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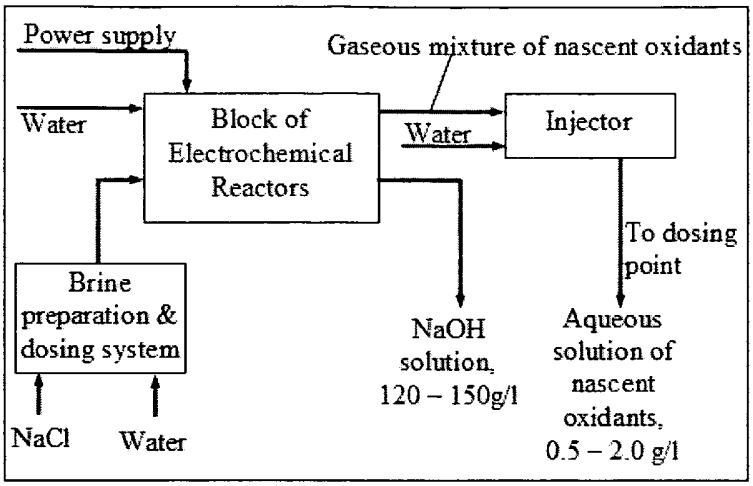
(54) **Title:** WOUND HEALING COMPOSITIONS



(57) **Abstract:** The present invention relates to compositions comprising an anolyte of electrochemilly activated water for treating a wound, and preferably for reducing and/or preventing infection of the wound and the surrounding areas. The compositions may also promote or accelerate wound healing.

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Wound Healing Compositions

The present invention relates to improved wound healing compositions for cleaning and irrigating wounds, treating and preventing wound infections and accelerating the rate at which wounds heal. The invention also relates to the use of such compositions for cleansing intact or uncompromised skin, as a preventative measure against the development of skin infections.

As used herein, the term "wound" includes surgical incisions as well as wounds caused by accidental trauma or disease, burns, abrasions and minor irritations of the skin.

Wound healing is a complex process involving a highly regulated cascade of biochemical and cellular events with the object of restoring tissue integrity following injury. The repair process in human tissue combines aspects of tissue repair and regeneration in response to tissue damage or loss. Wound healing is a concert of simultaneously occurring processes rather than a series of discrete steps, which, for convenience, can be characterised as haemostasis, inflammation, proliferation and remodeling.

Early wound healing events include coagulation and inflammation. Fibrin is the end product of the coagulation pathway and, besides inducing haemostasis, is also the primary component of the tissue matrix seen in the early phases of wound healing. It provides a framework for the migration of inflammatory and mesenchymal cells.

Monocytes transform into macrophages as they migrate from capillaries into extra vascular space. Macrophages phagocytose bacteria and tissue debris and secrete enzymes (collagenase and elastase) that break down damaged matrix. They also secrete cytokines, prostaglandins, oxygen free radicals and other regulators of wound healing.

Intermediate wound healing events include mesenchymal cell migration and proliferation, angiogenesis and epithelialisation. Fibroblasts from adjacent

undamaged tissue migrate into the wound matrix under the influence of cytokines and secrete collagen and proteoglycans of connective tissue matrix. Angiogenesis is the process by which blood flow across a wound is re-established. Capillaries sprout from existing venules on each side of the wound and connect with each other. In unclosed wounds, however, the new capillaries fuse only with neighbours migrating in the same direction and form granulation tissue. Epithelialisation begins within hours of injury and results in the resurfacing of any denuded area by cell migration and proliferation, creating a multi-layered epithelium.

Late wound healing events include collagen synthesis by fibroblasts, which have migrated into the wound. Collagen provides structural configuration, strength and a matrix for cellular movement in the wound.

There are many potential causes of disruption in the healing process which may lead to a prolonging of the inflammatory phase and the generation of a cascade of tissue responses that prevents healing and leads to the formation of a chronic wound. A major risk, especially with open wounds, is the development of infection, particularly bacterial infection. Wounds that remain infected cannot proceed to the granulation tissue phase of wound healing.

A particular hazard, especially for hospitalised patients, is the infection of a wound by an antibiotic-resistant bacterial strain. Three of the most common are Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococcus spp.* (VRE) and *Clostridium difficile* (C. difficile). An individual can become a carrier of MRSA in the same way that they can become a carrier of ordinary *Staphylococcus aureus*, namely by physical contact with the organism. Most commonly, the organism is transmitted by physical contact with someone who is either infected with the organism or carries it on his or her skin. If the organism is in the nose or is associated with the lungs rather than the skin then it may be passed around by droplet spread from the mouth and nose. One of the most important precautions to prevent spread of MRSA from a person infected with it is that they are placed in contact isolation. This type of isolation requires everyone in contact with the patient to be very careful about hand washing after touching either the patient or

anything in contact with the patient. If the organism is in the nose or lungs it may also be necessary to have the patient in a room to prevent spread to others by droplet spread. Because dust and surfaces can become contaminated with the organism, the cleaning of surfaces is also important.

Repeated trauma, foreign bodies, pressure necrosis, ischaemia and tissue hypoxia due to a poor blood supply are just some of the other factors which may promote a chronic inflammatory state in a wound. Some examples of wounds which may be slow- or non-healing are diabetic ulcers, pressure sores, ulcerated wounds, venous stasis ulcers and burns.

Alternatively, a subject may have an inherently impaired wound response. For example, patients with cirrhosis of the liver suffer chronic wounds due to a deficiency of coagulation factor XIII (FXIII). FXIII is the last enzyme to be activated in the blood coagulation pathway. It functions to cross-link fibrin chains, resulting in a stronger clot with an increased resistance to fibrinolysis. A deficiency of FXIII thus impairs clotting, so promoting the formation of chronic wounds. Other immunocompromised patients who may suffer impaired wound healing include those on immunosuppressive drugs and those suffering an immune cell deficiency (such as B or T cell deficiency).

According to the National Institutes of Health, chronic wounds such as venous leg ulcers, pressure sores, ischaemic ulcers and diabetic foot ulcers, affect more than 4 million Americans each year and cost about $9 billion to treat.

Conventionally, disinfectants and other chemotherapeutic agents are topically applied to wounds in order to restore the normal healing process and prevent infection. Many conventional disinfectants are toxic, flammable and irritating to the skin, eyes and throat and so must be used with extreme caution. Cleaning the wound with a conventional disinfectant may be effective to kill bacteria but it may also create a layer of superficial necrosis that can delay wound healing. Additionally, many known disinfectants such as chlorhexidine, providine-iodine, alcohol, hydrogen peroxide (in high concentrations, as it is normally used) are toxic

to fibroblasts and epithelial cells and so are likely to delay or prevent the healing of open wounds and ulcers.

McCauley et al (Differential in vitro Toxicity of Topical Antimicrobial Agents to Human Keratinocytes. J Surg Res, 1992; 52:276-285) have shown that both silver sulfadiazine and Sulfamylon®, which are used in the treatment of skin graft donor sites, wounds, burns, leg ulcers and pressure sores are toxic to fibroblasts in tissue culture at concentrations of 0.005% and 0.1%, respectively. Leitch et al (Inhibition of Wound Contraction by Topical Antimicrobials. Aust. New Zealand J Surg, 1993 Apr; 63(4):289-93) have presented data showing that silver sulfadiazine, Sulfamylon® and silver sulfadiazine with chlorohexadine significantly retarded wound healing in the acute wound model.

Furthermore, many of the disinfectants conventionally used to clean and irrigate wounds are harmful to the environment and their use should therefore be kept to a minimum. Many of the commonly used disinfectants have an extremely high chemical load and therefore are potentially very harmful to the environment. The chlorine content of disinfectants in particular has come under scrutiny in Europe.

In the light of the foregoing, it is clear that whilst the application of topical chemotherapeutic agents is essential in the prevention and treatment of infection and enhancement of wound healing, there is a need for an agent which effectively prevents or treats infection of the wound but does not retard and possibly even accelerates the healing process.

Electrochemically activated water is water which has undergone electrochemical activation (ECA). Such treatment involves the exposure of water and the natural salts therein or salts added to it, to a substantial electrical potential difference.

If an anode (+) and a cathode (-) are placed in pure water and a direct current is applied, electrolysis of the water will occur at the poles leading to a breakdown of the water into its constituent elements, producing gaseous hydrogen and oxygen. However, if sodium chloride is added to the water to form a solution, the dominant

electrolysis end product is chlorine, hypochlorite or hypochlorous acid (HOCl), a chlorine-based reagent which may be used to kill micro-organisms.

The electrochemical activation process is improved by interposing an ion-permeable membrane between the positive and negative electrodes, forming an anode chamber and a cathode chamber. Preferably, the aqueous sodium chloride solution is fed into both the anode chamber and the cathode chamber and the sodium chloride, which is in its ionised form in solution (Na^+ and Cl^-), is exposed to the controlled electrical potential difference between the cathode and the anode. This potential difference causes the Na^+ ions to migrate to the cathode and the Cl^- ions to migrate to the anode. The membrane which separates the anode chamber and the cathode chamber allows ions to pass unimpeded, whilst the un-ionised water and any organic molecules in the water are unable to pass through the membrane.

The presence of an ion-permeable non-ceramic membrane in the electrolysis apparatus allows the necessary ions to be concentrated in the anode and cathode chambers, which results in the formation of metastable species with high biocidal activity and very low chlorine levels. Although a similar process takes place in conventional electrochemical activation processes, the presence of an ion-permeable membrane prevents the complex reactive species formed at the cathode and anode from reacting with one another and being neutralised. The specific choice of a non-ceramic membrane further refines the chemical processes.

As the electrical potential is applied, high concentrations of Cl^- and OH^- build up on the anode side of the membrane and Na^+ and H^+ build up on the cathode side of the membrane. The unstable chemical state results in complex reactions which produce a metastable solution containing a wide variety of very reactive ions and molecules, such as those set out in Table 1.

Table 1

	Active species
Anolyte	Cl_2 (main)
	HOCl
Anolyte/Catholyte combination	Cl_2
	HOCl (main)
	OCl^-

It is the formation of these complex chemical species which leads to the formation of solutions described as electrochemically activated water or hydroactive water. The reactive constituents formed in the electrolysis of sodium chloride solution include hypochlorous acid (HOCl), chlorine (Cl_2) and hypochlorite (OCl-). These species are formed via chloride (Cl-) which is produced at the anode. The neat anolyte has a pH of 2.4 to 4, whereas the pH of the catholyte which contains NaOH is in the order of 10 to12. Anolyte and catholyte are mixed to produce a solution neutral or near-neutral pH (6 to 8), containing hypochlorous acid (HOCl) as predominant active species. The chemical equilibrium shifts towards chlorine (Cl_2) at low pH and towards hypochlorite (OCl-) at high pH. The selection of pH therefore allows manipulation of the active mix composition and stability profile required for the desired application.

The anolyte and catholyte produced by the electrochemical activation of an aqueous sodium chloride solution also exhibit opposing potentials. In an embodiment, the anolyte has a redox potential of approximately +1050mV, while the catholyte has a redox potential of approximately -850mV. This is compared to the redox potential of the starting material which, in an embodiment, is approximately +300 to 400mV.

In the apparatus with an ion-permeable membrane, two distinct solutions are formed, the catholyte and the anolyte, and these solutions can be separately extracted or they can be mixed.

The anolyte solution exhibits oxidative power and can destroy micro-organisms, and therefore has useful sterilizing and disinfectant properties. The catholyte solution

has properties which make it useful as a detergent and as a surfactant. Its reducing power also means that the catholyte is effective in precipitating metal ions out of water and it can be used to soften hard water.

Whilst the oxidative and reductive properties of the anolytes and catholytes of electrochemically activated water have previously been recognised, it has now unexpectedly been found that compositions comprising the anolyte of electrochemically activated water may be used to treat and prevent wound infection and to promote wound healing. It has even been found that the anolyte of electrochemically activated water can be used to promote wound healing in subjects who are predisposed to an impaired wound response, such as those patients suffering cirrhosis of the liver. This has not been disclosed in the prior art and is quite remarkable. Without treatment, such patients suffer chronic wounds that do not heal over a time period of weeks, if not months. Upon treating the wound daily with the anolyte of electrochemically activated water, we have found that a chronic wound may heal within a matter of days (Example 1). These results hold true for compositions having a pH between 2.5 and 9, including compositions having a pH between 4 and 8

The observation that a wound, once cleared of infection, may heal quicker following treatment with the compositions of the invention than it otherwise would by nature taking its course, provides a further advantage over the compositions described in the prior art.

These results have important implications for the treatment of wounds in other immunocompromised patients. Burns victims, transplant recipients or cancer patients on immunosuppressive drugs and patients suffering diseases such as pneumonia are all immunocompromised. Treating wounds in patients such as these with electrochemically activated water may therefore help to lessen the load on an already deficient immune system.

What is more, the use of such anolyte solutions in this manner is safe. Whilst the anolyte solutions are capable of killing a broad spectrum of micro-organisms,

including MRSA, the solutions are benign in terms of their effect on the skins of humans and animals. The anolyte of electrochemically activated water is not toxic to fibroblasts or epithelial cells and so wound healing is effected immediately upon its application. This is in stark contrast to traditional disinfectants, which can cause necrosis at the skin's surface and can consequently delay or even prevent wound healing.

In the past, it was generally believed that the disinfectant activity of electrochemically activated water is a function of the hypochlorite concentration of the water. As the concentration of hypochlorite increases, so does the redox potential (ORP) and the disinfectant activity of the electrochemically activated water. As a result, it was generally considered that the greater the hypochlorite content of the electrochemically activated water, the greater its disinfectant activity. Indeed, it was generally thought that the electrochemically activated water must include at least 50 to 200ppm hypochlorite in order for it to exhibit acceptable disinfectant activity. Where electrochemically activated water has been used in the past as a disinfectant, the hypochlorite or chlorine concentration has been greater than 50ppm and is usually much greater, sometimes as high as 650ppm.

International Publication No. WO 01/13926 (Sterilox Medical (Europe) Ltd) relates to "super-oxidised" water based on hypochlorous acid, such as that obtained by electrochemical activation of saline solution, for treating open wounds such as leg ulcers. Although the disclosure includes very little technical information about the make-up of the super-oxidised water, its activity is said to be due to the hypochlorous acid. International Publication No. WO 2003/028741 (Aquilabs S.A.) relates to compositions comprising hypochlorous acid for use in prophylactic and therapeutic medical applications. The hypochlorous acid is said to be the bactericidal agent and the claimed compositions comprise 6.5 to 7.3% hypochlorous acid. Compositions with a hypochlorous acid concentration of 5000ppm were used in one study described in the application.

Therefore, it was previously believed that electrochemically activated water having a chlorine content of 50ppm or less would not exhibit any disinfectant activity and

would not be effective against micro-organisms and certainly not effective against antibiotic resistant bacterial strains. However, it has now been discovered that electrochemically activated water with a chlorine content of no more than 50ppm does exhibit disinfectant activity. Indeed, electrochemically activated water with a chlorine content as low as 8ppm or less has been shown to exhibit disinfectant activity.

The disinfectant activity of this low chlorine electrochemically activated water is not a result of the hypochlorite in the water, but rather it appears to be due to the presence of hypochlorous acid, chlorine or chlorite, depending on the pH of the solution. This activity is thought to be "masked" in conventional electrochemically activated water by the high levels of chlorine and the activity of the hypochlorite. However, it has now been found that the hypochlorous acid, chlorine and chlorite produce a redox potential high enough for the water to have disinfectant activity (greater than +900mV), whilst having a low chlorine content. Indeed, it has been found that electrochemically activated water with a chlorine content of less than 130ppm, less than 100ppm, less than 80ppm, less than 50ppm, or even of no more than 8ppm has disinfectant activity and can be used in the present invention to kill or destroy a broad spectrum of bacterial species including MRSA. This chlorine content is extremely low compared to the hypochlorous acid concentrations disclosed as being necessary in prior art such as WO 2003/028741, as discussed above.

The particular active species present in the electrochemically activated water depends on the pH of the solution. At low pH, chlorine predominates, while at a more neutral pH, hypochlorous acid predominates. At higher pH, chlorite is the predominant active species. Low pH solutions were not, therefore, traditionally believed to have disinfectant properties, due to the absence of hypochlorous acid. However, we have shown that, surprisingly, electrochemically activated water at low pH (for example, at a pH lower than about 4 or not more than 3.5 or 3) does have disinfectant properties, due to the presence of chlorine, which is also capable of killing micro-organisms in the concentrations present.

Moreover, we have shown that electrochemically activated water has biocidal properties at all pHs and works equally well across the range of pH 2.5-9 with no impact on effectiveness.

The disinfectant activity of electrochemically activated water is thought to assist the body's own infection control mechanisms. Bacterial infection of a wound site induces the release of neutrophils, which produce hypochlorous acid to kill the invading bacteria. Upon treating a wound with electrochemically activated water, the active species in the water take on the role of native hypochlorous acid, acting as an exogenous supply of bactericide.

The overload of bactericide to the wound site upon treatment with electrochemically activated water enables the body to quickly combat the bacterial infection, so promoting an environment in which wound healing is the sole focus and the body does not also need to tackle the invading bacteria. It is believed that the electrochemically activated water thus allows normal wound healing to occur by minimising the body's involvement in infection control. Treatment of a wound with the compositions of the invention may also promote and/or accelerate wound healing.

As discussed above, it has also been found that the anolyte of electrochemically activated water can be used to promote wound healing in subjects who have an impaired wound response. Presumably, the overload of bactericide to the wound site upon treatment with electrochemically activated water assists the body in combating the bacterial infection, so relieving the pressure on the immune system and enabling what little wound healing capacity there is to function.

Therefore, according to a first aspect of the present invention, a composition comprising an anolyte of electrochemically activated water is provided, for use in treating a wound, the composition having a chlorine content of no more than about 130ppm, no more than about 100ppm, no more than about 80ppm or no more than about 50ppm.

Herein, the treatment of a wound means encouraging wound healing and preferably includes reducing and/or preventing infection of the wound and the surrounding areas.

In a particularly preferred embodiment, the composition is also for use in promoting wound healing. The promotion of wound healing refers to accelerating wound healing, the wound taking less time to heal than would have been required without the application of the compositions according to the present invention and preferably less time than if the wound was disinfected using conventional disinfectants or physical debridement, as discussed above.

As is clear from Example 1, acceleration of wound healing has even been demonstrated in patients having an inherently impaired wound response. As discussed above, wounds will not heal in these and other immunocompromised patients over a time period of weeks, or even months, despite conventional treatment. However, upon daily treatment of a chronic wound with a composition according to the present invention, the wound may heal completely in a matter of days.

In one embodiment of the invention, the composition comprises a combination of the anolyte and the catholyte of electrochemically activated water. In a particular embodiment, the catholyte is added to the anolyte in order to adjust the pH of the composition to a desired value, namely between 4 and 8, preferably between 6 and 8 and more preferably approximately 7. Alternatively, the pH of the compositions according to the present invention is adjusted to the aforementioned values by other means, for example by adding a buffer.

The compositions of the present invention have the advantage that their pH may be adjusted without the loss of disinfectant and biocidal activity. Combinations of the anolyte and the catholyte of electrochemically activated water retain the disinfectant and biocidal activity of the anolyte.

In preferred embodiments of the present invention, the pH of the composition remains constant, with variations of 0.5 of a unit or less, and preferably of 0.1 of a unit or less. Compositions which have a stable pH value between 6 and 8 are particularly useful in the present invention, as they are advantageous for use in physiological systems, as proposed herein. However, as disclosed above, the invention works equally well using compositions of pH less than about 6, and preferably less than about pH 4 or no more than about 3.5 or no more than about 3, with no impact on effectiveness. Accordingly, in other preferred embodiments of the invention, compositions which have a stable pH value of less than about 6, more preferably less than about 4 or no more than about 3.5 or no more than about 3, and most preferably between about pH 2.5 and about 4 or between about pH 2.5 and about 3.5 are particularly useful.

In another embodiment of the present invention, the compositions have a chlorine content of no more than about 130ppm, no more than about 100ppm, no more than about 80ppm, no more than about 50ppm, no more than about 35ppm or no more than about 8ppm. The chlorine content may be from about 0.3 to about 50ppm, preferably from about 1 to about 35ppm, and more preferably from about 5 to about 20ppm. It is desirable to have electrochemically activated water with a minimal chlorine content for a number of reasons. The water is so benign that, at effective doses, it is safe for use on humans and animals, even on open wounds. The low chlorine content of the compositions is important for promoting wound healing.

In one embodiment, the compositions of the invention have a chlorine content of no more than about 130ppm, and a pH of less than about 3.8 or less than about 3.5.

In another embodiment, the compositions according to the present invention preferably have a redox potential of at least about +900mV, and preferably of at least about +1000mV or at least about +1050mV.

In a further preferred embodiment, the level of hypochlorite in the compositions is less than about 10ppm, preferably less than about 5ppm, most preferably less than

about 1ppm. For example, the level of hypochlorite is no more than about 9, 8, 7, 6, 5, 4, 3, 2 or 1ppm.

The electrochemically activated water used in the present invention can be produced in a number of ways, including those well known in the art.

Conventional electrolysis processes operate at voltages and/or power densities at which $HClO^-$ and/or ClO_2 are produced. Indeed, it is these species in conventional electrochemically activated water which is traditionally relied upon for the biocidal activity.

However, as discussed above, electrochemically activated water with a minimal chlorine and hypochlorite content is to be used in the present invention. The chlorine and hypochlorite content of the electrochemically activated water is affected by the amount of sodium chloride in the water prior to the electrochemical activation process, the current used to electrolyse the solution (the size of the current and the length of time the water is exposed to it) and the rate of flow of the salt solution through the different chambers of the electrolytic cell.

Compositions for use in the present invention may be produced by running the electrolysis process at a voltage and/or power density which is higher than that at which O_2 and Cl_2 are produced, but which is also lower than that at which $HClO^-$ and ClO_2 are produced. Preferably, the voltage adjacent to the electrode face is between 1.35 and 1.63V, more preferably between 1.4 and 1.5V and most preferably between 1.42 and 1.47V. It is important to note that these values do not refer to the voltage across the whole system. The chemical reactions are taking place at the face of the electrodes and so it is the voltage here that is significant.

A person skilled in the technical field of the present invention would have no difficulty adjusting the parameters of the electrolysis process in order to achieve the voltage and/or power density at the electrodes required to produce the low chlorine and low hypochlorite water used in the compositions of the present invention, once

he is aware of the required voltage and/or power density at the electrodes relevant to the membrane in use.

The ratio of the anolyte:catholyte flow can be from 5:95 to 95:5 with the optimum flow ratio being 60:40, whilst the current should be between 1 and 20 amps with the preferred range being 6 to 12 amps.

The variation of the chlorine concentration in ppm with water flow at constant current is shown in Table 2.

Table 2

Total Flow	**Anolyte**				**Catholyte**	
litres/hr	Flow	Cl (ppm)	ORP	pH	Flow (l/hr)	pH
37.2	37.2	0	n/a	6.78	0	n/a
37.2	31.2	200	1053	3.16	6	12.28
39	27.6	250	1060	3.05	11.4	12.19
42	24	250	1069	2.87	18	11.98
42	22.8	300	1072	2.7	19.8	11.82
42	16.2	375	1093	2.45	25.8	11.89

In addition, the application of a low current during the electrochemical activation process also results in a solution with a reduced chlorine and hypochlorite content. This is likely to be due to the fact that the lower magnetic field has a lower ability to attract the negative chloride ions during the brief period that any specific chloride ion is within the electromagnetic field. As a result there is a far greater probability that the electrolysis will involve the water molecules which lie alongside the electrodes and produce oxygen based species than chlorine-based species.

Where the chlorine and hypochlorite content of the electrochemically activated water is to be limited by using a low sodium chloride concentration in the water fed into the apparatus carrying out the electrochemical activation, the water fed into the apparatus and electrochemically activated preferably has a sodium chloride

concentration of between 1000 and 5000ppm, and preferably between 2000 and 3000ppm chloride ion concentration.

The conventional electrochemical activation processes apply a current of approximately 10-20 amps to the sodium chloride solution. In order to reduce the chlorine and hypochlorite content of the electrochemically activated water, it is recommended that the current be reduced to between 1 and 10 amps with the preferred range being between 5 and 9 amps.

Regardless of the method used to produce the electrochemically activated water with low chlorine and hypochlorite content, the water has the beneficial properties utilised by the present invention.

The low chlorine electrochemically activated water used in the present invention may be generated using the ECA 2000 Series generator equipment available from Forum Bioscience Holding, Redhill, Surrey, UK. This system significantly reduces the formation of undesirable trihalomethane and chloroamine by-products.

The main principle of the ECA 2000 machines is an electrochemical synthesis of gaseous mixture of oxidants from a dilute solution of sodium chloride under pressure in diaphragm modular electrochemical elements, each of which is a separate electrochemical rector. The block diagram of such a device is presented in Figure 1.

As shown in Figure 1, sodium chloride solution is delivered into the electrochemical reactor. The process results in a partial division of sodium chloride solution into activated chlorine and oxygen based oxidants in the anode chamber, and hydrogen and sodium hydroxide formed in the cathode chamber. The oxidants produced in the anode chamber together with micro-droplets of water are collected (preferably in a darkened acid resistant air tight chemical drum). The electrochemically activated water is then delivered by the injection pump into the part of water to be processed resulting in a dilute solution of oxidants in the final treated water. Hydrogen is

generated in the cathode chambers of electrochemical elements and is vented to air through the catholyte discharge tube.

Previous equipment used a ceramic membrane to separate the solutions resulting from close contact with the electrodes. The ECA 2000 includes a newly developed "flat" core which consists of two half cells working at +15 to 0 volts and 0 to -15V, working back to back where the water to be processed flows across the electrodes and the electrode pairs (and thus the solutions) are separated by an ion exchange membrane. It is this core which can preferably be used to produce the electrochemically activated water which is described herein. It is non-ceramic.

The apparatus preferably has a feedback mechanism to maintain a constant current, which is dependent upon the ionic strength of the water, which is in turn dependent upon the salt content and the nature of the water used.

Provided that the storage conditions are suitable, electrochemically activated water, and the compositions comprising an anolyte of this water according to the present invention, can be stored for periods of weeks or months. In general, the compositions can be stored for more than 72 hours. Indeed, experiments show the compositions of the present invention to be stable even after storage for between 4 and 8 weeks. However, the stability of the compositions depends upon their pH; the higher the pH, the more stable the solution and so the longer the period of storage possible. Compositions having a pH of above 7.5 may be stored for a considerably long time, in excess of 12 months.

As discussed above, the disinfectant activity of the minimal chlorine electrochemically activated water is due to the chlorine in the water. It is clear that if gas is allowed to escape from the water during its storage or use, the activity will be diminished or lost. Therefore, it is necessary to ensure that the water is stored in a closed system which will keep the loss of gas from the water to a minimum. Any stirring or other agitation of the water should also be avoided, as this will encourage gas to escape from the water.

The compositions of the present invention are ecologically friendly and present no problems for the environment. This is shown by the concentrations of chlorite (ClO_2), chlorate (ClO_3) and perchlorate (ClO_4) measured in neat, freshly prepared electrochemically activated water, as recorded in Table 3.

Table 3 - Concentrations of chlorine species found in neat electrochemically activated water as produced in ECA 2000 machine

Species		**Concentration**
Chloride	Cl^-	2600-2800 mg/kg
Hypochlorite	ClO^-	9 mg/l
Chlorite	ClO_2^-	<0.2 mg/l
Chlorate	ClO_3^-	0.3-1.2 mg/l
Perchlorate	ClO_4^-	8-13 ng/l

The activity of compositions according to the present invention against bacteria, viruses and fungi species can be seen from Tables 4, 5 and 6 below.

Table 4 - Anti-microbial efficacy of electrochemically activated water with minimal chlorine content as produced by ECA 2000 generator

Dilution-neutralisation method for 1 minute contact time.			
Test organism	Viable count (cfu/ml) for test mixture (N_a) at concentrations:		
	Neat (approx 140ppm)	50% v/v (approx 70ppm)	5% v/v (approx 7ppm)
Pseudomonas aeruginosa	$<1.5 \times 10^2$	$<1.5 \times 10^2$	$<1.5 \times 10^2$
Staphylococcus aureus	$<1.5 \times 10^2$	$<1.5 \times 10^2$	$>3.0 \times 10^3$
Salmonella (enteritidis) abony	$<1.5 \times 10^2$	$<1.5 \times 10^2$	$<1.5 \times 10^2$
Escherichia coli	$<1.5 \times 10^2$	$<1.5 \times 10^2$	$<1.5 \times 10^2$
Campylobacter jejuni	$<1.5 \times 10^2$	$<1.5 \times 10^2$	$<1.5 \times 10^2$
Test organism	Reduction in viability at test concentration:		
	Neat	50% v/v	5% v/v
Pseudomonas aeruginosa	2.6×10^5	2.6×10^5	2.6×10^5
Staphylococcus aureus	3.3×10^5	3.3×10^5	1.7×10^4
Salmonella (enteritidis) abony	3.3×10^5	3.3×10^5	3.3×10^5
Escherichia coli	3.1×10^5	3.1×10^5	3.1×10^5
Campylobacter jejuni	1.3×10^5	1.3×10^5	1.3×10^5

Table 5 - Electrochemically activated water produced by an ECA 2000 generator: Virucidal efficacy against Influenza A virus: Reduction in virus infectivity

Sample	**Reduction in Infectivity (log_{10}) / Contact time (minutes)**			
	1	5	10	30
Test substance Neat	≥ 5.6	≥ 5.5	≥ 5.7	≥ 5.8
Test substance (approx 14ppm Cl)	3.7	4.2	4.9	≥ 5.8
Test substance (approx 1.5ppm Cl)	0.3	1.0	1.3	1.6
Test substance (approx 0.6 ppm Cl)	0 (-0.2)	0 (-0.1)	0.4	0.4

Table 6 - Measurement of inhibition of growth of *Saprolegnia parasitica* by electrochemically activated water with 24 hour incubation, 1 minute contact time.

Hydroactive concentration (%)	Colony diameter (mm)			Mean colony diameter (mm)	% Inhibition of growth
	Replicate A	Replicate B	Replicate C		
0 (SDW control)	36.30	34.55	35.25	35.37	N/A
100 (Neat)	33.30	32.60	28.40	31.43	11.14
50	32.95	27.80	29.40	30.05	15.04
25	33.35	33.45	32.30	33.03	6.62
10	34.25	32.15	34.70	33.70	4.72
5	33.50	34.40	34.35	34.08	3.65
1	34.80	35.20	33.80	34.60	2.18

The wound healing compositions of the present invention can be used directly on the wound, either to clean or irrigate it, or in combination with a wound dressing. Some examples of wound dressings in current use include conventional materials such as gauze, cotton, wool, lint, gamgee, sponges, and other absorbent materials, as well as low-adherent dressings, vapour permeable films/membranes, hydrogels, hydrocolloids, polysaccharide dressings, alginates, foams, de-odorisers, paste bandages, tulles, plain and medicated, desloughing agents and replaces the need to use anti-microbials. Where appropriate, the wound dressing may be soaked in a wound healing composition of the present invention. However it is used, a composition of the present invention would be expected to kill any bacteria already present in the wound and prevent any further bacteria from taking up residence in it.

It is envisaged that a fresh wound would be cleaned with a composition according to the first aspect of the present invention. Further washing could take place whenever the wound dressing is changed or at predetermined intervals. Although a wounded area may be soaked in, or even submersed in, a composition of the present invention, this manner of prolonged contact of the wound with the composition is not necessary for the desired wound treatment and promotion of wound healing.

In fact, accelerated wound healing has even been observed in cattle suffering dermatitis of the hoof, where lesions treated with the anolyte of electrochemically activated water healed in a matter of days in spite of the cattle being kept in their normal environment (Example 2). Non-obvious lesions in a lame cow have also been treated with electrochemically activated water, with the result that the cow was no longer lame. This proves that wound healing with electrochemically activated water does not have to be performed in an aseptic environment. While healing may be more rapid under aseptic conditions, this is not a prerequisite for achieving satisfactory results. Daily exposure of a wound to electrochemically activated water is sufficient for restoration of tissue integrity, even if the treated wound is subsequently exposed to an environment populated by bacteria or other infective micro-organisms.

These results also demonstrate that treatment with electrochemically activated water has a preventative effect, reducing an infection even if a wound is exposed to dirt, bacteria or other micro-organisms. This has important implications for patients who may have difficulty maintaining a clean environment, such as the elderly, immobile or otherwise incapacitated, and for those being treated in hospitals or other care homes where bacterial infections may be present or rife.

These results hold true for compositions of the invention having a pH from about 2.5 to about 9, including from about pH 4 to about pH 8.

The compositions according to the present invention are effective in treating even severely infected wounds. The treatment may, for example, involve washing the wound 1 to 3 times a day for a period of 2 to 5 days, or for as long as the wound takes to heal.

When using compositions having a chlorine content of approximately 50ppm, a wound will, for example, typically be washed using a volume of approximately 50 to approximately 250ml, and preferably a volume of approximately 100ml. Naturally, the volume should be adjusted according to the size of the wound. Where compositions with a lower chlorine content are used, the volume used to wash the

wound is preferably increased in order to achieve an equivalent therapeutic or prophylactic effect.

For convenience, the compositions according to the invention may be applied to the wound using immersion of the affected body-part, a squeezy or spray bottle, or dripped directly onto the wound, allowing the composition to be accurately directed.

Without wishing to be bound by any particular theory, it is thought that, beyond its role as an antibiotic and nutrient, molecular oxygen may support vital processes in wound healing such as angiogenesis, cell motility and extracellular matrix formation. In order to promote fibroblast proliferation and the production of collagen, oxygen must be present in sufficient quantities. Additionally, active oxygen species contribute as cellular messengers to several important processes that support wound healing, such as signalling and promoting the inflammatory response, killing bacteria and promoting angiogenesis. The active oxygen species in the electrochemically activated water of the present invention are thought to add to the endogenous production of neutrophils, macrophages and endothelial cells, thus accelerating the wound healing process.

In some embodiments of the present invention, the compositions of the present invention further comprise one or more therapeutically active agents, for administration to the wound with the anolyte of the electrochemically activated water. Suitable active agents include, for example, agents known to help fight infection, such as antibiotics, or those known to promote wound healing, such as hepatocyte growth factors, as well as agents which accelerate wound healing.

Alternatively, the compositions according to the present invention may be used in combination with one or more therapeutically active agents, such as agents known to help fight infection (for example, antibiotics), or those known to promote or accelerate wound healing (for example, hepatocyte growth factors). Preferably, the compositions of the invention and the therapeutically active agents are administered simultaneously, sequentially or separately.

As discussed above, the treatment of a wound includes preventing infection of the wound and the surrounding areas. Therefore, the treatment of a wound may also involve using the compositions according to the present invention to cleanse and/or wash uncompromised and/or uninjured skin, in order to disinfect such skin, for example prior to incision of the skin in a surgical setting, and therefore reduce the risk of contamination of the wound.

Electrochemically activated water could also be used preventatively, to cleanse and/or wash uncompromised and/or uninjured skin in individuals who are more prone to chronic wounds and associated skin infections, but who have not yet developed any such afflictions. For example, elderly or immobile individuals who may be at risk of suffering chronic wounds such as venous leg ulcers, pressure sores and ischaemic ulcers could be bathed or washed with the anolyte of electrochemically activated water to prevent any wound-related skin infections developing. The addition of the anolyte of electrochemically activated water to the non-potable water supply of homes for such individuals would be a satisfactory means for achieving such preventative effects, as the individuals would be able to bathe, shower or wash in the compositions of the invention.

Alternatively, electrochemically activated water could be used preventatively in individuals suffering non-wound related skin infections, or in healthy individuals as a measure to prevent skin infections developing at all (for example, as an infection control measure in care homes or in cattle to prevent transmission of diseases such as foot and mouth).

These results hold true for compositions of the invention having a pH from about 2.5 to about 9, including from about pH 4 to about pH 8.

The present invention also provides a composition comprising an anolyte of electrochemically activated water, for use in cleansing and/or washing uncompromised and/or uninjured skin, the composition having a chlorine content

of no more than about 50ppm. The compositions used in this way may have the various features discussed above.

The wound healing compositions of the present invention comprising electrochemically activated water are non-toxic, ecologically friendly, shelf-stable and anti-microbial. This is in stark contrast to conventional biocides based on chlorine, which are highly oxidative in their action and rely on this property to kill bacteria. In contrast, it is thought that the mildly oxidative compositions of the present invention act gradually over an extended period of time to kill a broad spectrum of bacteria including antibiotic-resistant strains such as MRSA and VRE. The described compositions therefore lend themselves well to use on wounded skin, or intact/uncompromised skin as a preventative measure against the development of wound-related skin infections. A further advantage of the compositions of the invention is their ability to promote the healing of wounds even in individuals who have poor wound healing ability.

Examples

Example 1: Wound healing in a patient suffering cirrhosis of the liver

This study demonstrates the ability of the compositions of the invention to promote wound healing in patients having an impaired wound response.

A patient with cirrhosis of the liver had suffered a chronic wound on her foot for three months, despite conventional treatment. Upon immersing her foot in one litre of a composition of the invention comprising 80ppm chlorine at pH 7 for five minutes, twice a day, for five days, the wound was healed by the end of the fourth day.

Example 2: Wound healing in cattle suffering pododermatitis

This study demonstrates the ability of the compositions of the invention to promote wound healing in a non-aseptic environment. It also demonstrates the prevention of infections in sound cows in a non-aseptic environment.

The herd studied was a zero graze herd on a large dairy farm with a history of lameness. Zero graze herds are more susceptible to foot problems owing to the anaerobic nutrient-rich environment created by the accumulation of slurry in the cattle stalls. The environmental conditions in the stalls are perfect for *Bacillus necrophorus*, which is the bacterial strain responsible for pododermatitis (dermatitis of the hoof) in cows.

The aim of the study was three-fold: to treat pododermatitis in infected cows, to prevent dermatitis and resolve lameness in a lame cow and to prevent infection, inflammation and lameness in a sound cow.

Four cows were studied; two having pododermatitis (cow Nos. 2751 and 2832), one which was lame but showed no signs of dermatitis (cow No. 195) and one which appeared sound (cow No. 3860).

Before treatment began, the cows' hooves were studied for signs of dermatitis and each cow was assigned a gait score. The gait score is a value between 1 and 5 denoting the level of lameness (1 being normal gait and 5 being almost completely crippled).

All cows were footbathed twice daily, pre-milking, in a footbath of a composition of the invention comprising 160ppm chlorine at pH 3. For cows showing clinical signs of dermatitis, wounds were washed as thoroughly as possible during milking by spraying a composition of the invention comprising 15% anolyte, 15% catholyte and approximately 25ppm chlorine, at pH 7.2. Twice a week, the wounds were washed extremely thoroughly with this composition.

Results

a) Treatment of pododermatitis in cow No. 2751

	Day 1 Before treatment	Day 3 Treatment begins	Day 8	Day 13
Wound appearance	Cursory examination reveals a fairly classic latter stage of pododermatitis.	Thorough examination reveals a severe case of dermatitis.	Good degree of wound resolution. Wound has gone and surrounding tissue is granulated and healthy. Base of right-hand digit is inflamed to some degree.	Almost complete resolution of dermatitis. Tissue has granulated well and swelling in right-hand digit has abated.
Gait	2	2-3	3	1

These results are illustrated in Figure 2. Figure 2A is a photograph of the cow's hoof before treatment (Day 1), Figure 2B is a photograph of the cow's hoof on Day 3, Figure 2C is a photograph of the cow's hoof on Day 8 and Figure 2D is a photograph of the cow's hoof on Day 13.

b) Treatment of pododermatitis in cow No. 2832

	Day 1 Before treatment	Day 3 Treatment begins	Day 10
Wound appearance	Cursory examination reveals fairly early clinical signs of pododermatitis.	Thorough examination reveals a raw wound with infected tissue sloughed away. Although wound is relatively clean, it is likely to quickly become re-infected in the unhygienic stalls of a zero graze herd.	Excellent wound resolution - only a scar remaining where the wound had been.
Gait	1	2	1

Treatment of this cow was so impressive that the cow had been sold by Day 15.

These results are illustrated in Figure 3. Figure 3A is a photograph of the cow's hoof before treatment (Day 1), Figure 3B is a photograph of the cow's hoof on Day 3 and Figure 3C is a photograph of the cow's hoof on Day 10.

c) Treatment of lameness and prevention of dermatitis in cow No. 195

	Day 1 Before treatment	Day 6 (Five days of treatment)
Hoof appearance	No discernable signs of dermatitis, past or present. However, particularly pronounced inflammation around bulb of right digit	No discernable signs of dermatitis. Inflammation has subsided
Gait	4-5 (very severe lameness).	2-3

Importantly, this cow did not go on to develop clinical dermatitis from the original inflammation observed.

These results are illustrated in Figure 4. Figure 4A is a photograph of the cow's hoof before treatment (Day 1) and Figure 4B is a photograph of the cow's hoof on Day 6.

d) Prevention of dermatitis in sound cow No. 3860

	Day 1 Before treatment	Day 6 (Five days of treatment)
Hoof appearance	Sound	Sound
Gait	1	1

The results are illustrated in Figure 5. Figure 5A is a photograph of the cow's hoof before treatment (Day 1) and Figure 5B is a photograph of the cow's hoof on Day 6.

The importance of regular foot washing with compositions of the invention in cattle is thus clear from this study. The compositions of the invention promote wound healing, even in a non-aseptic environment such as that of zero graze herds. The compositions can also be used preventatively to avoid the development of infection, inflammation and lameness in a sound cow, even in a non-aseptic environment.

Claims

1. A composition comprising an anolyte of electrochemically activated water for treating a wound, the composition having a chlorine content of no more than about 130ppm.

2. A composition as claimed in claim 1, for treating or preventing a wound infection.

3. A composition as claimed in claim 2, wherein the infection is a bacterial infection.

4. A composition as claimed in claim 3, wherein the bacterial wound infection is caused by Methicillin-resistant *Staphylococcus aureus* or Vancomycin-resistant *Enterococcus spp.*

5. A composition as claimed in any one of the preceding claims, for use in promoting wound healing.

6. A composition as claimed in claim 5, for use in an individual suffering an impaired wound response.

7. A composition as claimed in claim 5, for use in a non-aseptic environment.

8. A composition as claimed in claim 4, for increasing the supply of oxygen to a wound.

9. A composition as claimed in any one of the preceding claims, wherein the composition further comprises a catholyte of electrochemically activated water.

10. A composition as claimed in any one of the preceding claims, wherein the composition has a pH of between about 6 and 8.

11. A composition as claimed in any one of claims 1-9, wherein the composition has a pH of less than about 4, less than about 3.8 or less than about 3.5 and preferably has a pH of between about 2.5 and about 3.8.

12. A composition as claimed in any one of the preceding claims, wherein the composition has a chlorine content of less than about 100ppm, less than about 80ppm, less than about 50ppm, less than about 35ppm or less than about 8ppm.

13. A composition as claimed in any one of the preceding claims, wherein the composition has a hypochlorite concentration of no more than about 10ppm

14. A composition as claimed in any one of the preceding claims, further comprising one or more therapeutically active agents.

15. A composition as claimed in any one of the preceding claims, for cleansing uncompromised skin.

16. Use of electrochemically activated water in the manufacture of a medicament for treating a wound, wherein the medicament comprises a composition as claimed in any one of claims 1-14.

17. A use as claimed in claim 16, wherein the medicament is for administration with one or more therapeutically active agents.

18. A wound dressing comprising a composition as claimed in any one of claims 1-13 for treating a wound.

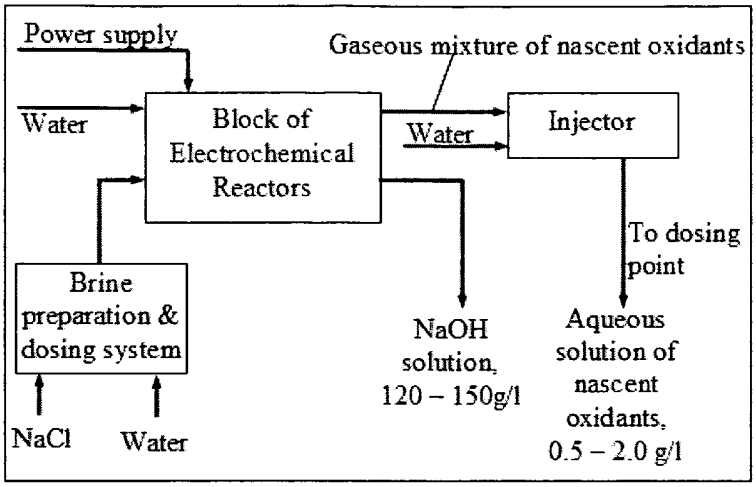
Power supply
Gaseous mixture of nascent oxidants
Water
Block of Electrochemical Reactors
Water
Injector
To dosing point
Brine preparation & dosing system
NaOH solution, 120 – 150g/l
Aqueous solution of nascent oxidants, 0.5 – 2.0 g/l
NaCl
Water

Figure 1

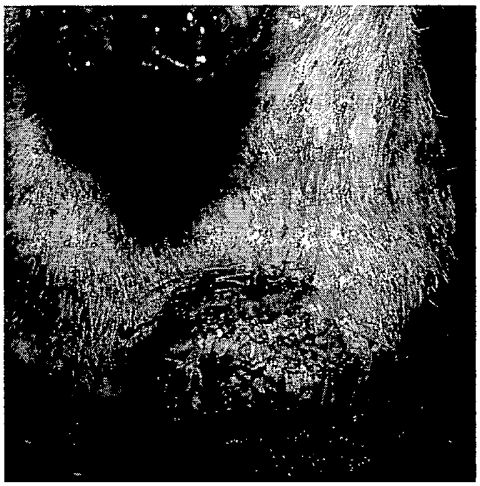

Figure 2A

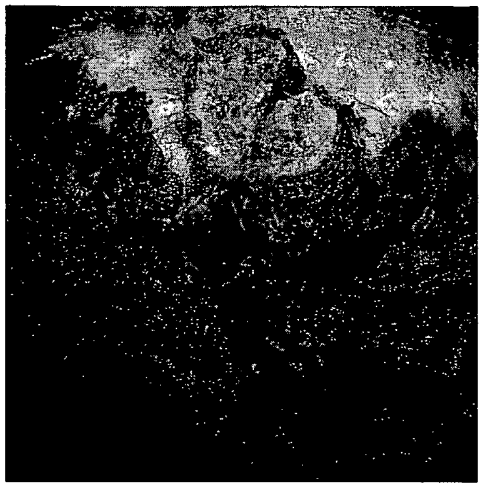

Figure 2B

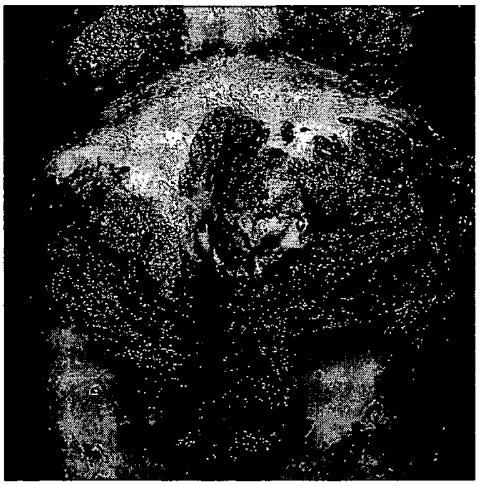

Figure 2C

Figure 2D

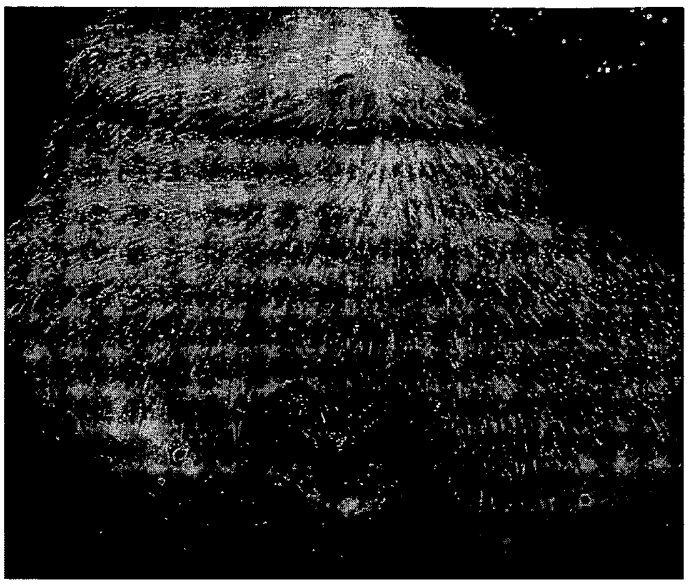

Figure 3A

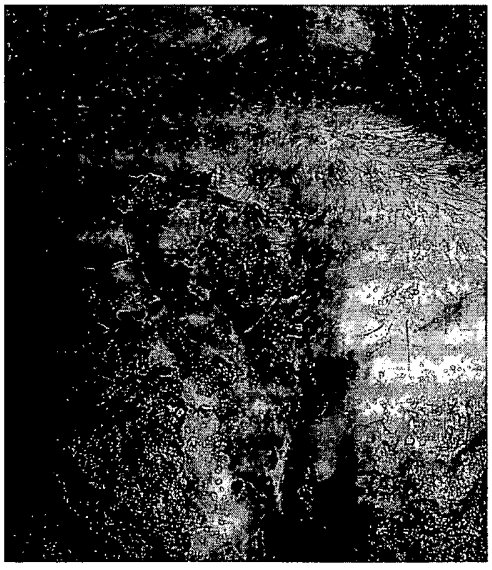

Figure 3B

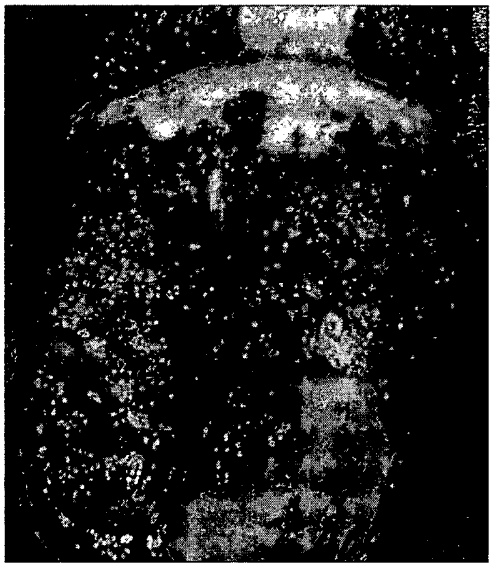

Figure 3C

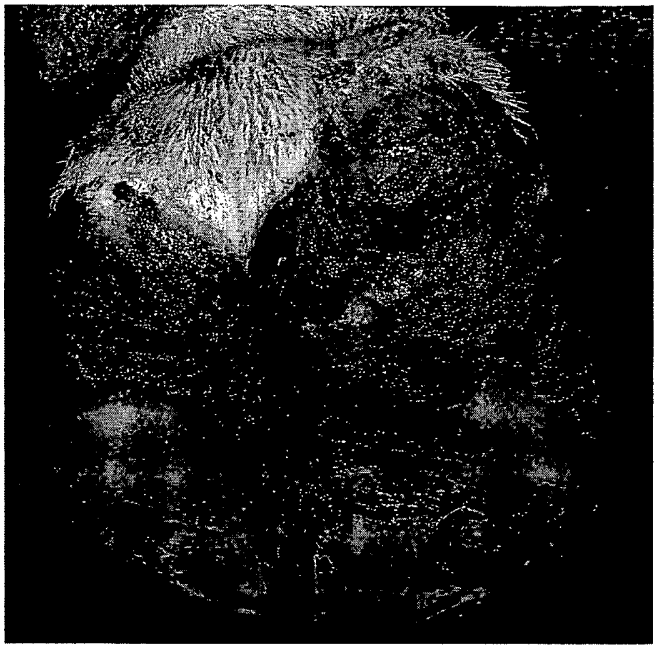

Figure 4A

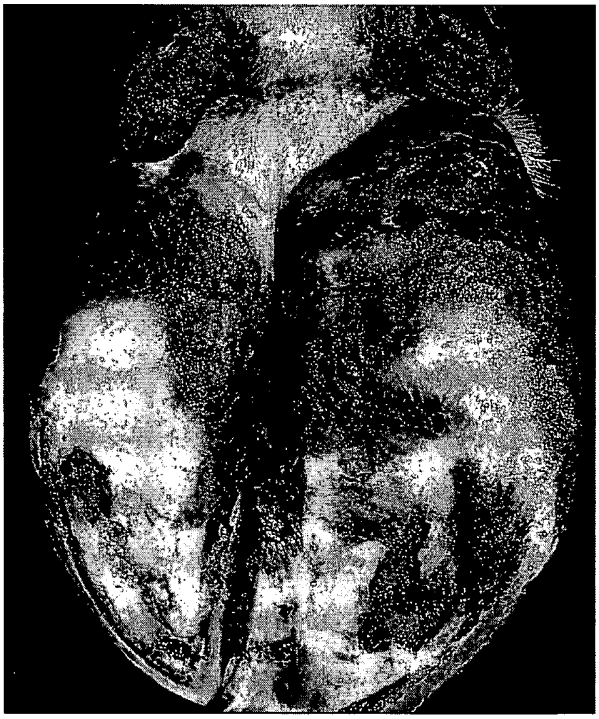

Figure 4B

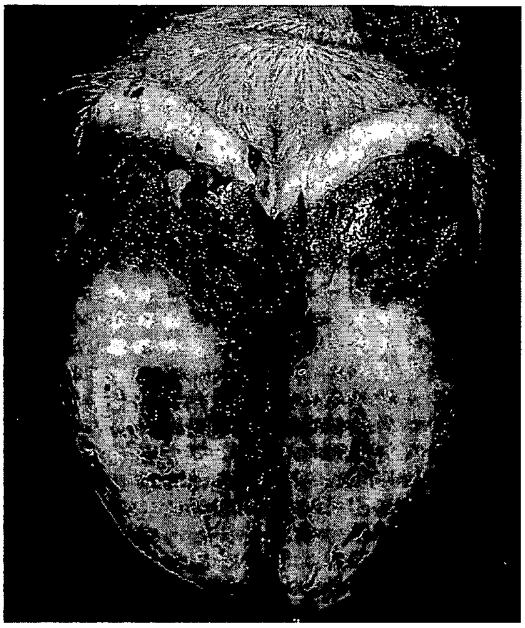

Figure 5A

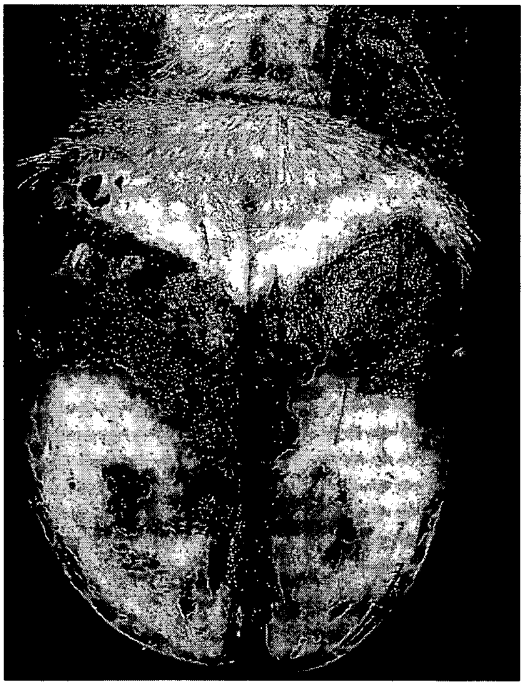

Figure 5B

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2007/050606

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K33/00 A61K33/20 A61P17/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X,Y	US 2006/169575 A1 (SUMITA OSAO [JP]) 3 August 2006 (2006-08-03) claims 1,14,18 examples 1-10,12	1-18

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 February 2008	18/02/2008
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Authorized officer Peris Antoli, Berta

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2007/050606

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	WO 01/13926 A (STERILOX MEDICAL EUROP LTD [GB]; SELKON JOE B [GB]) 1 March 2001 (2001-03-01) cited in the application claims 1,4,11,13,27 page 2, lines 21-29 page 5, lines 1-24 page 6, lines 24-30 page 7, lines 14-19	1-18
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Y	LANDA-SOLIS ET AL: "Microcyn<tm>: a novel super-oxidized water with neutral pH and disinfectant activity" JOURNAL OF HOSPITAL INFECTION, ACADEMIC PRESS, LONDON, GB, vol. 61, no. 4, December 2005 (2005-12), pages 291-299, XP005179446 ISSN: 0195-6701 abstract page 295, column 1, paragraph 2 - page 297, column 1, paragraph 2	1-18

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2007/050606

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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