

DEVICE AND RELATED METHODS FOR DEBRIDEMENT OF WOUNDS

FIELD

The present invention relates to a device, a kit, and a method for debriding and/or cleaning wounds.

5 BACKGROUND

WO 2019/028057 relates to methods of debriding a wound by applying a material in frictional contact with the surface of the wound to remove tissue from the wound surface. The material comprises a solid porous foam soaked by a fluid. Suitable materials include silicone elastomers and copolymers.

- 10 WO 2019/057256 relates to a device for debriding wounds. The material used is polyurethane and the device has a slitted surface with ridges.

- EP 3 560 544 relates to a device for debriding wounds. The device comprises a recess for a NO-releasing formulation. The abrasive segment of the device may be a sponge, but the carrier/device may be flexible and made from a textile fabric, or layers that form a
15 unitary sheet or web.

US 2022/0016246 relates to hypertonic antimicrobial therapeutic compositions.

SUMMARY

There is a need for improvements in devices and methods for handling sores, ulcers and wounds.

- 20 Accordingly, a device for debriding and/or cleaning a wound, a sore or an ulcer is provided, the device comprising a first part comprising an anti-septic composition, the first part optionally made of a water-absorbing material; a second part e.g., comprising a water absorbing material and thereby to function as a water-reservoir; a top surface, wherein optionally the top surface is abrasive; and optionally a water-permeable bottom surface.
- 25 The first part and the second part may be separated from each other e.g., by a water-impermeable membrane also denoted primary membrane.

Further, a method for debriding and/or cleaning wounds, sores and/or ulcers is provided, the method comprising providing a device as disclosed herein; adding water to the device

and/or a container holding the device, optionally until the device is saturated with water for dissolving water-soluble components of the first part; applying the bottom surface or top surface to the wound, sore or ulcer, whereby the water-soluble components of the first part are released from the device; rubbing the wound, sore or ulcer and optionally the
5 surroundings of the wound, sore or ulcer with the bottom surface or top surface of the device to remove biofilm and/or necrotic tissue; optionally squeezing the second part of the device to allow water to rinse the wound, sore or ulcer; optionally rubbing the wound, sore or ulcer and the surroundings of the wound, sore or ulcer with the top surface or the bottom surface to remove remaining unwanted tissue; and optionally rinsing the wound,
10 sore or ulcer with water.

A kit for debriding and/or cleaning wounds, sores and/or ulcers is provided, the kit comprising packaging and a device as disclosed herein, the packaging comprising a container and a seal, the container forming a cavity accommodating the device.

It is an advantage of the present disclosure that improved point-of-care cleaning of wound
15 is provided.

Further, an improved tool for debriding and/or cleaning wounds, sores and/or ulcers is provided.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other features and advantages of the present disclosure will become
20 readily apparent to those skilled in the art by the following detailed description of examples thereof with reference to the attached drawings, in which:

Fig. 1A is a top view of an example device according to the present disclosure,

Fig. 1B is a side view of an example device according to the present disclosure,

Fig. 1C is a side view of an example kit,

25 Figs. 2A-2C illustrate conformation of bacteria morphology by gram staining,

Fig. 3A shows growth curves of ESBL and two strains of MRSA prior to the antibacterial testing,

Fig. 3B shows broth microdilution on blood agar plates in duplicate,

Fig. 4 illustrates broth microdilution on MSB agar plates in duplicate,

Fig. 5 shows kinetics analysis at different time points on antibiofilm activity,

Fig.6 illustrates a summary (mean of the 1-way ANOVA) for all agents representing the best dose response in activating the neutrophils (NETS assay),

5 Fig. 7 shows the VEGF concentration (activated immune system) from biopsy samples,

Fig. 8 illustrates cytotoxicity by the LDH assay of different agents on human white blood cells,

Fig 9. shows the difference in outcome (healed / not healed) correlated with the different concentration of VEGF, IL-1L, IL-8 between different baselines,

10 Fig. 10 is a table illustrating bactericidal minimum concentration after 5 min of exposure of the different agents (at 70 mmol/l) and after recultivating it on agar plates, where – is lethality and + is living,

Fig. 11 shows different gel formulations,

Fig. 12 shows six different gel applications mixed and further analyzed regarding
15 gravity/travel distance and droplet heights,

Fig. 13A shows gravity testing,

Fig. 13B shows droplets height in mm,

Fig. 14A-G show development for an example leg ulcer,

Fig. 15A-H show development for an example plantar diabetic foot ulcer,

20 Fig. 16A-G show development for an example leg ulcer,

Fig. 17A-D show development for an example diabetic foot ulcer,

Fig. 18A is a top view of an example device according to the present disclosure, and

Fig. 18B is a side view of an example device according to the present disclosure.

DETAILED DESCRIPTION

Various exemplary embodiments and details are described hereinafter, with reference to the figures when relevant. It should be noted that the figures may or may not be drawn to scale and that elements of similar structures or functions are represented by like reference numerals throughout the figures. It should also be noted that the figures are only intended to facilitate the description of the embodiments. They are not intended as an exhaustive description of the disclosure or as a limitation on the scope of the disclosure. In addition, an illustrated embodiment needs not have all the aspects or advantages shown. An aspect or an advantage described in conjunction with a particular embodiment is not necessarily limited to that embodiment and can be practiced in any other embodiments even if not so illustrated, or if not so explicitly described.

The figures are schematic and simplified for clarity, and they merely show details which aid understanding the disclosure, while other details have been left out. Throughout, the same reference numerals are used for identical or corresponding parts.

The present disclosure relates to devices, methods, and kits for debriding and/or cleaning a wound, a sore or an ulcer.

A device for debriding and/or cleaning a wound, a sore or an ulcer is disclosed, the device comprising a first part comprising an anti-septic composition, and a second part, wherein the first part and the second part are separated from each other by a water-impermeable membrane. The water-impermeable membrane separating the first part and the second part is also denoted primary membrane.

The device comprises a top surface, wherein the top surface optionally is abrasive. The device comprises a bottom surface, such as a water-permeable bottom surface.

The device optionally has a first end and a second end. The device may have a first end surface comprising the first end, and a second end surface comprising the second end. The first end surface may be a planar first end surface. The second end surface may be a planar second end surface. The first end surface and the second end surface may be parallel.

The device optionally comprises a first side surface and/or a second side surface. The first side surface may be planar or curved. The second side surface may be planar or curved. The first side surface and the second side surface may be parallel. The device may be box-shaped optionally with one or more rounded corners and/or one or more rounded edges.

The device may have a length (distance between first end and second end) along a longitudinal axis in the range from 40 mm to 150 mm, such as in the range from 70 mm to 120 mm, e.g., 100 mm.

The device may have a height (distance between top surface and bottom surface) in the range from 5 mm to 50 mm, such as in the range from 10 mm to 30 mm.

The device may have a width (distance between first side surface and second side surface) in the range from 30 mm to 100 mm, such as in the range from 40 mm to 80 mm.

The first part may have a (first) volume in the range from 10 ml to 100 ml. In one or more examples, the first part has a volume in the range from 20 ml to 50 ml, such as 30 ml.

The first part may have a first length also denoted L_1 in the range from 20 mm to 60 mm, such as 30 mm, 35 mm, 40 mm, 45 mm, 50 mm or any range there between. In one or more examples, the first length is in the range from 30 mm to 50 mm, such as 40 mm.

The first part may be configured to soak up a first liquid volume or may have a first liquid capacity in the range from 5 ml to 50 ml. In one or more examples, the first part may be configured to soak up a first liquid volume or may have a first liquid capacity in the range from 10 ml to 30 ml, such as 15 ml, 20 ml, or 25 ml.

The second part of the device may be designed to absorb water and/or may function as a water-reservoir, e.g., used for rinsing.

The second part may have a (second) volume in the range from 20 ml to 120 ml, such as in the range from 20 ml to 90 ml. In one or more examples, the second part has a volume in the range from 60 ml to 80 ml, such as 70 ml. The second volume may be larger than the first volume, e.g., to provide sufficient rinsing capacity from water soaked by the second part.

In one or more examples, a ratio between the second volume and the first volume is in the range from 1 to 5. In one or more examples, a ratio between the second volume and the first volume is larger than 1.5.

5 The second part may have a second length also denoted L_2 in the range from 20 mm to 120 mm, such as in the range from 40 mm to 100 mm, e.g., 45 mm, 50 mm, 55 mm, 60 mm, 65 mm, 70 mm, 75 mm, 80 mm, 85 mm, 90 mm or any range therebetween. In one or more examples, the second length is in the range from 50 mm to 90 mm, such as 70 mm.

10 In one or more examples, a ratio between the second length and the first length is in the range from 1 to 3.

The second part may be configured to soak up a second liquid volume or may have a second liquid capacity in the range from 10 ml to 60 ml. In one or more examples, the second part may be configured to soak up a second liquid volume or may have a second liquid capacity in the range from 10 ml to 40 ml, such as 20 ml, 25 ml, or 30 ml. The
15 second liquid capacity may be larger than the first liquid capacity.

In one or more examples, a ratio between the second liquid capacity and the first liquid capacity is in the range from 1 to 5. In one or more examples, a ratio between the second liquid capacity and the first liquid capacity is larger than 1.5.

20 The first part and the second parts are dimensioned to provide an effective amount of anti-septic and allow sufficient rinsing capacity while providing a device that is easy to handle.

In one or more examples, the device comprises a first part comprising an anti-septic composition; a second part comprising a water absorbing material and thereby to function as a water-reservoir; a top surface, wherein the top surface is abrasive; and a water-
25 permeable bottom surface, wherein the first part and the second part are separated from each other by a water-impermeable primary membrane.

The primary membrane may be or comprise a glue layer and/or a polymer film.

30 In one or more examples, the first part is made of a water-permeable material and/or a water-absorbing and/or water-permeable material. The water-permeable material may be compatible with the anti-septic composition, such as one or more active chlorine agents.

The first part may comprise a foam material. The anti-septic composition may be embedded in the foam material and/or arranged in a first cavity of the foam material. Accordingly, the first part may comprise or define a first cavity accommodating the anti-septic composition.

- 5 In one or more examples, the anti-septic composition comprises one or more anti-septic agents. In one or more examples, the anti-septic composition comprises one or more anti-microbial agents. In one or more examples, the anti-septic composition comprises one or more detaching agents. In one or more examples, the anti-septic composition comprises one or more anti-septic agents, one or more anti-microbial agents, and one or more
10 detaching agents. A detaching agent is an agent that softens or loosens attachment of necrotic tissue/biofilm to healthy tissue. An example of a detaching agent is chlorine and other oxidative agents thereof.

- In one or more examples, the anti-septic composition comprises an emulsifying agent also denoted an emulsifier. An emulsifying agent is also denoted a gelling agent that
15 forms a gel when mixed with water or other liquid. In other words, the emulsifying agent increases viscosity of the liquid. This may be advantageous to secure or increase contact between the wound surface and the anti-septic composition. The emulsifying agent may comprise one or more of glycerine, cellulose, agar, and povidone. In one or more examples, the emulsifying agent comprises cellulose.

- 20 The anti-septic composition, when dissolved in water or other liquid, helps and contributes to loosening the attachment of necrotic tissue to healthy tissue to facilitate debridging.

- In one or more examples, the anti-septic composition comprises powdered agents for debridging one or more of wounds, sores and ulcers. In other words, the anti-septic composition may be or comprise a powder. In one or more examples, the anti-septic
25 composition comprises a tablet or pellets.

- In one or more examples, the anti-septic composition comprises an active chlorine agent or a plurality of active chlorine agents. The active chlorine agent or active chlorine agents of the plurality of active chlorine agents may be selected from Cl_2 , hypochlorite, chlorite, chlorate, perchlorate, and alkaline or alkali earth salts thereof. In one or more examples,
30 the anti-septic composition comprises at least 100 mg active chlorine agent, such as in the range from 200 mg to 2.0 g active chlorine agent.

In one or more examples, the anti-septic composition comprises calcium hypochlorite, e.g., in a concentration less than 25 g/l, such as in a range of from 2 g/l to 20 g/l, such as in the range from 4 g/l to 12 g/l. The anti-septic composition may comprise calcium hypochlorite in a concentration of 10 g/l.

- 5 In one or more examples, the ratio between the content of active chlorine agent in the anti-septic composition and the first liquid capacity is larger than 30 millimolar, such as in the range from 50 millimolar to 150 millimolar.

In one or more examples, the active chlorine agent is hypochlorite, such as a salt of hypochlorite, e.g., calcium hypochlorite. For example, the anti-septic composition may
10 comprise calcium hypochlorite of in the range from 0.2 g to 2 g, such as in the range from 1 g to 1.8 g.

In one or more examples, the anti-septic composition comprises a pH adjusting agent to adjust pH, such as to adjust pH to a range of from 8.00 to about 9.00. The pH adjusting agent may be sodium hydrogen carbonate (bicarbonate) or hydrogen borate salt. The pH
15 adjusting agent may be NaOH or HCl. Thereby a good activity of the chlorine agent(s) after application on a wound, sore or ulcer is provided.

The first part, such as the anti-septic composition, of the device may comprise one or more pharmaceutically acceptable excipients, such as one or more of colloidal silicon particles, sodium chloride, and potassium chloride.

- 20 In one or more examples, the first part, such as the anti-septic composition, comprises a drying agent, such as one or more of colloidal silicon particles.

In one or more examples, the first part, such as the anti-septic composition, comprises enhancer or initiator, such as sodium chloride and/or potassium chloride, e.g. for inducing and/or increasing enzymatic activity. The anti-septic composition may comprise sodium
25 chloride of in the range from 50 mg to 200 mg, such as 140mg. The anti-septic composition may comprise potassium chloride of in the range from 30 mg to 150 mg, such as 70 mg.

In one or more examples, the first part, such as the anti-septic composition, comprises one or more of colloidal silicon particles, sodium chloride and potassium chloride.

In one or more examples, the anti-septic composition comprises sodium hydrogen carbonate also denoted sodium bicarbonate. The concentration of sodium hydrogen carbonate may be in a range of from 1 to 3 g/l, such as 2 g/l.

5 In one or more examples, the anti-septic composition comprises potassium chloride. The concentration of potassium chloride may be in a range of from 0.5 to 3 g/l, such as 1 g/l.

In one or more examples, the anti-septic composition comprises sodium chloride. The concentration of sodium chloride may be in a range of from 1 to 4 g/l, such as 2 g/l.

10 In one or more examples, the anti-septic composition comprises potassium chloride and sodium chloride in a combined concentration of in the range from 2 to 4 g/l, e.g. 3 g/l, for example 1 g/l of potassium chloride and 2 g/l of sodium chloride.

In one or more examples, the anti-septic composition comprises titanium dioxide and one or more of silicium dioxide, silicon dioxide, and fumed silica. The concentration of titanium dioxide may be in a range of from 0.1 to 1 g/l, such as 0.4 g/l. The concentration of silicium dioxide, silicon dioxide or fumed silica may be in a range of from 0.2 to 1.0 g/l, 15 such as 0.6 g/l.

In one or more examples, the anti-septic composition comprises sodium hydrogen carbonate also denoted sodium bicarbonate; potassium chloride; sodium chloride; titanium dioxide; one or more of silicium dioxide, silicon dioxide, and fumed silica; optionally sodium hydroxide or other pH adjusting agent, e.g., to adjust pH to a range 20 from 8.00 to 9.00; and calcium hypochlorite.

In one or more examples, the concentration of sodium hydrogen carbonate is in a range of from 1 to 3 g/l, such as 2 g/l; the concentration of potassium chloride is in a range of from 0.5 to 3 g/l, such as 1 g/l; the concentration of sodium chloride is in a range of from 1 to 4 g/l, such as 2 g/l; the concentration of titanium dioxide is in a range of from 0.1 to 1 25 g/l, such as 0.4 g/l; the concentration of silicium dioxide, silicon dioxide or fumed silica is in a range of from 0.2 to 1.0 g/l, such as 0.6 g/l, and the concentration of calcium hypochlorite is in a range of from 5 to 25 g/l, such as 10 g/l, 15 g/l, or 20 g/l.

30 It is noted that when referring to a concentration of the anti-septic agent or components thereof, reference is made to the concentration in the liquid of the first part, when the device has been soaked with liquid, such as water. The liquid, which may be a gel, with anti-septic composition dissolved therein is also referred to as the anti-septic liquid. In

other words, when referring to a concentration of the anti-septic composition or components/agents thereof, reference may be made to the weight of the anti-septic agent or components/agents thereof divided by the first liquid capacity of the first part.

5 In one or more examples, the device comprises a first layer, e.g., of abrasive material, forming the top surface. In other words, the first layer may have a first surface forming the top surface of the device. The first layer may have a thickness in the range from 0.3 mm to 5 mm. The first layer may be porous.

10 In one or more examples, the device comprises a first membrane. The first membrane, e.g., a first region of the first membrane, may be between the first layer, such as a second primary surface of the first layer, and the first part, such as a first surface of the first part. Thus, a first region of the first membrane may be between a second primary surface of the first layer and a first surface of the first part. The first membrane may be a water-impermeable membrane separating the first layer and the first part. In other words, the first membrane may be configured to prevent water and/or liquid containing the anti-septic
15 composition from travelling from the first part to the first layer, thereby ensuring that the first layer used for final rubbing and/or rinsing during use is not contaminated with the anti-septic composition. In one or more examples, the anti-septic agent may be arranged between foam material of the first part and the first membrane.

20 In one or more examples, the first membrane covers the second part or at least parts thereof. In other words, the first membrane, e.g., a second region of the first membrane, may be between the first layer, such as a second secondary surface of the first layer, and the second part, such as a first surface of the second part. Thus, a second region of the first membrane may be between a second secondary surface of the first layer and a first surface of the second part. The first membrane may be a water-impermeable membrane
25 separating the first layer and the second part. In other words, the first membrane may be configured to prevent liquid containing the anti-septic composition from travelling from the first layer to the second part and/or prevent water or other liquid from travelling from the second part to the first layer, thereby ensuring that the water in the second part does not leak into the first layer. Thus, in one or more examples, the first membrane is between the
30 first layer, such as a second secondary surface of the first layer, and the second part.

The first membrane may be a polymer film or sprayed on the first layer or on the first part and/or the second part. In one or more examples, the first membrane may be or comprise a glue for gluing the first layer to the first part and/or to the second part.

In one or more examples, the first part comprises one or more of silicone, polysiloxane, a polyurethan, polyester, cellulose, and non-woven fabric. The silicone, polysiloxane or polyurethan of the first part may be in the form of a foam. Thus, the first part may comprise a foam material.

- 5 In one or more examples, the second part comprises one or more of silicone, polysiloxane, a polyurethan, polyester, cellulose, and non-woven fabric. The silicone, polysiloxane or polyurethan of the second part may be in the form of a foam. Thus, the second part may comprise a foam material.

- 10 In one or more examples, the device, such as the second part and/or the first layer optionally comprises an anti-septic neutralizer, such as an acid, to neutralize the anti-septic composition. The anti-septic neutralizer may be a powder, a tablet, or pellets.

- Also disclosed is a method for debriding and/or cleaning wounds, sores and/or ulcers. In one or more examples, the method comprises providing a device as described herein; adding water or other liquid to the device, e.g. until the device is saturated with water, for
15 dissolving water-soluble components of the first part/anti-septic composition; applying, such as pressing, the bottom surface or the top surface to the wound, sore or ulcer, whereby the water-soluble components of the first part are released from the device; rubbing the wound, sore or ulcer and/or the surroundings of the wound, sore or ulcer with the bottom surface or top surface of the device to remove biofilm and necrotic tissue;
20 optionally squeezing the second part of the device to allow water to rinse the wound, sore or ulcer; optionally rubbing the wound, sore or ulcer and the surroundings of the wound, sore or ulcer with the top surface or the bottom surface to remove remaining unwanted tissue; and optionally rinsing the wound, sore or ulcer with water.

- Further a kit for debriding and/or cleaning wounds, sores and/or ulcers is disclosed, the kit
25 comprising packaging and a device as described herein. The packaging comprising a container and a seal, the container and the seal forming a cavity also denoted primary cavity accommodating the device. The cavity may have a volume, e.g., in the range from 150 ml to 400 ml. The container may comprise a secondary cavity for accommodating liquid, such as a liquid container with the liquid. In other words, the kit may comprise a
30 liquid container e.g., arranged in a secondary cavity or attached to the container. The secondary cavity may be closed or sealed by a sealing. A kit including a liquid container/liquid allows for a self-contained kit that is ready to use without access to water, such as tap water.

To generate a sterile wound composition for application on a wound, sore or ulcer, the bottom surface and/or the ends and/or sides of the device may contain a sterile filter. Such a sterile filter ensures that bacteria present in the wound cannot enter the filter and the device and thereby contamination of the device can be avoided.

- 5 The device may comprise a second layer forming the bottom surface. The second layer may be a sterile filter, such as a non-woven filter, e.g. with a maximum pore size in the range from 0.1 μm to 0.6 μm , such as 0.2 μm or 0.45 μm .

The second layer may be water-impermeable.

- 10 The second layer may be or form a second membrane, such as a polymer film or sprayed on the first part and/or the second part.

In one or more examples, the foam material or parts thereof may be or constitute a sterile filter, e.g. by selecting a suitable pore size of the foam.

The anti-septic composition, such as one or more of anti-septic agents, anti-microbial agents, and detaching agent, contained in the first part may be present in powder form.

- 15 The anti-septic composition may be distributed in a porous material, such as a sponge or foam, to retain water in the first part – when the device is used – so that the anti-septic composition or agents thereof are released from the device in dissolved form or at least together with water. The water that enters into the second part – when the device is used – may also transport water to the wound, sore or ulcer, but then the agent only can be
20 dissolved by such water upon contact with the wound, sore or ulcer.

The second part may form a second cavity and/or may contain a material, such as a foam material, that can absorb water and release water upon contact with the wound, sore or ulcer. The release of water from the first part and/or the second part may be facilitated by squeezing or pressing the device, such as the first part and/or the second part, manually.

- 25 Before using the device, the device is soaked in water or other liquid so that water/liquid can enter the first part and the second part. The second part should be configured to contain or hold sufficient water to allow for rinsing of the wound, sore or ulcer after release of the anti-septic composition of the first part.

- 30 After rinsing of the wound, sore or ulcer, the device is turned around and the top surface is contacted with the wound, sore or ulcer and mechanically and manually rubbed on the

wound, sore or ulcer. Hereby the wound, sore or ulcer is mechanically cleaned, and necrotic tissue is removed.

Before use, the device may be placed in a container and water is poured in the container/on the device so that the device absorbs water, and the device is then ready to use. Preferably, the device is packaged in suitable packaging with a container closed with a seal, such as a removable foil, e.g., where water directly can be poured into the container when the seal or foil is removed by the user.

Fig. 1A illustrates a top view of an example device according to the present disclosure. The device 2 is a device for one or more of debriding, rubbing, and cleaning a wound, a sore or an ulcer, the device 2 comprising two or more parts including a first part 4 and a second part 6, a top or first surface 8, and a bottom or second surface (not shown in Fig. 1 but elsewhere indicated with reference number 10). The first part 4 has a first length L_1 optionally of 30 mm, and the second part 6 has a second length L_2 optionally of 70 mm, wherein the first part 4 and the second part 6 are separated by a primary membrane 12, such as a water-impermeable membrane or layer. The first part 4 comprises an anti-septic composition 26, e.g., embedded in foam material and/or arranged in a first cavity of a foam material. The anti-septic composition 26 comprises one or more agents, such as one or more anti-septic agents including active chlorine agent 26A, one or more anti-microbial agents and/or one or more detaching agents. The anti-septic composition 26 comprises a pH adjusting agent 26B. The first part 4 is designed to absorb liquid, such as water e.g. with a first volume of in the range from 5 ml to 30 ml, whereby the anti-septic composition 26, such as the anti-septic agent, is dissolved to form an anti-septic liquid that can be squeezed out of the first part for application to the wound, sore, or ulcer. The second part 6 is designed to absorb liquid, such as water e.g., with a second volume of in the range from 10 ml to 40 ml, and thereby to function as a liquid-reservoir, such as a water-reservoir. The top surface 8 is an abrasive or rough surface, e.g., formed by a first layer 14 of abrasive material, and the bottom surface 10 is water permeable. In other words, the device 2 optionally comprises a first layer 14 of abrasive material.

The device 2 may comprise a second layer (not shown in Fig. 1A but elsewhere indicated with reference number 16) of optionally water permeable material. Optionally, the device 2 has a first end 18 forming a first end surface, and a second end 20 forming a second end surface optionally with a length L of 100 mm. Optionally, the device 2 has a width W of 50 mm with a first side 22 forming a first side surface, and a second side 24 forming a

second side surface. The device 2 is box-shaped optionally with rounded corner(s) and/or edge(s).

The device 2, such as the first part 4 and/or the second part 6, comprises a water-permeable material that is compatible with active chlorine agents, such as one or more of polyester, cellulose, silicone, polysiloxane or a polyurethan. The device 2, such as the first
5 part 4 and/or the second part 6, optionally comprises a non-woven material.

The device 2, such as the first part 4 and/or the second part 6, optionally comprises a porous material, such as a foam material or a non-woven material. For example, the device 2, such as the first part 4 and/or the second part 6, comprises one or more of a
10 silicone foam, a polysiloxane foam, and a polyurethan foam.

The anti-septic composition 26 of the first part 4 comprises one or more, optionally powdered, agents, e.g., for debriding of one or more of wounds, sores and ulcers. For example, the anti-septic composition 26 comprises an active chlorine agent 26A, such as an active chlorine agent selected from Cl_2 , hypochlorite, chlorite, chlorate, perchlorate,
15 and alkaline or alkali earth salts thereof. In other words, the first part optionally comprises an active chlorine agent selected from Cl_2 , hypochlorite, chlorite, chlorate, perchlorate, and alkaline or alkali earth salts thereof. In an example device as illustrated, the active chlorine agent 26A is hypochlorite, such as calcium hypochlorite.

The first part 4/anti-septic composition 26 optionally comprises a pH adjusting agent 26B,
20 e.g., to adjust pH to a range of from 8.00 to about 9.00. The pH adjusting agent 26B may be or comprise one or more of sodium hydrogen carbonate, a hydrogen borate salt.

Optionally, the first part 4 comprises colloidal silicon particles, e.g., in combination with sodium chloride and/or potassium chloride.

In one or more examples, the first part 4 comprises i) sodium hydrogen carbonate also
25 denoted sodium bicarbonate, ii) potassium chloride, iii) sodium chloride, iv) titanium dioxide, v) one or more of silicium dioxide, silicon dioxide and fumed silica, vi) optionally, sodium hydroxide to adjust pH to from 8 to 9, and vii) calcium hypochlorite.

Fig. 1B is a first side view of device 2. The device 2 has a height H optionally of 20 mm and comprises a first membrane 28 optionally including a first region 28A between the
30 first layer 14 and the first part 4. The first membrane 28 may be a water-impermeable membrane separating the first layer 14 and the first part 4. In other words, the first

membrane 28 may be configured to prevent anti-septic liquid from travelling from the first part 4 to the first layer 14, thereby ensuring that the first layer 14 used for rubbing and/or rinsing during use is not contaminated with the anti-septic composition/liquid. In one or more examples, the anti-septic composition 26 may be arranged between foam material of the first part 4 and the first membrane 28. In one or more examples, the anti-septic composition 26 may be arranged between foam material of the first part 4 and the second layer 16. The second layer 16 may be dissolvable by water or water-permeable to ensure water being soaked by the first part.

The first membrane 28 may comprise a second region 28B between the first layer 14 and the second part 28. The first membrane 28 may be a water-impermeable membrane separating the first layer 14 and the second part 6. In other words, the first membrane may be configured to prevent anti-septic liquid from travelling from the first layer 14 to the second part 6 and/or prevent water or other liquid from travelling from the second part 6 to the first layer 14, thereby ensuring that the water in the second part 6 does not leak into the first layer 14.

Fig. 1C shows a cross section of an example kit according to the present disclosure, the kit 50 comprising packaging and a device as disclosed herein, such as device 2. The packaging of the kit 50 comprises a container 52 and a seal 54, the container 52 forming a cavity 56 accommodating the device 2. Water or other liquid added to the container 52 after removing the seal 54 will soak the device 2 and dissolve the anti-septic agent of the first part in the device. The container may have dimension L x W x H of 105 mm x 55 mm x 2.5 mm.

WOUND HEALING

Wound healing is a well-orchestrated process, which can be described into four phases:

Hemostasis – the thrombocytes and coagulations system are activated, and the bleeding is stopped.

Inflammatory phase – where neutrophilic granulocytes are activated to eradicate microorganism, biofilm and necrotic tissue.

Proliferation phase – where fibroblasts, keratinocytes and endothelial cells are activated to angiogenesis, ECM production (new tissue) and epithelialization.

Remodeling phase – reinforcement of the tissue so the wound does not reappear mainly by formation of collagen.

The process of wound healing usually takes 3-4 weeks. Wounds that have not healed within 6 weeks are chronic wounds or ulcers (also called hard-to-heal wounds or ulcers) .

- 5 The healing may halt in the inflammatory phase due to incapability to eradicate microorganisms, biofilm and necrotic tissue. Hypochlorite and hypochlorous acid are the active substances that neutrophilic granulocytes use to eradicate microorganisms, biofilm and necrotic tissue.

- 10 When the wound/ulcer is clean, the proliferations phase can start. The cutaneous nervous system plays a crucial role in wound healing by starting the proliferations phase. The production and secretion of IL-1, IL-6, IL-8, IL-10 and TNF α are known as mediator of proliferation phase.

- Both in diabetic foot ulcer and ulcer in persons with neuropathy for other causes show impaired wound healing. If the production IL-1, IL-6, IL-8, IL-10 and TNF α can be
15 increased even with existing neuropathy, the wound healing will improve.

Studies of the present disclosure have shown that cleaning wounds and ulcer with hypochlorite and hypochlorous acid increase the level of neuropeptides such as IL-1, IL-6, IL-8, IL-10 and TNF α .

- Fig. 2A-2C illustrate conformation of bacteria morphology by gram staining, ESBL (red)
20 and MRSA (blue). *K. pneumoniae* ESBL (A), MRSA CCUG 31966 (B) and MRSA CCUG 35600 (C). ESBL (red) and MRSA (blue). *K. pneumoniae* ESBL (Fig. 1A), MRSA CCUG 31966 (Fig. 1B) and MRSA CCUG 35600 (Fig. 1C)

- Fig. 3A shows example growth curves of ESBL and MRSA strains. Bacteria reach the lag-phase after 20 hours, no matter the specimen tested. In addition, OD measurements and
25 viable counting was performed of the exponential phase of each bacteria strain to calculate the cell concentration (CFU/ml). Furthermore, Fig. 2 shows that the bacteria are doing well prior to the testing – without any addition of chemical, i.e., without sodium hypochlorite and sodium hydrogen carbonate (actually, that the chemical and/or test method is not the one killing of the bacteria). All strains had reached stationary phase
30 after 15 hours incubation at 37°C when cultured in MH broth (Mueller Hinton). In addition,

the optical density (OD) of the overnight cultures with corresponding viable counts of inoculums was established to log 5.5 CFU/ml (ESBL) and 6.2 CFU/ml, respectively.

Fig. 3B show growth inhibition of ESBL and two strains of MRSA on agar plates. The sodium hypochlorite gel was diluted by a two-fold serial titration applied at the highest concentration from the top 70 mM down to 0.03 mM (millimolar). The left plates show a minimum microbicidal concentration for ESBL at 1.1mM, the middle and right plates on the two different strains of MRSA with MBC values at 0.6mM. Bacterial growth appears as dense gray dots as seen for the growth controls, i.e., three vertical dots to the left side of each plate, while no growth appears as empty agar surface which is seen for the sterility controls that were applied as three drops vertically, to the right side of each plate.

Fig. 4 illustrates growth inhibition of *Streptococcus mutans* IB (2 top rows) and *Streptococcus mutans* OMZ65 (2 bottom rows) (10^8 CFU/ml) on Mitis Salivarius Bacitracin (MSB) agar by test agents at different concentrations from the left: Plate A: 70, 35, 17.5, 8.8, 4.4, 2.2 mmol/l; Plate B: 1.1, 0.5, 0.3, 0.14, 0.07, 0.03 mmol/l. Drops without any colonies (or empty agar surface) equal the MMC99 value indicating that the test solution has reduced $\geq 99\%$ of the bacteria. Positive and negative controls worked as expected, not shown. MBC of the CHL (the chlorine agent) were observed at 2.2mM.

Fig. 5 shows time-kill analysis with Live/Dead staining of MRSA biofilm treated with sodium hypochlorite solution (Ch), Microdacyn (Mi.), and Prontosan (Pr.) for 5, 10, 30, and 60 minutes shown in sequential order from top to bottom row. The microphotographs are taken with x10 magnification. – green is survival – reddish is dead. Live/dead at 5 min showed log CFU values at 0 (CHL), 7.44 (MI) and 6.89 (Pr): 30 min 0 (CHL) 7.43 (Mi) and 2.87 (Pr). Live/Dead images (10X) shown in sequential order from top (5 min) to bottom row (60 min).

Fig. 6 illustrates a summary (mean of the 1-way ANOVA) for all agents representing the best dose response in activating the neutrophils (NETS assay). Positive controls used was with added PMA and the negative control without PMA. A summary of agent's concentration (Absorbance) giving the highest induction over 160 (min). The amino acids with PMA at 1.06 mM showed to be statically significant compared with the positive control (cells + PMA). Likewise, the sodium hypochlorite at 7.5 mM with PMA ($p < 0.0001$) and chloramines at 1.325 mM were all statically significant.

Fig. 7 shows the VEGF concentration (activated immune system) from biopsy samples. Graph shows medians, 10-90 percentile, MIN and MAX values. No significant difference was found between and within the groups.

Fig. 8 illustrates the LDH cytotoxicity of different agents on human serum (white blood cells, 10^7 cells/well). The x-axis represents the different agent's concentration (mM) vs the y-axis represents the cytotoxicity (%), calculated from the formula below). Control (white blood cells in RPMI media), yellow curve; chloramines, red curve; sodium hypochlorite, blue curve; sodium borohydride, grey curve. Cytotoxicity appeared already at 4 mM for chloramines and started to affect the white blood cells at 8 mM for the chlorine solution.

Fig 9. Shows the mean with 95% CI, for the difference in outcome (healed / not healed) correlated with the concentration of VEGF, IL1L, IL8 between week 4 – baseline, week 8 – baseline, week 8 – week 4, week 12- week 8 and week 12 – baseline. The significance ($p < 0.05$) was found using Mann-Whitney t-test (unpaired, as Gaussian distribution was not assumed, a non-parametric test was used). Significant values were revealed at week 0-4 and at week 8-12 for IL-1L and IL8.

EXAMPLES

EXAMPLE 1 – MIC microdilution assay

EVALUATION CRITERIA

MIC (minimal inhibitory concentration) value is the lowest concentration of the substances that cause the optical density reduction by more than 90% compared with control growth results OR the MIC value is the concentration at which the optical density equals the negative control.

Aim

To evaluate the antibacterial inhibitory activity of hypochlorite solution (0.5%, 70mM) in one strain of ESBL (Extended Spectrum Beta-Lactamase) producing bacterium and two strains of MRSA (Methicillin resistant Staphylococcus aureus) in Minimum Inhibitory Concentration (MIC). These bacteria are known to be difficult to kill.

Materials

TEST ARTICLE DESCRIPTION

Test solution comprise of equal volumes calcium hypochlorite 1 % (140 mM) and sodium hydrogen carbonate 2% (238 mM) with small amounts of salt, titan dioxide and silica. All solutions were filtered through a Chromacol nylon filter (0.2 µm) to be equivalent prior to the antibacterial analysis with the benchmark products Microdacyn and Prontosan in liquid forms.

In the device, all chemicals are present in powdered form to be mixed with water at the treatment site. The final concentration of hypochlorite is 0.5%. Carbonate is present in a concentration of 1% (buffer).

10 Strains

Klebsiella pneumoniae subsp. *Pneumoniae* CCUG 59413

Methicillin resistant *Staphylococcus aureus* (MRSA) CCUG 31966

Methicillin resistant *Staphylococcus aureus* (MRSA) CCUG 35600

Media

15 Horse blood agar plates

MH (Mueller-Hinton Broth) at pH 7.2-7.4 at RT (25°C) cation adjusted

Gram-stain

Equipment; Epoch Microplate reader (BioTek instruments, Inc., Winooski, VT)

Methods

20 Test organism – Preparation of bacterial inoculums (=working solution)

Streak plates were done on horse agar plates and characterized by gram-staining.

Each strain was then cultured to assess when stationary phase is reached.

Isolated colonies were inoculated to 5 ml MH broth and incubated for 15 hours (overnight) at 37°C.

OD measurements were done using a spectrophotometer and over-night suspensions adjusted to correspond to OD 0.28 at 475 nm. This corresponds to $1.5 - 3.0 \times 10^8$ CFU/ml with most bacterial species.

Then working solutions were done by diluting the suspension in MH broth to equal 1 to 2×10^6 CFU/ml in order to give a final inoculum at 1 to 2×10^5 CFU in each well.

This working solution was inoculated to each well within 15 minutes.

Plate counts were done on the inoculums to confirm the inoculum size of the working solution.

Test Specimen – Preparation

10 The hypochlorite solution was prepared by combining the antimicrobial agent 1% calcium hypochlorite (AM) with (sodium hydrogen carbonate 2%, Na-CMC, 30 %, pH 9). The prepared solution (0,5 % active chlorine, 70mM) was transferred to a sterile Eppendorf tube and filtered by a (0.2 μ m) sterile nylon filter to be compare with the benchmark products Microdacyn and Prontosan.

15 Procedure – MIC microdilution assay

The AM substance was titrated in sterile water in 96 well plates according to below plate layout.

Then 100 μ l of respective bacterial suspension in triplicates at $1-2 \times 10^6$ cells/ml was added to each well followed by incubation for 24 hours at $35 \pm 2^\circ\text{C}$.

20 Controls were included:

Growth control/positive ctrl = MH broth with bacteria. Add 100 μ l of inoculum + 100 μ l of MH broth.

Sterility control = MH broth with AM substance. Add 100 μ l of AM 140 mM + 100 μ l of MH broth.

25 No growth control = broth including both substance and bacteria but stored in fridge until analysis. This is used as blank. Add 100 μ l of inoculum + 100 μ l of AM 140 mM

The plates were analyzed after 22 – 24 h in a microtiter plate reader at OD₅₉₅.

Bacterial characterization and exponential phase (growth) of bacteria

GRAM STAINING

Gram staining of the included strains confirmed the morphologies of *K. pneumoniae* (ESBL) and methicillin resistant *S. aureus* (MRSA) strains as well as that they were gram
5 stained as anticipated, ESBL red and MRSA blue, see Fig. 1.

GROWTH CURVE

The growth curves of each strain when grown in MH broth was established by measuring the OD at different time points until stationary phase had been confirmed, see Fig. 2. All strains had reached stationary phase after 15 hours incubation at 37°C when cultured in
10 MH broth. Fig. 2 shows that the bacteria are doing well in the testing – without any addition of chemical, i.e., without calcium hypochlorite and sodium hydrogen carbonate. (Actually, in this test, no chemicals are used)

MIC microdilution assay

MIC microdilution assays were run in triplicate to assess the MIC values for Sodium
15 hypochlorite solution (pH 9) against one strain of ESBL and two strains of MRSA (Table 1). The tests show the functionality of the chlorine solution.

Table 1. Minimum inhibitory concentrations (MIC) for sodium hypochlorite solution against ESBL and 2 strains of MRSA.

Strain	Collection nr	MIC (mM)
ESBL	CCUG 59413	17.5
MRSA	CCUG 31966	4.4
	CCUG 35600	4.4

20 Conclusion

The calcium hypochlorite solution (0.5%, 70mM) pH 9, inhibited growth of the clinical isolates of ESBL, one strain, and MRSA, two strains. The MIC values of the Sodium hypochlorite solution against ESBL was 17.5 mM and against MRSA 4.4 mM (Table 1)

EXAMPLE 2 – ANTIBACTERIAL ACTIVITY

5 MMC

Aim

To evaluate the antibacterial killing activity of calcium on one strain of ESBL and two strains of MRSA in Minimum Microbicidal Concentration (MMC₉₉) assays.

Materials

- 10 Test solution comprise of equal volumes calcium 1 % (140 mM) and sodium hydrogen carbonate 2% (238 mM) with small amounts of salt, titan dioxide and silica. After mixing the test solution was filtered through a Chromacol nylon filter (0.2 µm) to be equivalent with the benchmark products Microdacyn and Prontosan in liquid forms.

Methods

- 15 Test organism – Preparation of bacterial inoculums (=working solution)
1. Streak plates were done on horse agar plates and characterized by gram-staining
 2. Isolated colonies were inoculated to 5 ml MH broth for over-night growth at 37°C.
 3. OD measurements were done using a spectrophotometer and over-night suspensions adjusted to correspond to OD 0.28 at 475 nm. This corresponds to 1.5 -3.0 X
20 10⁸ CFU/ml with most bacterial species.
 4. Then working solutions were done by diluting the suspension in MH broth to equal 1 to 2 x 10⁷ CFU/ml in order to give a final inoculum at 5 X 10⁵ CFU/ml in each well.
 5. This working solution was inoculated to each well within 15 minutes.
 6. Plate counts were done on the inoculums to confirm the inoculum size of the
25 working solution.

Test Specimen – Preparation

The calcium was prepared just before titration, by combining the antimicrobial agent (AM, 1% calcium hypochlorite) with (NaHCO₃ 2%, salt, titanium dioxide, silica). The prepared two-component solution was transferred to a sterile Eppendorf tube.

5 Procedure – MMC₉₉

The AM substance was titrated in a two-fold dilution series horizontally in sterile water in 96 well plates starting at 70 mM (0.5% calcium hypochlorite) of the AM solution and ending at 0.07 mM, see Fig. 3.

10 Directly thereafter, to each well 5 µl of respective bacterial suspension at $1-2 \times 10^7$ cells/ml was added and controls included Growth control = broth with bacteria, 5×10^4 CFU per well and sterility control = broth with AM substance, 70 mM, but no bacteria

The 96 well plates were then sealed with an adhesive plastic film and incubated for 2 hours at $36 \pm 1^\circ\text{C}$.

15 After incubation, 5 µl or about 2.5×10^3 CFU from each well was applied as drops in triplicate onto horse agar plates starting with the highest concentration of the AM substance on top row on first plate and ending with the lowest concentration on the second plate bottom row. There were two horse agar plates for each strain.

EVALUATION CRITERIA

20 Drops without any colonies equal the MMC₉₉ value indicating that the test solution has reduced $\geq 99\%$ of the bacteria. MMC value is minimal microbiocidal concentration. MMC₉₉ value means that 99% of the colonies are dead.

RESULTS

25 The calcium hypochlorite solution (0.5%, 70mM) had killed *K. pneumoniae* ESBL at 2.2 mM, MRSA CCUG 31966 at 2.2 mM for one replicate and the other replicate at 1.1 mM, and MRSA CCUG 35600 at 1.1 mM (Figure 3). The controls worked as expected.

The agar plates were photographed after 24 hours incubation at $36 \pm 1^\circ\text{C}$, see Fig. 3.

Conclusion

Sodium hypochlorite solution (70 mM, pH 9) killed the clinical isolates of *K. pneumoniae* ESBL, one strain, and MRSAs, two strains at the MMC_{99} values 2.2 and 1.1 mM, respectively.

- 5 The conclusion based on Example 1 and 2 is that the 0.5% chlorine solution tested is very effective in killing even very resistant bacteria in very low concentrations – lower than 5 mM.

EXAMPLE 3 – EFFICACY OF CHLORINE SOLUTION

- 10 AIM: The efficacy of different chlorine solutions on bacteria strains using the same concentrations of chlorine by MIC, MBC and kinetics

MATERIALS AND METHODS

- Different irrigants (majority of chlorine solutions; buffered calcium hypochlorite, chloramines and Chlorhexidine) were tested to determine the inhibition or the elimination of ten different strains of bacteria. Bacteria strains at set concentrations were compared using different agar dilution methods: A) the minimal inhibitory concentration (MIC) and B) the minimum bactericidal concentration (MBC)
- 15

Preparation of the irrigants

- Free chlorine was determined spectrophotometrically (Spectroquant®, chlorine test, 1.00599.0001, Merck Darmstadt, Germany) at 557 nm. The utilised standard curve gave a molar absorptivity, epsilon: ϵ value at 23 000 (mol/l⁻¹)⁻¹*cm⁻¹, further used to calculate the free chlorine in agents using the Beer-Lambert law. The chloramine-based products (e.g., Perisolv and Carisolv) hold an excess of free chlorine ~ 80 mM after subtracting the stoichiometric component A (135 $\pm$ 25 mmol/l) and component B (53 mmol/l amino acids) before mixing, respectively. All irrigants used were adjusted to the same concentration (70 mmol/l) and are presented in Table 2. Calcium hypochlorite, Chlorhexidine and Hydrogen peroxide were handled in accordance with the legalisation of EU from a safety and biocompatible perspective (the MDR directive in ISO 10993). Furthermore, preparation and handling did follow the local restriction in the laboratories, the material safety data sheet (MSDS) and/or instructions for use (IFU), when using commercial products.
- 20
- 25

Table 2. List of irrigants used for MIC (Minimal Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) tests. The final concentration of chemicals was adjusted to 70 mM

Agent	Active part	pH	Abbrevia-tion	Manufacturer
Carisolv [®] (gel) A+B	RNHCl ⁺	11.0	CAR	RLS Global AB, Gothenburg, Sweden
Perisolv [®] (gel) A+B	-RNHCl ⁺	11.0	PER	RLS Global AB, Gothenburg, Sweden
Perisolv [®] (liquid) A+B	-RNHCl ⁺	11.0	NOG	RLS Global AB, Gothenburg, Sweden
Perisolv [®] , component A (liquid), *	-Ocl ⁻	11.0	OCL	RLS Global AB, Gothenburg, Sweden
Amino acids of Perisolv [®] , comp. B (gel) **	-NH ₂	11.0	AAP	RLS Global AB, Gothenburg, Sweden
Buffered chlorine solution	-Ocl	9.6	CHL	Univ. Gothenburg, Sweden
Hydrogen Peroxide solution	-O ₂ ⁻	4.0	H ₂ O ₂	MERCK KgaA, Darmstadt, Germany
Chlorhexidine digluconate	-C ₅ H ₁₁ N ₅	7.0	CHX	MERCK KgaA, Darmstadt, Germany

5 Table 3. Denomination of bacteria specimens in the different MIC or MBC test used in the study. The CFU/ml in growing phase are represented in the columns to the right in the table.

Bacterial strains	Abbreviation	Agar diffusion & Time to kill	Micro dilution
Lactobacillus spp	LBc	5.2×10^7	1.5×10^9
Streptococcus mutans IB	SM-IB	8.8×10^7	2.5×10^9
Streptococcus mutans OMZ65	SM-65	5.2×10^7	2.5×10^9
Streptococcus sobrinus B13	SS-B13	3.4×10^7	1.5×10^9
Prevotella nigrescens ATCC33563	PN	3.6×10^7	5.0×10^8
Prevotella gingivalis	PG	2.1×10^7	1.8×10^8
Streptococcus salivarius ATCC8618	SS-8618	4.4×10^6	4.5×10^8
Prevotella intermedia ATCC25611	PI	2.0×10^7	1.8×10^8
Actinomyces actinomycetemcomitans	AA	2.8×10^7	1.1×10^8
Fusobacterium nucleatum ATCC10953	FN	1.1×10^7	5.0×10^8

METHODS MIC (see table 2)

METHODS – MBC

(A) - Macro dilution of the agents and time-to-kill

5 The antimicrobial agents were added into seven test tubes demonstrating single volumes of the agent (2.5, 5.0, 7.5, 10.0, 20.0, 50.0 and 100.0 μ l). The initial concentration of the five agents were adjusted and to controlled by the Spectroquant®, chlorine test, to ~ 70 mmol/l representing 0.5% w/w of the active substance in CHL, PER and CAR. Thus, CHX and H2O2 was performed within the same concentration (70 mmol/l). Aliquots of each bacterial suspension (1ml) was collected in their exponential growth phase checked by

10 OD500, (See Table 3) and further placed in the single test tubes. The different volumes of the agent were added to each tube with a Hamilton syringe (0-50 μ l). A droplet of the agent was placed on the neck of the tube until all seven volumes were added. At an exact

time, all the tubes were mixed with a vortex for 5 s (time 0 min at the start) and stopped after five and ten minutes of reaction, at which time the bacteria were sampled and inoculated on blood agar plates. The plates were incubated at 37°C for three days. The purity and numbers of the bacterial cells were confirmed under the microscope with plate counting on agar, where the symbols (-) and (+) represented no colonies (100% lethality) or one or more colonies respectively (See Fig. Table 4).

RESULTS – MBC

Time-to-kill – MBC

Macro-dilution of the five agents in aliquots of 1 ml bacterial suspension with known concentration (CFU in Table 3) showed the lowest instant killing concentrations for CHL at 0.18 mmol/l (PG and PN), followed by PER and CAR at 0.35 mmol/l (FN and Aa) and for CHX at 1.4 mmol/l (LB, Aa) after 5 min exposure. Summarising the total minimum volumes required to kill all the 10 specimens per agent (70 mmol/l) indicated an average killing volume at 37.5 µl for CHL, 305 µl PER, 340 µl CHX and 475 µl CAR, respectively (See Table 4). Furthermore, recalculation with no consideration taken of the bacteria specimens killed showed the following concentration per specimens; 0.26 mmol/l DAK, 2.14 mmol/l PER, 2.38 mmol/l CHX and 3.33 mmol/l for CAR. Consequently, taking the different molar mass into reconsideration for the substances gave a minimum lethality number at 0.2 mg/ml for CHL and 12 mg/ml for the CHX, respectively.

The killing effect were more pronounced after 10 minutes than after 5 minutes; however, the lethality pattern was similar no matter the exposure time. Comparing the average killing volume per agent (70 mmol/l) for the 10- and 5-min exposure revealed volumes at; 28.5 µl vs 37.5 µl

for CHL, 162.5 µl vs 305 µl for PER, 320 µl vs 340 µl for CHX and 342.5 µl vs 475 µl for CAR, that after transferring into molarity became 0.2 mmol/l CHL, 1.13 mmol/l, PER 2.2 mmol/l CHX and CAR 2.39 mmol/l CAR after 10 minutes of exposure.

The results are shown in Fig. 10, and they show that the CHL solution is the most efficient to kill bacteria within 5- 10 min and in very low concentrations.

METHODS – MIC

(A) - Microdilution on agar plates (A)

Microdilution determines the lowest concentration of an antimicrobial agent that inhibits the visible growth of a bacterial strain, after two-fold dilutions of the agent. The present technique corresponds with the Minimum Microbicidal Concentration MMC99 assay in the exposure time and lethality of the micro-organism, but subculturing on agar plates

5 involved the agent and was therefore deemed to belong to the MIC technique (MIC90).

The antibacterial agents were prepared from a stock solution (140 mmol/l, adjusted to 70 mmol/l (see preparation in Table 2) and further diluted into twelve test tubes in a two-fold manner with a total volume of 2 ml per tube.

10 The bacterial suspension (Mueller Hinton Broth, supplemented with 20.25 mg Ca²⁺ and 10-12.5 mg Mg²⁺ per litre) adjusted to 0.5 at OD550 was added to 96-well microtiter plates (ThermoFisher Scientific, UK) (100µl/well) in duplicates. Ten specimens are described in Table 2. The two-fold dilutions of the agents (CAR, PER, CHL, CHX, H₂O₂) were then added to the 96-well microtiter plate in aliquots of 100 µl, starting from column 1 at 140 mmol/l) to column 12 at 0.06 mmol/l), that after the addition of the bacteria
15 suspension resulted in 70 mmol/l) in column 1 and 0.03 mmol/l) in column 12 and a final working solution of bacteria per well are presented in Table 2. The 96 well plates were then sealed with an adhesive plastic film and incubated for two hours at 36 ± 1°C. After incubation, 10 µl of each solution (reagent/bacteria) were plated on agar plates (blood agar, Mitis Salivarius and Brucella) suitable for each specimen starting with the highest
20 concentration of the agent on the left side in rows of duplicates. As a result, three bacterial specimens were fitted on each agar plate with the net result of four agar plates per agent including controls: positive control (broth with bacteria, 1-2 x 10⁷ CFU per well) and negative control (broth with the agent, 70 mmol/l), but no bacteria). For the construction of the agar plates, see the results section. Agar plates were finally incubated
25 for 24 hours at 37 °C. The plates were photographed the day after.

RESULTS – MIC

Results – (A) MIC by serial dilution on agar plates

From a two-fold serial dilution, all agents inoculated in microbial suspensions with a working solution at 1.5 – 3 x 10⁵ CFU/ml showed an antimicrobial effect that was
30 observed on agar plates.

Starting from the highest 70 mM concentration of the agent on the left side of the agar plates to the lowest 0.04 mM on the right side, the SM-IB and SM-65 colonies seemed to resist the antimicrobial treatment of CHX at 0.03 mmol/l and were observed as dense colonies, resembling the positive control (plate B, Fig. 3). Higher concentrations were all lethal to the bacteria cells and resulted as empty, cauterized agar spots. The MMC99 value of CHL was observed at 2.4 mmol/l, 17.5 mmol/l for the chloramines (CAR and PER) and 35 mmol/l for H₂O₂ on corresponding bacteria specimen (plate A, Fig. 4). For the remaining specimens (LBc, SM-B13, Ss, FN, PI, PN, PG, Aa), the MMC99 was nearly the same for CHX at 0.04 mmol/l. However, for the other agents such as CHL, the MMC99 varied between 2.2 and 4.4 mmol/l and regarding the chloramines (CAR and PER), the MMC99 values varied between 8.8 mmol/l (mainly, PI, PN and PG) and 17.5 mmol/l, depending on the species. See Fig. 4.

EXAMPLE 4 – TIME-KILL ANALYSIS ON BIOFILMS –

Background

Time-kill analyses on biofilms were proposed to be done quantitatively with plate count and qualitatively with microscopy by staining live and dead cells with the fluorophores Syto 9 and Propidium Iodide (PI) included in the Live/Dead staining kit.

In order to be sure that the antimicrobial activity analyzed is corresponding the certain time points of choice, the antimicrobial activity must be neutralized. Therefore, before time kill analyses were performed, validation of neutralization buffers was done to ascertain that the neutralization buffers could neutralize the activity of the different antimicrobial substances and at the same time not being toxic to the specific strains tested. The neutralization buffers were selected based on literature survey.

Time-kill analysis is in general done on bacterial cells in planktonic phase. It is also described as a standard, ASTM E2315 – 03 Assessment of Antimicrobial Activity Using a Time-Kill Procedure. Although, bacterial cells as biofilm are clinically relevant to study. Therefore, the present study has evaluated time-kill analysis on biofilm. The method of choice was recently published by Barber KE, Werth BJ, McRoberts JP, Rybaka MJ. – A novel approach utilizing biofilm time-kill curves to assess the bactericidal activity of ceftaroline combinations against biofilm-producing methicillin-resistant staphylococcus aureus. Antimicrob Agents Chemother. 2014;58(5):2989–92.

Aim

To evaluate kinetically (5-, 10-, 30-, and 60-min time-points) the activity on *Pseudomonas aeruginosa* and methicillin resistant *Staphylococcus aureus* (MRSA) of hypochlorite solution and the benchmark products Prontosan and Microdacyn.

5 Strains.

Pseudomonas aeruginosa CCUG 56489, ATCC 15692 (PAO1)

Methicillin resistant *Staphylococcus aureus* (MRSA) CCUG 35600

10 The neutralization buffers were not toxic when tested against *P. aeruginosa* and MRSA and they could neutralize the activity of the antimicrobial substances (Table 4). Thus, the requirement of more than 50% bacterial survivors of the bacterial inoculum was met and the neutralizers were assessed as suitable.

Table 4. Toxicity of the stop solutions on the different specimens

Test	Sample	CFU/ml	% Survive	Pass criteria
	<i>P. aeruginosa</i> Bacterial inoculum	1,6E+03		
Toxicity	<i>P. aeruginosa</i> + N1	2,0E+03	>100	yes
	<i>P. aeruginosa</i> + N2	2,0E+03	>100	yes
Neutralization efficiency	<i>P. aeruginosa</i> + N1 + Dakin, gel	2,4E+03	>100	yes
	<i>P. aeruginosa</i> + N1 + Microdacyn	2,0E+03	>100	yes
	<i>P. aeruginosa</i> + N2 + Prontosan	2,0E+03	>100	yes

	S. aureus Bacterial inoculum	1,3E+03		
Toxicity	S. aureus+ N1	1,5E+03	>100	yes
	S. aureus+ N2	1,6E+03	>100	yes
Neutralization efficiency	S. aureus+ N1 + CHL	1,6E+03	>100	yes
	S. aureus+ N1 + Microdacyn	1,5E+03	>100	yes
	S. aureus+ N2 + Prontosan	1,7E+03	>100	yes

Time to kill of biofilm – plate count.

Bacterial inoculums

The bacterial concentration in the inoculums were for *P. aeruginosa* log 5.91 CFU/ml and
5 for *S. aureus* log 6.80 CFU/ml (Table 5).

Table 5. Viable counts of the bacterial inoculums presented as mean log CFU/ml and STDEV calculated from duplicate plates.

Strain	Log CFU/ml	Stdev
<i>P. aeruginosa</i>	5,91	0,13
<i>S. aureus</i>	6,80	0,01

Table 6. Viable counts of PAO1 biofilm, presented as mean log CFU/ml and STDEV
10 calculated from three replicates and their duplicate plates.

PAO1	**Sodium hypochlorite		Microdacyn		Prontosan		Pos ctrl*	
	Log cfu/ml	Stdev	Log cfu/ml	Stdev	Log cfu/ml	Stdev	Log cfu/ml	Stdev
5 min	2,61	0,58	7,73	0,03	7,08	1,61	7,78	0,04
10 min	2,27	0,51	7,79	0,05	4,74	2,59		
30 min	0,33	0,58	7,64	0,04	5,26	1,17		
60 min	0,00	0,00	7,79	0,08	3,37	2,31		

*The positive control, treated with PBS, was assessed at 60 min analysis, and assumed to be equal at all time points of analysis.

** The hypochlorite solution was filtered through a Chromacol nylon filter (0.2 µm) to equal a liquid to be compare with the benchmark products Microdacyn and Prontosan later

5 Table 7. Viable counts of MRSA biofilm, presented as mean log CFU/ml and STDEV calculated from three replicates and their duplicate plates.

MRSA	Sodium hypochlorite		Microdacyn		Prontosan		Pos ctrl*	
	Log cfu/ml	Stdev	Log cfu/ml	Stdev	Log cfu/ml	Stdev	Log cfu/ml	Stdev
5 min	0,00	0,00	7,44	0,11	6,89	0,43	7,49	0,18
10 min	1,17**	2,03	7,43	0,18	7,03	0,26		
30 min	0,00	0,00	7,43	0,11	2,87	3,24		
60 min	0,00	0,00	7,55	0,05	1,34	1,75		

*The positive control, treated with PBS, was assessed at 60 min analysis, and assumed to be equal at all time points of analysis.

**Two replicates had log 0.00 CFU/ml

Table 8 CFU over time on a 48 hr biofilm treated with antimicrobial products

Time	Sodium hypochlorite		Microdacyn		Prontosan	
	PAO1	MRSA	PAO1	MRSA	PAO1	MRSA
5 min	5,18	7,78	0,06	0,35	0,70	0,90
10 min	5,51	6,61	0,00	0,36	3,04	0,76
30 min	7,45	7,78	0,15	0,36	2,53	4,91
60 min	7,78	7,78	0,00	0,24	4,42	6,44

Time kill analysis was evaluated also qualitatively with wide-field fluorescence microscopy. A 48 hour biofilm was then treated with respective antimicrobial product for 5, 10, 30, and 60 minutes, i.e., corresponding timepoints as when analyzed with plate count. At each time point of analysis, the antimicrobial activity was stopped by neutralizing it with neutralization buffer. The treatment and neutralization buffer were then discarded, and the biofilm rinsed before the Live/Dead stain was added and the samples placed under the microscope. Live cells stain green with Syto 9 and dead cells red with PI. Syto 9 penetrates the membranes of live cells while PI penetrates cells with damaged membranes.

The hypochlorite solution was seen to have killed PAO1 cells after 30 minutes treatment and Prontosan after 60 minutes treatment while the majority of the cells remained green at all timepoints of analysis when treated with Microdacyn except for after 60 minutes treatment when part of the biofilm stained red (not Shown). In addition,

Sodium hypochlorite solution was seen to have killed the MRSA cells in the biofilm after 30 minutes treatment while the majority of the cells remained green at all timepoints of analysis when treated with Microdacyn (Mi) and Prontosan (Pr) (Figure 5).

Conclusion

The validation study showed that the neutralization buffers were not toxic but efficient in neutralizing the activities of hypochlorite solution, Microdacyn, and Prontosan when tested

against *P. aeruginosa* (ATCC 15692 (PAO1)) and Methicillin resistant *S. aureus* (CCUG 35600).

Sodium hypochlorite_killed biofilm cells of PAO1 and MRSA already after 5 minutes shown with plate count analysis and after 30 minutes when analyzed with Live/Dead stain.

Microdacyn treated PAO1 and MRSA biofilm cells were equal in number to the control that was treated with placebo or PBS. The lack of antibiofilm activity was confirmed with Live/Dead staining. Although, some antibiofilm activity could be detected after 60 minutes treatment of PAO1.

- 10 Prontosan showed antibiofilm activity, however not immediate, when analyzed with plate count. The Live/Dead staining of MRSA biofilm showed no antibiofilm activity but for PAO1 biofilm after 60 minutes treatment.

EXAMPLE 5

NETS reaction (immune response)

- 15 Formation of NETS (i.e., formation of neutrophil extra cellular traps consisting of neutrophil DNA, intracellular proteins, and histones) is induced by bacterial activity, their products, cytokines and/or chemicals (Guimaraes-Costa AB et al, 2012. DOI no: 10.1155/2012/929743). Activity of NETs reaction could be performed in vitro is by PMA (Phorbol myristate acetate). Formation of NETs can be measured by Sytox green assay
- 20 by staining the extra cellular DNA of activated neutrophils. Initiated NETs by PMA increase in Sytox green fluorescence is visualized after 2-3 hr.

- Neutrophiles (5×10^4 cells/well) in RPMI solution (without phenol red, 80 μ l) with Sytox green DNA stain (1.25 μ M; molecular probes in PMN, 100 μ l) and PMA (stimuli 20 μ l) were added to a 96 well tissue plate and incubated at 37°C, CO₂ for 15 min. Sytox green
- 25 fluorescence was measured at every 15 min for 3hr at ex 485 / em 535 nm in a Clariostar plate reader (BMG Labtech)

- Cells (6x) were added with or without PMA (1mM), with Triton (10%) for fully plasma membrane destruction, agents with initial concentration ranging from 100mM with dilution 100x, 50x 20x and finally blanks representing both RPMI (culture media, Invitrogen) and
- 30 PMN (includes the stain)

Kinetics was measured for neutrophils treated with different agent overtime (min) vs the absorbance of Sytox green (AU). See Fig. 6.

Sixteen 96 plates (4 plates x 4) were tested in wide range of concentrations. Amino acids (Leu, Lys, Glu) were tested in concentrations 0.053, 0.53, 1.06 and 5.3 mM, respectively.

5 Sodium hypochlorite in 0.18, 3.2, 7.5, 18 and 180 mM and chloramines 0.0265, 0.09, 0.265, 0.53, 0.9, 1.325 and 9 mM. The best dose response concentration activating the neutrophils are shown in Fig. 6. In addition, response values. Obtained at 160 min were normalized to positive control (neutrophil + PMA), 100% and difference between groups were checked using 1-way ANOVA with Turkey's test. The statistics was performed for
10 each agent at all concentrations tested. A mean of all response values was the plotted enable a statistical comparison among the agents with the most effective concentration activating the neutrophils (See Fig. 6).

From kinetic testing the best concentration activating the neutrophils was the hypochlorite solution (7mM+PMA) followed by sodium borohydride (75mM -PMA), Chloramines
15 (1.325mM + PMA) in comparison with neutrophils + PMA, at 160 min. Neutrophils without PMA indicated the lowest activation, thus, absorbance at 160 min.

Fig. 6 show amino acids at 1.06 mM with PMA to be statically significant compared with the positive control (cells + PMA). Likewise, the sodium hypochlorite at 7.5 mM with PMA and chloramines at 1.325 mM were all statically significant.

20 In all, hypochlorite solution in concentrations at 7.5 mM seems to be the most efficient agent for activating the NETS reaction in white blood cells. However, chloramines showed the largest cytotoxicity around 3.5 mM whereas sodium hypochlorite was still not cytotoxic against neutrophils at 8mM whereas sodium borohydride did not indicate any cytotoxicity See the LDH assay (Figure 8)

25 NETs reaction reflects the healing process

Background

The ROS reaction involves both the NETS and MPO reaction which is both an extracellular event and/or phagocytosis with the aim to killing of microbes. Example 1-3 defines the antimicrobial effect of hypochlorous acid. Thus, the ROS including NETs
30 reaction are activated. Furthermore, nerve signals are linked to an active immune

response including the activity of the ROS reaction, why measuring the levels of immune markers might be an indication of the healing process or time to heal.

Hypochlorite and hypochlorous acid are the active substances that neutrophilic granulocytes use to eradicate microorganisms and necrotic tissue. Furthermore, to

- 5 introduce the proliferations phase, where production of IL-1, IL-6, IL-8, IL-10 and $\text{TNF}\alpha$ is a result of the proliferations phase to start.

When the wound/ulcer is clean the proliferations phase should start. The cutaneous nervous system plays a crucial role in wound healing by starting the proliferations phase. The production and secretion of IL-1, IL-6, IL-8, IL-10 and $\text{TNF}\alpha$ are known as mediator
10 of proliferation phase.

Both in diabetic foot ulcer and ulcer in persons with neuropathy for other causes show impaired wound healing. If the production IL-1, IL-6, IL-8, IL-10 and $\text{TNF}\alpha$ can be increased even with existing neuropathy the wound healing will improve.

- From our biopsy data combined with deduced healing by skin area (mm^2) before and
15 after treatment levels of IL-1L (8-4 weeks), IL8 (0-4 weeks) were significant, the VEGF levels was not significant, but seemed work in parallel with the healing process. See Fig. 7 and 9.

EXAMPLE 6

Cytotoxicity

- 20 From an earlier clinical trial and case reports sodium hypochlorite and chloramines (1%, 140mM active chlorine resp. 0.5%, 70mM active chlorine) have been tested on ulcers without any adverse side effects (Bergqvist et al, 2016). Below results represent solely cell lines in suspension. Then cytotoxicity appeared already at 4mM for chloramines and 8mM for sodium hypochlorite in solutions

- 25 Background of the assay

Information adopted from the instructions for use of the LDH assay kit.

The Thermo ScientificTM PierceTM LDH Cytotoxicity Assay Kit provides a simple, reliable colorimetric method for quantifying cellular cytotoxicity assays. The kit can be used with

different cell types for measuring cytotoxicity mediated by chemical compounds as well as assaying cell-mediated cytotoxicity.

Lactate dehydrogenase (LDH) is a cytosolic enzyme present in many different cell types. Plasma membrane damage releases LDH into the cell culture media. Extracellular LDH in the media can be quantified by a coupled enzymatic reaction in which LDH catalyzes the conversion of lactate to pyruvate via NAD^+ reduction to NADH. Diaphorase then uses NADH to reduce a tetrazolium salt (INT) to a red formazan product that can be measured at 490nm. The level of formazan formation is directly proportional to the amount of LDH released into the medium, which is indicative of cytotoxicity.

- 10 The cultured cell line i.e., white blood cells (10^5 CFU) prepared from a standard protocol at the department of Rheumatology, Sahlgrenska university hospital, Gothenburg Sweden were kept at 4°C no more than 1 hr. after the final centrifugation.

- For appropriate results the optimum number of cells per well (96-well plate) was determined to ensure LDH signal is within a linear range. Thus, prior to the LDH assay cells (10^5 , log 5) were transferred by multichannel pipettes to a 96-well plate supplemented 1640 medium (Sigma, St Louis MO) and 10% fetal bovine serum in serial dilution. Lysis buffer were added release LDH or the level of formazan that is directly proportional to LDH measured spectrophotometrically at 490 nm. The cell line was tested towards a positive control in 1 % BSA (bovine serum albumin) in PBS buffer (phosphate buffer saline). (Pierce LDH Cytotoxicity Assay kit. Thermo, Scientific, Rockford, IL, US)

In addition to ensure the viability of the cells a separate set of wells within the same 96-well plate, was measured using Thermo Scientific alamar Blue Cell Viability Assay Reagent (Product No. 88951-2). (Thermo, Scientific, Rockford, IL, US)

- For the different agents mediated cytotoxicity the optimal number of cells ($100\ \mu\text{l}$; 10^4 cell) in RPMI 1640 medium and 10% were plated in triplicates in a 96 well culture plate and incubated overnight at 37°C .

Next spontaneous LDH in water, maximal LDH with triton and the effect of the agent were all tested in triplicates.

- The different treatments and controls were transferred to a new plate by aliquots of $50\ \mu\text{l}$ together with $50\ \mu\text{l}$ reaction mixtures incubated 30 minutes. Yet, a stop solution was

added before measuring the absorbance at 490 nm. The cytotoxicity is measured in (%) from agent treated LDH activity minus spontaneous LDH activity divided by maximum LDH activity minus Spontaneous LDH activity. About 45% and higher is considered cytotoxic.

- 5 To conclude chloramines was the most cytotoxic compound at 45% at 4mM followed by hypochlorite solution at 25% at 8mM. (Figure 9) Sodium borohydride (reducing agent) was used as a control to the oxidative agents (Chloramines and hypochlorite) seemed to have a cytotoxic effect at 10% whereas the control (solely cells) showed a cytotoxicity at 5 % (Figure 9).

10 **EXAMPLE 7 – Some clinical examples of wound healing with existing neuropathy:**

A 47 year old man with diabetes since he was a child and plantar ulcer for seven years. He had diabetic neuropathy, no sense of vibration, touch, pain or pressure in his feet the last ten years. His diabetes was out of order HbA1c 87 (long term blood sugar level which should be under 46), he smoked and did not use his offloading aid. All the above details
15 make healing very difficult. The size of the ulcer was 36 x 22 mm. His ulcer was cleaned with hypochlorite ones a week and surprisingly the ulcer healed within 9 weeks.

A 60 year old man paralyzed from waist down after a car accident in his twenties. He was in a wheelchair and had a deep pressure ulcer on his buttock since months. Daily cleaning with different solution and offloading had not improved the ulcer it just went
20 worse. His ulcer was 9 x 12 cm and 6 cm deep. The ulcer was cleaned with hypochlorite twice a week and healed in 15 weeks.

A 64 year old man with diabetes since his teenage, diabetic neuropathy and a plantar ulcer for five years. The ulcer size was 18 x 15 mm and 5 mm deep. The ulcer was cleaned once a week with hypochlorite and the ulcer healed in 7 weeks.

- 25 No dressing has proved to enhance the healing.

It is the local immune system that heals the wound. If the wound/ulcer is properly cleaned from microorganisms, biofilm and necrosis (dead tissue) the proliferations phase can start.

- 30 The dressings are to absorb secretion (plasma that contains degrading enzyme and bacteria) and to protect the wound from further damage.

Bacteria need three things to survive and duplicate: Food, warmth and moisture.

Most new dressings are occlusive or semi occlusive and the moisture will be left in the wound. With occlusive or semi occlusive dressing the wound/ulcer become a bacterial culture.

- 5 All cases above and earlier written cases have had a dry absorbent airy dressing with a non-sticking surface to protect the wound/ulcer.

- All cases have been treated with a dissolved calcium hypochlorite. The hypochlorite solution only needs 20 seconds to break biofilm, kill bacteria and soften necrosis. The hypochlorite solution is left on while debriding is performed which enable disinfection of
10 newly exposed healthy tissue. The hypochlorite solution is cleaned away with tap water to leave the wound/ulcer free to heal.

- The dry component (anti-septic composition) in the device also denoted sponge is meant to be dissolved by adding of water (any water will do because of the chlorite instant bactericidal effect) to a gel or fluid also denoted anti-septic liquid, which is then applied to
15 the wound surface via one or more of the bottom surfaces, first end surface, first side surface, and second side surface of the device/sponge. The device/sponge is left on the wound for a suitable time, such as 20 – 30 seconds and can then be used for debridement. The top surface of the sponge has a rougher surface (abrasive surface) which can be used for tougher debridement. Hypochlorite is basic. The device, such as
20 the second part and/or the first layer optionally comprises an acid to neutralize the anti-septic composition, such as hypochlorite of the anti-septic composition. The skin has a weak acid pH so to further enhance the epithelialization of the wound an acidic pH will be performed.

EXAMPLE 8

- 25 Gel formulations

EVALUATION CRITERIA

- Standardized droplets of different gels shown in table 9 by weight (μg) and volume (μl) before and after mixing with equal volumes CaOCl_2 (1 %). Volumes at 20(μl) were added stoichiometrically 1:1 20(μl) and estimated at 0 min, 10, 30 and 60 min by the droplets
30 shape, height, diameter and travelled distance (gravity). Drops with conserved shaped

were considered unaffected by the chlorine solution and droplets with short travel distance in a vertical plane were considered not affected by gravity and having a better consistence.

RESULTS

5 Fig. 11 shows different gel formulations.

Fig. 12 shows six different gel applications mixed and further analyzed regarding gravity /travel distance and droplet heights. The table shows gravity and evaporation tests (0-60 min and 24 hr) of the different gel formulations before and after mixing with the chlorine agent.

The two most promising gels (DAK_4 and DAK_6) were seen as least affected by the chlorine agent after mixing, which means the diameter (mm) of the droplets were similar to the initial value of gel droplet (mm). In addition, showed the lowest travel distance (cm/min) during 60 min after mixing and hold a lower density (g/L). Droplets height and evaporation seems to be a consequence of the additives, such as TiO₂ and SiO₂, especially DAK_3 that consists of both TiO₂ and SiO₂. To be noted the DAK_6 has a much lower pH (6.7) in comparison with the other at 9 and 11.5 (ref, CAR and PER).

Fig. 13A shows gravity testing (std droplets 0.02g) on a vertical plane measured by travel distance in cm during 60 min and Fig. 13B shows droplets height (mm), during 60 min.

20 RESULTS

Gravity seemed proportional to the height of the droplet, meaning the gel with the largest height also travelled the longest distance under shortest time (8 cm, 5 mi). In addition, also represented one of the heavier gels. Whereas the lower density gels merely travel at all (0 cm, 60 min). Furthermore, the height of the droplet may indicate the degree of viscosity or bonding's holding the gel together. Gels with best performance is gel 1, 4 and 6.

EXAMPLE 9

Clinical cases with hypochlorite in gel form

In one more example the hypochlorite solution, the antimicrobial agent (AM, 1% calcium hypochlorite) with (NaHCO₃ 2%, salt, titanium dioxide, silica) was prepared with a gel carrier to facilitate the treatment of wound/ulcer.

45 years old woman with rheumatoid arthritis, treated with methotrexate and having
5 several round ulcers, size 3-4 cm in diameter and 1-2 cm deep, on both lower legs for seven weeks. The ulcers had occurred due to inflammation in the arteries leading to ischemia.

The rheumatologist and the dermatologist had prescribed cleansing of the ulcers with Chlorhexidine.

10 On the left lower leg the ulcers were cleaned with Chlorhexidine twice a week and on the right lower leg the ulcers were cleaned with Chlorine gel 0,5% and rubbed with polyurethan sponge twice a week.

The ulcers on left lower leg did not improve and the ulcers on the right lower leg healed completely within six weeks. After six weeks the treatment of the ulcers on left lower leg
15 was changed to Chlorine gel 0,5% and polyurethan sponge and the ulcers healed in five weeks. No photographs.

A 70-years old man, earlier operated because of varices otherwise healthy. Two years earlier he had run marathon. In April 2019 he had laceration on the left lower leg when cutting a rosebush. The lacerations were cleaned two to three times a week according to
20 standard treatment but it didn't heal. The lacerations developed into a deep ulceration with black necrosis involving 3/4 of the lower leg by August. On the 15th of August the new treatment started by cleaning the ulcer with 0,5% chlorine gel. The ulcer was cleaned and debrided twice a week with the chlorine gel. After three weeks all black and brown necrosis were gone but yellow necrosis remained. Ig. 14A shows the ulcer when first
25 presented at the out clinic. Fig. 14B shows ulcer after three weeks still yellow slough and yellow necrosis.

After another two weeks most of the yellow necrosis was gone and new red healthy tissue could be seen.

Fig. 14C shows the status at five weeks and Fig. 14D at seven weeks. The same treatment was used and the dressing being non-active absorbent non occlusive and non-sticking. After eight weeks a compression bandage 20 mmHg was introduced.

Fig. 14E shows the status at nine weeks and Fig. 14F shows the status at eleventh weeks. Complete healing (Fig. 14G) occurred week 12 and it has remained healed ever since.

A 78-years old woman with diabetes and a foot ulcer. She had had a pressure ulcer on her heel for ten months. In spite of intense treatment like correcting her blood sugar levels with insulin, correcting her blood circulation by endovascular revascularization, intense cleaning and debridement of the ulcer, two different antibiotics, first intravenous and later orally and high doses of morphine, there was no improvement.

Specialists in diabetes, surgery, dermatology, infection diseases and orthopedics all concluded that she had to have her lower leg amputated, which she didn't want.

When she first visited the out clinic, she was sitting in a wheelchair, somnolent from all morphine she had to take because of severe pain, not being able to walk. She had long term treatment of two different antibiotics.

After four weeks of cleaning and debridement the ulcer with 0,5% Chlorine gel all black necrosis were gone. The ulcer over the Achilles tendon healed within eight weeks. The plantar ulcer slowly turned to more red healthy tissue. Within 16 weeks only skin covering was needed before the ulcer was totally healed. One orthopedic surgent misjudged the subdermal tissue of being slough. He cut a hole all the way into the bone discovering that the bone was hard without osteit. The hole had a diameter of 3,5 cm and depth of 4 cm. It took another 16 weeks before the hole healed. Diabetic foot ulcer often reoccurs but this ulcer has remained healed for three years. She is now able to walk and is on no other medication than the diabetes one.

If a person with diabetes, foot ulcer and age over 75 years, has to be amputated the mortality rate is 50%.

Fig. 15A shows the ulcer when presented at the outclinic. The redness around the ulcer is 5-8 cm.

Fig. 15B shows the ulcer after 8 weeks. All black necrosis was gone and the ulcer over the Achilles tendon was completely healed

Fig. 15C shows the ulcer at 10 weeks where no slough or yellow necrosis remained.

Fig. 15D shows the ulcer at 12 weeks where the subdermal tissue is beginning to develop

- 5 Fig. 15E-15F: The orthopedic surgent misjudged the subdermal tissue of being slough. He cut a hole all the way into the bone discovering that the bone was hard without osteit. The hole had a diameter of 3,5 cm and depth of 4 cm. It was hard to get the carved hole to heal.

- 10 Fig. 15G shows the ulcer at 16 weeks – without the hole the ulcer would have been healed within 17 weeks.

Fig. 15H shows the ulcer healed at week 28 and has remained healed.

- 15 A 87-years old man who spent ten hours travelling to Bosnia. He ended up with a venous thrombosis from foot to groin in the left leg. He was taken into hospital and treated with anticoagulants. While in hospital he developed an ulcer on the left lower leg due to pressure and venous insufficiency. The leg ulcer was cleaned according to standard care. When returning to Sweden he had black necrosis on the back of his left lower leg and several ulcers on the front with black necrosis. The patient was offered amputation of the left lower leg, which the patient rejected.

- 20 He turned to the out clinic and was treated, cleaned and debrided twice a week with 0,5% Chlorine gel, absorbent, nonsticking, nonocclusive dressing and within 17 weeks he was completely healed and there was no need for compression. No photo was taken when most of the leg was black for ethical reasons as it was believed it could not be cured.

Fig. 16A: Four weeks yellow necrosis and yellow slough. All black necrosis gone.

- 25 Fig. 16B: After 5 weeks of treatment with 0,5% Chlorine gel more red tissue could be seen.

Fig. 16C: At 7 weeks of treatment new skin was developing from the sides of the ulcer and from skin islands in the middle of the ulcer.

Fig. 16D shows ulcer after 9 weeks, Fig. 16E after 11 weeks, Fig. 16F after 13 weeks, and Fig. 16G after 16 weeks.

A 60-years old woman with three different rheumatic diseases and immune modifying medication with impaired local immune system. She had ulcers on her feet for 23 years.

5 She had been treated with standard methods; the ulcers sometimes reduced in size at times but never healed. She was treated, cleaned and debrided with 0,5% Chlorine gel. After three months the ulcers had reduced significantly and her pain was gone. She was now able to walk for an hour for the first time in 20 years. After another four months of treatment with 0,5% Chlorine gel she was completely healed. No photographs

10 A 70-years old man who has had diabetes for four years. He had a motorcycle accident at the age of 18. The accident gave him an ulcer over the Achilles tendon that never healed in spite of being treated with the best methods offered each year. The last treatment included transplantation of skin over the Achilles tendon. The hole skin transplant died, the black necrosis had to be debrided, the infection treated and the healing of the ulcer
15 induced. The treatment with 0,5% Chlorine gel was done only one time a week why the healing took longer time than for other persons. After a year of treatment with 0,5% Chlorine gel the ulcer was completely healed. He was amazed by the fact that it didn't heal when he was young and healthy but did heal when he was old and had diabetes.

0,5% Chlorine gel is antimicrobial within seconds, breaks biofilm and softens necrosis,
20 which loosens its connection to healthy tissue. This means that every ulcer can easily be properly debrided.

Fig. 17A shows ulcer at the first appointment, where most of the black necrosis could be removed.

Week 11 all black and yellow necrosis gone and new red healthy tissue.

25 Fig. 17C Week 24 the ulcer is slowly reducing in size and get skin covered

Fig. 17D After ten months of treatment at the out clinic he preferred to handle the treatment himself. He reported that the ulcer was completely healed after nearly a year and it has remained healed.

Fig. 18A illustrates a top view of an example device according to the present disclosure.

30 The device 2A is a device for one or more of debriding, rubbing, and cleaning a wound, a

sore or an ulcer, the device 2A comprising two or more parts including a first part 4 and a second part 6, a top or first surface 8, and a bottom or second surface (not shown in Fig. 1 but elsewhere indicated with reference number 10). The first part 4 has a first length L₁ optionally of 30 mm, and the second part 6 has a second length L₂ optionally of 70 mm, wherein the first part 4 and the second part 6 are separated by a primary membrane 12, such as a water-impermeable membrane or layer. The first part 4 comprises an anti-septic composition 26 optionally in form of a tablet, e.g., embedded in foam material and/or arranged in a first cavity 27, see Fig. 18B of a foam material and arranged at a distance from the second surface, e.g. within 15 mm, such as within 10 mm, from the second surface 10. Thereby the risk of the anti-septic composition not being dissolved is reduce. The anti-septic composition 26 comprises one or more agents, such as one or more anti-septic agents including active chlorine agent 26A, one or more anti-microbial agents and/or one or more detaching agents. The anti-septic composition 26 comprises a pH adjusting agent 26B. The first part 4 is designed to absorb liquid, such as water e.g. with a first volume of in the range from 5 ml to 50 ml, whereby the anti-septic composition 26, such as the anti-septic agent, is dissolved to form an anti-septic liquid that can be squeezed out of the first part for application to the wound, sore, or ulcer. The second part 6 is designed to absorb liquid, such as water e.g., with a second volume of in the range from 10 ml to 40 ml, and thereby to function as a liquid-reservoir, such as a water-reservoir. The top surface 8 is abrasive, e.g., formed by a first layer 14 of abrasive material, and the bottom surface 10 is water permeable. In other words, the device 2A optionally comprises a first layer 14 of abrasive material.

The device 2A may comprise a second layer (not shown in Fig. 18A but elsewhere indicated with reference number 16) of optionally water permeable material. Optionally, the device 2A has a first end 18 forming a first end surface, and a second end 20 forming a second end surface optionally with a length L of 100 mm. Optionally, the device 2A has a width W of 50 mm with a first side 22 forming a first side surface, and a second side 24 forming a second side surface. The device 2A is box-shaped optionally with rounded corner(s) and/or edge(s).

The device 2A, such as the first part 4 and/or the second part 6, comprises a water-permeable material that is compatible with active chlorine agents, such as one or more of polyester, cellulose, silicone, polysiloxane or a polyurethan. The device 2A, such as the first part 4 and/or the second part 6, optionally comprises a non-woven material.

The device 2A, such as the first part 4 and/or the second part 6, optionally comprises a porous material, such as a foam material or a non-woven material. For example, the device 2A, such as the first part 4 and/or the second part 6, comprises or is made of one or more of a silicone foam, a polysiloxane foam, and a polyurethane foam.

- 5 Fig. 18B is a first side view of device 2A. The device 2A has a height H optionally of 20 mm and comprises a first membrane 28 optionally including a first region 28A between the first layer 14 and the first part 4. The first membrane 28 may be a water-impermeable membrane separating the first layer 14 and the first part 4. In other words, the first
- 10 the first part 4 to the first layer 14, thereby ensuring that the first layer 14 used for rubbing and/or rinsing during use is not contaminated with the anti-septic composition/liquid. The anti-septic composition 26 is arranged as a tablet or powder in a first cavity 27 in the first part 4, wherein the first membrane 28 seals the first cavity 27. The tablet anti-septic composition 26 is arranged near the second surface 10, such as within 10 mm from the
- 15 second surface 10. In other words, a bottom surface of the first cavity 27 is within 10 mm, such as within 7 mm and even within 4 mm, from the bottom surface 10. In one or more examples including device 2, 2A, the second layer 16 may be water-impermeable. Thereby water or liquid may only be soaked into the first part 4 and the second part 6 via one or more of the first end 18, first side 22, and second side 24. In one or more
- 20 examples, the second layer 16 (if present) may cover the first cavity 27. In other words, the first cavity 27 may in one or more examples be formed as a recess in the second surface 4B or a through-going bore in the first part 4. In one or more examples including device 2, 2A, the first side 22 and/or the second side 24 or at least parts thereof may be water-impermeable, e.g. to reduce risk of the second part 6 being contaminated with the anti-septic liquid. A water-impermeable second layer 16 may contribute to reduce risk of
- 25 the second part 6 being contaminated with the anti-septic liquid.

It is noted that features described in relation to Fig. 1A and 1B may also apply to Fig. 18A, and 18B.

- 30 All cases have been treated with a gel containing hypochlorite. The gel stays on the surface of the wound/ulcer at least a minute and also goes down in holes, gaps and undermining. The hypochlorite gel only needs 20 seconds to break biofilm, kill bacteria and soften necrosis. The gel is left on while debridement is performed which enable

disinfection of newly exposed healthy tissue. The gel is cleaned away with tap water to leave the wound/ulcer free to heal.

This shows that treatment with hypochlorite can heal all different types of wounds/ulcers, venous ulcers, pressure ulcers, ischemic ulcers, autoimmune ulcers, neuropathic ulcers.

- 5 Also disclosed are devices, methods, and kits according to any of the following items

Item 1. A device for debriding and/or cleaning a wound, a sore or an ulcer, the device comprising:

a first part comprising an anti-septic composition;

- 10 a second part comprising a water absorbing material and thereby to function as a water-reservoir;

a top surface, wherein the top surface is abrasive; and

a water-permeable bottom surface,

wherein the first part and the second part are separated from each other by a water-impermeable primary membrane.

- 15 Item 2. A device according to Item 1, wherein the first part is made of a water-permeable material that is compatible with one or more active chlorine agents.

Item 3. A device according to Item 1 or 2, wherein the anti-septic composition comprises one or more anti-septic agents, one or more anti-microbial agents and/or one or more detaching agents.

- 20 Item 4. A device according to any of Items 1-3, wherein the anti-septic composition comprises powdered agents for debriding one or more of wounds, sores and ulcers.

Item 5. A device according to any of Items 1-4, wherein the anti-septic composition comprises an active chlorine agent selected from Cl_2 , hypochlorite, chlorite, chlorate, perchlorate, and alkaline or alkali earth salts thereof.

- 25 Item 6. A device according to Item 5, wherein the active chlorine agent is hypochlorite, such as calcium hypochlorite.

Item 7. A device according to any of Items 1-6, wherein the anti-septic composition comprises a pH adjusting agent to adjust pH to a range of from 8.00 to about 9.00.

Item 8. A device according to any of Items 1-7, wherein the first part comprises one or more of colloidal silicon particles, sodium chloride and potassium chloride.

5 Item 9. A device according to any of Items 1-8, wherein the anti-septic composition comprises:

i) sodium hydrogen carbonate also denoted sodium bicarbonate,

ii) potassium chloride,

iii) sodium chloride,

10 iv) titanium dioxide,

v) one or more of silicium dioxide, silicon dioxide, and fumed silica,

vi) optionally, sodium hydroxide to adjust pH to from 8 to 9, and

vii) calcium hypochlorite.

Item 10. A device according to Item 9, wherein

15 i) the concentration of sodium hydrogen carbonate is in a range of from 1 to 3 g/l (2 g/l)

ii) the concentration of potassium chloride is in a range of from 0.5 to 3 g/l (1 g/l),

iii) the concentration of sodium chloride is in a range of from 1 to 4 g/l (2 g/l),

iv) the concentration of titanium dioxide is in a range of from 0.1 to 1 g/l (0.4 g/l),

v) the concentration of silicium dioxide, silicon dioxide or fumed silica is in a range of from
20 0.2 to 1.0 g/l (0-6 g/l), and

vii) the concentration of calcium hypochlorite is in a range of from 5 to 25 g/l (20 g/l).

Item 11. A device according to any of Items 1-10, wherein the device comprises a first layer of abrasive material forming the top surface.

Item 12. A device according to Item 11, wherein the device comprises a first membrane between the first layer and the first part, wherein the first membrane is a water-impermeable membrane separating the first layer and the first part.

Item 13. A device according to any of Items 1-12, wherein the first part and/or the second
5 part comprises one or more of silicone, polysiloxane, a polyurethan, polyester, cellulose, and non-woven fabric.

Item 14. A device according to Item 13, wherein the silicone, polysiloxane or polyurethan is in the form of a foam.

Item 15. A method for debriding and/or cleaning wounds, sores and/or ulcers, the method
10 comprising:

providing a device as defined in any one of Items 1-14;

adding water to the device until the device is saturated with water for dissolving the water-soluble components of the first part;

applying the bottom surface to the wound, sore or ulcer, whereby water-soluble
15 components of the first part are released from the device;

rubbing the wound, sore or ulcer and the surroundings of the wound, sore or ulcer with the bottom surface of the device to remove biofilm and necrotic tissue;

optionally squeezing the second part of the device to allow water to rinse the wound, sore or ulcer;

20 optionally rubbing the wound, sore or ulcer and the surroundings of the wound, sore or ulcer with the top surface to remove remaining unwanted tissue; and

optionally rinsing the wound, sore or ulcer with water.

Item 16. A kit for debriding and/or cleaning wounds, sores and/or ulcers, the kit comprising packaging and a device according to any one of Items 1-14, the packaging
25 comprising a container and a seal, the container forming a cavity accommodating the device.

The use of the terms "first", "second", "third" and "fourth", "primary", "secondary", "tertiary" etc. does not imply any particular order, but are included to identify individual elements.

Moreover, the use of the terms "first", "second", "third" and "fourth", "primary", "secondary", "tertiary" etc. does not denote any order or importance, but rather the terms "first", "second", "third" and "fourth", "primary", "secondary", "tertiary" etc. are used to distinguish one element from another. Note that the words "first", "second", "third" and "fourth", "primary", "secondary", "tertiary" etc. are used here and elsewhere for labelling purposes only and are not intended to denote any specific spatial or temporal ordering. Furthermore, the labelling of a first element does not imply the presence of a second element and vice versa.

It may be appreciated that the figures comprise some elements, circuitries or operations which are illustrated with a solid line and some elements, circuitries or operations which are illustrated with a dashed line. Elements, circuitries or operations which are comprised in a solid line are elements, circuitries or operations which are comprised in the broadest example embodiment. Elements, circuitries or operations which are illustrated in a dashed line are examples which may be comprised in, or a part of, or are further elements, circuitries or operations which may be taken in addition to elements, circuitries or operations of the solid line examples. It should be appreciated that these operations need not be performed in order presented. Furthermore, it should be appreciated that not all of the operations need to be performed. The example operations may be performed in any order and in any combination.

It is to be noted that the word "comprising" does not necessarily exclude the presence of other elements or steps than those listed. It is to be noted that the words "a" or "an" preceding an element do not exclude the presence of a plurality of such elements. It should further be noted that any reference signs do not limit the scope of the claims.

Although features have been shown and described, it will be understood that they are not intended to limit the claimed disclosure, and it will be made obvious to those skilled in the art that various changes and modifications may be made without departing from the scope of the claimed disclosure. The specification and drawings are, accordingly to be regarded in an illustrative rather than restrictive sense. The claimed disclosure is intended to cover all alternatives, modifications, and equivalents.

LIST OF REFERENCES

	2	device
	4	first part
	4A	first surface of first part
5	4B	second surface of first part
	6	second part
	6A	first surface of second part
	6B	second surface of second part
	8	top surface
10	10	bottom surface
	12	primary membrane, membrane
	14	first layer
	16	second layer
	18	first end
15	20	second end
	22	first side
	24	second side
	26	anti-septic composition
	26A	active chlorine agent
20	26B	pH adjusting agent
	27	first cavity
	28	first membrane

	28A	first region of first membrane
	28B	second region of first membrane
	50	kit
	52	container
5	54	seal
	56	cavity
	H	height of device
	W	width of device
	L	length of device
10	L_1	length of first part
	L_2	length of second part

CLAIMS

1. A device for debriding and/or cleaning a wound, a sore or an ulcer, the device comprising:

5 a first part comprising an anti-septic composition, wherein the first part is made of a water-absorbing material;

a second part comprising a water absorbing material and thereby to function as a water-reservoir;

a top surface, wherein the top surface is abrasive; and

a water-permeable bottom surface,

10 wherein the first part and the second part are separated from each other by a water-impermeable membrane.

2. A device according to claim 1, wherein the first part is made of a water-permeable material that is compatible with one or more active chlorine agents.

3. A device according to claim 1 or 2, wherein the anti-septic composition comprises one
15 or more anti-septic agents, one or more anti-microbial agents and/or one or more detaching agents.

4. A device according to any of the preceding claims, wherein the anti-septic composition comprises powdered agents for debriding one or more of wounds, sores and ulcers.

5. A device according to any of the preceding claims, wherein the anti-septic composition
20 comprises an active chlorine agent selected from Cl₂, hypochlorite, chlorite, chlorate, perchlorate, and alkaline or alkali earth salts thereof.

6. A device according to claim 5, wherein the active chlorine agent is hypochlorite, such as calcium hypochlorite.

7. A device according to any of the preceding claims, wherein the anti-septic composition
25 comprises a pH adjusting agent to adjust pH to a range of from 8.00 to about 9.00.

8. A device according to any of the preceding claims, wherein the first part comprises one or more of colloidal silicon particles, sodium chloride and potassium chloride.

9. A device according to any of the preceding claims, wherein the anti-septic composition comprises:

i) sodium hydrogen carbonate also denoted sodium bicarbonate,

ii) potassium chloride,

5 iii) sodium chloride,

iv) titanium dioxide,

v) one or more of silicium dioxide, silicon dioxide, and fumed silica,

vi) optionally, sodium hydroxide to adjust pH to from 8 to 9, and

vii) calcium hypochlorite.

10 10. A device according to claim 9, wherein

i) the concentration of sodium hydrogen carbonate is in a range of from 1 to 3 g/l (2 g/l)

ii) the concentration of potassium chloride is in a range of from 0.5 to 3 g/l (1 g/l),

iii) the concentration of sodium chloride is in a range of from 1 to 4 g/l (2 g/l),

iv) the concentration of titanium dioxide is in a range of from 0.1 to 1 g/l (0.4 g/l),

15 v) the concentration of silicium dioxide, silicon dioxide or fumed silica is in a range of from 0.2 to 1.0 g/l (0-6 g/l), and

vii) the concentration of calcium hypochlorite is in a range of from 5 to 25 g/l (20 g/l).

11. A device according to any of the preceding claims, wherein the device comprises a first layer of abrasive material forming the top surface.

20 12. A device according to claim 11, wherein the device comprises a first membrane between the first layer and the first part, wherein the first membrane is a water-impermeable membrane separating the first layer and the first part.

13. A device according to any of the preceding claims, wherein the first part and/or the second part comprises one or more of silicone, polysiloxane, a polyurethan, polyester, cellulose, and non-woven fabric.

14. A device according to claim 13, wherein the silicone, polysiloxane or polyurethan is in
5 the form of a foam.

15. A method for debriding and/or cleaning wounds, sores and/or ulcers, the method comprising:

providing a device as defined in any one of claims 1-14;

10 adding water to the device until the device is saturated with water for dissolving the water-soluble components of the first part;

applying the bottom surface to the wound, sore or ulcer, whereby water-soluble components of the first part are released from the device;

rubbing the wound, sore or ulcer and the surroundings of the wound, sore or ulcer with the bottom surface of the device to remove biofilm and necrotic tissue;

15 optionally squeezing the second part of the device to allow water to rinse the wound, sore or ulcer;

optionally rubbing the wound, sore or ulcer and the surroundings of the wound, sore or ulcer with the top surface to remove remaining unwanted tissue; and

optionally rinsing the wound, sore or ulcer with water.

20 16. A kit for debriding and/or cleaning wounds, sores and/or ulcers, the kit comprising packaging and a device according to any one of claims 1-14, the packaging comprising a container and a seal, the container forming a cavity accommodating the device.