

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
1 March 2001 (01.03.2001)

PCT

(10) International Publication Number
WO 01/13926 A2

- (51) International Patent Classification⁷: **A61K 33/00**
- (21) International Application Number: **PCT/GB00/03264**
- (22) International Filing Date: **23 August 2000 (23.08.2000)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data:
9919951.5 **23 August 1999 (23.08.1999)** **GB**
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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **IMPROVEMENTS IN OR RELATING TO STERILISING PREPARATIONS**

(57) Abstract: Super-oxidised water based on hypochlorous acid, such as is obtained by the electrochemical treatment of a saline solution, may be used in the treatment of leg ulcers or other open wounds. Preferably the pH of the super-oxidised water is within the range of 4 to 7 and the water has a redox potential of >950mV. Medicaments based on the super-oxidised water may be in liquid or gel form. The super-oxidised water is able to control the microbial population within the wound and at the same time permit cell proliferation.

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IMPROVEMENTS IN OR RELATING TO STERILISING PREPARATIONS

This invention relates to mixtures of oxidants which are referred to in this specification as "super-oxidised water", a term which is known in the art.

5

Super-oxidised water may be used as a sterilising, disinfecting and biocidal solution. One form of super-oxidised water is produced by the applicant under the trade mark STERILOX®. This STERILOX super-oxidised water is generated at the point of use, for example in a hospital, by passing saline solution over coated titanium electrodes separated by a semi-permeable ceramic membrane at a current about 6 to 9Amps. An apparatus having coated titanium electrodes separated by a semi-permeable ceramic membrane is disclosed in the specifications of UK Patent Nos. 2253860 and 2274113. The basic structure of the apparatus is disclosed in GB2253860 and can be used to produce the STERILOX super-oxidised water.

10

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STERILOX super-oxidised water contains a mixture of oxidising species predominantly hypochlorous acid (HOCl) and sodium hypochlorite. The STERILOX super-oxidised water has a pH of 5-7 and an oxidation reduction potential (redox) of around 1000mV. The high redox potential allows for the quick and efficient destruction of microbes (bacteria, viruses, fungi and spores). Hypochlorous acid and hypochlorite are in equilibrium and the position of the equilibrium is determined solely by the pH.

20

Applicant has found that the resultant super-oxidised water is non-hazardous, non-irritating and non-sensitising to the skin, non-irritating to the eyes, not harmful if swallowed and shows no evidence of mutagenic activity.

25

It is considered that hypochlorous acid exerts its biocidal effect by attacking the surface and plasma membrane proteins, impairing transport of solutes and the salt balance of bacterial cells (Pieterse *et al*, Water SA (1996) 22(1); 43-48). However, it is believed that HOCl does not enter freely into eukaryotic cells which may explain the selectivity of hypochlorous solutions.

30

The STERIOX process produces an extremely effective sterilising cold non-toxic solution which is free from highly toxic chemicals and acts against a wide variety of bacteria, fungi, viruses and spores. The generation of STERIOX solutions requires
5 only water, electricity and pure, vacuum-dried crystalline salt. Applicant considers that the STERIOX super-oxidised water will be suitable for a broad range of applications in both medical and non-medical environments such as the preservation of poultry and fish and general agricultural and petrochemical uses, the breaking down of bacterial biofilm, water treatment and general disinfection in medical and
10 veterinary applications. The STERIOX super-oxidised water has been found to be particularly useful for the disinfection of endoscopes which are sensitive to other cold disinfectants, such as peracetic acid, which are commonly used.

While glutaraldehyde may be used as a reliable disinfection agent of flexible fibre-
15 optic endoscopes and other heat-sensitive instruments, although being widely practised in many hospitals, its use can cause asthma and dermatitis in healthcare staff as a result of exposure to glutaraldehyde fumes, hence a predilection to the use of peracetic acid and the relatively recent move towards the use of STERIOX super-oxidised water in such applications.

20 STERIOX super-oxidised water has been tested and is the subject of two scientific papers by Selkon *et al* (Journal of Hospital Infection (1999) 41; 59-70) and Shetty *et al* (Journal of Hospital Infection (1999) 41; 101-105). In these studies, freshly produced STERIOX super-oxidised water was found to be highly active against
25 *Mycobacterium tuberculosis*, *Mycobacterium avium-intracellulare*, *Mycobacterium chelonae*, *Escherichia coli* (including type 0157), *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Bacillus subtilis* var *niger* spores, methicillin-resistant *Staphylococcus aureus*, *Candida albicans*, poliovirus type 2 and human immunodeficiency virus HIV-1.

30 There has been a recent upsurge in interest in the use of super-oxidised water as a disinfectant because of its rapid and highly biocidal activity against a wide range of bacteria. Tanaka *et al* (Journal of Hospital Infection (1996) 34; 43-49) report the

electrolysis of a saline solution to produce a super-oxidised water with a highly acidic pH of 2.3-2.7 which limits its suitability for many applications, particularly the disinfection of endoscopes. The acidic pH of the super-oxidised water produced by the method described by Tanaka also precludes its use in other medical indications.

5

Having carried out trials in a large number of applications, including those mentioned above, Applicant turned its attention to the use of STERIOX super-oxidised water as a disinfectant of mammalian tissue, in particular the treatment of open wounds such as leg ulcers.

10

An article by 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 two types of leg ulcers; arterial and venous. Arterial ulcers, which are much harder to treat, are caused by ischaemia, while venous ulcers are caused by blood stasis in the veins.

There are many proposals for the management of ulcers, all of which have varying degrees of success. Successful ulcer management is very much dependent on the rigid adherence to a program of treatment in combination with effective disinfection of the wound which reduces bacterial infection and promotes the regeneration of dermal fibroblasts and keratinocytes in the bed of the ulcer which are essential for healing of the wound and the growth of new tissue. If the bacterial growth is not controlled, the wound cannot heal.

30

The most useful treatment for venous ulcers is the use of compression bandages together with elevation of the leg(s). This mimics the pumping action of the calf muscles which return the blood back to the body and maximises the removal of blood

from the leg(s). In conjunction with this, other treatment strategies include the use of topical treatments such as GRANUFLEX® to aid granulation and skin repair, algenates 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. Whilst antibiotics have been used to reduce infection in the past, nowadays this is not a treatment of choice due to the increased risk of antibiotic resistance.

Whilst potassium permanganate (KMnO_4) is an oxidant which has stood the test of time in the treatment of leg ulcers, it still nevertheless has the disadvantages of irritating and injuring newly grown skin and causing skin discolouration. Known hypochlorites, such as EUSOL (Edinburgh University Solution Of Lime) and Daikin's solution, rely on a high concentration of hypochlorite ions for their disinfectant properties. 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, resulting in these preparations falling out of use. Attempts have been made to reduce the alkaline effect of the high hypochlorite ion content of these solutions e.g. by the use of suitable buffers, but have been found to be ineffective in such circumstances.

All this has militated against the use of preparations including hypochlorites for the treatment of leg ulcers. However, the success in disinfection and sterilisation of endoscopes and the known non-irritant effects of the STERIOX super-oxidised water, have led the Applicant to re-address the treatment of open wounds such as leg ulcers.

As a first step, *in vitro* tests were carried out on single layers of cultured human dermal fibroblast cells and keratinocyte cells to ascertain whether or not super-oxidised water had any effect on the viability of the cells. The cells were incubated under sterile conditions in a super-oxidised water based on hypochlorous acid and including sodium hypochlorite and other oxidised chlorine species, having a pH range from 4 to 7 and a redox potential of around 1000mV.

Surprisingly, Applicant found that there is an optimum pH at which cell growth is not-inhibited despite there being other pH levels within the range at which the viability of the cells is impaired. Indeed, in view of the findings of Tanaka *et al*, Applicant
5 expected that the lower pH range would be more effective.

The next step was crucial because the applicant then had to ascertain whether or not the *in vitro* results could not only be replicated *in vivo* but also that there would be a sufficient biocidal effect to counteract any bacterial activity which would prejudice the
10 viability of new cells. Accordingly, a clinical trial was carried out on a patient with chronic venous leg ulcers using freshly produced super-oxidised water based on hypochlorous acid having a pH of 5.4.

The patient did not experience any pain, and in fact commented that the treatment was
15 comfortable and had a soothing effect. There was a positive effect on the bacterial flora as well as the clinical appearance of the wounds. No adverse effect was observed on the surrounding skin which, in a number of patients with long-standing ulcers, is often sensitive.

20 Applicant believes that the effects which have been observed can be explained by the low concentration of oxidised (free available) chlorine present in a super-oxidised water based on hypochlorous acid. This is in contrast to commonly known hypochlorite solutions which owe their biocidal activity to a high concentration of free chlorine (including hypochlorite) as indicated by their characteristic smell.

25 It could be said that Applicant has discovered a principle which is that a balance between the biocidal effect and non-inhibition of cell growth enables the microbial population present in a wound to be controlled such as to allow cell growth to occur.

30 In order to carry this principle into effect and from one aspect of the invention there is provided a super-oxidised water which is based on hypochlorous acid, acts as a biocide and allows cell growth.

Expressed in another way, the present invention resides in a super-oxidised water in which the biocidal effect is due to hypochlorous acid and the non-inhibitory effect on cell growth is dependent on the level of the pH of the hypochlorous acid solution.

5

From a still further aspect of the invention, in a super-oxidised water based on hypochlorous acid and having a pH of 4 to 7, the said solution has a pH selected from within the range of 4.3 to 6.2.

10 In a further aspect of the invention, there is provided, for use in the treatment of, or medicament for, skin ulcers or other open wounds, a super-oxidised water or other formulation such as a gel, which is based on hypochlorous acid, acts as a biocide and allows cell growth.

15 The super-oxidised water or other preparation based on hypochlorous acid may have a pH of 4 to 7, with the pH preferably being selected from within the range of 4.0 to 6.5, and more preferably within the range of 4.0 to 6.2.

20 In any of the aspects of the invention defined above, the said super-oxidised water has a biocide rate (D Value) of approximately 1 log unit reduction unit of *bacillus subtilis* spores in less than 1 minute with a 9:1 super-oxidised water: innoculum mix. A biocide rate of about 3.4 seconds may be achieved and is particularly preferred.

25 The super-oxidised water based on hypochlorous acid may be obtained by the electrolysis of a saline solution. Accordingly, from another aspect of the invention there is provided a method of obtaining a super-oxidised water based on hypochlorous acid, said method comprising passing the saline solution through an electrochemical cell having electrodes separated by a semi-permeable membrane and operating in such manner as to produce a super-oxidised water based on hypochlorous acid which acts
30 as a biocide and allows cell growth.

In a preferred method of carrying out the invention, the super-oxidised water is obtained and the pH level of the said solution is adjusted by a buffering action which

involves the use of an alkaline feed from the said cell. By using an alkaline solution in this way, the addition of conventional buffers and the consequent expense is avoided.

5 Whilst the pH of the said super-oxidised water is preferably adjusted to be within the range of 4.3 to 6.5, Applicant has found that, in the case of wounds such as leg ulcers for example, it is advantageous if the pH is adjusted to 5.4 or thereabouts.

10 From a still further aspect, the invention resides in a method of producing a medicament containing a super-oxidised water as defined above for use in the treatment of leg ulcers or other open wounds.

The invention also comprehends, in a super-oxidised water or other formulation having a pH of 4 to 7, the selection of a pH in the range of 4.3 to 6.5, and preferably 5.4, or thereabouts that is used for the treatment of leg ulcers or other open wounds.

15 By means of any of the aspects of the invention defined above, the disinfection of wounds such as leg ulcers is readily achieved without irritation and pain, the spread of infection is reduced, cell growth and regeneration are facilitated, thereby enhancing healing.

20 An added advantage is that there is no resistance or tolerance developed to disinfection which would occur with antibiotics.

25 Furthermore, the super-oxidised water of the invention lends itself readily to the treatment being carried out in many ways, although it has been found that a hydrobath in which the leg is immersed is soothing and generally pleasant for the patient. However, it should be appreciated that preparations other than solutions may be used, for example gels.

30 From yet another aspect, the invention also resides in a method of treating a human or animal body having a leg ulcer or other open wound using any of the super-oxidised water based on hypochlorous acid herein referred to above and any of the methods defined hereinabove.

Applicant believes that the present invention, in any or all of its aspects is a breakthrough and will revolutionise the treatment of open wounds, in particular leg ulcers, in a way which has been found to be impossible hitherto.

5

In order that the invention may be more readily understood, reference will now be made, by way of example, to the accompanying examples, in which:

Example 1 describes an *in vitro* study which investigated the effect of super-oxidised water based on hypochlorous acid on the proliferation of cultured human dermal fibroblast cells.

10

Example 2 describes an *in vitro* study which investigated the cytotoxic effect of super-oxidised water based on hypochlorous acid on cultured human dermal fibroblast cells.

15

Example 3 describes an *in vitro* study which investigated the effect of super-oxidised water based on hypochlorous acid on the proliferation of cultured human keratinocyte cells.

Example 4 describes a clinical trial of super-oxidised water on a patient with chronic leg ulcers.

20

Example 1

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.

25

Three *in vitro* trials of super-oxidised water based on hypochlorous acid were carried out on single layer cell cultures of human dermal fibroblast (HDF) cells to ascertain whether the super-oxidised water affected HDF cell proliferation. A range of dilutions of super-oxidised water at different pH levels were investigated.

30

Method

The super-oxidised water used in the trials was the product of the electrolysis of an aqueous saline solution passed over a mixture of proprietary catalysts on titanium electrodes to give a mixture of oxidising species, particularly hypochlorous acid (HOCl) at a concentration of about 144mg/l to 400mg/l available free chlorine (Cl). The super-oxidised water was produced as required for each test; the apparatus (supplied by Sterilox Medical Limited, Abingdon, UK) was operated to give a final solution redox potential of >950mV as recommended by the company.

Appropriate dilutions of the super-oxidised water were made and the pH of the final solution was adjusted using a phosphate buffer.

For the proliferation assay, HDF cells were seeded in normal (10%) foetal calf serum (FCS) and Dulbecco's Modified Eagle Medium (DMEM) at 1.5×10^3 cells/well. After 24 hours, the medium was changed to low (0.4%) FCS/DMEM. After a further 48 hours incubation, super-oxidised water at varying concentrations was added to the cells. The viability of the cells was observed, using a standard absorption assay, 3 and 6 days after the application of super-oxidised water.

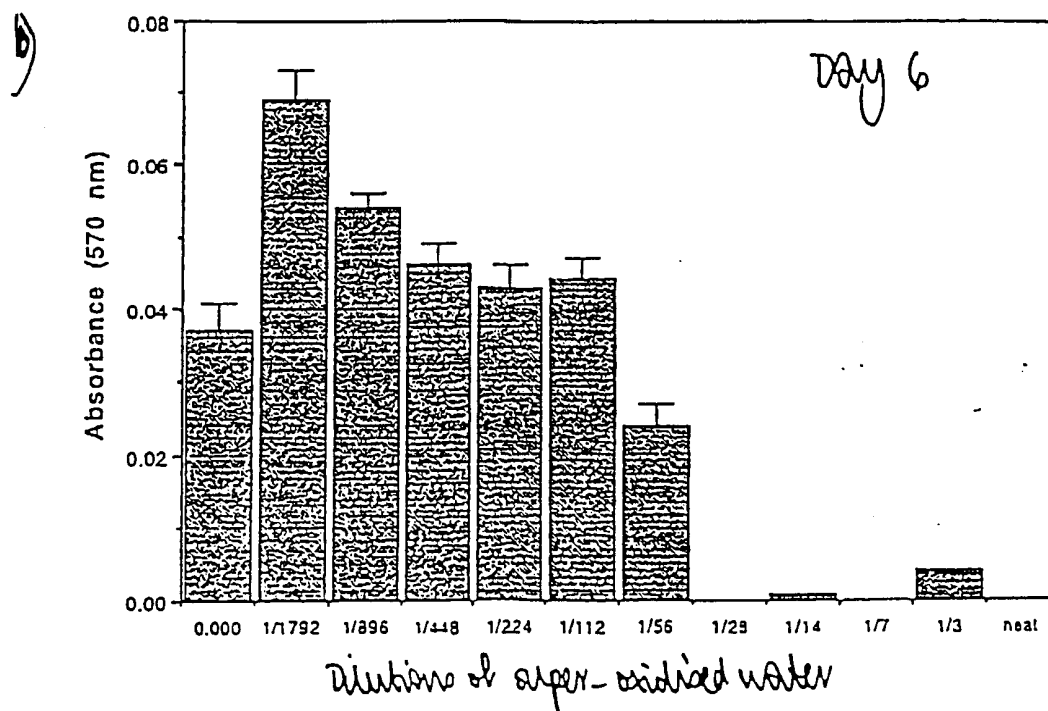
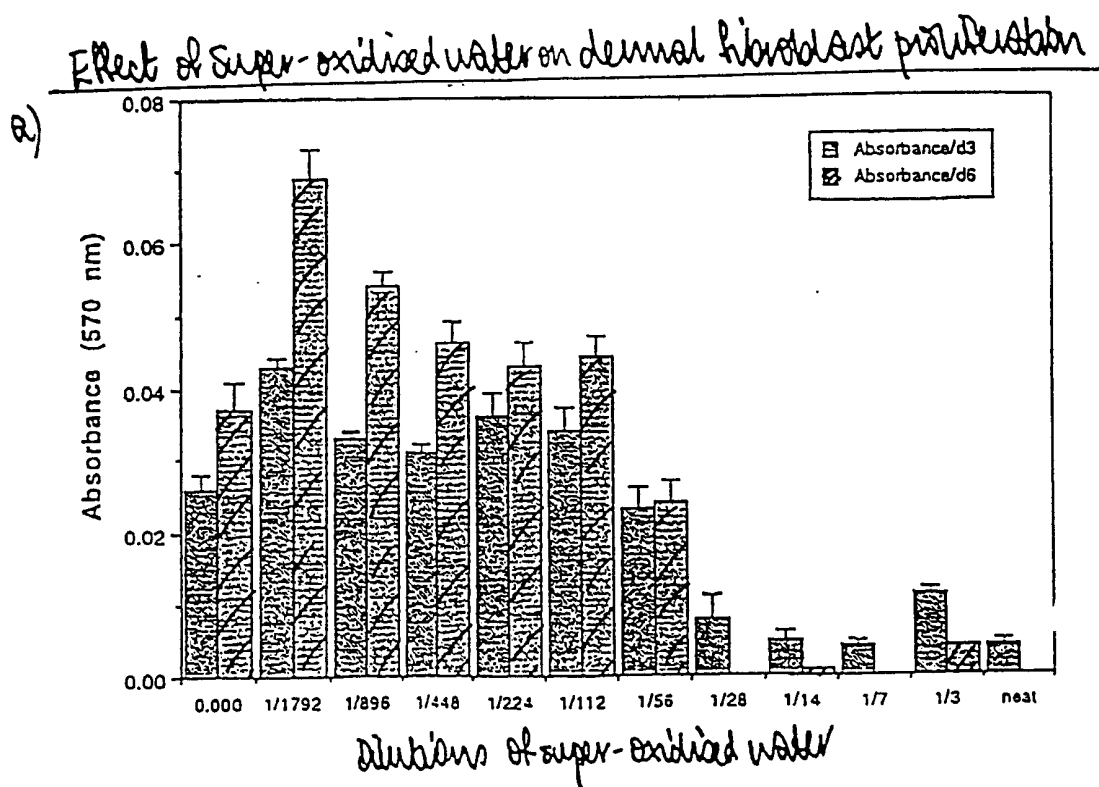
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Results

i) Trial 1 HDF cells were incubated with super-oxidised water in a range of dilutions at a pH of 4.3. The dilutions used were: 1, 1/3, 1/7, 1/14, 1/28, 1/56, 1/112, 1/224, 1/448, 1/896, 1/1792 and 0.

25

As shown in the accompanying graphs, on both day 3 and day 6, super-oxidised water dilutions of 1/28 or less significantly inhibited HDF proliferation or killed the cells. Slight inhibition of proliferation was seen at a dilution of 1/56. Dilutions of 1/112 to



1/448 showed no effect on proliferation, while the 1/896 dilution showed some cell proliferation and the dilution of 1/1792 showed significant proliferation of HDF cells.

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

10 ii) Trial 2 Using the same conditions as trial 1, HDF cells were incubated with super-oxidised water in a range of dilutions at a pH of 6.2. The dilutions used were: 1/20, 1/40, 1/60, 1/80, 1/100, 1/120, 1/1000, 1/1500, 1/2000, 1/3000, 1/4000 and 0.

15 No stimulation of proliferation was seen and indeed, inhibition of HDF growth was seen with cells incubated with a dilution of super-oxidised water of 1/20. However, the higher dilution of 1/40 showed no cell toxicity.

20 This trial was repeated with the HDF cells seeded in super-oxidised water 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 was seen at dilutions of 1/20 and below. However, dilution of more than 1/20 showed 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.

25 iii) Trial 3 In this trial, two plates of cells were grown always in normal growth medium (10% FCS/DMEM). One plate of cells was treated as described in trial 1 but after three days of incubation with the said super-oxidised water, the growth medium was changed from 0.4% FCS/DMEM to 10% FCS/DMEM in order to observe the recovery of the cells.

30

HDF cells were incubated at 31°C with super-oxidised water in a range of dilutions at a pH of 5.4. The dilutions used were: 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 was seen in cells incubated with super-oxidised water at a dilution of 1/20 or higher in either 0.4% or 10% FCS/DMEM. Some levels of proliferation had reached statistical significance.

5

A dilution of 1/10 super-oxidised water inhibited 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 super-oxidised water,
10 the medium was changed to 10% FCS/DMEM. The cells that had been incubated with super-oxidised water showed the same ability to recover from depression of cell growth seen whilst growing in 0.4% FCS/DMEM as control cells.

This trial was repeated with the HDF cells seeded in super-oxidised water at dilutions
15 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.

Again, on both day 3 and day 6, cell proliferation was 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 was seen, even at the dilution of 1/7 super-
20 oxidised water. Stimulation of cell growth was also seen in cells grown in 10% FCS/DMEM in the presence of super-oxidised water, however, the levels of proliferation did not reach statistical significance. Where cell growth had been impaired by incubation with super-oxidised water, recovery was seen, confirming the observations from the first set of experiments.

25

In summary, HDF cells incubated with super-oxidised water at pH 5.4 showed no inhibition of cell growth, even in the presence of a 1/7 dilution of super-oxidised water.

30 Conclusion

The presence of super-oxidised water at a pH of 5.4 does not inhibit HDF cell growth *in vitro*.

Example 2

Three *in vitro* trials were carried out to investigate the cytotoxic effect of super-oxidised water based on hypochlorous acid on HDF cells.

Method

The super-oxidised water based on hypochlorous acid was identical to that described in Example 1.

10

HDF cells were seeded in 10% FCS/DMEM at 5×10^3 cells/well. After incubation at 31°C for 72 hours, dilutions of super-oxidised water were prepared in HBSS and added to the cells. The viability of the cells was ascertained by a standard absorption assay at time intervals of 15 minutes up to one hour from the addition of the super-oxidised water.

15

Results

i) Trial 1 HDF cells were incubated with super-oxidised water in a range of dilutions at a pH of 4.3. The dilutions used were: 1, 1/3, 1/7, 1/14, 1/28, 1/56, 1/112, 1/224, 1/448, 1/896, 1/1792 and 0.

20

No effect on cell viability was seen in the presence of super-oxidised water at dilutions of 1/28 or more at any of the time points. A dilution of 1/14 induced mild damage to the cells while dilutions of 1/7 and less killed the cells.

25

ii) Trial 2 Using the same conditions as trial 1, HDF cells were incubated with super-oxidised water in a range of dilutions at a pH of 6.2. The dilutions used were: 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 was seen on the viability of HDF cells in the presence of super-oxidised water at dilutions of 1/20 or more at any time point. However, dilution of 1/16 or less induced cell damage.

30

iii) Trial 3 Using the same conditions as trials 1 and 2, HDF cells were incubated with super-oxidised water in a range of dilutions at a pH of 4.0. The dilutions used were: 1, 1/4, 1/8, 1/12, 1/16, 1/20, 1/24, 1/28, 1/50, 1/100, 1/200 and 0.

5

Dilutions of super-oxidised water at 1/24 and 1/20 induced slight damage to the cells while dilution of 1/16 or less induced cell death.

Conclusion

10 The results of these trials support the results shown in Example 1 in that, while super-oxidised water 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

15

In view of the results described in Examples 1 and 2, two *in vitro* trials were carried out to investigate the effect super-oxidised water on human keratinocyte (HK) cell proliferation. The super-oxidised water used in these trials was identical to that described in Example 1.

20

Keratinocytes are epidermal skin cells that synthesise keratin and, together with dermal fibroblasts, are essential for skin healing.

Trial 1

25 HK cells (subcultured, P2, FS, 7 years) were seeded at 8×10^3 cells/well and incubated at 31°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
30 supplements, hereinafter referred to as keratinocyte basal medium (KBM).

After a further 48 hours incubation, super-oxidised water diluted in KBM at pH 5.4 was added to plates 1 and 2, and super-oxidised water diluted in KGM was added to

plates 3 and 4. The dilutions of super-oxidised water were: 1/10, 1/20, 1/50, 1/100, 1/150, and 0. The pH of the final super-oxidised water solution was adjusted using a phosphate buffer.

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

10 The absorbance assay showed that, on both day 3 and day 5, cell proliferation had occurred in the presence of all dilutions of super-oxidised water. At day 5, the level of cell proliferation had reached a significant level compared to cell growth in the absence of super-oxidised water.

15 Trial 2

In view of the fact that the HK cells did not grow in KBM and showed significant proliferation in the presence of super-oxidised water in KGM, it was decided to use KBM with lower concentrations of supplements as a holding medium, with or without super-oxidised water.

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

25 After incubation for a further 24 hours, super-oxidised water diluted in KBM with 20% supplements was added to plates 1 and 2, super-oxidised water diluted in KBM with 50% supplements was added to plates 3 and 4, and plates 5 and 6 received super-oxidised water diluted in complete KGM. The dilutions of super-oxidised water were:
30 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 were incubated for a further 3 days in the presence of super-oxidised water, after which time, a standard absorbance assay was carried out on plates 1, 3 and 5 to

ascertain the viability of the cells, and the medium in plates 2, 4 and 6 was changed to KGM. Plates 2, 4 and 6 were assayed after a further 48 hours of incubation.

5 Stimulation of cell proliferation on both day 3 and day 5 was seen in all percentages of KGM supplements. However, the level of stimulation was not significantly different when compared to control cell growth. No cytotoxicity was seen even at the low dilution of 1/7 super-oxidised water.

Conclusion

10 Dermal keratinocytes cultured in the presence of KGM and super-oxidised water showed enhanced cell proliferation and no cytotoxicity was seen in the presence of super-oxidised water.

Example 4

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

20

Method

The patient's legs were immersed in 40 litres of super-oxidised water in a hydrobath for fifteen minutes before being allowed to dry.

25 An intermediate assessment without treatment was carried out after one week. A second treatment with super-oxidised water was repeated after two weeks in which the patient was subjected to three fifteen-minute washes at approximately three hour intervals.

30 Post-treatment clinical evaluation was carried out one and several days after the second treatment.

Semi-quantitative microbiological analysis of the leg ulcers was carried out on swabs taken before and fifteen minutes after treatment with super-oxidised water.

Results

- 5 After the first treatment, the patient reported that the treatment was comfortable and free from pain. The appearances of the ulcers on both legs were markedly improved when assessed five hours after treatment.

10 As shown in Table 1, quantitative microbiology showed 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.

15 Table 1 - semi-quantitative microbiological analysis of leg ulcers before and after treatment with super-oxidised water based on hypochlorous acid. Figures quoted indicate the number of colony forming units (cfu) per ml.

	Right Leg	Left Leg
Pre-treatment	1×10^7	1×10^7
Post-treatment	1×10^5	1×10^3

20 The patient was seen one week after the first treatment and was treated with conventional therapy, including potassium permanganate. The effect of these on the appearance of the leg ulcers following treatment did not appear to be as striking as that seen with super-oxidised water.

25 A second treatment with super-oxidised water was repeated a further week later and similar beneficial results were obtained. In between the treatment periods the ulcers had become sloughy on both legs. Immediately after the first wash, the ulcer bed was whitish due to effervescence. A cleansing effect was seen after the later two washes and a marked improvement was seen with the state of the ulcer 18 hours after the first wash.

The patient reported no discomfort to the treatment, the solution in the bath was soothing and the skin felt a bit tight afterwards. The patient commented that the tightness started to be felt once cold air was accessible to the skin.

- 5 Referring to Table 2, quantitative microbiology showed 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.

10 Table 2 - semi-quantitative microbiological analysis of leg ulcers before and after treatment with super-oxidised water based on hypochlorous acid. Figures quoted indicate the number of colony forming units (cfu) per ml.

	Right leg		Left leg	
	(cfu/ml)	organisms found	(cfu/ml)	organisms found
pre-treatment 1	1.9×10^8	Coliforms Proteus spp skin flora	4.5×10^6	Coliforms Proteus spp skin flora
post-treatment 1	1.2×10^5	Coliforms skin flora Proteus spp	4.5×10^4	skin flora β -haemolytic streptococci Coliforms Proteus spp
pre-treatment 2	3.0×10^3	Coliforms Proteus spp skin flora	1.5×10^4	coliforms skin flora
post-treatment 2	1×10^3	Coliforms Proteus spp skin flora	<10	no growth
pre-treatment 3	<10	no growth	6.3×10^3	Coliforms β -haemolytic streptococci skin flora
post-treatment 3	3.0×10^2	Coliforms skin flora	3.0×10^3	β -haemolytic streptococci

post 24 hours	2.7×10^2	Proteus spp	1.5×10^6	β -haemolytic streptococci
		Coliforms		
		Proteus spp		
		skin flora		
				Coliforms
				Proteus spp

Conclusion

The main objectives of the clinical study were to examine patient comfort and safety as well as the efficiency of a treatment of super-oxidised water based on hypochlorous acid.

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 did not experience pain and, in fact, commented on a soothing effect. There was a positive effect on the bacterial flora as well as the clinical appearance of the wounds. There was no adverse effect on the surrounding skin which, in a number of patients with long-standing ulcers, is often sensitive.

Whilst the invention has been described with reference to the example 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, for disinfecting valve-replacements and to surface wounds, open wounds and plural cavity infections which are exhibiting drug-resistance.

CLAIMS:

1. A medicament for the treatment of open wounds comprising super-oxidised water
5 based on hypochlorous acid.
2. A medicament according to claim 1, wherein the super-oxidised water is in liquid form for application to the wound by bathing or spraying.
- 10 3. A medicament according to claim 1, wherein the super-oxidised water is in gel form for topical application to the wound.
4. A medicament according to any preceding claim, wherein the super-oxidised water or preparation derived therefrom has a pH of 4 to 7.
- 15 5. A medicament according to claim 4, wherein the pH is selected from within the range of 4.0 to 6.5.
6. A medicament according to claim 5, wherein the pH is selected from within the
20 range of 4.0 to 6.2.
7. A medicament according to claim 6, wherein the pH is selected from within the range of 4.3 to 6.2.
- 25 8. A medicament according to claim 7, wherein the pH is about 5.4.
9. A medicament according to any preceding claim, wherein the super-oxidised water has a redox potential of >950mV.
- 30 10. A medicament according to claim 9, wherein the super-oxidised water has a redox potential of about 1000mV.

11. A medicament according to any preceding claim, wherein the super-oxidised water or preparation derived therefrom has a biocide rate (D Value) of approximately 1 log reduction unit of *bacillus subtilis* spores in less than 1 minute with a 9:1 super-oxidised water: innoculum mix.
- 5 12. A medicament according to any preceding claim, wherein the super-oxidised water is diluted to the extent that it does not inhibit cell proliferation in use.
- 10 13. A medicament according to claim 12, wherein the super-oxidised water is diluted to the extent that it promotes cell proliferation in use.
14. A medicament as claimed in any preceding claim, wherein the super-oxidised water comprises an output solution obtained by electrochemical treatment of a saline solution.
- 15 15. A medicament as claimed in claim 14, wherein the pH of the super-oxidised water is adjusted to the desired level by using an alkaline solution output from the electrochemical cell in which the saline solution is treated.
- 20 16. A medicament as claimed in claim 14, wherein the output solution further comprises a phosphate buffer to adjust the pH to the desired level.
17. Super-oxidised water based on hypochlorous acid for use in the treatment of the human or animal body.
- 25 18. Super-oxidised water as claimed in claim 16 having a pH of 4 to 7.
19. Super-oxidised water as claimed in claim 18 having a pH selected from within the range of 4.0 to 6.5.
- 30 20. Super-oxidised water as claimed in claim 19 having a pH selected from within the range of 4.0 to 6.2.

21. Super-oxidised water as claimed in claim 20 having a pH selected from within the range 4.3 to 6.2.
- 5 22. Super-oxidised water as claimed in claim 21 having a pH of about 5.4.
23. Super-oxidised water as claimed in any of claims 17 to 22 having a redox potential of >950mV.
- 10 24. Super-oxidised water as claimed in claim 23 having a redox potential of about 1000mV.
25. Super-oxidised water as claimed in any of claims 17 to 24 having a biocide rate (D Value) of approximately 1 log unit reduction of *bacillus subtilis* spores in less than 1
15 minute with a 9:1 super-oxidised water: innoculum mix.
26. Use of super-oxidised water based on hypochlorous acid in the preparation of a medicament for treatment of the human or animal body.
- 20 27. Use of super-oxidised water as claimed in any of claims 17 to 25 in the preparation of a medicament for treatment of leg ulcers or other open wounds.
28. Use of super-oxidised water based on hypochlorous acid in the preparation of a medicament for treatment of human or animal body by controlling the microbial
25 population and permitting cell growth.
29. Use of super-oxidised water as claimed in claim 26, wherein the water or preparation derived therefrom has a pH as claimed in any one of claims 18 to 22, and/or a redox potential as claimed in claim 23 or claim 24, and/or a biocide rate as
30 claimed in claim 25.
30. A method of preparing a medicament according to any one of claims 1 to 16 comprising passing a saline solution through an electrochemical cell having electrodes

separated by a semipermeable membrane, operating the cell in such manner as to produce super-oxidised water based on hypochlorous acid, and incorporating the super-oxidised water in a carrier preparation at a sufficient concentration to impart biocidal properties and allow cell proliferation when applied to a human or animal
5 body.

31. A method according to claim 30, wherein the pH of the super-oxidised water generated in the cell is controlled within the cell by a buffering action involving re-feeding alkaline solution also generated in the cell.

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32. A method of treating a leg ulcer or other open wound on a human or animal body by applying to the wound a medicament according to any of claims 1 to 16, or a super-oxidised water according to any of claims 17 to 25.

15 33. A method according to claim 32, wherein the wound is immersed in a hydrobath containing said medicament or said super-oxidised water.

34. A hydrobath preparation for the treatment of leg ulcers or other open wounds comprising super-oxidised water based on hypochlorous acid.

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35. A method of treating a leg ulcer substantially as hereinbefore described with reference to Example 4 of the accompanying description.

25 36. A medicament for the treatment of leg ulcers or open wounds substantially as hereinbefore described with reference to any one of Examples 1 to 3 of the accompanying description.