.) The results (FIG. 7) demonstrate that degranulation is significantly reduced with increasing concentrations of the ORP water solution.

[0232] Surprisingly, the inhibitory effect of the ORP water solution (Microcyn) on mast cell degranulation is similar to that observed with the clinically effective "mast cell stabilizer" and established anti-allergic compound sodium cromoglycate (Intel™). Degranulation was again measured by β-hexosaminidase enzymatic activity in the pellet and supernatant of stimulated cells, using a colorimetric reaction based on the capacity of this enzyme to hydrolize distinct carbohydrates. Cells loaded with anti-DNPmonocional IgE were stimulated with or without a 15 minute pre-incubation with sodium cromoglycate (Intel™). Cromoglycate was no more effective than the ORP water solution in reducing degranulations (Compare FIG. 7 with FIG. 8; both achieving at least about 50% reduction in degranulation.)

EXAMPLE 19

[0233] This example demonstrates the inhibitory activity of an exemplary ORP water solution on mast cell activation by a calcium ionophore.

[0234] Mast cells can be stimulated via the activation of calcium fluxes induced by a calcium ionophore. Signaling pathways activated by calcium ionophores have been characterized using bone marrow-derived mast cells (BMMC), the rat leukemia cell line RBL 2H3, mouse and rat peritoneal mast cells, and other mast cell lines, such as MC-9. In all of these systems the calcium mobilization causes mast cell degranulation (e.g., histamine release), cytoskeletal re-arrangements, and activation of different transcription factors (e.g., NFAT, NFκB, AP-1, PU.1, SP1, Ets.) which activate cytokine gene transcription that culminate with cytokine production and secretion.

[0235] Mature murine BMMC were loaded with a monoclonal anti-Dinitrophenol IgE (300 ng/million cell) during 4 hours at 37°C. Culture media was removed and cells were resuspended in physiological buffer (Tyrode's Buffer/BSA). Cells were then treated for 15 minutes at 37°C with distinct concentrations of the ORP water solution (Microcyn). Buffer was removed and cells were resuspended in fresh Tyrode's/BSA and stimulated with calcium ionophore (100 mM A23187) during a 30 minute incubation at 37°C. Degranulation was measured by β-hexosaminidase activity determination in supernatants and pellets of the stimulated cells, using a colorimetric reaction based on the capacity of this enzyme to

hydrolyze distinct carbohydrates. (β-hexosaminidase has been shown to be located in the same granules that contain histamine in mast cells.) The results (FIG. 9) demonstrate that degranulation is significantly reduced with increasing concentrations of the ORP water solution.

[0236] These results suggest that ORP water solution is a non- specific inhibitor of histamine release. Thus, ORP water solution –even at different concentrations- will inhibit the degranulation of mast cells independently of the stimulus (e.g. antigen or ionophore). While not desiring to be bound by any theory, ORP water solution probably modifies the secretory pathway system at the level of the plasma membrane and/or cytoskeleton. Because the mechanism of action of ORP water solution is believed to be non-specific, it is believed that ORP water solution can have broad potential clinical applications.

EXAMPLE 20

[0237] This example demonstrates the effect of an exemplary ORP water solution on the activation of mast cell cytokine gene transcription.

[0238] FIG.s 10A and 10B is an RNAase protection assay from mast cells treated with ORP water solution at different concentrations for 15 minutes and further stimulated by antigen as described in Example 20. After stimulation, mRNA was extracted using affinity chromatography columns (RNAeasy kit, Qiagene) and the RNAse Protection Assay was performed using standard kit conditions (Clontech, Becton & Dickinson) in order to detect mRNA production of distinct cytokines after antigen challenge. The cytokines included TNF-α, LIF, IL13, M-CSF, IL6, MIF and L32.

[0239] FIG. 10A and 10B show that the ORP solution water (Microcyn) did not modify cytokine mRNA levels after antigen challenge in mast cells irrespective of the concentrations of ORP water solution or antigen used for the experiment.

[0240] In this study, the level of transcripts (i.e., the RNA content of stimulated mast cells) of proinflammatory genes was not changed in ORP water solution-treated mast cells after being stimulated with various concentrations of antigen. Thus, the ORP water solution inhibited the secretory pathway of these cytokines without affecting their transcription.

EXAMPLE 21

[0241] This example demonstrates the inhibitory activity of an exemplary ORP water solution on mast cell secretion of TNF-α.

[0242] Mast cells were treated with different concentrations of ORP water solution for 15 minutes and further stimulated by antigen as described in Example 20, Thereafter, the tissue culture medium was replaced and samples of the fresh medium were collected at various periods of time (2-8 hours) for measuring TNF-α levels. Samples were frozen and further analyzed with a commercial ELISA kit (Biosource) according to the manufacturer's instructions.

[0243] FIG. 11 shows that the level of secreted TNF-α to the medium from ORP water solution-treated cells after antigen stimulation is significantly decreased in comparison to the untreated cells.

[0244] Thus, the ORP water solution inhibited TNF-α secretion of antigen-stimulated mast cells. These results are in agreement with clinical observations that the use of ORP water solutions can decrease the inflammatory reaction in various wounds after surgical procedures.

EXAMPLE 22

[0245] This example demonstrates the inhibitory activity of an exemplary ORP water solution on mast cell secretion of MIP 1-α.

[0246] Mast cells were treated with different concentrations of an exemplary ORP water solution (Microcyn) for 15 minutes and further stimulated by antigen as described in Example 20. Thereafter, the tissue culture medium was replaced and samples of the fresh medium were collected at various periods of time (2-8 hours) for measuring MIP 1-α levels. Samples were frozen and further analyzed with a commercial ELISA kit (Biosource) according to the manufacturer's instructions.

[0247] FIG. 12 shows that the level of secreted MIP 1-α to the medium from ORP water solution-treated cells after antigen stimulation was significantly decreased in comparison to the untreated cells.

[0248] Thus, the ORP water solution inhibited MIP 1-α secretion of antigen-stimulated mast cells. These results are in agreement with clinical observations that the use of ORP water solutions can decrease the inflammatory reaction in various wounds after surgical procedures.

[0249] The results of analogous studies measuring IL-6 and IL-13 secretion are depicted in FIGS. 13 and 14.

[0250] Examples 19-21 and this example further demonstrate that the ORP water solution is able to inhibit early and late phase allergic responses initiated by IgE receptor crosslinking.

EXAMPLE 22

[0251] This example demonstrates the safety of an exemplary ORP water solution (Microcyn) when sprayed into the nasal cavity of rabbits.

[0252] Forty-two rabbits were randomly assigned to four groups; Groups I, II, III, and IV (Table 6). Rabbits were treated as follows: on Day 0, sterile saline was administered to the Group I rabbits and benzalkonium chloride was administered to Group II rabbits. Also on Day 0, Microcyn was administered to Groups III and IV at 40 ppm and 80 ppm, respectively. All articles were dosed by nasal spray into the right nostril. On Day 7, following the 8th dose, one-third of the rabbits from each group were sacrificed and necropsied. The remaining

animals were dosed daily through Day 14 when half of the remaining animals from each group were sacrificed and necropsied. On Day 21, after seven days without dosing, the remaining rabbits were sacrificed and necropsied.

Table 6.

Group No.	Treatment	No. of Rabbits Sacrificed per day		
		Day 7	Day 14	Day 21
I	Sterile Saline	3	3	3
II	Benzalkonium Chloride	1	1	1
III	Microcyn, 40 ppm	5	5	5
IV	Microcyn, 80 ppm	5	5	5

[0253] Samples of the nasal mucosa from both nostrils were collected from each rabbit and preserved in formalin for histopathological analysis. Formalin-preserved tissues were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. A board-certified veterinary pathologist examined all tissues listed above. The nasal cavity was sectioned at three levels called nasal cavity levels I, II, and III and the left and right sides were evaluated. Level I was the most anterior section, with some, but not all of the sections including hair follicle containing epithelium. Most of this region is lined by stratified squamous epithelium. Level II is about 1/3 of the way posterior from the nares and includes the vomeronasal organ and large sections of turbinate. Level III is about 2/3 of the way posterior from the nares and often includes smaller sections of turbinate. At each level the nasal cavity was evaluated for epithelial integrity, epithelial cilia, inflammatory cells, edema, goblet cells, glandular hyperplasia, and blood vessels. Sections were graded as follows: "minimal" represents the least amount of change and usually requires careful searching to identify; "mild" is a small, but readily identifiable lesion; "moderate" is widespread or large lesions that do not occupy me major portion of the tissue; and "marked" is a severe lesion that is large and often occupies a major portion of the tissue.

[0254] Pathologic changes in the nasal tissues were generally limited to levels II and III and were not seen until Day 14 with the exception of a single rabbit in Group IV at Day 7. At Day 14, non-dose-related increases in either incidence or severity of minimal to mild focal epithelial necrosis, hyperplasia, and/or epithial ciliary atrophy occurred in some rabbits from both Group III and Group IV when compared to those seen in rabbits of Groups I or II. Accompanying the epithelial lesions were focal infiltrates of lymphocytic inflammatory cells, the presence of which persisted until Day 21. At Day 21, the epithelial lesions of necrosis or ciliary atrophy were no longer observed in the treated rabbits. The minimal focal epithelial hyperplasia seen in two sections at the time is considered to be a renewal/recovery change, as

it was during the treatment phase of the study. Some Microcyn-treated rabbits had either goblet cell hypocellularity or goblet cell hyperplasia at the 21 day period, but these changes were no different from those found in controls. Lymphocytic inflammatory infiltrates were the main findings at the end of the 21 day test period. A single control rabbit had minimal focal epithelial necrosis at Day 21 which was considered to be an incidental finding.

[0255] There was no clear dose-related effect upon incidence or severity of the minimal to mild lesions. The mild lymphocytic infiltrates and focal epithelial hyperplasia were considered to be normal and expected changes associated with healing/recovery. Although the Microcyn was administered into the right nostril of all rabbits, there was no substantive difference in the lesion pattern/incidence between the sides.

[0256] This example demonstrates that daily intranasal administration of 40 or 80 ppm of Microcyn to rabbits caused minimal to mild lesions of focal nasal epithelial necrosis, hyperplasia, and/or epithelial ciliary atrophy by 14 (but not 7) days of treatment.

EXAMPLE 24

[0257] This study demonstrates the lack of toxicity of an exemplary ORP water solution, Dermacyn.

[0258] This study was done in accordance with ISO 10993-5:1999 standard to determine the potential of an exemplary ORP water solution, Dermacyn , to cause cytotoxicity. A filter disc with 0.1 mL of Dermacyn was placed onto an agarose surface, directly overlaying a monolayer of mouse fibroblast cells (L-929). The prepared samples were observed for cytotoxic damage after 24 hours of incubation at 37 °C in the presence of 5% CO_2. Observations were compared to positive and negative control samples. The Dermacyn containing samples did not reveal any evidence of cell lysis or toxicity, while positive and negative control performed as anticipated.

[0259] Based on this study Dermacyn was concluded not to generate cytotoxic effects on murine fibroblasts.

EXAMPLE 25

[0260] This study was conducted with 16 rats to evaluate the local tolerability of an exemplary ORP water solution, Dermacyn, and its effects on the histopathology of wound beds in a model of full-thickness dermal wound healing. Wounds were made on both sides of the subject rat. During the healing process skin sections were taken on either the left or the right sides (e.g., Dermacyn™-treated and saline-treated, respectively).

[0261] Masson's trichrome-stained sections and Collagen Type II stained sections of the Dermacyn and saline-treated surgical wound sites were evaluated by a board-certified veterinary pathologist. The sections were assessed for the amount of Collagen Type 2 expression as a manifestiation of connective tissue proliferation, fibroblast morphology and

collagen formation, presence of neoepidermis in cross section, inflammation and extent of dermal ulceration.

[0262] The findings indicate that Dermacyn was well tolerated in rats. There were no treatment-related histopathologic lesions in the skin sections from either sides' wounds (Dermacyn-treated and saline-treated, respectively). There were no relevant histopathologic differences between the saline-treated and the Dermacyn™-treated wound sites, indicating that the Dermacyn-treatement was well tolerated. There were no significant differences between Collagen Type 2 expression between the saline-treated and the Dermacyn-treated wound sites indicating that the Dermacyn does not have an adverse effect on fibroblasts or on collagen elaboration during wound healing.

EXAMPLE 26

[0263] This example demonstrates the use of an exemplary oxidative reductive potential water, Microcyn, in accordance with the invention as an effective antimicrobial solution.

[0264] An In-Vitro Time-Kill evaluation was performed using Microcyn oxidative reductive potential water. Microcyn was evaluated versus challenge suspensions of fifty different microorganism strains -- twenty-five American Type Culture Collection (ATCC) strains and twenty-five Clinical Isolates of those same species -- as described in the Tentative Final Monograph, Federal Register, 17 June 1994, vol. 59:116, pg. 31444. The percent reductions and the Log_{10} reductions from the initial population of each challenge strain were determined following exposures to Microcyn for thirty (30) seconds, one (1) minute, three (3) minutes, five (5) minutes, seven (7) minutes, nine (9) minutes, eleven (11) minutes, thirteen (13) minutes, fifteen (15) minutes, and twenty (20) minutes. All agar-plating was performed in duplicate and Microcyn was evaluated at a 99 % (v/v) concentration. All testing was performed in accordance with Good Laboratory Practices, as specified in 21 C.F.R. Part 58.

[0265] The following table summarizes the results of the abovementioned In-Vitro Time-Kill evaluation at the thirty second exposure mark for all populations tested which were reduced by more than 5.0 Log_{10}:

Table 7. 30-Second In-Vitro Kill.

No.	Microorganism Species	Initial Population (CFU/mL)	Post-Exposure Population (CFU/mL)	Log_{10} Reduction	Percent Reduction
1	*Acinetobacter baumannii* (ATCC #19003)	2.340×10^9	$< 1.00 \times 10^3$	6.3692	99.9999
2	*Acinetobacter baumannii* Clinical Isolate BSLI #061901Ab3	1.8150×10^9	$< 1.00 \times 10^3$	6.2589	99.9999
3	*Bacteroides fragilis* (ATCC #43858)	4.40×10^{10}	$< 1.00 \times 10^3$	7.6435	99.9999

4	*Bacteroides fragilis* Clinical Isolate BSLI #061901Bf6	2.70×10^{10}	$< 1.00 \times 10^{3}$	7.4314	99.9999
5	*Candida albicans* (ATCC #10231)	2.70×10^{10}	$< 1.00 \times 10^{3}$	6.3345	99.9999
6	*Candida albicans* Clinical Isolate BSLI #042905Ca	5.650×10^{9}	$< 1.00 \times 10^{3}$	6.7520	99.9999
7	*Enterobacter aerogenes* (ATCC #29007)	1.2250×10^{9}	$< 1.00 \times 10^{3}$	6.0881	99.9999
8	*Enterobacter aerogenes* Clinical Isolate BSLI #042905Ea	1.0150×10^{9}	$< 1.00 \times 10^{3}$	6.0065	99.9999
9	*Enterococcus faecalis* (ATCC #29212)	2.610×10^{9}	$< 1.00 \times 10^{3}$	6.4166	99.9999
10	*Enterococcus faecalis* Clinical Isolate BSLI #061901Efs2	1.2850×10^{9}	$< 1.00 \times 10^{3}$	6.1089	99.9999
11	*Enterococcus faecium* VRE, MDR (ATCC #51559)	3.250×10^{9}	$< 1.00 \times 10^{3}$	6.5119	99.9999
12	*Enterococcus faecium* Clinical Isolate BSLI #061901Efm1	1.130×10^{9}	$< 1.00 \times 10^{3}$	6.0531	99.9999
13	*Escherichia coli* (ATCC #11229)	5.00×10^{8}	$< 1.00 \times 10^{3}$	5.6990	99.9998
14	*Escherichia coli* Clinical Isolate BSLI #042905Ec1	3.950×10^{8}	$< 1.00 \times 10^{3}$	5.5966	99.9997
15	*Escherichia coli* (ATCC #25922)	6.650×10^{8}	$< 1.00 \times 10^{3}$	5.8228	99.9998
16	*Escherichia coli* Clinical Isolate BSLI #042905Ec2	7.40×10^{8}	$< 1.00 \times 10^{3}$	5.8692	99.9998
17	*Haemophilus influenzae* (ATCC #8149)	1.5050×10^{9}	$< 1.00 \times 10^{4}$	5.1775	99.9993
18	*Haemophilus influenzae* Clinical Isolate BSLI #072605Hi	1.90×10^{9}	$< 1.00 \times 10^{4}$	5.2788	99.9995
19	*Klebsiella oxytoca* MDR (ATCC #15764)	1.120×10^{9}	$< 1.00 \times 10^{3}$	6.0492	99.9999
20	*Klebsiella oxytoca* Clinical Isolate BSLI #061901Ko1	1.810×10^{9}	$< 1.00 \times 10^{3}$	6.2577	99.9999
21	*Klebsiella pneumoniae* subsp. *ozaenae* (ATCC #29019)	1.390×10^{9}	$< 1.00 \times 10^{3}$	6.1430	99.9999

22	*Klebsiella pneumoniae* Clinical Isolate BSLI #061901Kpn2	9.950×10^8	$< 1.00 \times 10^3$	5.9978	99.9999
23	*Micrococcus luteus* (ATCC #7468)	6.950×10^8	$< 1.00 \times 10^3$	5.8420	99.9999
24	*Micrococcus luteus* Clinical Isolate BSLI #061901Ml2	1.5150×10^9	$< 1.00 \times 10^3$	6.1804	99.9999
25	*Proteus mirabilis* (ATCC #7002)	1.5950×10^9	$< 1.00 \times 10^3$	6.2028	99.9999
26	*Proteus mirabilis* Clinical Isolate BSLI #061901Pm2	2.0950×10^9	$< 1.00 \times 10^3$	6.3212	99.9999
27	*Pseudomonas aeruginosa* (ATCC #15442)	6.450×10^8	$< 1.00 \times 10^3$	5.8096	99.9999
28	*Pseudomonas aeruginosa* Clinical Isolate BSLI #072605Pa	1.3850×10^9	$< 1.00 \times 10^3$	6.1414	99.9999
29	*Pseudomonas aeruginosa* (ATCC #27853)	5.550×10^8	$< 1.00 \times 10^3$	5.7443	99.9999
30	*Pseudomonas aeruginosa* Clinical Isolate BSLI #061901Pa2	1.1650×10^9	$< 1.00 \times 10^3$	6.0663	99.9999
31	*Serratia marcescens* (ATCC #14756)	9.950×10^8	$< 1.00 \times 10^3$	5.9978	99.9999
32	*Serratia marcescens* Clinical Isolate BSLI #042905Sm	3.6650×10^9	$< 1.00 \times 10^3$	6.5641	99.9999
33	*Staphylococcus aureus* (ATCC #6538)	1.5050×10^9	$< 1.00 \times 10^3$	6.1775	99.9999
34	*Staphylococcus aureus* Clinical Isolate BSLI #061901Sa1	1.250×10^9	$< 1.00 \times 10^3$	6.0969	99.9999
35	*Staphylococcus aureus* (ATCC #29213)	1.740×10^9	$< 1.00 \times 10^3$	6.2405	99.9999
36	*Staphylococcus aureus* Clinical Isolate BSLI #061901Sa2	1.1050×10^9	$< 1.00 \times 10^3$	6.0434	99.9999
37	*Staphylococcus epidermidis* (ATCC #12228)	1.0550×10^9	$< 1.00 \times 10^3$	6.0233	99.9999
38	*Staphylococcus epidermidis* Clinical Isolate BSLI #072605Se	4.350×10^8	$< 1.00 \times 10^3$	5.6385	99.9998
39	*Staphylococcus haemolyticus* (ATCC #29970)	8.150×10^8	$< 1.00 \times 10^3$	5.9112	99.9999
40	*Staphylococcus haemolyticus* Clinical Isolate BSLI #042905Sha	8.350×10^8	$< 1.00 \times 10^3$	5.9217	99.9999

41	*Staphylococcus hominis* (ATCC #27844)	2.790×10^8	$< 1.00 \times 10^3$	5.4456	99.9996
42	*Staphylococcus hominis* Clinical Isolate BSLI #042905Sho	5.20×10^8	$< 1.00 \times 10^3$	5.7160	99.9998
43	*Staphylococcus saprophyticus* (ATCC #35552)	9.10×10^8	$< 1.00 \times 10^3$	5.9590	99.9999
44	*Staphylococcus saprophyticus* Clinical Isolate BSLI #042905Ss	1.4150×10^9	$< 1.00 \times 10^3$	6.1508	99.9999
45	*Streptococcus pneumoniae* (ATCC #33400)	2.1450×10^9	$< 1.00 \times 10^4$	5.3314	99.9995
46	*Streptococcus pyogenes* (ATCC #19615)	5.20×10^9	$< 1.00 \times 10^3$	6.7160	99.9999
47	*Streptococcus pyogenes* Clinical Isolate BSLI #061901Spy7	2.5920×10^9	$< 1.00 \times 10^3$	6.4141	99.9999

[0266] While their microbial reductions were measured at less than 5.0 Log_{10}, Microcyn also demonstrated antimicrobial activity against the remaining three species not included in Table 8. More specifically, a thirty second exposure to Microcyn reduced the population of *Streptococcus pneumoniae* (Clinical Isolate; BSLI #072605Spn1) by more than 4.5 Log_{10}, which was the limit of detection versus this species. Further, when challenged with *Candida tropicalis* (ATCC #750), Microcyn demonstrated a microbial reduction in excess of 3.0 Log_{10} following a thirty second exposure. Additionally, when challenged with *Candida tropicalis* (BSLI #042905Ct), Microcyn demonstrated a microbial reduction in excess of 3.0 Log_{10} following a twenty minute exposure.

[0267] The exemplary results of this In-Vitro Time-Kill evaluation demonstrate that Microcyn oxidative reductive potential water exhibits rapid (i.e., less than 30 seconds in most cases) antimicrobial activity versus a broad spectrum of challenging microorganisms. Microbial populations of forty-seven out of the fifty Gram-positive, Gram-negative, and yeast species evaluated were reduced by more than 5.0 Log_{10} within thirty seconds of exposure to the product.

EXAMPLE 27

[0268] This example demonstrates a comparison of the antimicrobial activity of an exemplary oxidative reductive potential water, Microcyn, used in accordance with the invention versus HIBICLENS® chlorhexidine gluconate solution 4.0 % (w/v) and 0.9 % sodium chloride irrigation (USP).

[0269] An In-Vitro Time-Kill evaluation was performed as described in Example 26 using HIBICLENS® chlorhexidine gluconate solution 4.0 % (w/v) and a sterile 0.9 % sodium chloride irrigation solution (USP) as reference products. Each reference product was

evaluated versus suspensions of the ten American Type Culture Collection (ATCC) strains specifically denoted in the Tentative Final Monograph. The data collected was then analyzed against the Microcyn microbial reduction activity recorded in Example 26.

[0270] Microcyn oxidative reductive potential water reduced microbial populations of five of the challenge strains to a level comparable to that observed for the HIBICLENS® chlorhexidine gluconate solution. Both Microcyn and HIBICLENS® provided a microbial reduction of more than 5.0 Log_{10} following a thirty second exposure to the following species: *Escherichia coli* (ATCC #11229 and ATCC #25922), *Pseudomonas aeruginosa* (ATCC #15442 and ATCC #27853), and *Serratia marcescens* (ATCC #14756). Further, as shown above in Table 7, Microcyn demonstrated excellent antimicrobial activity against *Micrococcus luteus* (ATCC #7468) by providing a 5.8420 Log_{10} reduction after a thirty second exposure. However, a direct *Micrococcus luteus* (ATCC #7468) activity comparison to HIBICLENS® was not possible because after a thirty second exposure, HIBICLENS® reduced the population by the detection limit of the test (in this specific case, by more than 4.8 Log_{10}). It is noted that the sterile 0.9 % sodium chloride irrigation solution reduced microbial populations of each of the six challenge strains discussed above by less than 0.3 Log_{10} following a full twenty minute exposure.

[0271] Microcyn oxidative reductive potential water provided greater antimicrobial activity than both HIBICLENS® and the sodium chloride irrigation for four of the challenge strains tested: *Enterococcus faecalis* (ATCC #29212), *Staphylococcus aureus* (ATCC #6538 and ATCC #29213), and *Staphylococcus epidermidis* (ATCC #12228). The following table summarizes the microbial reduction results of the In-Vitro Time-Kill evaluation for these four species:

Table 8. Comparative Results

Microorganism Species	Exposure Time	Log_{10} Reduction		
		Microcyn	HIBICLENS®	NaCl Irrigation
Enterococcus faecalis (ATCC #29212)	30 seconds	6.4166	1.6004	0.3180
	1 minute	6.4166	2.4648	0.2478
	3 minutes	6.4166	5.2405	0.2376
	5 minutes	6.4166	5.4166	0.2305
	7 minutes	6.4166	5.4166	0.2736
	9 minutes	6.4166	5.4166	0.2895
	11 minutes	6.4166	5.4166	0.2221
	13 minutes	6.4166	5.4166	0.2783
	15 minutes	6.4166	5.4166	0.2098
	20 minutes	6.4166	5.4166	0.2847
Staphylococcus aureus (ATCC #6538)	30 seconds	6.1775	1.1130	0.0000
	1 minute	6.1775	1.7650	0.0191
	3 minutes	6.1775	4.3024	0.0000
	5 minutes	6.1775	5.1775	0.0000
	7 minutes	6.1775	5.1775	0.0000
	9 minutes	6.1775	5.1775	0.0000
	11 minutes	6.1775	5.1775	0.0267
	13 minutes	6.1775	5.1775	0.0000
	15 minutes	6.1775	5.1775	0.0191
	20 minutes	6.1775	5.1775	0.0000
Staphylococcus aureus (ATCC #29213)	30 seconds	6.2405	0.9309	0.0000
	1 minute	6.2405	1.6173	0.0000
	3 minutes	6.2405	3.8091	0.0460
	5 minutes	6.2405	5.2405	0.0139
	7 minutes	6.2405	5.2405	0.0000
	9 minutes	6.2405	5.2405	0.0113
	11 minutes	6.2405	5.2405	0.0283
	13 minutes	6.2405	5.2405	0.0000
	15 minutes	6.2405	5.2405	0.0000
	20 minutes	6.2405	5.2405	0.0615
Staphylococcus epidermidis (ATCC #12228)	30 seconds	5.6385	5.0233	0.0456
	1 minute	5.6385	5.0233	0.0410
	3 minutes	5.6385	5.0233	0.0715
	5 minutes	5.6385	5.0233	0.0888
	7 minutes	5.6385	5.0233	0.0063
	9 minutes	5.6385	5.0233	0.0643
	11 minutes	5.6385	5.0233	0.0211
	13 minutes	5.6385	5.0233	0.1121
	15 minutes	5.6385	5.0233	0.0321
	20 minutes	5.6385	5.0233	0.1042

[0272] The results of this comparative In-Vitro Time-Kill evaluation demonstrate that Microcyn oxidative reductive potential water not only exhibits comparable antimicrobial

activity to HIBICLENS® against *Escherichia coli* (ATCC #11229 and ATCC #25922), *Pseudomonas aeruginosa* (ATCC #15442 and ATCC #27853), *Serratia marcescens* (ATCC #14756), and *Micrococcus luteus* (ATCC #7468), but provides more effective treatment against *Enterococcus faecalis* (ATCC #29212), *Staphylococcus aureus* (ATCC #6538 and ATCC #29213), and *Staphylococcus epidermidis* (ATCC #12228). As shown in Table 8, Microcyn exemplifies a more rapid antimicrobial response (i.e., less than 30 seconds) in some species. Moreover, exposure to Microcyn results in a greater overall microbial reduction in all species listed in Table 8.

EXAMPLE 28

[0273] This example demonstrates the effectiveness of an ORP water solution against Penicillin Resistant *Streptococcus pneumoniae* (ATCC 51915).

[0274] A culture of *Streptococcus pneumoniae* was prepared by using a frozen culture to inoculate multiple BAP plates and incubating for 2-3 days at 35-37°C with CO2. Following incubation 3-7 mL of sterile diluent/medium was transferred to each agar plate and swabbed to suspend the organism. The suspensions from all plates were collected and transferred to a sterile tube and compared to a 4.0 McFarland Standard. The suspension was filtered through sterile gauze and vortex mixed prior to use in the testing procedure.

[0275] An inoculum of 0.1 ml of the organism suspension was added to 49.9 ml of the Microcyn or control substance. At each exposure period, the test mixture was mixed by swirling. The test mixture was exposed for 15 seconds, 30 seconds, 60 seconds, 120 seconds, 5 minutes, and 15 minutes at 25.0°C.

[0276] A 1.0 ml sample was removed from the test mixture and added to 9.0 ml of neutralizer representing a 100 dilution of the neutralized inoculated test mixture. A 5 ml aliquot of the 100 neutralized inoculated test mixture was transferred to a 0.45 microliter filter apparatus pre-wetted with 10 ml of Butterfield's Buffer. The filter was rinsed with approximately 50 mL of Butterfield's Buffer, asepticaliy removed from the apparatus, and transferred to a BAP plate. Additional 1:10 serial dilutions were prepared and one (1.0) ml aliquots of the 10-3- 10-4 dilutions of neutralized inoculated test mixture were plated in duplicate on BAP.

[0277] The bacterial subculture plates were incubated for 48±4 hours at 35-37°C in C02. Subculture plates were refrigerated for two days at 2-8°C prior to examination. Following incubation and storage, the agar plates were observed visually for the presence of growth. The colony forming units were enumerated and the number of survivors at each exposure time was determined. Representative subcultures demonstrating growth were appropriately examined for confirmation of the test organisms.

[0278] The exemplary ORP water solution, Microcyn, demonstrated a >99.93197279% reduction of Penicillin Resistant *Streptococcus pneumoniae* (ATCC 51915) after 15 second, 30 second, 60 second, 120 second, 5 minute, and 15 minute contact times at 25.0°C.

EXAMPLE 29

[0279] The objective of this Example is to determine the microbial activity of an exemplary ORP water solution (Dermacyn) versus Bacitracin using a bacterial suspension assay.

[0280] Dermacyn is a ready to use product, therefore performing dilutions during testing was not required. Bacitracin is a concentrated re-hydrated solution requiring a dilution to 33 Units/ml.

[0281] A purchased spore suspension of B. atropheus at 2.5 x 107 /ml was used for testing. In addition fresh suspensions of Pseudomonas aeruginosa, and Staphylococcus aureus were prepared and measured using a spectrophotometer to ensure the titer was acceptable

[0282] Nine microliters of test substance was added to 100 ul of microbe suspension. The test mixture was held at 20°C for the contact times of 20 seconds, 5 minutes, and 20 minutes. 1.0 ml of the test mixture (entire mixture) was added to 9,0 ml of neutralizer for 20 minutes (this is the original neutralization tube or ONT) 1.0 ml of the neutralized test mixture was plated on Tryptic Soy Agar in duplicate for the 5 minute and 20 minute contact times. Additional dilutions and spread plates were used for the 20 second time point, to achieve countable plates.

[0283] All plates were incubated at 30°C -35°C for a total of 3 days and were evaluated after each day of incubation. To determine the number of microbes exposed to Dermacyn and Bacitracin during testing the suspensions Four 10-fold dilutions were performed and 1.0 ml of the final 2 dilutions was plated in duplicate, where applicable.

[0284] Dermacyn when challenged with the test organisms showed total eradication (>4 log reduction) of the vegetative bacteria at all time points and for spores at the 5, and 20 minute time points. Bacitracin only produced approximately 1 log reduction. Microcyn at the 20 second time point showed some reduction in spores. Bacitracin showed no evidence of lowering the bacterial or spore populations over the time periods tested.

EXAMPLE 30

[0285] This example demonstrates the effectiveness of two exemplary ORP water solutions (M1 and M2) against bacteria in biofilms.

[0286] The parental strain for all studies is P. aeruginosa PAO1. All planktonic strains were grown aerobically in minimal medium (2.56 g Na_2HPO_4, 2.08 g KH_2PO_4, 1.0 g NH_4Cl, 0.04 g $CaCl_2$ •2 H_2O, 0.5 g $MgSO_4$ • $7H_2O$, 0.1 mg $CuSO_4$ • $5H_2O$, 0.1 mg $ZnSO_4$ • H_2O, 0.1 mg $FeSO_4$ • $7H_2O$, and 0.004 mg $MnCl_2$ • $4H_2O$ per liter, pH 7.2) at 22 °C in shake flasks at

220 rpm. Biofilms were grown as described below at 22°C in minimal medium. Glutamate (130 mg/liter) was used as the sole carbon source.

[0287] Biofilms were grown as described previously (Sauer et.al., J. Bacteriol. 184:1140–1154 (2002),which is hereby incorporated by reference). Briefly, the interior surfaces of silicone tubing of a once-through continuous flow tube reactor system were used to cultivate biofilms at 22°C. Biofilms were harvested after 3 days (maturation-1 stage), 6 days (maturation- 2 stage), and 9 days (dispersion stage) of growth under flowing conditions. Biofilm cells were harvested from the interior surface by pinching the tube along its entire length, resulting in extrusion of the cell material from the lumen. The resulting cell paste was collected on ice. Prior to sampling, the bulk liquid was purged from the tubing to prevent interference from detached, planktonic cells.

[0288] The population size of planktonic and biofilm cells was determined by the number of CFU by using serial dilution plate counts. To do so, biofilms were harvested from the interior surface after various periods of time of exposure to SOSs. Images of biofilms grown in once-through flow cells were viewed by transmitted light with an Olympus BX60 microscope (Olympus, Melville, NY) and a _100 magnification A100PL objective lens. Images were captured using a Magnafire cooled three-chip charge-coupled device camera (Optronics Inc., Galena, CA) and a 30-ms exposure. In addition, confocal scanning laser microscopy was performed with an LSM 510 Meta inverted microscope (Zeiss, Heidelberg, Germany). Images were obtained with a LD-Apochrome 40_/0.6 lens and with the LSM 510 Meta software (Zeiss).

[0289] A 2-log reduction was observed for M1-treated biofilms within 60 min of treatment. The finding indicates that every 10.8 min (+/- 2.8 min), treatment with M1 results in a 50% reduction in biofilm viability.

Table 9. M1 Killing.

Time (min)	Viability (%)
0	100
10	50
20	25
34	12.5
47	6.25
54	3.125

[0290] However, overall M2 was somewhat more effective in killing biofilms than M1 because the results indicated that every 4.0 min (+/- 1.2 min), treatment with M2 results in a 50% reduction in biofilm viability.

Table 10. M2 Killing.

Time (min)	Viability (%)
0	100
2.5	50
7	25
12	12.5
15	6.25
20	3.125

[0291] Thus, ORP water is effective against bacteria in bioflims.

[0292] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0293] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0294] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIMS:

1. A method of treating or preventing sinusitis in a patient, the method comprising administering to the patient a therapeutically effective amount of an oxidative reductive potential water solution, wherein the solution is stable for at least about twenty-four hours and the solution has a pH of from about 6.4 to about 7.8.

2. The method of claim 1, comprising administering the oxidative reductive potential water solution to the upper respiratory airway.

3. The method of claim 1, comprising contacting one or more tissues in the upper respiratory airway with the oxidative reductive potential water solution.

4. The method of claim 1, comprising contacting tissue in one or more cranial sinuses with the oxidative reductive potential water solution.

5. The method of claim 4, wherein the one or more cranial sinuses are selected from the group consisting of frontal sinuses, maxillary sinuses, ethmoid sinuses, and sphenoid sinuses, and combinations thereof.

6. The method of claim 1, comprising administering the oxidative reductive potential water solution to one or more ethmoid sinuses.

7. The method of claim 1, comprising contacting one or more tissues in the ethmoid sinuses with the oxidative reductive potential water solution.

8. The method of claim 1, comprising intranasally administering the oxidative reductive potential water solution.

9. The method of claim 1, comprising administering the oxidative reductive potential water solution through one or more openings of the mouth or nose.

10. The method of claim 1, comprising delivering the oxidative reductive potential water solution in the form of a liquid, spray, mist, aerosol or steam.

11. The method of claim 1, wherein the oxidative reductive potential water solution is administered by aerosolization, nebulization or atomization.

12. The method of claim 1, wherein the oxidative reductive potential water solution is administered in the form of droplets having a diameter in the range of from about 0.1 micron to about 100 microns.

13. The method of claim 1, wherein the sinusitis is acute sinusitis.

14. The method of claim 1, wherein the sinusitis is chronic sinusitis.

15. The method of claim 1, wherein the sinusitis results from an allergic reaction.

16. The method of claim 1, wherein the sinusitis results from asthma.

17. The method of claim 1, wherein the sinusitis results from an infection.

18. The method of claim 17, wherein the infection is by one or more microorganisms selected from the group consisting of viruses, bacteria, and fungi.

19. The method of claim 18, wherein the infection is by one or more viruses selected from the group consisting of coxsackie viruses, adenoviruses, rhinoviruses, and influenza viruses.

20. The method of claim 18, wherein the infection is by one or more bacteria selected from the group of *Streptococcus pneumoniae, Haemophilus influenzae,* staphylococci, non-pneumococcal streptococci, corynebacterium, and anaerobes.

21. The method of claim 18, wherein the infection is by one or more fungi selected from the group of zygomycetes, aspergillus, and candida.

22. The method of claim 1, wherein the oxidative reductive potential water solution is administered in combination with up to about 25% of one or more carriers.

23. The method of claim 1, wherein the oxidative reductive potential water solution is administered in combination with up to about 50% of one or more carriers.

24. The method of claim 1, wherein the oxidative reductive potential water solution is administered in combination with up to about 75% of one or more carriers.

25. The method of claim 1, wherein the oxidative reductive potential water solution is administered in combination with up to about 90% of one or more carriers.

26. The method of claim 1, wherein the oxidative reductive potential water solution is administered in combination with up to about 95% of one or more carriers.

27. The method of claim 1, wherein the oxidative reductive potential water solution is administered in combination with up to about 99% of one or more carriers

28. The method of claim 22, wherein the one or more carriers is selected from the group consisting of sterile water, saline and combinations thereof.

29. The method of claim 23, wherein the one or more carriers is selected from the group consisting of sterile water, saline and combinations thereof.

30. The method of claim 24, wherein the one or more carriers is selected from the group consisting of sterile water, saline and combinations thereof.

31. The method of claim 25, wherein the one or more carriers is selected from the group consisting of sterile water, saline and combinations thereof.

32. The method of claim 26, wherein the one or more carriers is selected from the group consisting of sterile water, saline and combinations thereof.

33. The method of claim 27, wherein the one or more carriers is selected from the group consisting of sterile water, saline and combinations thereof.

34. The method of claim 1, wherein the pH of the oxidative reductive potential water solution is from about 7.4 to about 7.6.

35. The method of claim 1, wherein the oxidative reductive potential water solution is stable for at least about two weeks.

36. The method of claim 1, wherein the oxidative reductive potential water solution is stable for at least about two months.

37. The method of claim 1, wherein the oxidative reductive potential water solution is stable for at least about six months.

38. The method of claim 1, wherein the oxidative reductive potential water solution is stable for at least about one year.

39. The method of claim 1, wherein the oxidative reductive potential water solution comprises cathode water in an amount of from about 10% to about 50% by volume of the solution.

40. The method of claim 1, wherein the oxidative reductive potential water solution comprises cathode water in an amount of from about 20% to about 40% by volume of the solution.

41. The method of claim 1, wherein the oxidative reductive potential water solution comprises anode water in an amount of from about 50% to about 90% by volume of the solution.

42. The method of claim 1, wherein the oxidative reductive potential water solution comprises from about 10% by volume to about 50% by volume of cathode water and from about 50% by volume to about 90% by volume of anode water.

43. The method of claim 1, wherein the oxidative reductive potential water solution comprises at least one free chlorine species selected from the group consisting of hypochlorous acid, hypochlorite ions, sodium hypochlorite, chlorite ions, and combinations thereof.

44. The method of claim 1, wherein the oxidative reductive potential water solution comprises from about 15 ppm to about 35 ppm hypochlorous acid.

45. The method of claim 1, wherein the oxidative reductive potential water solution comprises from about 25 ppm to about 50 ppm sodium hypochlorite.

46. The method of claim 1, wherein the oxidative reductive potential water solution comprises from about 15 ppm to about 35 ppm hypochlorous acid, from about 25 ppm to about 50 ppm sodium hypochlorite, a pH of from about 6.2 to about 7.8, and the solution is stable for at least about one week.

47. The method of claim 1, wherein the oxidative reductive potential water solution has a potential from about –400 mV to about +1300 mV.

48. The method of claim 1, further comprising administering at least one additional therapeutic agent from the group consisting of antihistamines, decongestants, anti-infective agents, anti-inflammatory agents, and combinations thereof.

FIG. 1

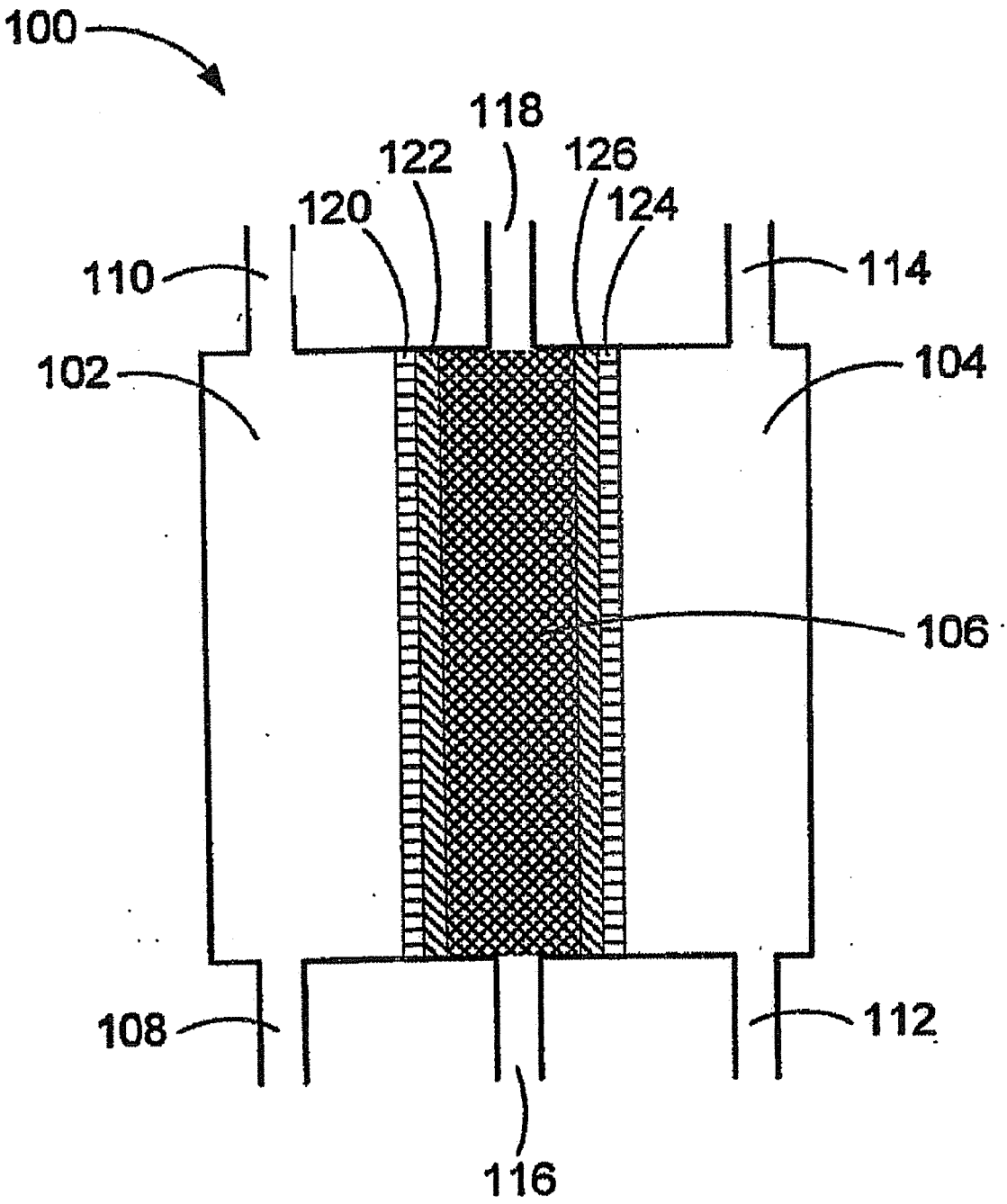
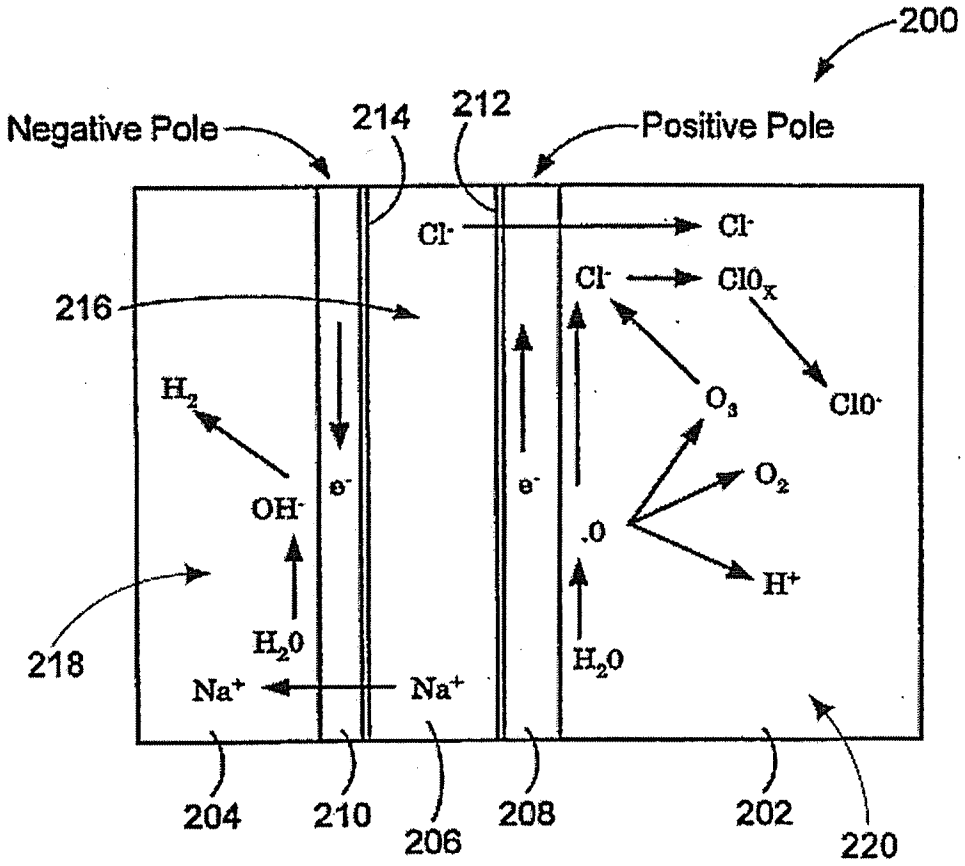


FIG. 2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Negative Pole
214
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Positive Pole
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Cl-
Cl-
ClOx
216
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O3
ClO-
e-
e-
OH-
O2
.O
H+
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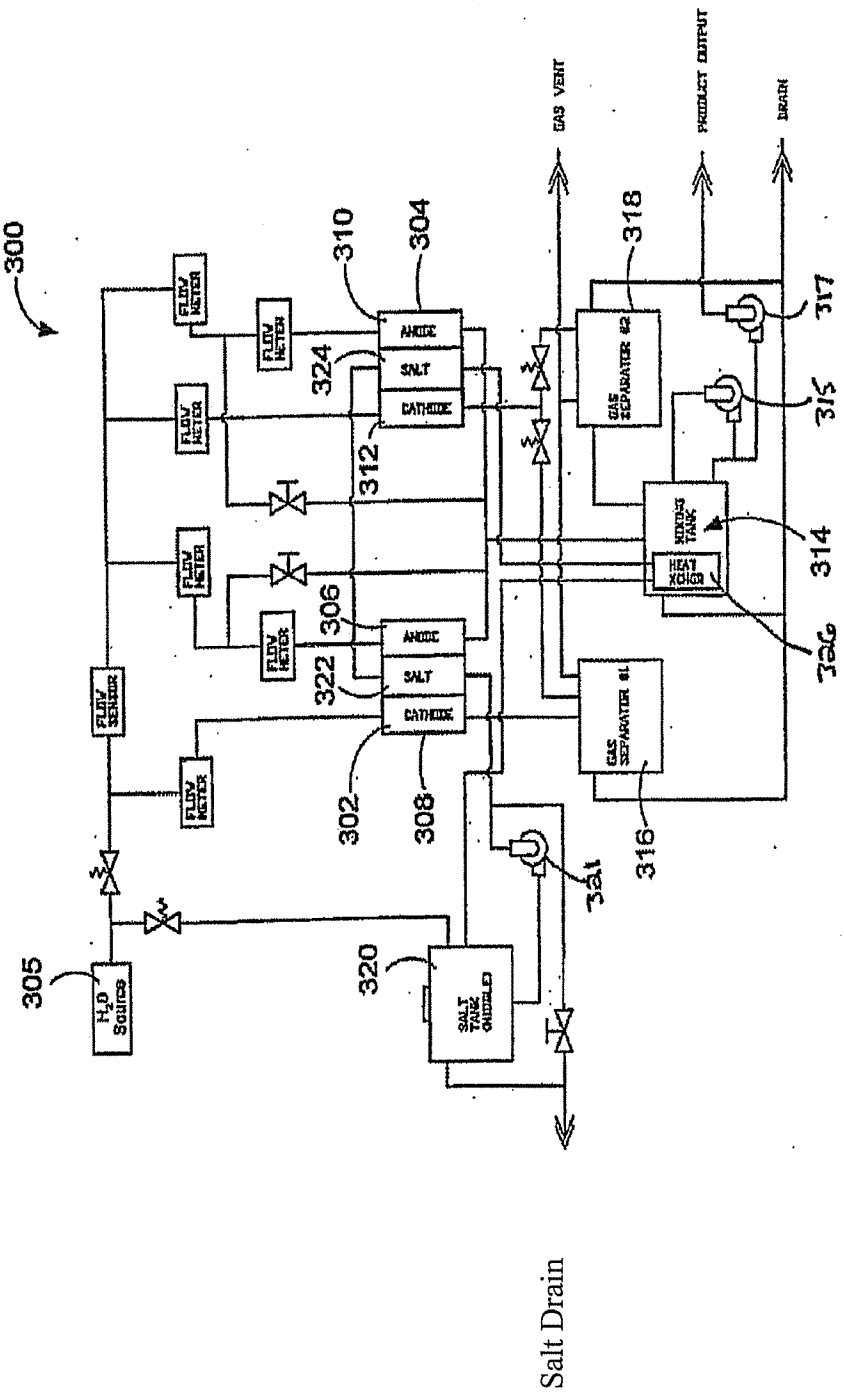
H2O
Na+
Na+
204
210
206
208
202
220

3/17

FIG. 3


300
305
H2O Source
FLOW SENSOR
FLOW METER
320
SALT TANK GRIDDLED
302
308
ANODE
SALT
CATHODE
304
306
310
312
322
324
316
GAS SEPARATOR #1
318
GAS SEPARATOR #2
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MIXING TANK
HEAT XCHGR
317
315
GAS VENT
PRODUCT OUTPUT
DRAIN
Salt
Salt Drain

FIG. 4A

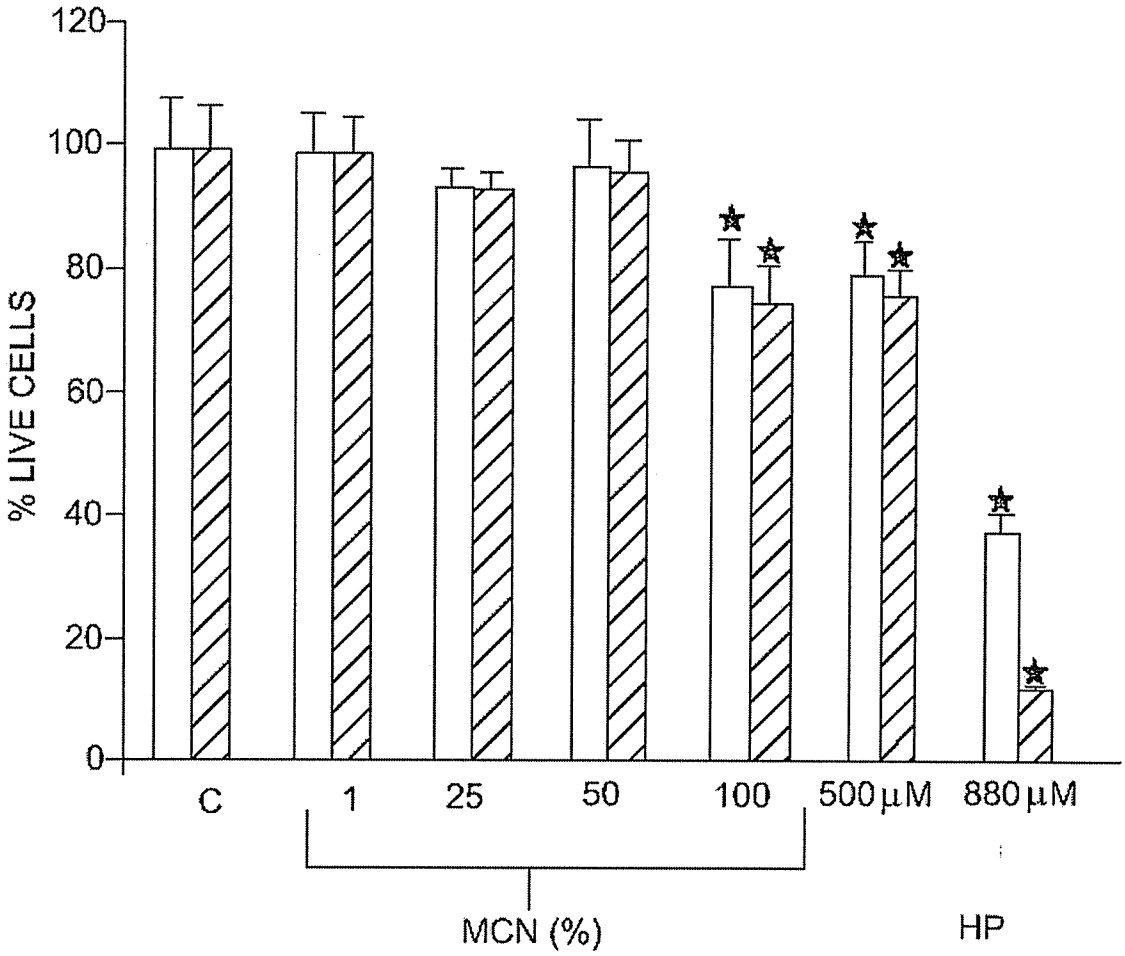


FIG. 4B

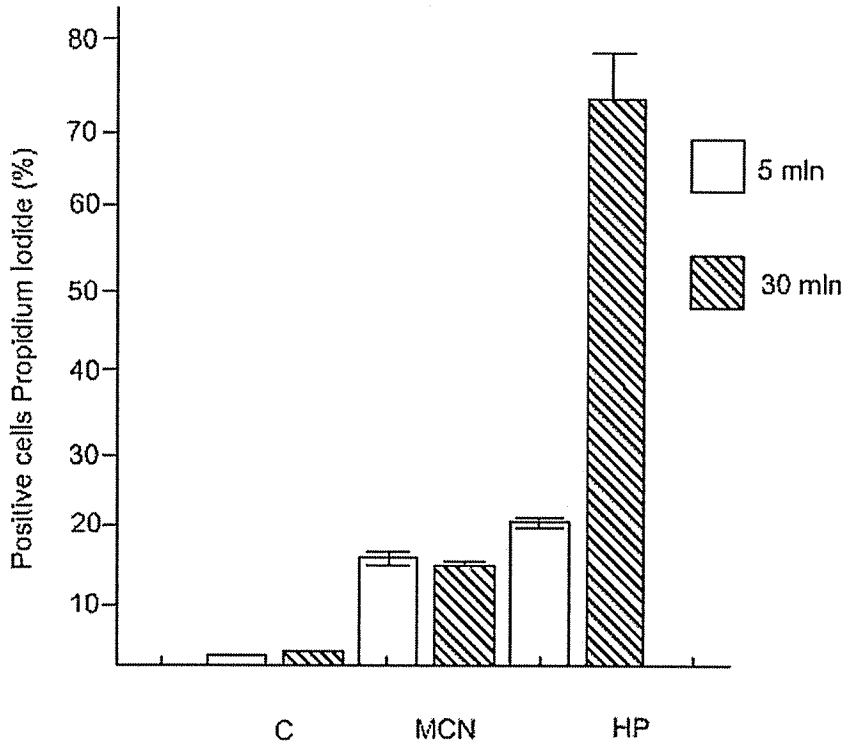

Positive cells Propidium Iodide (%)
80
70
60
50
40
30
20
10
C
MCN
HP
5 mln
30 mln

FIG. 4C

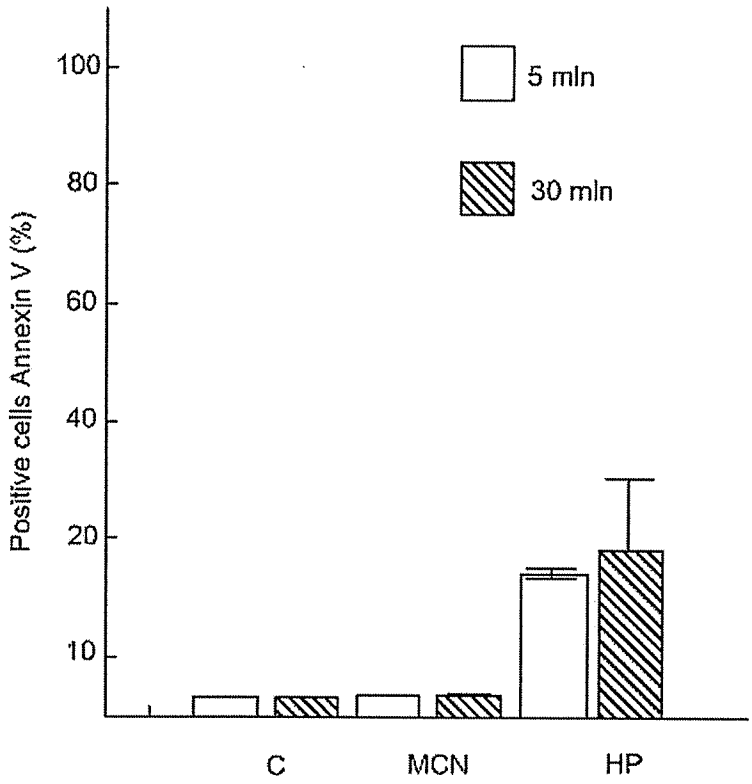


FIG. 5

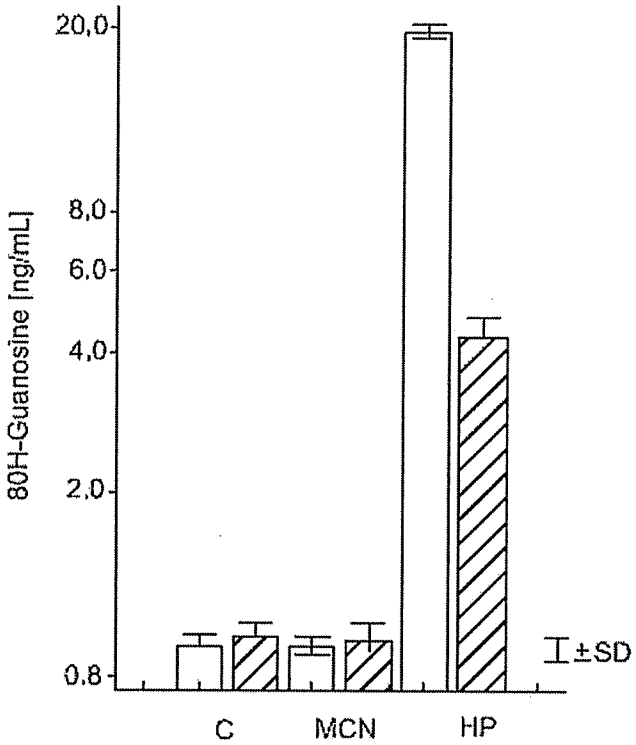


FIG. 6

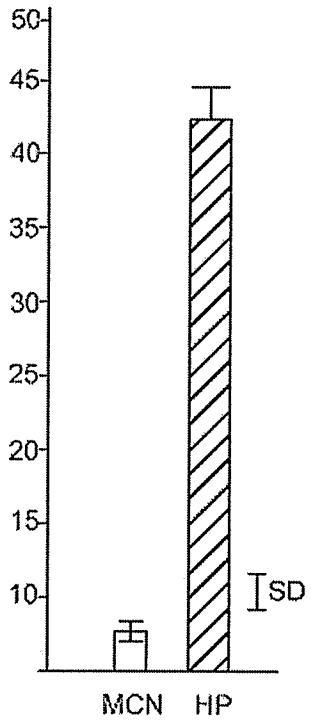
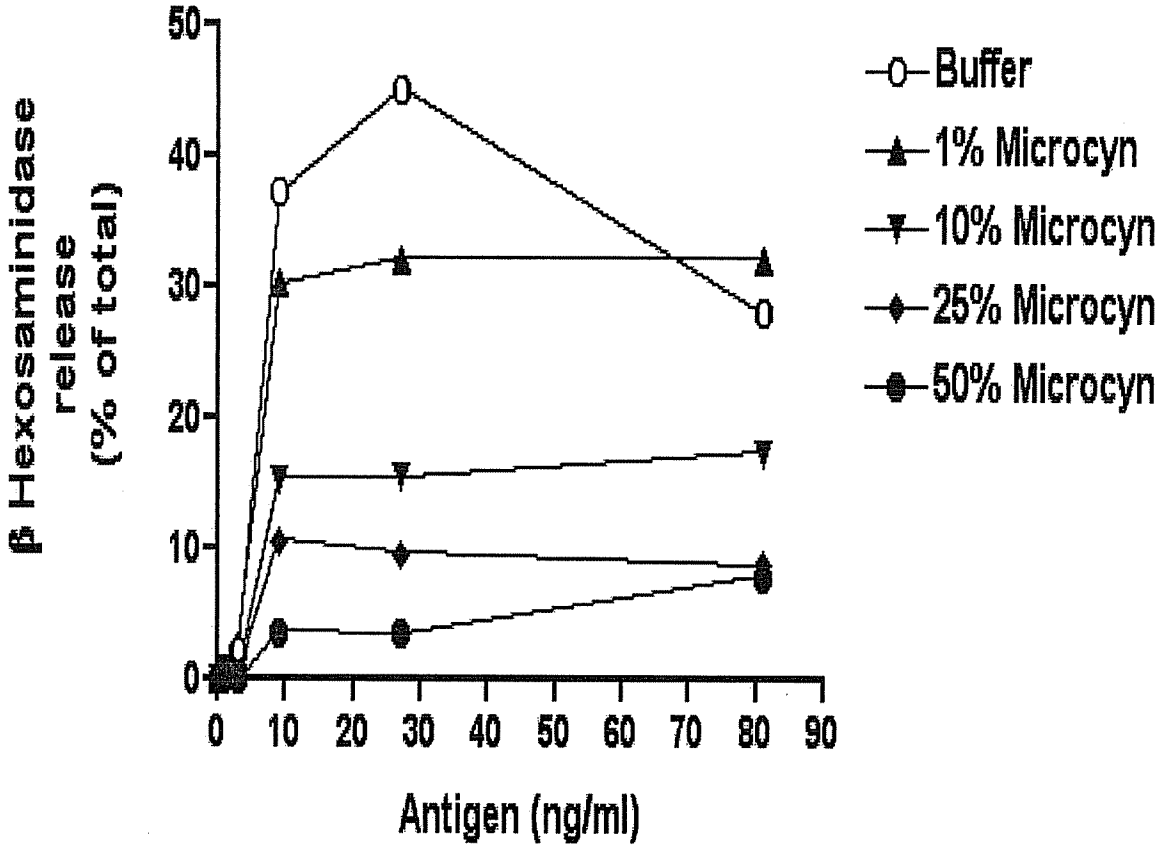
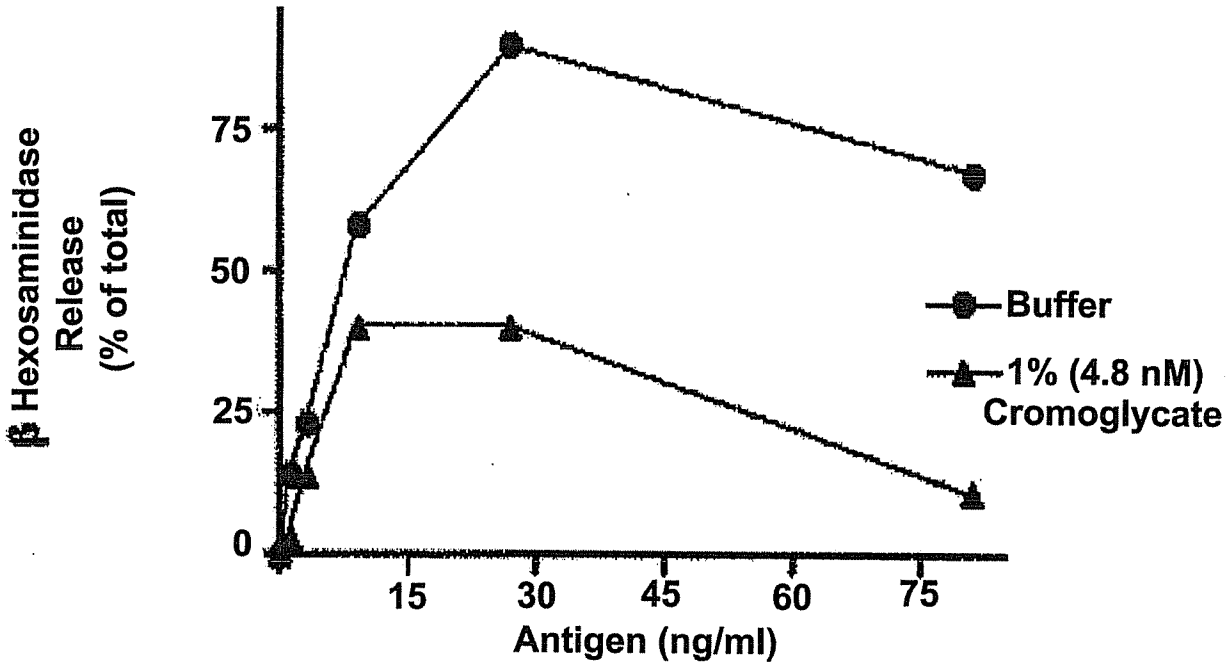


FIG. 7


β Hexosaminidase release (% of total)
50
40
30
20
10
0
0 10 20 30 40 50 60 70 80 90
Antigen (ng/ml)
Buffer
1% Microcyn
10% Microcyn
25% Microcyn
50% Microcyn

10/17
FIG. 8


β Hexosaminidase Release (% of total)
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15
30
45
60
75
Antigen (ng/ml)
Buffer
1% (4.8 nM) Cromoglycate

FIG. 9

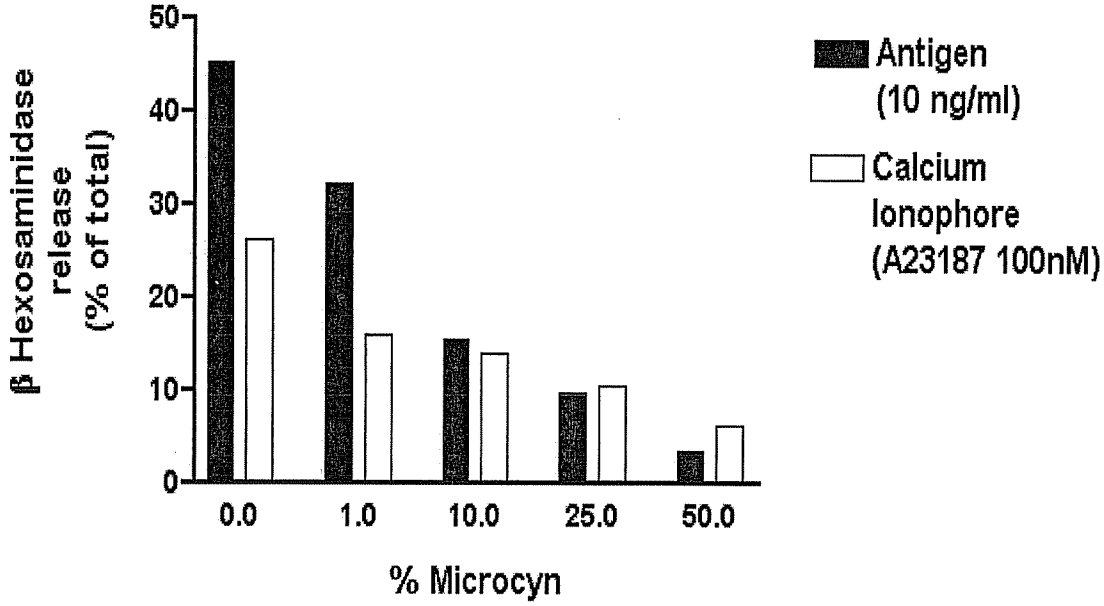

β Hexosaminidase release (% of total)
50
40
30
20
10
0
0.0
1.0
10.0
25.0
50.0
% Microcyn
Antigen (10 ng/ml)
Calcium Ionophore (A23187 100nM)

FIG. 10A

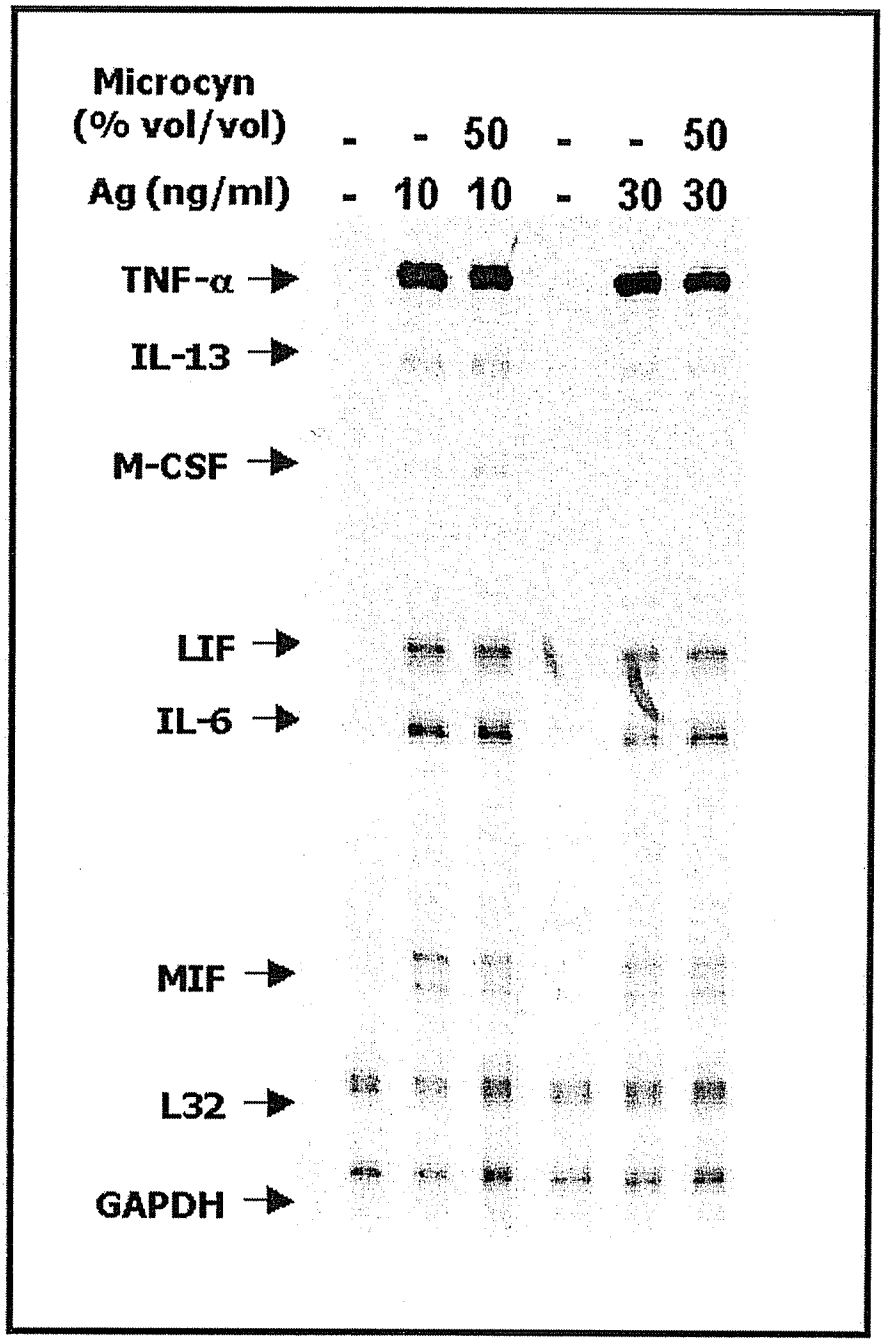

Microcyn
(% vol/vol)
- - 50 - - 50
Ag (ng/ml)
- 10 10 - 30 30
TNF-α
IL-13
M-CSF
LIF
IL-6
MIF
L32
GAPDH

FIG. 10B

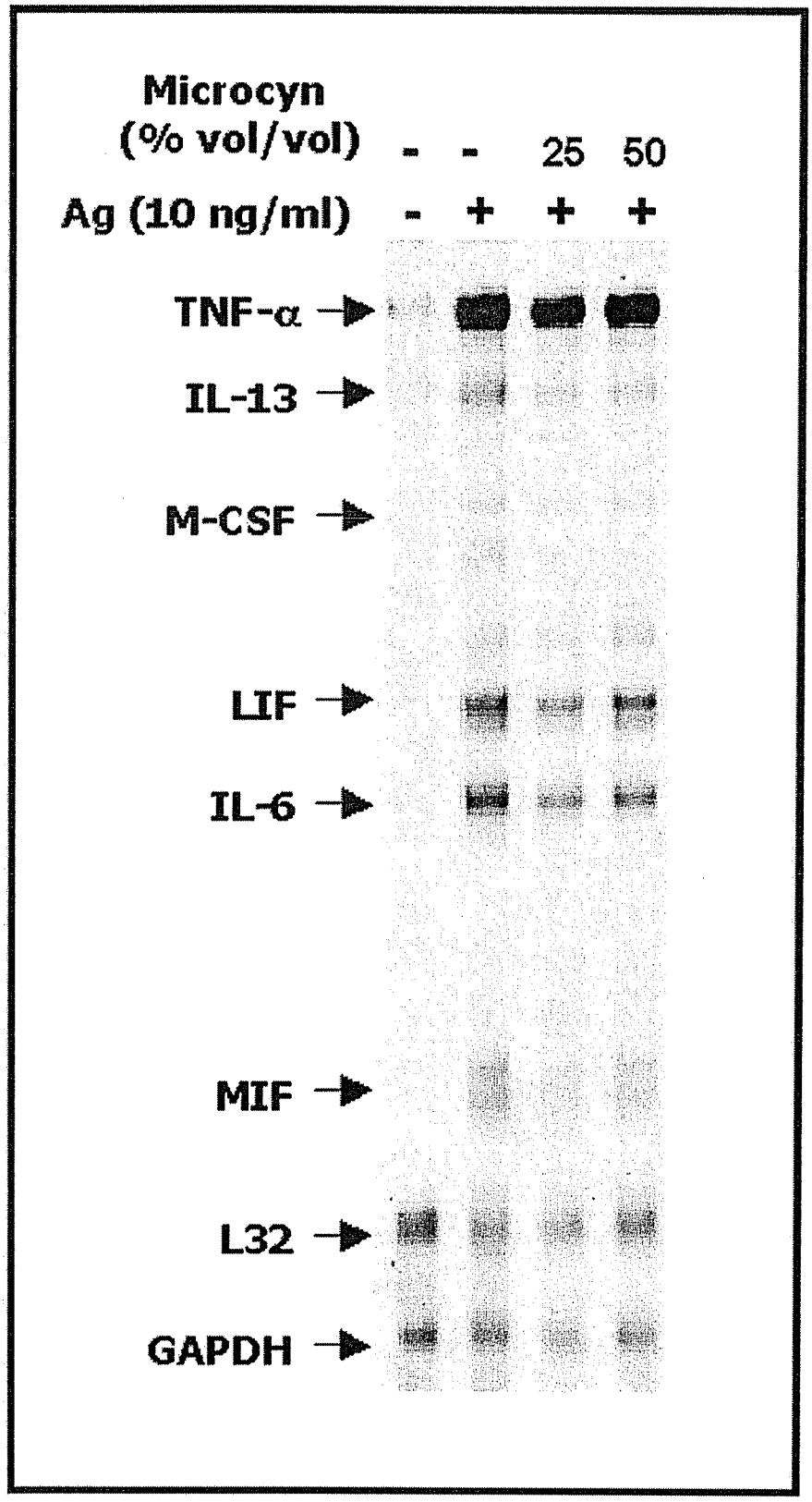

Microcyn
(% vol/vol) - - 25 50
Ag (10 ng/ml) - + + +
TNF-α
IL-13
M-CSF
LIF
IL-6
MIF
L32
GAPDH

FIG. 11

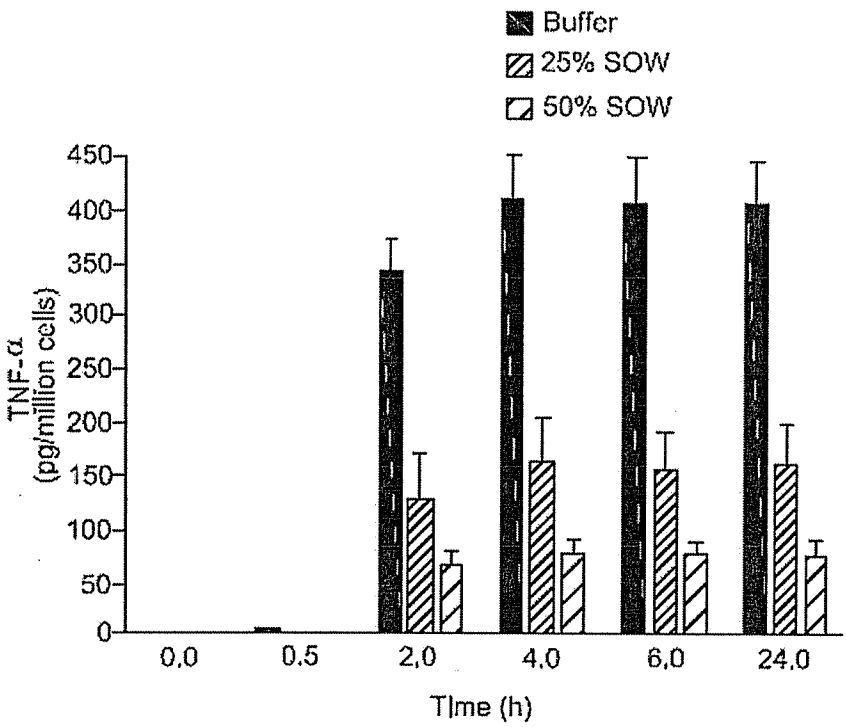
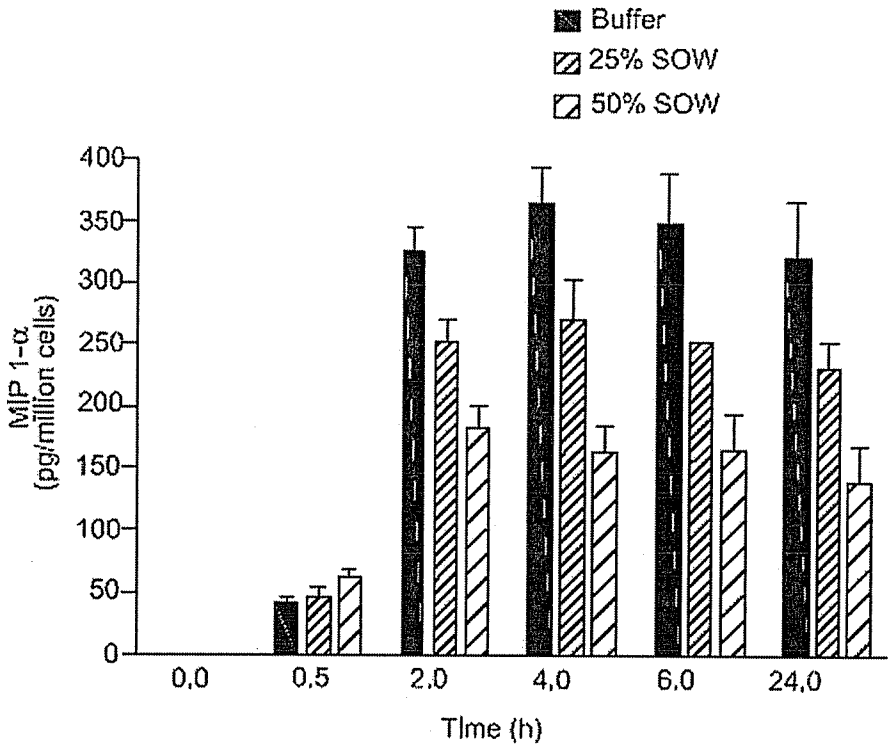

Buffer
25% SOW
50% SOW
TNF-α (pg/million cells)
450
400
350
300
250
200
150
100
50
0
0.0
0.5
2.0
4.0
6.0
24.0
Time (h)

FIG. 12


Buffer
25% SOW
50% SOW
MIP 1-α (pg/million cells)
400
350
300
250
200
150
100
50
0
0,0
0,5
2,0
4,0
6,0
24,0
Time (h)

16/17
FIG. 13

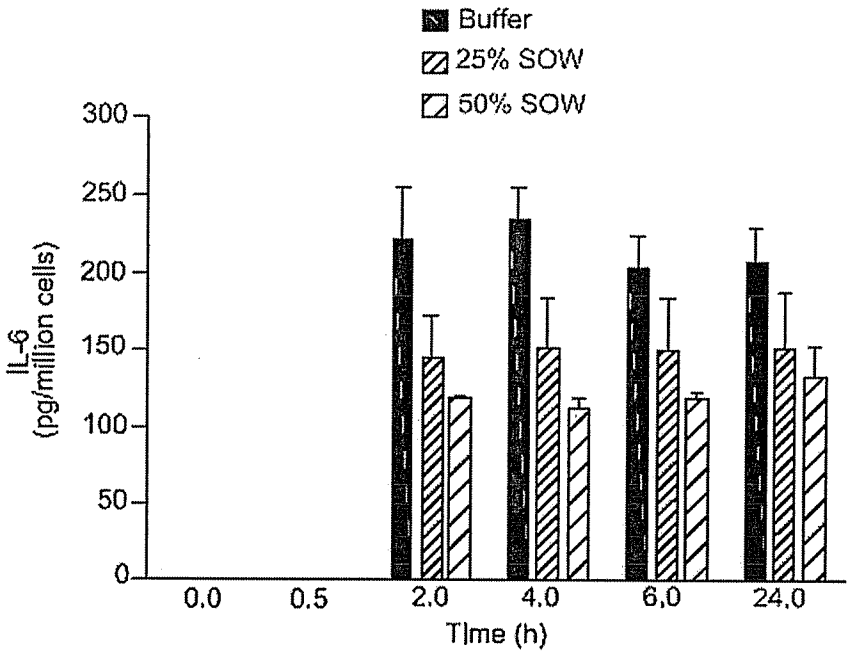
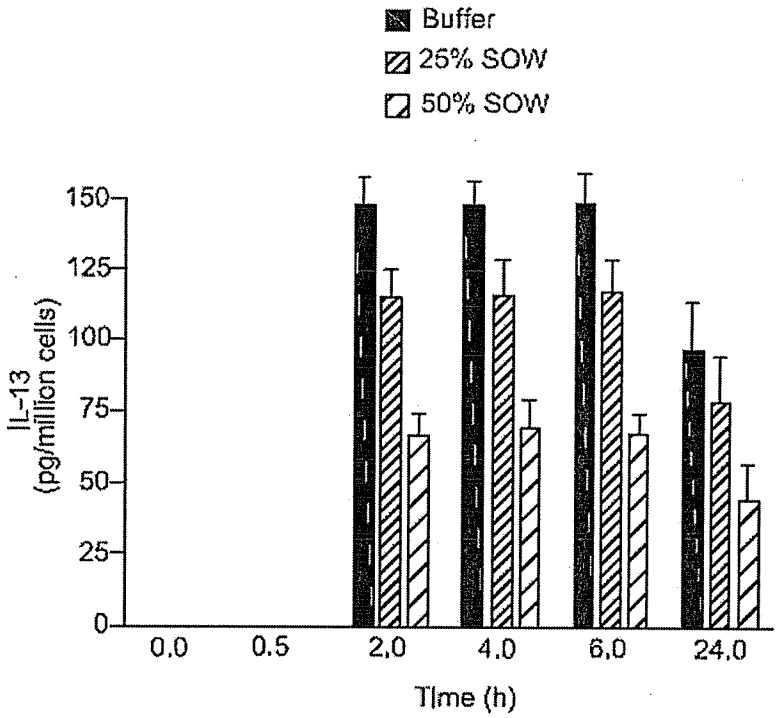

Buffer
25% SOW
50% SOW
IL-6 (pg/million cells)
300
250
200
150
100
50
0
0.0
0.5
2.0
4.0
6.0
24.0
Time (h)

FIG. 14


Buffer
25% SOW
50% SOW
IL-13 (pg/million cells)
150
125
100
75
50
25
0
0.0
0.5
2.0
4.0
6.0
24.0
Time (h)