Title: METHOD OF TREATING OPEN WOUNDS USING HYPOCHLOROUS ACID

Abstract: Methods for treating open wounds, such as chronic refractory open wound, by administering an electrolyzed saline solution comprising hypochlorous acid are provided. Method of alleviating the pain associated with open wounds by administering an electrolyzed saline solution are also provided. Combination treatment methods are also provided where an electrolyzed saline solution is administered subsequent to or concurrently with standard compression bandaging.
METHOD OF TILEATING OPEN WOUNDS USING HYPOCHLOROUS ACID

CROSS-EJEFERENCE TO RELATED APPLICATIONS
This application is a continuation-in-part of U.S. Application No. 10/830,878, which was filed on April 23, 2004, which is a divisional of U.S. Pat. Ser. No. 10/084,518, filed Feb. 25, 2002, now abandoned, which is a continuation of International Patent Application No. PCT/GB00/03264, filed Aug. 23, 2000, the disclosures of which are incorporated herein by reference. This application also claims priority to U.S. Provisional Application No. 60/749,582, which was filed on December 13, 2005, which is incorporated by reference herein.

FIELD OF THE INVENTION
This invention relates to methods of treating wounds and associated symptoms using an electrolyzed saline solution containing hypochlorous acid.

BACKGROUND OF THE INVENTION
Symptoms of many open wounds are chronic inflammation, debility, pain, and social stigma from infection. According to the National Institutes of Health, chronic wounds such as venous leg ulcers, pressure sores, ischemic ulcers and diabetic foot ulcers, affect more than 4 million Americans each year and cost about $9 billion to treat. An article by George Cherry in The Prescriber (May 1996) entitled "GP guide to the care of patients with leg ulcers" states "leg ulcers are notoriously difficult to treat successfully and can seriously reduce the patient's quality of life." Indeed, according to Cherry, "epidemiological studies have shown that at any given time there are approximately 100,000 patients in the UK that have leg ulceration. In treating these patients it has been estimated that over £39 million per year alone is spent on materials used in their ulcer care."

There are many proposals for the management of open wounds such as ulcers, all of which have varying degrees of success. Successful wound management depends on rigidly adhering to a treatment program in combination with effective wound disinfection. Such wound management reduces bacterial infection and promotes the regeneration of dermal fibroblasts and keratinocytes in the bed of the ulcer, which are essential for wound healing and new tissue growth. If the bacterial growth is not controlled, the wound cannot heal.
One treatment option for venous ulcers is using compression bandages and elevating the legs. This mimics the pumping action of the calf muscles, which return the blood back to the body, and maximizes the removal of blood from the leg(s). However around 30% of patients fail to respond to this treatment and require multiple and costly alternative therapies to alleviate their symptoms. Other treatment strategies include the use of topical treatments such as GRANUFLEX®, to aid granulation and skin repair, alginates to clean the wound of debris, dry inert dressings to protect the wound (but which do not promote healing), and bacteriostatic or bactericidal ointments to reduce the infection. While antibiotics have been used to reduce infection in the past, nowadays this is often not a treatment of choice due to the increased risk of antibiotic resistance.

Another treatment option, potassium permanganate (KMnO₄), is an oxidant which has stood the test of time in the treatment of leg ulcers but still has the disadvantages of irritating and injuring newly grown slhn and causing skin discoloration. Similarly, other disinfectants such as EUSOL (Edinburgh University Solution of Lime) and Daikin's solution, rely on a high concentration of hypochlorite ions for their disinfectant properties and are also irritating and painful. In fact, these compounds are no longer recommended for use due to their irritant and painful effects and impairment of cell growth which outweigh their therapeutic value. Although there have been attempts to reduce the alkaline effect of the high hypochlorite ion content of these solutions, these attempts have been generally ineffective.

All this has militated against the use of preparations including hypochlorites for the treatment of open wounds, such as leg ulcers. Accordingly, a need exists for another method to treat open wounds.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic outline of the main processing stages of a method for producing an electrolyzes saline solution for application in certain embodiments of a method according to the present invention.

FIG. 2 is a flow diagram depicting a method of producing an electrolyzed saline solution for application according to certain embodiments of a method of the present invention.
FIG. 3 is a schematic illustration of an electrochemical cell that can be used to produce an electrolyzed saline solution for application in certain embodiments of a method of the present invention.

FIG. 4a is a bar graph illustrating the effect on dermal fibroblast proliferation, measured by absorption assay at 3 and 6 days, of various concentrations of an electrolyzed saline solution according to the present invention.

FIG. 4b is a bar graph similar to FIG. 4a, showing the results of the absorption assay at 6 days.

DRAFT SUMMARY OF THE INVENTION

In an embodiment, the present invention provides a method of treating a chronic, refractory open wound by applying to the wound an electrolyzed saline solution comprising hypochlorous acid having a pH of from about 4 to about 7 and an available free chlorine content of from about 50 to 1000 parts per million (ppm) when produced by an electrochemical cell system.

In another embodiment, the present invention provides a method of reducing pain associated with an open wound by applying to the wound an electrolyzed saline solution comprising hypochlorous acid having a pH of from about 4 to about 7 and an available free chlorine content of from about 50 to about 1000 ppm when produced by an electrochemical cell system.

In an embodiment, the present invention provides a method of treating an open wound comprising (i) applying a compression bandage to the wound and applying subsequently or concurrently to the wound an electrolyzed saline solution comprising hypochlorous acid having a pH of from about 4 to about 7 and an available free chlorine content of from about 50 to 1000 ppm when produced by an electrochemical cell system.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides various methods of treating open wounds and associated symptoms in patients. An open wound, as is generally known in the art, is a wound that breaks the skin or mucous membrane. Non-limiting examples of open wounds include ulcers, pressure sores, burns, lacerations, surface wounds, and incisions. Non-limiting examples of ulcers
include diabetic ulcers and foot and leg ulcers (including arterial and venous leg ulcers). The term "patient," as used herein, includes human and animal patients. The term "treating," as used herein refers to improving, preventing the occurrence or worsening of, or alleviating the symptoms or pathologic effects of an open wound. For example, treating can include decreasing the size of the open wound, decreasing the duration of the open wound, reducing the spread of infection, healing the open wound by facilitating cell growth and regeneration, decreasing the microbial count in the open wound, improving the clinical appearance of the open wound, and/or reducing the sensitivity of skin surrounding the open wound. In a preferred embodiment, treating the open wound comprises healing the wound by reducing the microbial count in the wound and permitting cellular proliferation. Specifically, in a preferred embodiment, an electrolysed saline solution of the present invention has no adverse effect on fibroblasts and keratinocytes and thus acts as a microbiocide to aid in the healing process by not inhibiting normal cell growth and, in fact, allowing normal cell growth to occur.

In an embodiment, the present invention provides a method of treating a chronic, refractory open wound in a patient by applying to the wound an electrolyzed saline solution. Although the type of wounds that are classified as refractory open wounds are known in the art, in an embodiment, the type of refractory open wounds that are treated according to this aspect of the present invention is a wound that does not experience at least a 44% reduction in size after three weeks of standard treatment with compression bandaging. In another embodiment, the chronic, refractory wound is a wound that is over 5cm² in size and/or that is older than six months. In an preferred embodiment, the open wound is a venous leg ulcer.

In another embodiment, the present invention provides a method of reducing pain associated with an open wound in a patient by applying to the wound an electrolyzed saline solution. One method of determining whether the patient has experienced a reduction in pain is by initially assessing the pain prior to treatment and then assessing the pain after treatment. For example, the patient can participate in a questionnaire, such as a McGill questionnaire, to determine the patient's level of pain. Alternatively, a reduction in pain can be determined by comparing the patient's level of pain after receiving treatment against another patient's level of pain who has not been treated with an electrolyzed saline solution of the present invention.

In another embodiment, the present invention provides a method of treating an open wound by applying a compression bandage to the wound and then applying an electrolyzed
saline solution to the wound. In another embodiment, the electrolyzed saline solution is applied
to the wound in conjunction with a compression bandage.

In a further embodiment, the present invention provides a medicament for open wounds,
which contains an electrolyzed saline solution according to the embodiments describes herein,
that is used for treating chronic refractory open wounds, reducing the pain associated with an
open wound, and/or that is used subsequent to or concurrently with standard compression
bandaging. The medicament can be in any appropriate form, such as a solution, a gel, cream,
ointment, paint, micronized spray or other form.

In yet another embodiment, the present invention provides a kit for treating an open
comprising an electrolyzed saline solution according to any of the embodiments described herein
and a compression bandage.

In any of the embodiments of the present invention, an electrolyzed saline solution can be
administered in combination with other treatments such as antibiotics, compression bandaging or
other therapeutic agents;

An electrolyzed saline solution of the present invention may be administered as
frequently as necessary in order to obtain the desired therapeutic effect. Frequency of
administration will depend, for example, on the stage of the open wound, the route of
administration or associated symptom being treated. Suitable doses of an electrolyzed saline
solution can be determined by a physician or qualified medical professional and depend on
factors such as the nature of the open wound or symptom, the route of administration, the
duration of treatment, and the condition of the patient. An electrolyzed saline solution of the
present invention can be applied in different forms such as a liquid or gas and in a number of
ways such as by way of a gel, cream, ointment, or micronized spray. Alternatively or in
addition, the open wound can be immersed in a bath, such as a hydrobath.

The pH of an electrolyzed saline solution of the present invention is between about 4 to 7,
including all intermediate numbers therebetween. In a preferred embodiment, the pH is between
about 5.4 to 5.8. In a more preferred embodiment, the pH is 5.4. The available free chlorine
(AFC) concentration of an electrolyzed saline solution of the present invention is between about
50-1000 parts per million (ppm). In a preferred embodiment, the AFC concentration is about
150-180 ppm. The main active component in an electrolyzed saline solution of the present
invention is hypochlorous acid and preferably the hypochlorous acid is present at 95%. In an
embodiment, the electrolyzed saline solution has a biocide rate (D Value) of approximately 1 log unit reduction of bacillus subtilis spores in less than 1 minute with a 9:1 electrolyzed saline solution: inoculum mix. In a preferred embodiment, the biocide rate is about 3.4 seconds.

An electrolyzed saline solution can be prepared by any suitable method that results in the electrolyzed saline solution having the above-described characteristics. FIG. 1 provides a schematic outline of the main processing stages of a non-limiting, exemplary method for producing an electrolyzed saline solution of the present invention. Such a method involves an input and pre-processing stage; a production stage; and a storage and dispensing stage. In the input and pre-processing stage, water can be passed through a water softener zone where excess magnesium and calcium ions are removed. The resultant softened water can be passed as process water to a brine generation zone where a salt (such as NaCl) can be added to produce a dilute salt solution. Preferably, the salt is vacuum dried crystalline salt which is commercially produced to a consistent standard. The dilute salt solution can be a substantially constant concentration since a known quantity of salt is added to a known quantity of softened water to achieve a desired concentration of the dilute salt solution. Another method may involve mixing a known amount of a salt, such as, for example, NaCl or KCl, with de-ionized or de-mineralized water. This water can be used as delivered by a deionizer or demineralizer or can be dosed with a known amount of a buffering agent, such as, for example, sodium bicarbonate. This electrolyte can then be introduced to the production stage.

In the production stage, the dilute saline solution or prepared electrolyte can be passed to one or more electrolytic cell systems, such as the electrolytic cell depicted in FIG. 2 (a preferred embodiment of which is described in more detail in FIG. 3). The electrochemical cell includes a cathode and an anode chamber across which a substantially constant electric current is applied. The applied electric current can be maintained constant via an energy control and monitoring zone. Catholyte and anolyte are produced from the cathode and anode chambers respectively as a result of electrochemical treatment of the saline solution in the cells. Catholyte and anolyte can be prevented from mixing using a separator. For example, a semi-permeable membrane can be used in the case of parallel plate technology (for example, NAFION® membrane) or a porous ceramic membrane. In some embodiments, the catholyte is not required for the final solution and is directed to drain. In other embodiments, all or part of the catholyte is re-introduced into the anode chamber (referred to in the art as catholyte recirculation). Catholyte which is not
recirculated can be directed to waste and anolyte, otherwise referred to as output solution, is passed to a buffer storage and quality subsystem. The output solution can be tested in the buffer storage and quality subsystem, and, if it fails to meet the quality standards, can also be directed to waste. If the output solution falls within specification, the output solution can be permitted to pass to an output solution storage zone from where it can be subsequently dispensed for use.

FIG. 2 is a flow diagram or "hydraulic map" showing in more detail a non-limiting, exemplary method of producing an electrolyzed saline solution of the present invention. Potable water can be passed through an external water softener containing a cation exchange resin (not shown) thereby exchanging hardness ions of calcium and magnesium onto the resin and releasing sodium ions into the water. The softened water can be fed through a valve 16 into a softened water tank 14 which may include a plurality of level detectors for monitoring and controlling the softened water level in it. For example, tank 14 may include a level detector 20, which is a safety device which is activated only when the softened water in tank 14 reaches a predetermined extra high level to stop the charging of tank 14 with further softened water. Tank 14 may also include a level detector 22 which ensures that tank 14 has a correct volume of softened water to prepare the appropriate concentration of saline solution. Tank 14 may also include a level detector 26 and softened water will begin to re-charge tank 14 when the softened water drops below a predetermined low level determined by level detector 26 and at the end of production of one batch of electrolyzed saline solution. Tank 14 may also include a valve 28 which allows liquid to be drained.

To produce a saline solution from the softened water in tank 14, a salt, such as, for example, vacuum dried crystalline salt can be added to tank 14 via dispensing wheel 21. Dispensing wheel 21 contains many tablets of known salt mass, a pre-determined number of which are dispensed through a hole in the top of tank 14 at the start of each electrolyzed saline solution production cycle. Preferably, the saline solution has a salt concentration range of 2.0 to 90.0 g/L.

Pump 59 can pump the saline solution towards an electrolytic cell pack 63. The flow rate of the saline solution can be monitored by a sensor 10. The sensor can ascertain whether the incoming saline solution is at a temperature within the range under which the process can reasonably operate, such as between 5 and 35°C. Other parameters such as the incoming solution's pressure, softness, alkalinity, pH, conductivity, and microbial count can be monitored,
modulated and/or controlled to establish that the solution falls within acceptable levels for the process or for desired characteristics of the resulting solution. For example, as the salt concentration of the solution is increased, the conductivity can be increased and other parameters, such as the current, would change. The various parameters can be modified to correspond to the desired salt concentration of the solution. A person of skill in the art can appreciate whether the incoming water is not suitable for processing according to the present invention. If sensor 10 detects that the properties of the incoming saline solution do not fall within acceptable limits, the solution can be diverted through a waste discharge manifold (not shown) to a drain via valve 12. On the other hand, if the incoming saline solution is acceptable, it can be allowed to flow into the cells through valve 13.

The saline solution can then split into two streams 58 and 60 before being fed through electrochemical cell pack 63. In certain embodiments, electrochemical cell pack 623 can include eight electrolytic cells, with two sets of four cells connected hydraulically in parallel. For simplicity, only one cell is illustrated. In general, the number of cells in the cell pack can be determined by the output volume required from the particular system. Each cell has an anode chamber and a cathode chamber and the flow of saline solution can be split such that the greater portion is fed to the anode chamber and the lesser portion is fed to the cathode chamber. In certain embodiments, approximately 90% of the saline solution can be passed through the anode chamber and the remainder can be passed through the cathode chamber. The flow rate of saline solution through the cathode chamber can be much lower than for the anode chamber and the pressure in the cathode chamber can also be lower. The flow rate of saline solution into the cathode chamber, which also has an influence on the pH of the output solution, can be controlled by a flow regulator 68. Flow regulator 68 can be manually adjusted if there is a variation in input water quality.

In certain embodiments, the flow rate supplied to the anode is from 50% to 95%, inclusive of all intermediate values, of the solution applied to the electrolytic cell pack 63. In certain embodiments, the flow rate to the anode is from 85% to 95% of the solution supplied to the electrolytic cell.

As the saline solution flows through the electrolytic cells, a fixed current of from 0.1 to 25 amps, preferably 15 to 25 amps, and more preferably 18-19 amps, can be applied to each cell causing electrolysis of the saline solution thereby generating available free chlorine in the
resulting anolyte, elsewhere generally referred to as the output solution. In order to produce output solution at a certain pH, namely between 4 to 7 (acidic to neutral), the pH of the output solution may be at least partially controlled by dosing a portion of the catholyte to the inlet stream for the anode chambers. The catholyte may be dosed to the inlet stream 58 by an adjustable pump and valve system 66 and the dosing rate is increased or decreased to achieve the target pH. The remaining catholyte which is not dosed into the input stream 58 for the anode chambers can be directed to waste, if necessary diluting it prior to disposal. As just described, in certain embodiments, the catholyte can be dosed into the anode stream 58 before this stream enters the anode. However, the catholyte can also be dosed into the anode stream after it has been electrolyzed. In those application where the electrolyte is prepared by mixing the various salts with de-ionized or de-mineralized water, mixing of the catholyte may not be performed, in which case all the catholyte is diverted to drain. If a proportion of the catholyte is used for pH control, then the catholyte can be dosed to the anode stream either before or after it enters the anode chamber.

The output solution can then be directed to tank 70. The pH of such output solution can be measured by a meter 72. If the pH does not fall within the desired parameters, a valve 76 can be opened and the contents of tank 70 can be drained to waste. Meter 72 can be linked to a pump and valve system 66 to adjust the level of catholyte dosed to the anode chambers thereby enabling the pH of the output solution to be adjusted to bring the output solution within the desired pH range. If the pH of the output solution is determined to fall within the desired parameters, valve 76 can be kept closed and the output solution can be allowed to fill tank 70. Other properties of the output solution, such as redox potential or AFC, could also form the basis of the measurement and control system consisting of meter 72 and adjustable pump and valve system 66.

Storage tank 70 may include various level detectors for monitoring liquid levels in the tank. For example, a level detector 90 may be activated by an extra high level of output solution within the tank, raising; an alarm and stopping production. Low level detector 94 may be activated when the level of the output solution falls to a low level, raising an alarm and preventing further dispensing to the appropriate receptacle. As the output solution is dispensed and after a period of time below the level of detector 94, production of output solution may be re-commenced. From the storage tank, the output solution can be distributed in individual
nebulizers, inhalers, or ampules. Of course, the above-described processing steps of producing an electrolyzed saline solution are only exemplary and other electrochemical processes could be used to produce an electrolyzed saline solution of the present invention.

**FIG. 3** shows an embodiment of an electrolytic cell 300 used in certain methods of producing an electrolyzed saline solution according to an embodiment of the present invention. In this embodiment, cell 300 comprises co-axial cylindrical and rod electrodes 302, 304 respectively, separated by a semi-permeable ceramic membrane 306 co-axially mounted between the electrodes thus splitting the space between the electrodes to form two chambers 308 and 310. Cylindrical electrode 301, which is this embodiment forms the anode, is typically made from commercially pure titanium coated with a ruthenium oxide and iridium oxide-based electrocatalytic (active) coating suitable for the evolution of chlorine from a chloride solution. Rod electrode 304, which in this embodiment forms the cathode, is typically made from titanium and can be machined from an 8mm stock bar to a uniform cross-section over its effective length, which is typically about 210mm ± 0.5mm. Of course, it will be understood by one of skill in the art that other suitable materials and configurations can be used to fabricate electrodes 302 and 304 to allow these electrodes to perform their necessary function. Also, either electrode can serve as the anode and similarly either electrode can serve as the cathode. If the rod is used as an anode, it is coated with a coating, such as ruthenium oxide and iridium oxide based electrocatalytic (active) coating, for example, suitable for the evolution of chlorine from a chloride solution. Semi-permeable ceramic membrane 306 forming a separator and creating the anode and cathode chambers 308 and 310 can be composed of aluminium oxide (80%), zirconium oxide (18.5%) and yttrium oxide (1.5%), and preferably has a porosity of about 50-70%, a pore size of 0.3 to 0.5 microns and a wall thickness of 0.5mm ±0.3mm/-0.1mm. The ceramic of certain embodiments of membrane 306 is described in the specification of patent application GB 2354478 (Sterilox Medical (Europe) Limited), the subject matter of which is incorporated herein by reference. Ceramic membrane 306 can be made of any other suitable semi-permeable or ion-selective material of ceramics other than the aluminium oxide, zirconium oxide and yttrium oxide ceramic described above.

Generally, the surface area of the anode can be largely defined by the quantities of output solution desired to be produced and available free chlorine content desired in that solution. However, in order to provide a system that is of a size appropriate for commercial installation
and to produce the quantities of biocidal solution of the invention often required, an anode surface area of 0.065 to 0.095 m², inclusive of all intermediate values, can be utilized. Such a surface area can be made up by a number of electrolytic cells working in parallel. An anode area of 0.070 to 0.090 m² is more preferable, and an anode surface area of 0.075 to 0.085 m² is even more preferable. In certain embodiments, eight cells are arranged in parallel and the current density on the surface of each anode is within the range 1.5 to 2.5 kAm⁻², more preferably 1.7 to 2.2 kAm⁻², and still more preferably 1.85 to 1.95 kAm⁻².

In this embodiment, cell 300 is provided with entry passages 312 and 314 to permit the saline solution to enter cell 300 and flow upwards through the anode and cathode chambers 308 and 310 to be discharged as anolyte and catholyte through exit passages 316 and 318 respectively. The anolyte containing available free chlorine constitutes the output solution. As previously described in reference to FIG. 2, in certain embodiments, in order to provide a preferred amount of output solution within a reasonable period of time, a group of cells can be connected together to form a cell pack 63. For example, a cell pack comprising eight cells connected together in parallel hydraulically and in series electrically may generate about 200 litres/hour of output solution.

In certain embodiments of the invention, the flow rate through the anode chamber may vary between 100 to 220 l/h. A flow rate of 150 to 210 l/h is more preferred and a flow rate of 185 to 205 l/h is even more preferred. The flow rate can also be any value within the expressed ranges. The person skilled in the art will appreciate that the flow rate can be altered beyond such a range but still produce the solution of the invention by varying the number of cells/surface area of anode. For example, the flow rate per anode surface area of 1.25 × 10³ to 2.75 × 10³ l/h/m² can be used produce an embodiment of an electrolyzed saline solution of the invention. The flow rate can also take any value with the aforementioned range. Preferably, the flow rate is 1.87 × 10³ to 2.63 × 10³ l/h/m² and more preferably the flow rate is 2.31 × 10³ to 2.56 × 10³ l/h/m². The skilled person can obtain the required current to produce a suitable solution by setting the flow rate to that just described and varying the current until the solution produced has the suitable specifications.

In certain embodiments, the current range is 15 to 25 A, inclusive of all intermediate values. In certain embodiments, a current range of 17 to 22 A is used and in certain embodiments a current range of 18.5 to 19.5 A is used.
The residual salt concentration of an embodiment of an electrolyzed saline solution can be from 2.0 g/1 to 90 g/1. In certain embodiments, the residual salt concentration is greater than 9 g/1 and in certain embodiments, the residual salt concentration is 70-90 g/1. This residual salt concentration can result from the entire desired amount of salt being added during the input and pre-processing stage or less than the entire desired amount of salt being added during the input and pre-processing stages and the remainder of the desired amount of salt being added after the production stage.

EXAMPLES

Example 1:

Three in vitro trials are carried out on single layers of cultured human dermal fibroblast cells and keratinocyte cells to ascertain whether or not an electrolyzed saline solution produced according to the above-described method has any effect on the viability of the cells. Fibroblasts are flattened, irregular-shaped, connective tissue cells which are ubiquitous in fibrous connective tissue. They secrete components of the extracellular matrix, including collagen, and play an important role in tissue regeneration.

The cells are incubated under sterile conditions in an electrolyzed saline solution containing primarily hypochlorous acid and including sodium hypochlorite and other oxidized chlorine species, having a pH range from 4 to 7 and a redox potential of around 1000 mV. A range of dilutions of electrolyzed saline solution at different pH levels is investigated.

Specifically, the electrolyzed saline solution used in the trials is the product of the electrolysis of an aqueous saline solution passed over a mixture of catalysts on titanium electrodes to give a mixture of oxidizing species, particularly hypochlorous acid (HOCl) at a concentration of about 144 mg/l to 400 mg/l available free chlorine (Cl). The electrolyzed saline solution is produced as required for each test; the apparatus (supplied by Sterilox Medical Limited, Abingdon, UK) is operated to give a final solution redox potential of >950 mV. Appropriate dilutions of the electrolyzed saline solution are made, and the pH of the final solution is adjusted using a phosphate buffer.

For the proliferation assay, HDF cells are seeded in normal (10%) fetal calf serum (FCS) and Dulbecco’s Modified Eagle Medium (DMEM) at 1.5 x 10^3 cells/well. After 24 hours, the medium is changed to low (0.4%) FCS/DMEM. After a further 48 hours incubation, electrolyzed saline solution at varying concentrations is added to the cells. The viability of the cells is
observed, using a standard absorption assay, 3 and 6 days after the application of electrolyzed saline solution.

i) Trial 1: HDF cells are incubated with electrolyzed saline solution in a range of dilutions at a pH of 4.3. The dilutions used are: 1, 1/3, 1/7, 1/14, 1/28, 1/56, 1/112, 1/224, 1/448, 1/896, 1/1792 and 0.

As shown in the accompanying graphs, on both day 3 (FIG. 4a) and day 6 (FIG. 4b) electrolyzed saline solution dilutions of 1/28 or less significantly inhibits HDF proliferation or kills the cells. Slight inhibition of proliferation is seen at a dilution of 1/56. Dilutions of 1/112 to 1/448 shows no effect on proliferation, while the 1/896 dilution shows some cell proliferation and the dilution of 1/1792 shows significant proliferation of HDF cells.

These results show that high concentrations of electrolyzed saline solution significantly inhibit HDF proliferation, probably because of the acidity, and therefore toxicity, of the electrolyzed saline solution. The high level of proliferation seen with a concentration of electrolyzed saline solution at 1/1792 may be attributed to other factors.

ii) Trial 2: Using, the same conditions as Trial 1, HDF cells are incubated with electrolyzed saline solution in a range of dilutions at a pH of 6.2. The dilutions used are: 1/20, 1/40, 1/60, 1/80, 1/100, 1/120, 1/1000, 1/1500, 1/2000, 1/3000, 1/4000 and 0.

No stimulation of proliferation is seen and indeed, inhibition of HDF growth is seen with cells incubated with a dilution of electrolyzed saline solution of 1/20. However, the higher dilution of 1/40 shows no cell toxicity.

This trial is repeated with the HDF cells seeded in electrolyzed saline solution at dilutions of 1, 1/4, 1/8, 1/12, 1/16, 1/20, 1/24, 1/28 and 1/32. After both day 3 and day 6, cell damage or inhibition of proliferation is seen at dilutions of 1/20 and below. However, dilution of more than 1/20 shows no damage or inhibition or proliferation.

In conclusion, while the more alkaline pH appears to be less toxic to HDF cells, proliferation of HDF cells is not seen at this pH.

iii) Trial 3: In this trial, two plates of cells are grown in normal growth medium (10% FCS/DMEM). One plate of cells is treated as described in Trial 1 but after three days of incubation with the electrolyzed saline solution, the growth medium is changed from 0.4% FCS/DMEM to 10% FCS/DMEM in order to observe the recovery of the cells.
HDF cells are incubated at 31°C with electrolyzed saline solution in a range of dilutions at pH of 5.4. The dilutions used are: 1/10, 1/20, 1/40, 1/60, 1/80, 1/100, 1/150, 1/1000, 1/1500, 1/2000, 1/4000 and 0.

On day 3 and day 6 cell proliferation is seen in cells incubated with electrolyzed saline solution at a dilution of 1/20 or higher in either 0.4% or 10% FCS/DMEM. Some levels of proliferation have reached statistical significance. A dilution of 1/10 electrolyzed saline solution inhibits cell growth in HDF cells grown in 0.4% FCS/DMEM but not in 10% FCS/DMEM.

After 3 days incubation in 0.4% FCS/DMEM with or without electrolyzed saline solution, the medium is changed to 10% FCS/DMEM. The cells that have been incubated with electrolyzed saline solution show the same ability to recover from depression of cell growth seen while growing in 0.4% FCS/DMEM as control cells.

This trial is repeated with the HDF cells seeded in electrolyzed saline solution at dilutions of 1/7, 1/10, 1/15, 1/20, 1/40, 1/60, 1/100, 1/500, 1/1000, 1/2000, 1/4000 and 0. 100621 Again, on both day 3 and day 6, cell proliferation is seen with HDF cells grown in 0.4% FCS/DMEM with the difference seen being statistically significant at most dilutions. No inhibition of cell growth is seen, even at the dilution of 1/7 electrolyzed saline solution. Stimulation of cell growth is also seen in cells grown in 10% FCS/DMEM in the presence of electrolyzed saline solution. However, the levels of proliferation does not reach statistical significance. Where cell growth has been impaired by incubation with electrolyzed saline solution, recovery is seen, confirming the observations from the first set of experiments.

In summary, HDF cells incubated with electrolyzed saline solution at pH 5.4 show no inhibition of cell growth, even in the presence of a 1/7 dilution of electrolyzed saline solution.

**Example 2:**

Three in vitro trials are carried out to investigate the cytotoxic effect of electrolyzed saline solution based on hypochlorous acid on HDF cells. The electrolyzed saline solution based on hypochlorous acid is identical to that described in Example 1.

HDF cells are seeded in 10% FCS/DMEM at 5.10^5 cells/well. After incubation at 31°C for 72 hours, dilutions of electrolyzed saline solution are prepared in HBSS and added to
the cells. The viability of the cells is ascertained by a standard absorption assay at time intervals of 15 minutes up to one hour from the addition of the electrolyzed saline solution.

i) Trial 1: HDF cells are incubated with electrolyzed saline solution in a range of dilutions at a pH of 4.3. The dilutions used are: 1, 1/3, 1/7, 1/14, 1/2, 1/56, 1/12, 1/224, 1/448, 1/896, 1/1792 and 0.

No effect on cell viability is seen in the presence of electrolyzed saline solution at dilutions of 1/28 or more at any of the time points. A dilution of 1/14 induces mild damage to the cells while dilutions of 1/7 and less killed the cells.

ii) Trial 2: Using the same conditions as trial 1, HDF cells are incubated with electrolyzed saline solution in a range of dilutions at a pH of 6.2. The dilutions used are: 1, 1/4, 1/8, 1/12, 1/16, 1/20, 1/24, 1/28, 1/32, 1/36, 1/40 and 0.

No significant effect is seen on the viability of HDF cells in the presence of electrolyzed saline solution at dilutions of 1/20 or more at any time point. However, dilution of \( \frac{1}{16} \) or less induces cell damage.

iii) Trial 3: Using the same conditions as Trials 1 and 2, HDF cells are incubated with electrolyzed saline solution in a range of dilutions at a pH of 4.0. The dilutions used are: 1, 1/4, 1/8, 1/12, 1/16, 1/20, 1/24, 1/28, 1/50, 1/100, 1/200 and 0.

Dilutions of electrolyzed saline solution at 1/24 and 1/20 induces slight damage to the cells while dilution of 1/16 or less induces cell death.

The results of these trials support the results shown in Example 1 in that, while electrolyzed saline solution at pH 4.0 to 4.3 and pH 6.2 induce damage to cultured HDF cells, greater cytotoxic effects; are seen at the lower pH.

**Example 3:**

In view of the results described in Examples 1 and 2, two in vitro trials are carried out to investigate the effect electrolyzed saline solution on HK cell proliferation. The electrolyzed saline solution used in these trials is identical to that described in Example 1. Keratinocytes are epidermal skin cells that synthesize keratin and, together with dermal fibroblasts, are essential for skin healing.

**Trial 1**

HK cells (subcultured, P2, FS, 7 years) are seeded at \( 8 \times 10^3 \) cells/well and incubated at
3 °C in CLONETICS® (Biowhittaker, US) serum-free medium with complete supplements, hereinafter referred to as keratinocyte growth medium (KGM), in four 24-well plates. After 24 hours incubation the medium in plates 1 and 2 was changed to CLONETICS® (Biowhittaker, US) serum-free medium without complete supplements, hereinafter referred to as keratinocyte basal medium (KBM).

After a further 48 hours incubation electrolyzed saline solution diluted in KBM at pH 5.4 is added to plates 1 and 2, and electrolyzed saline solution diluted in KGM is added to plates 3 and 4. The dilutions of electrolyzed saline solution are: 1/10, 1/20, 1/50, 1/100, 1/150, and 0. The pH of the final electrolyzed saline solution solution is adjusted using a phosphate buffer.

After incubation for a further 3 days a standard absorption assay is carried out on plate 3 to observe the viability and growth of the cells. The absorption assay was carried out on plate 4 after a still further two days. Since the cells incubated in KBM do not grow well, plates 1 and 2 are discarded.

The absorption assay shows that, on both day 3 and day 5, cell proliferation has occurred in the presence of all dilutions of electrolyzed saline solution. At day 5, the level of cell proliferation has reached a significant level compared to cell growth in the absence of electrolyzed saline solution.

Trial 2

In view of the fact that the HK cells do not grow in KBM and show significant proliferation in the presence of electrolyzed saline solution in KGM, KBM with lower concentrations of supplements is used as a holding medium, with or without electrolyzed saline solution.

HK cells (thawed, P2, FS, 7 years) are seeded to six 96-well plates at 3 times 10^3 cells/well in KGM. After incubation for 24 hours, the medium in plates 1 and 2 is changed to KBM with 20% supplements, and the medium in plates 3 and 4 is changed to KBM with 50% supplements.

After incubation for a further 24 hours, electrolyzed saline solution diluted in KBM with 20% supplements is added to plates 1 and 2, electrolyzed saline solution diluted in KBM with 50% supplements is added to plates 3 and 4, and plates 5 and 6 received electrolyzed saline solution diluted in complete KGM. The dilutions of electrolyzed saline solution are: 1/7, 1/10, 1/15, 1/20, 1/40, 1/60, 1/100, 1/500, 1/1000, 1/2000, 1/4000 and 0.
The cells are incubated for a further 3 days in the presence of electrolyzed saline solution, after which time, a standard absorption assay is carried out on plates 1, 3 and 5 to ascertain the viability of the cells, and the medium in plates 2, 4 and 6 is changed to KGM. Plates 2, 4 and 6 are assayed after a further 48 hours of incubation.

Stimulation of cell proliferation on both day 3 and day 5 is seen in all percentages of KGM supplements. However, the level of stimulation is not significantly different when compared to control cell growth. No cytotoxicity is seen even at the low dilution of \{fraction (1/7)} electrolyzed saline solution.

Conclusion

Dermal keratinocytes cultured in the presence of KGM and electrolyzed saline solution show enhanced cell proliferation, and no cytotoxicity is seen in the presence of electrolyzed saline solution.

Example 4:

A preliminary clinical evaluation of electrolyzed saline solution based on hypochlorous acid is carried out on one patient with chronic venous ulcers on both left and right legs. The aim of the trial is to determine whether the bacterial status of the ulcers is altered and the bed of the ulcer improved by treatment with electrolyzed saline solution.

The patient's legs are immersed in 40 liters of electrolyzed saline solution in a hydrobath for fifteen minutes before being allowed to dry. An intermediate assessment without treatment is carried out after one week.

A second treatment with electrolyzed saline solution is repeated after two weeks in which the patient is subjected to three fifteen-minute washes at approximately three-hour intervals. Post-treatment clinical evaluation is carried out one and several days after the second treatment.

Semi-quantitative microbiological analysis of the leg ulcers is carried out on swabs taken before and fifteen minutes after treatment with electrolyzed saline solution.

After the first treatment, the patient reports that the treatment is comfortable and free from pain. The appearances of the ulcers on both legs are markedly improved when assessed five hours after treatment.

As shown in Table 1, quantitative microbiology shows a reduction in the number of
colony-forming units in the order of $10^2$ in the right leg ulceration and a reduction in the order of $10^4$ on the left leg.

**TABLE 1**

Semi-quantitative microbiological analysis of leg ulcers before and after treatment with electrolyzed saline solution based on hypochlorous acid

<table>
<thead>
<tr>
<th></th>
<th>Right Leg</th>
<th>Left Leg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Treatment</td>
<td>$1 \times 10^7$</td>
<td>$1 \times 10^7$</td>
</tr>
<tr>
<td>Post-Treatment</td>
<td>$1 \times 10^5$</td>
<td>$1 \times 10^3$</td>
</tr>
</tbody>
</table>

Figures quoted indicate the number of colony-forming units (cfu) per mL.

The patient is seen one week after the first treatment and is treated with conventional therapy, including potassium permanganate. The effect of these on the appearance of the leg ulcers following treatment does not appear to be as striking as that seen with electrolyzed saline solution.

A second treatment with electrolyzed saline solution is repeated a further week later, and similar beneficial results are obtained. In between the treatment periods the ulcers have become sloughy on both legs. Immediately after the first wash, the ulcer bed is whitish due to effervescence. A cleansing effect is seen after the later two washes, and a marked improvement is seen with the state of the ulcer 18 hours after the first wash.

The patient reports no discomfort to the treatment, the solution in the bath is soothing, and the skin feels a bit tight afterwards. The patient comments that the tightness starts to be felt once cold air was accessible to the skin.

Referring to Table 2, quantitative microbiology shows a reduction in the number of colony-forming units in the order of $10^2$ in the right leg ulceration and a reduction in the order of $10^4$ on the left leg.

**TABLE 2**

Semi-quantitative microbiological analysis of leg ulcers before and after treatment with electrolyzed saline solution based on hypochlorous acid

<table>
<thead>
<tr>
<th></th>
<th>Right Leg (cfu/ml)</th>
<th>Organisms found</th>
<th>Left Leg (cfu/ml)</th>
<th>Organism found</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-treatment</td>
<td>$1.9 \times 10^8$</td>
<td>Coliforms</td>
<td>$4.5 \times 10^6$</td>
<td>Coliforms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proteus spp</td>
<td></td>
<td>Proteus spp</td>
</tr>
<tr>
<td></td>
<td>Skin Flora</td>
<td>Coliforms</td>
<td>Proteus spp</td>
<td>Skin Flora</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------</td>
<td>-----------</td>
<td>-------------</td>
<td>------------------</td>
</tr>
<tr>
<td><strong>Post-treatment 1</strong></td>
<td>$1.2 \times 10^5$</td>
<td>$4.5 \times 10^4$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre-treatment 2</strong></td>
<td>$3.0 \times 10^6$</td>
<td>$1.5 \times 10^6$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Post-treatment 2</strong></td>
<td>$1 \times 10^3$</td>
<td>$&lt;10$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pre-treatment 3</strong></td>
<td>$&lt;10$</td>
<td>$6.3 \times 10^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Post-treatment 3</strong></td>
<td>$3.0 \times 10^2$</td>
<td>$3.0 \times 10^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Post 24 hours</strong></td>
<td>$2.7\times 10^{-2}$</td>
<td>$1.5 \times 10^6$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figures quoted indicate the number of colony-forming units (cfu) per mL.

The use of antiseptics for cleansing wounds is controversial, particularly with reference to the degree of pain associated with this kind of treatment. This patient do not experience pain and, in fact, commented on a soothing effect. There is a positive effect on the bacterial flora as well as the clinical appearance of the wounds. There is no adverse effect on the surrounding skin which, in a number of patients with long-standing ulcers, is often sensitive.

**Example 5:**

Patients were admitted for an initial three weeks of treatment with standard compression bandaging. All patients had previously failed standard treatment with compression bandaging, had an ulcer size of 5 cm² or more, had ulcer duration of at least six months, had no insulin-dependent diabetes mellitus, and had an ankle-brachial pressure index (ABPI) ≤3.8. According to previous studies, wound healing can be expected to occur in only 22% of patients who fail to achieve a 44% reduction in wound size after three week of standard treatment. Therefore, patients who did not achieve a 44% reduction in wound size at three weeks were offered a hypochlorous acid treatment (a HOCl wash for twenty minutes in a forced circulation leg bandage).
hydrobath) in addition to standard compression treatment. The HOCl solution used was generated by electrolyzing a concentrated solution of salt to a solution consisting of over 97% HOCl at pH 5.4-5.8, with a >950mV redox potential. The concentration of HOCl used was 150-180 parts per million chlorine radicals.

The HOCl wash was administered twice a week for three weeks and then once a week for nine weeks. During the nine week period, patients continued to have routine dressing changes and reapplication of the compression bandage at home once a week.

After completing this program of treatment, patients were followed up for a further six to eight weeks while they continued with their standard compression bandaging treatment.

At each visit, including all follow-up visits, the degree of pain was assessed using a modified McGill questionnaire and the ulcers were photographed and traced. A sterile swap was pressed laterally on the wound to express underlying tissue fluid and exudate was taken for semiquantitative microbiology at the start of treatment regimens and when deemed appropriate on clinical grounds. Routine blood samples were taken on admission for a biochemical screen and full blood count.

Based on the wound appearance, in particular erythema, increased exudate and odor, and supported by microbiological evidence of a heavy growth of pathogenic bacteria, topical antibacterial agents were applied during both phases of the trial when necessary. These were restricted to metronidazole, silver sulphadiazine, cadexomer iodine and Aquacel Ag (ConvaTec). The antibiotics were used sparingly, and when the data were analyzed appeared not to have had a major therapeutic effect on their own.

Systemic antibacterial chemotherapy was routinely restricted to the use of flucloxacillin orally for 10 days only for wounds that showed the presence of β-haemolytic streptococci of Lancefield groups A, B and G. This applied to only three patients with streptococcal group G infections.

The statistical analyses performed, including the Yates-corrected chi-square test, were restricted to the results, for patients whose ulcers were ≥5cm² and over six months' old.

As shown in Tables 3, all 10 patients who achieved a ≥44% reduction in ulcer size after three weeks of standard treatment continued to improve, and nine (90%) achieved full healing within the 12 weeks of continued standard treatment. One patient who achieved a 60% reduction in ulcer size after three weeks of initial treatment further improved to an 80% reduction by nine
weeks. However, this patient contracted methicillin-resistant Staphylococcus aureus (MRSA) in her wound which was associated with considerable enlargement of the ulcer and loss of dietary control of her diabetes mellitus. She was transferred to another department for insulin treatment and ulcer care, so was excluded from further follow-up.

TABLE 3

Ten patients who achieved a reduction in ulcer size of ≥44% after three weeks’ standard treatment with four-layer bandaging and appropriate local treatment

<table>
<thead>
<tr>
<th>Initial</th>
<th>Size of ulcer on admission (cm²)</th>
<th>Duration of ulcer (months)</th>
<th>Reduction of ulcer size after three weeks</th>
<th>Time to complete healing</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR/83</td>
<td>12.0</td>
<td>12</td>
<td>47%</td>
<td>healed at 6 weeks</td>
</tr>
<tr>
<td>AD/49M</td>
<td>8.6</td>
<td>9</td>
<td>Nearly healed</td>
<td>healed at 4 weeks</td>
</tr>
<tr>
<td>PH/84</td>
<td>13.5</td>
<td>6</td>
<td>Nearly healed</td>
<td>healed at 5 week</td>
</tr>
<tr>
<td>CR/63</td>
<td>9.2</td>
<td>7</td>
<td>60%</td>
<td>healed at 12 weeks</td>
</tr>
<tr>
<td>MH/84</td>
<td>6.4</td>
<td>5</td>
<td>48%</td>
<td>healed at 11 weeks</td>
</tr>
<tr>
<td>CT/53</td>
<td>4.2</td>
<td>7</td>
<td>53%</td>
<td>healed at 5 weeks</td>
</tr>
<tr>
<td>BG/88</td>
<td>8.8</td>
<td>12</td>
<td>60%</td>
<td>increased in size due to MRSA infection and failure of dietary control of DM healed at 10 weeks</td>
</tr>
<tr>
<td>MT/87</td>
<td>5.0</td>
<td>12</td>
<td>84%</td>
<td>healed at 8 weeks</td>
</tr>
<tr>
<td>GR/63</td>
<td>3.1</td>
<td>7</td>
<td>48%</td>
<td>healed at 8 weeks</td>
</tr>
<tr>
<td>HL/79</td>
<td>8.5</td>
<td>12</td>
<td>49%</td>
<td>healed at 10 weeks</td>
</tr>
</tbody>
</table>

MT=male; MRSA=methicillin-resistant Staphylococcus aureus; DM=diabetes mellitus

The twenty remaining patients received HOCl as well as standard four-layer compression. The benefits of HOCl treatment are shown in Table 4 and 5.

As shown in Table 4, in nine patients, the reduction in ulcer size with three weeks of standard therapy was less than 44% (mean reduction: 17%; range 0-34%). After HOCl treatment, there was a substantial improvement (mean reduction after five weeks: 58%; range 18-100%). Five ulcers healed within 12 weeks and four on follow-up within twenty weeks.

TABLE 4

Nine patients who failed to achieve ≥44% reduction in ulcer size after three weeks’ standard treatment, but who healed after subsequent treatment with HOCl for 12 weeks
As shown in Table 5, a further five patients experienced a substantial reduction in ulcer size of 60-88% by 12 weeks of HOCl treatment, but were not followed up. Thus, in fourteen of the twenty patients (70%), a substantial benefit in terms of healed or healing ulcers was obtained.

**TABLE 5**

Five patients who failed to achieve a ≥4% reduction in ulcer size with the initial standard treatment, but achieved a >60% reduction after HOCl wash plus standard treatment

<table>
<thead>
<tr>
<th>Initial age</th>
<th>Size of ulcer on admission (cm²)</th>
<th>Duration of ulcer (months/years)</th>
<th>Ulcer size after three weeks standard</th>
<th>Reduction of ulcer size after HOCl washes at 5 weeks</th>
<th>Time to complete healing after HOCl washes</th>
</tr>
</thead>
<tbody>
<tr>
<td>VW/85</td>
<td>71.6</td>
<td>5 years</td>
<td>Increased by 5.4%</td>
<td>49%</td>
<td>71%</td>
</tr>
<tr>
<td>BW/78</td>
<td>2.0</td>
<td>15 months</td>
<td>Reduced by 18.0%</td>
<td>50%</td>
<td>88%</td>
</tr>
<tr>
<td>KC1/29M</td>
<td>13.5</td>
<td>15 months</td>
<td>Reduced by 22.0%</td>
<td>30%</td>
<td>60%</td>
</tr>
<tr>
<td>SB/71</td>
<td>47.1</td>
<td>18 months</td>
<td>Reduced by 1.2%</td>
<td>77%</td>
<td>83%</td>
</tr>
<tr>
<td>JD/69</td>
<td>20.0</td>
<td>5 months</td>
<td>Reduced by 6.2%</td>
<td>74%</td>
<td>70%</td>
</tr>
</tbody>
</table>
M=male

As shown in Table 6, six out of 20 patients (30%) treated with HOCl failed to achieve either healing or a substantial reduction in ulcer size. The clinical background of these six patients was, overall, considerably poorer than those whose ulcers had responded. Five (83%) had hypertension, compared with only four (28%) of the 14 patients whose ulcers responded (Table 4 and 5).

**TABLE 6**

Five patients who failed to achieve a ≥4% reduction in ulcer size with the initial standard treatment, but achieved a >60% reduction after 12 weeks' additional treatment with HOCl

<table>
<thead>
<tr>
<th>Initial age (vrs)</th>
<th>Size of admission ulcer (cm²)</th>
<th>Duration of ulcerission (months/years)</th>
<th>Ulcer size after three weeks' standard</th>
<th>Reduction of ulcer size after 12 weeks' HOCl washes</th>
<th>Time to complete healing after 15 HOCl washes</th>
</tr>
</thead>
<tbody>
<tr>
<td>JC/85</td>
<td>25.5</td>
<td>16</td>
<td>Reduced by 23%</td>
<td>35%</td>
<td>21%</td>
</tr>
<tr>
<td>PE/48M</td>
<td>85.9</td>
<td>12</td>
<td>Increased by 33%</td>
<td>34%</td>
<td>41%</td>
</tr>
<tr>
<td>GK/72</td>
<td>4.5</td>
<td>3</td>
<td>Reduced by 17%</td>
<td>6.3%</td>
<td>39%</td>
</tr>
<tr>
<td>MA/71</td>
<td>9.8</td>
<td>23</td>
<td>Reduced by 13%</td>
<td>12.8%</td>
<td>13%</td>
</tr>
<tr>
<td>KH/83</td>
<td>5.6</td>
<td>7</td>
<td>No reduction</td>
<td>13%</td>
<td>29%</td>
</tr>
<tr>
<td>PG/57M</td>
<td>6.8</td>
<td>12</td>
<td>No reduction</td>
<td>12%</td>
<td>27%</td>
</tr>
</tbody>
</table>

M=male

**Reduction in Pain**

Fourteen of the 20 patients who initially had pain in the modified McGill questionnaire at levels three to five rapidly reduced these pain levels to nil to one. The remaining six patients did not have pain at the start of treatment above level one, that is minimal discomfort.

**Adverse Effects of BOCl Washes**
There was no discomfort from the HOCl washes, except for one patient who, after four weeks’ treatment, developed a low-grade eczema, suspected to be caused by the HOCl. The eczema progressed and, although the ulcer size had reduced by 46% at seven weeks, the HOCl treatment was discontinued. The patient's (BF, Table 2) eczema resolved and the ulcer healed at 19 weeks.

There were no other adverse effects. Of the twenty patients who received the washes, the first 14 had their blood biochemistry and haematological assessments repeated at the end of treatment. There were no abnormal changes.

The foregoing description and examples have been set forth merely to illustrate the invention and are not intended as being limiting. Each of the disclosed aspects and embodiments of the present invention may be considered individually or in combination with other aspects, embodiments, and variations of the invention. Further, while certain features of embodiments of the present invention may be shown in only certain figures, such features can be incorporated into other embodiments shown in other figures while remaining within the scope of the present invention. In addition, unless otherwise specified, none of the steps of the methods of the present invention are confined to any particular order of performance. Modifications of the disclosed embodiments incorporating the spirit and substance of the invention may occur to persons skilled in the art and such modifications are within the scope of the present invention. Furthermore, all references cited herein are incorporated by reference in their entirety.

Further, while the invention has been described at times with reference to the examples in relation to the treatment of leg ulcers, it should be appreciated that the invention has considerably wider applicability. For example, the invention has applicability to burns, to organ transplants in relation to which current practice is to disinfect organs with antibiotics for two weeks before they are used in a patient, to disinfection of valve-replacements, and to surface wounds, open wounds and plural cavity infections which are exhibiting drug-resistance.
What is claimed is:

1. A method of treating a chronic, refractory open wound comprising:
   applying to the wound an electrolyzed saline solution comprising hypochlorous acid
   having a pH of from about 4 to about 7 and an available free chlorine content of from about 50 to
   1000 parts per million (ppm) when produced by an electrochemical cell system.

2. The method of claim 1, wherein the open wound is an ulcer, burn, or surface wound.

3. The method of claim 2, wherein the ulcer is a venous leg ulcer.

4. The method of claim 1, wherein the electrolyzed saline solution is in a liquid form and is
   applied to the wound by bathing.

5. The method of claim 1, wherein the electrolyzed saline solution is applied to the wound
   by immersing the wound in a hydrobath containing the electrolyzed saline solution.

6. The method of claim 1, wherein the electrolyzed saline solution is in a liquid form and is
   applied to the wound by spraying.

7. The method of claim 1, wherein the pH of the electrolyzed saline solution is between 5.4
   and 5.8.

8. The method of claim 1, wherein treating comprises reducing the microbial count in the
   open wound and permitting cell proliferation.

9. The method of claim 1, wherein the electrolyzed saline solution has a biocidal rate (D
   value) of approximately 1 log reduction unit of bacillus subtilis spores in less than one minute
   with a 9:1 electrolyzed saline solution: inoculum mix.

10. The method of claim 1, wherein the wound is an ulcer and the size of the ulcer is at least
    5 cm² and the ulcer is at least six months old.
11. The method of claim 1, wherein the electrolyzed saline solution contains at least 95% hypochlorous acid.

12. The method of claim 1, wherein the available free chlorine concentration of the electrolyzed saline solution is 150-180 parts per million.

13. A method of reducing pain associated with an open wound comprising:
   applying to the wound an electrolyzed saline solution comprising hypochlorous acid having a pH of from about 4 to about 7 and an available free chlorine content of from about 50 to about 1000 parts per million (ppm) when produced by an electrochemical cell system.

14. The method of claim 13, wherein the open wound is a ulcer, burn, or surface wound.

15. The method of claim 13, wherein the ulcer is a venous leg ulcer.

16. A method of treating an open wound comprising
   (i) applying a compression bandage to the wound; and
   (ii) applying to the wound after or during step (i) an electrolyzed saline solution comprising hypochlorous acid having a pH of from about 4 to about 7 and an available free chlorine content of from about 50 to 1000 parts per million (ppm) when produced by an electrochemical cell system.

17. The method of claim 16, wherein the open wound is a ulcer, burn, or surface wound.

18. The method of claim 17, wherein the ulcer is a venous leg ulcer.
Fig. 4a
Effect of super-oxidized water on dermal fibroblast proliferation

Fig. 4b
Effect of super-oxidized water on dermal fibroblast proliferation

Dilutions of super-oxidized water