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(54) Title: FIBROUS WOUND DRESSING COMPRISING AN ANTISEPTIC

(57) Abstract: A fibrous wound dressing comprising a formulation of amphiphilic antiseptic and separate surfactants is provided.

FIBROUS WOUND DRESSING COMPRISING AN ANTISEPTIC

TECHNICAL FIELD

The present technology relates to a fibrous wound dressing comprising an antiseptic formulation.

BACKGROUND

Fibrous dressings for wound care are typically used for exuding wounds, including leg ulcers, pressure ulcers, diabetic foot ulcers, donor sites, postoperative wounds and skin abrasions.

A number of antiseptic compounds useful in wound treatment are amphiphilic, e.g. octenidine. Such compounds associate to surfaces, and have reduced mobility in a wound environment, or a hydrophilic matrix.

Additionally, challenges also exist when a formulation is exposed to a sensitive wound environment. In particular, the presence of ions and other components in the wound exudate can promote the undesirable precipitation of amphiphilic components.

As an amphiphilic molecule, octenidine has shown to associate to surfaces and thereby reduce mobility in a matrix. Early experiments documented that when octenidine is impregnated into a plain foam matrix, only a relatively low amount of octenidine was freely extractable (cf. experimental section). This strongly indicates that octenidine is attracted to the foam matrix, thereby restricting its release.

A need exists for a formulation of amphiphilic antiseptics, such as octenidine, in which the mobility of the amphiphilic antiseptic is increased in a wound environment. Additionally, the formulation should provide good solubility, mobility of the amphiphilic antiseptic and stability (i.e. lack of precipitation of the amphiphilic antiseptic). The present technology shows that the formulation of an amphiphilic antiseptic compound in a wound dressing can provide a major impact on the extractability, mobility and stability of said antiseptic.

SUMMARY

A fibrous wound dressing is therefore provided which comprises a formulation of (a) an amphiphilic antiseptic and (b) at least one separate non-ionic surfactant or (c) at least one

separate cationic surfactant or (d) at least one separate zwitterionic surfactant. The formulation can be coated on the surface of the fibrous wound dressing. The formulation may alternatively be comprised (i.e. impregnated) within the fibres of said fibrous wound dressing.

Additional aspects of the technology are presented in the following description, the examples and the dependent claims.

DETAILED DISCLOSURE

As set out above, a fibrous wound dressing is provided comprising a formulation of (a) an amphiphilic antiseptic and (b) at least one separate non-ionic surfactant or (c) at least one separate cationic surfactant or (d) at least one separate zwitterionic surfactant. The term "separate" is used to mean that the same component may not be considered as both antiseptic and surfactant, but that the formulation comprises two separate, different components.

The amphiphilic antiseptic (component a) in the formulation – being amphiphilic – has both hydrophilic and hydrophobic moieties. Examples are quaternary ammonium compounds such as benzalkonium chloride and benzethonium chloride. Biguanides such as chlorhexidine or polyhexanide (PHMB) or other cationic compounds such as octenidine and ethyl lauroyl arginate (LAE). The antiseptic is preferably octenidine. The term "amphiphilic antiseptic" includes salts thereof.

Experimental results have shown that when octenidine is impregnated into a hydrophilic matrix, using hydrophilic polyurethane foam as model system only relatively low amount of octenidine was freely extractable (see Example 1, Table 1). This strongly indicates that octenidine is being attracted to the foam matrix, which thereby restricts the release of octenidine.

The limited release of octenidine from the foam matrix can possibly be explained on the basis of the chemical structure of octenidine. Octenidine consist of two pyridines and two aliphatic tails and an aliphatic linker between the pyridinium structure. This results in an abnormal structure for a cationic detergent (see Figure 1) and a high degree of hydrophobicity. The high degree of hydrophobicity is expected to cause the attraction to surfaces and thereby low release. Similar reasoning can be applied to other amphiphilic antiseptics and to other substrates/products.

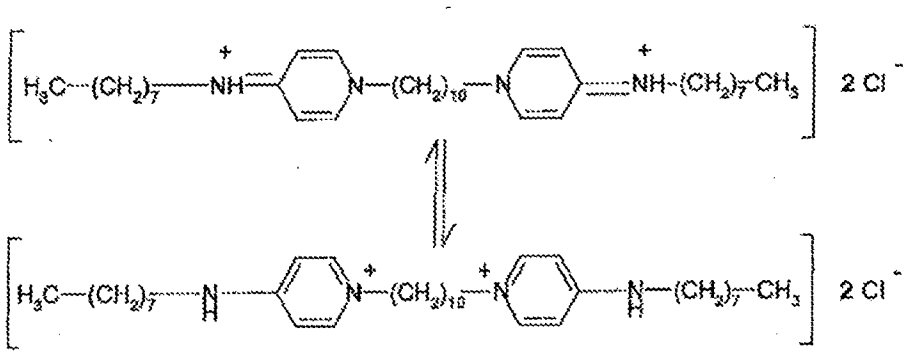


Figure 1: Chemical Structure of Octenidine.

Fibrous wound dressing

A fibrous wound dressing is provided. The term "fibrous" means comprised of fibres, typically in a nonwoven or woven structure, usually with physical entanglement between the fibres to maintain the integrity of the dressing.

In one alternative, the fibrous wound dressing comprises one or more layers of nonwoven material. Said layers of nonwoven material may be the same; or said layers may be different in terms of fibre type (natural, synthetic or semi-synthetic, or blends thereof), physical properties (e.g. hydrophilicity/hydrophobicity, physical dimensions or density) and/or type of nonwoven (e.g. airlaid, wetlaid, spunlace etc.). In one advantageous embodiment, the fibrous wound dressing comprises more than one layer of nonwoven material and/or more than one type of fibers.

Suitable fibres for the fibrous wound dressing include natural fibres such as wood, cotton, alginate collagen, or chitosan fibres, synthetic fibres such as polymeric fibres or semi-synthetic fibres such as rayon. Fibres may be staple fibres or continuous fibres.

Suitable nonwoven techniques for providing the layers of nonwoven material include airlaying, wet-laying and various spinning techniques.

The fibrous wound dressing may comprise additional components such as foams, super absorbent material or adhesive, typically in a layered construction. Alternatively, such components may be distributed throughout the fibrous dressing.

In another alternative, the fibrous wound dressing is a "stand alone" dressing in which the fibrous nonwoven layer is the sole component of the wound dressing.

The fibrous wound dressing has a wound-facing surface layer, which is defined as a sheet or layer arranged to be in direct contact to a wound, or peri-wound skin.

In embodiments, the fibrous wound dressing comprises carboxymethylcellulose (CMC) fibers and/or alginate-based fibers. Both CMC- and alginate-based fibrous dressings absorb water, thereby transforming from a distinct fiber structure to a more amorph gelling structure. Since this is an irreversible process that destroys the fiber structure, water cannot be used as a carrier for the impregnation solution for CMC- and alginate-based fibrous dressings. Therefore, the formulation for the fiber-based water absorbing platforms need to address this issue by not utilizing solvents that make the fibers transform and to choose a surfactant that following is dissolvable in the chosen solvent.

Ethanol or other polar organic solvents will not cause swelling alginate- and CMC-based fibers and is as such a good carrier solvent for the formulation since octenidine is readily dissolvable in ethanol. A range of relevant solvents are dissolvable in polar organic solvents Such as Tween 80. Tween 80 is a surfactant that has shown good performance together with Octenidine, both in relation to increasing release of octenidine, as well as stabilizing octenidine against precipitation with proteins and other wound bed compounds as well as being soluble in ethanol. Therefore, the octenidine formulation to be used for CMC and Alginate based fiber dressings is preferable composed from Ethanol as solvent, and Tween 80 as amphiphile surfactant. However, other surfactants dissolvable in alcohols, such as Tween 20, Empigen BB, benzalkonium chloride, or poloxamers, will potentially be candidates for this formulation system. In embodiments, the surfactant is Tween 80.

Formulation

The fibrous wound dressing comprises a formulation of (a) an amphiphilic antiseptic and (b) at least one separate non-ionic surfactant or (c) at least one separate cationic surfactant. Preferably the surfactant is (b) at least one separate non-ionic surfactant or (d) at least one separate zwitterionic surfactant.

By formulation is meant a formulation solution that is meant to be impregnated into the fibrous wound dressing. Following impregnation, the carrying solvent is evaporated off, leaving the formulation compounds within the fiber structure. Thereby, the percental concentrations within the impregnation formulation can be re-calculated into mass of compound per square (or cubic) area of fiber, depending on the absorbance capacity of the given fiber. Example: If a fiber has a absorbency of 0.3mL/square centimetre and the formulation holds 0.1% amphiphilic antiseptic and 1% non-ionic surfactant. Then, the fiber will be impregnated with 0,3mg amphiphilic antiseptic and 3 mg non-ionic surfactant per

square centimetre. This will lead to a finished fiber (dried) containing 0,3 mg amphiphilic antiseptic and 3 mg non-ionic surfactant per square centimetre. For reading this document, the relation between impregnation formulation and mass per square or cubic area fiber will be defined as in this section.

The antiseptic and the surfactant can either be blended into the matrix during formation of the fiber, applied during the formation of the fibrous sheet structure or applied as a coating or impregnation to the fibrous structure after formation of the fibrous structure.

The formulation is suitably a solution of said components in an appropriate solvent with good wettability to the applied fibrous dressing e.g. water and/or alcohols. Suitable alcohols may be methanol or ethanol or other polar organic solvents, when applying the said formulation to a hydrophilic fibrous dressing or mixtures with more apolar solvent such as hexane, ethyl acetate or volatile silicone fluids when applying said formulation to a hydrophobic fibrous dressing.

In one aspect, the formulation does not comprise surfactants other than the surfactants specified. In a further aspect, the formulation does not comprise antiseptics other than the antiseptic specified. In one aspect, the formulation consists of an amphiphilic antiseptic and at least one surfactant.

In one aspect, the formulation is free from inorganic salts. In particular, the formulation is free from halide salts of group I or II metals, e.g. NaCl, KCl, $MgCl_2$ or $CaCl_2$. Dissolution of the antiseptic is thereby improved. The formulation suitably comprises between 0.001 - 10% w/w, preferably between 0.05 - 5 wt% of said amphiphilic antiseptic. The formulation suitably comprises between 0.05 – 10% w/w, preferably between 0.01 – 5 wt%, more preferably between 0.1 - 5 wt% of said surfactant. The dressings and formulations can show antibacterial effects even at such low concentrations of antiseptic/surfactant. Meaning for a fiber dressing with an absorbency of 0.3mL/cm^2: 0.003-30 mg/cm^2 preferably between 0.15 - 3 mg/cm^2 of said amphiphilic antiseptic. The formulation suitably comprises between 0.15 – 30 mg/cm^2 w/w, preferably between 0.05 – 2.5 mg/cm^2, more preferably between 0.3 – 1.5 mg/cm^2 of said surfactant. By any deviation in exemplified absorbency (0.5mL/cm^2) the above-mentioned mass contents can be corrected.

In embodiments, the fibrous wound dressing comprises 0.003-30 mg/cm^2, preferably between 0.15 - 3 mg/cm^2, of said amphiphilic antiseptic. In embodiments, the fibrous wound dressing comprises between 0.15 – 30 mg/cm^2 w/w, preferably between 0.05 – 2.5 mg/cm^2, more preferably between 0.3 – 1.5 mg/cm^2 of said surfactant.

The formulation may be applied to a surface of the fibrous wound dressing which is arranged to face the user when in use (i.e. the opposite face to any backing layer). Alternatively, the formulation may be applied to a surface of the fibrous wound dressing which is arranged opposite the user when in use (i.e. the opposite face to the wound contact side). Alternatively or additionally, the formulation may be incorporated into the wound dressing (i.e. impregnated). Any known methods for applying the formulation into/onto the dressing may be used, such as rolling or spraying of the formulation onto a pre-formed fibrous wound dressing or incorporation by dipping/bathing the said fibrous wound dressing in the formulation.

In a first aspect, therefore a method for manufacturing a fibrous wound dressing is provided, said method comprising but not limited to

a. providing a formulation of (a) an amphiphilic antiseptic and (b) at least one separate non-ionic surfactant or (c) at least one separate cationic surfactant or (d) at least one separate zwitterionic surfactant, said formulation additionally including a solvent; and

b. applying the formulation to a pre-formed fibrous wound dressing, such that the formulation becomes coated on a surface of the fibrous wound dressing.

In another aspect, the formulation may be applied to free fibres, prior to formation of the fibrous wound dressing. A method for manufacturing a fibrous wound dressing is therefore provided, said method comprising

a. providing a formulation of (a) an amphiphilic antiseptic and (b) at least one separate non-ionic surfactant or (c) at least one separate cationic surfactant, or (d) at least one separate zwitterionic surfactant said formulation additionally including a solvent;

b. applying the formulation to fibers, such that the formulation becomes coated on said fibres; and

c. forming a fibrous wound dressing from said coated fibres.

As a further option, which may supplement the above options of coating the fibres/the wound dressing, the formulation may be comprised within the matrix of the fibres making up the wound dressing. In other words, the formulation (of antiseptic and surfactant) is blended with the fiber forming matrix, and then formed together with this material into the required

fibres. In this manner, the formulation is encapsulated within the fibres of the fibrous wound dressing, which could provide improved properties with respect to stability and release of the antiseptic.

In this aspect, therefore, a method for manufacturing a fibrous wound dressing is provided, said method comprising

a. providing a formulation of (a) an amphiphilic antiseptic and (b) at least one separate non-ionic surfactant or (c) at least one separate cationic surfactant or (d) at least one separate zwitterionic surfactant, said formulation additionally including a solvent;

b. providing a polymer composition;

c. mixing said formulation with said polymer composition and spinning the mixture to form fibers impregnated with said formulation;

d. forming a fibrous wound dressing from said impregnated fibres.

The term "surfactant" as used herein means organic compounds that are amphiphilic, meaning they contain both hydrophobic groups and hydrophilic groups. The surfactant in the formulation is preferably non-ionic; i.e. it comprises polar hydrophilic regions which are not charged. It has been found that non-ionic surfactants can provide benefits in terms of stability of the formulation and release of the antiseptic.

Alternatively, the surfactant is cationic. It has been found that cationic surfactants can provide benefits in terms of stability of the formulation.

It has also been discovered that certain anionic detergents such as SDS, can interact with the antiseptic via ionic interaction and may cause precipitation and/or undesired interaction with the fibrous wound dressing.

In one aspect, the surfactant comprises a single hydrophobic moiety, and a single hydrophilic moiety. Without being bound by theory, it is hypothesised that surfactants having one of each of such moieties can arrange optimally with the amphiphilic antiseptic. Additionally, testing of certain surfactants with e.g. more than one hydrophobic moiety did not provide the desired benefits.

In one aspect, the surfactant is a fatty acid monoester or fatty acid monoamide of a polyhydroxy compound. If a monoamide surfactant is used, it should be uncharged in the physiological conditions present in a wound.

According to this aspect, the fatty acid monoester or fatty acid monoamide may comprise a C2-C22 fatty acid moiety, e.g. a C4-C18 fatty acid moiety or a C6-C12 fatty acid moiety. In embodiments, the fatty acid moiety is saturated. In embodiments, the fatty acid is unsaturated.

In another aspect, the surfactant is a fatty alcohol monoether of a polyhydroxy compound. The fatty alcohol monoether may comprise a C2-C22 fatty alcohol moiety, e.g. a C4-C18 fatty alcohol moiety or a C6-C12 fatty alcohol moiety. The fatty alcohol moiety may be saturated or unsaturated.

In embodiments, the fatty acid moiety or said fatty alcohol moiety used herein is saturated. In embodiments, the fatty acid moiety or said fatty alcohol moiety used herein is unsaturated.

The polyhydroxy compound used as the hydrophilic moiety may be comprised of any multifunctional hydroxy- and/or amine compound (number of hydroxy groups + amine groups >= 2), that may or may not be derivatized by any combination of ethylene oxide and propylene oxide. Particular polyhydroxy compounds may be selected from glycerol, sorbitan, ethoxylated sorbitan, glucose, ethylene glycol, polyethylene glycol or amine derivatives thereof.

Most preferably, the non-ionic surfactants are a C6-C12 fatty alcohol monoether of glucose, or a C6-C12 fatty acid monoester of ethoxylated sorbitan. Suitable non-ionic surfactants are e.g. polysorbates (Tween) and decyl glucoside.

In a further aspect, the surfactant is a di-block copolymer (A-B), wherein one block of said copolymer (A) is hydrophobic, and the other block (B) of said copolymer is hydrophilic.

In a further aspect, the surfactant is a block copolymer and preferable di-block copolymer (A-B), wherein one block of said copolymer (A) is hydrophobic, and the other block (B) of said copolymer is hydrophilic and preferably nonionic

The hydrophobic block (A) may be selected from, but not limited to, polypropylene oxide, polypropylene ethylenoxide copolymers, polysiloxanes, polystyrene, polylactide, polycaprolactone and the like. Similarly, the hydrophilic block may be selected from, but not

limited to, polyethylene oxide, poly(ethylene oxide co-propylene oxide), polyoxazoline, poly(vinyl pyrolidone) and the like.

In embodiments, the surfactant is a zwitterionic surfactant, such as lauryl betaine (Empigen BB).

Overall, the surfactant may have a hydrophilic-lipophilic balance (HLB) between 10 and 17 inclusive.

EXAMPLES

As an amphiphilic molecule, octenidine has shown to associate to surfaces and thereby reduce mobility in a matrix. Previous studies have indicated that Octenidine did not diffuse freely in foam matrices, indicating a high degree of interactions between octenidine and foam matrix.

To address this, we have investigated formulations to increase the mobility of octenidine by co-formulating different surface active compounds and salts. Solubility and stability (no precipitation when interacting with e.g. salts or proteins) was tested in a solution. Release from a fibrous wound dressing can be tested by coating or impregnating the formulation onto or into a fibrous matrix, optionally drying the fibrous and following carrying out release studies.

1. **Octenidine in foam, no surfactant**

Discs of hydrophilic polyurethane foam were impregnated by applying a known volume of octenidine-containing solution to the surface of the foam and letting it soak into the foam matrix in a liquid:foam ratio which allowed the foam to be saturated with liquid. Afterwards, the impregnated foam was dried at RT overnight.

The dried foam disc was immersed in the extraction media for 24 h and the extracted octenidine concentration was determined by UV at 285nm

% Octenidine extractable after being impregnated into plain foam w% OCT in impregnation solution	Release % water	Release % phosphate buffer (23 mM)
0.1 w%	5%	7%

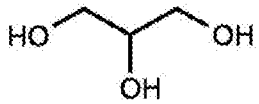
0.5 w%	1.5%	13%
1.0 w%	1.5%	20%

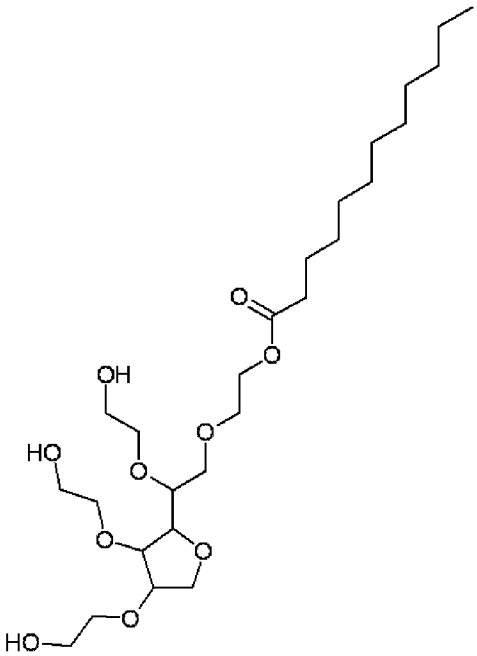
Table 1: These results show that when octenidine is impregnated into a matrix such as a plain foam matrix only relatively low amounts of octenidine were freely extractable.

2. Solubility of Octenidine with/without surface active compounds.

In these experiments, octenidine dihydrochloride was dissolved in different solutions to determine the solubility with/without the presences of surface active compound (surfactant).

To investigate the interaction between the dissolved octenidine and isotonic salt concentrations (0.9%), 0.9% NaCl was co-formulated with glycerol (A4), tween (A5), or both combined (A6).





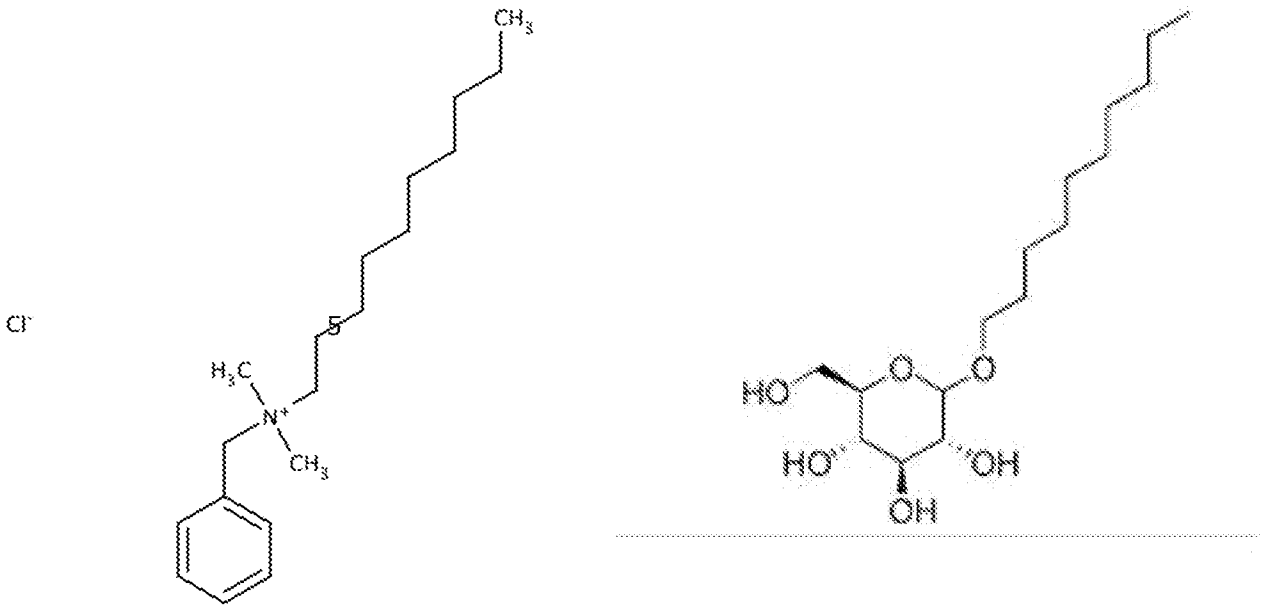


Figure 2: Chemical structures of glycerol, Tween 20, benzalkonium, decyl glucoside.

The solutions used were as follows:

- A1: 3 w% Tween-20
- A2: 5 w% glycerol
- A3: 3 w% tween-20, 5 w% glycerol
- A7. MQ water
- A8: PBS buffer 23 mM
- A9: 2% Benzalkonium chloride
- A10: 5% Plantacare 2000 UP (50% decyl glucoside solution)

Two concentrations of Octenidine were tested: 1% and 3%

Conc. 1%: 1.00g Octenidine + 100 ml solution

Conc. 3%: 3.00g Octenidine + 100 ml solution

All solutions were prepared in conical glass bottles, sealed with plastic film at room temperature and stirred. The solutions were inspected every 15 min. and observations were recorded.

The results from the solubility tests are shown in Table 2:

1% Octenidine	pH	Total time to dissolution	Dissolved after 1 week at RT
A1	3.23	1h	Yes
A2	5.06	1h 05min	Yes
A3	3.28	1h 15min	Yes
A7	4.99	1h 20min	Yes
A8	6.82	41min	Yes
A9	6.45	1h 15min	Yes
A10	10.14	51min	Yes

Table 2: overview of solubilities of 1% Octenidine co-formulated with different surfactant compounds.

All of the used solvent systems (H_2O, Glycerol, Phosphate, Tween20, Benzalkonium Cloride, and Plantacare (50% Decyl glucoside)) were able to dissolve 1 % Octenidine. The solubility of 3% Octenidine was also tested and only Plantacare (solution A10) was able to fully dissolve 3% Octenidine and keep it in the dissolution without precipitation (results not shown).

Solvent systems containing salts (A4, A5, A6) did not dissolve 1% octenidine. Also, if octenidine is dissolved in respectively Tween20 or Tween20/glycerol, the same solubility/stability is indicated, while glycerol alone did not show any better solubilisation capacity than water alone. This indicates that glycerol does not have any significant effect on the solubility of octenidine, neither negative nor positive.

3. Stability of solutions towards salts.

The solutions with 1% Octenidine from experiment 2 that were totally dissolved (A1, A2, A3, A7, A8, A9, A10), were tested in a new experiment. The solutions were diluted with 0.9% NaCl to different concentrations to observe whether the Octenidine precipitated in the solution. The ratios 2:1, 1:4 and 1:10 (test solution: 0.9%NaCl) were tested and all the solutions were heated to RT (37°C) for 1 hour. To challenge the solubility, the samples were also cooled to 4° and possible precipitation was observed.

The results are shown in Table 3:

1% Octenidine	Temp.	Addition of 0.9% NaCl solution 2:1	Addition of 0.9% NaCl solution 1:4	Addition of 0.9% NaCl solution 2:1
A1	37°C	No precipitation	No precipitation	No precipitation
	4°C	Precipitation	Precipitation	Precipitation
A2	37°C	Visible precipitation	Visible precipitation	-
	4°C	-	-	-
A3	37°C	No precipitation	No precipitation	No precipitation
	4°C	Precipitation	Precipitation	Precipitation
A7	37°C	Visible precipitation	Visible precipitation	-
	4°C	-	-	-
A8	37°C	No precipitation	Visible precipitation	-
	4°C	Precipitation	-	-
A9	37°C	No precipitation	No precipitation	No precipitation
	4°C	Precipitation	Precipitation	Precipitation
A10	37°C	No precipitation	No precipitation	No precipitation
	4°C	-	Less precipitation than other solutions	Precipitation

Table 3: Salt stability of octenidine solutions

If addition of salt is carried out after octenidine has been dissolved, the precipitating effect of NaCl is not seen at room temperature for solutions A1, A3, A9 and A10 (Table 3), indicating that an interaction between an amphiphile such as Tween20 or decyl glucoside and octenidine, protects octenidine from salt precipitation.

For all formulations except Plantacare precipitations was observed at octenidine: salt solution of 2:1 at increasing salt concentrations (1:4) slight precipitation was observed in the octentine:plantacare formulation and with even stronger precipitations at a ratio of 1:10. However, this show that decylglucoside has the best capacity to stabilize octenidine in relation to salting out.

Overall, the 3 amphiphiles (Tween 20, benzalkonium and decyl glucoside) all dissolve 1% octenidine. But most importantly, indicated by the salt additions, they are able to stabilize octenidine in a salt-containing solution such as a wound bed and avoid precipitation upon contact with salt. Based on the temperature experiments it is indicated that decyl glucoside (Plantacare) has the best capacity to stabilize the octenidine.

4. Protein binding and precipitation

The purpose of this experiment is to investigate the capability of surfactant to protect Octenidine from precipitation when mixed with a protein/salt media, such as simulated wound fluid (SWF), to further understand how Octenidine and the co-formulation with detergents will respond to being released into a wound bed environment.

The results show that surfactants can significantly reduce the interaction between a protein pool and Octenidine by reducing the agglomeration of octenidine and proteins/salts. This means that the surfactants will prevent unwanted precipitation, thereby making sure that a large portion of the Octenidine is available for acting in the wound environment.

The following surfactants were tested:

Solution no.	Surfactant:	INCI name	Batch no.
A	1% Tween 20	Polysorbate 20	Batch #094K0052
B	1% Tween 80	Polysorbate 80	Lot #BCBV7863
C	1% Plantacare 810 UP	Caprylyl / Capryl Glucoside	lot. 17483268
D	1% Plantacare 2000 UP	Decyl Glucoside	lot. 0019096298
E	1% Benzalkonium chloride	Benzalkonium chloride	Lot #BCBV7858
F	1% Empigen BB	Lauryl Betaine	Lot #BCBQ6967
G	1% Decanesulfonate	Decane-sulfonate	Lot #BCBT6967
H	1% Plantacare 1200 UP	Lauryl glucoside	lot. 19090815
I	Water	-	-

The experiment was done as follows:

i) 2 ml of solution A, B, C etc., each containing 1 mg/ml Octenidine, were mixed with 2 ml SWF or water. The mix of solutions were done twice (one for each filter type).

ii) The mix of solutions were incubated for 1 hour at room temp. on a shaking table at 100 rpm.

iii) The mix of solutions were filtrated through a 0.22 µm filter.

iv) The filtrated solution was diluted ten times in eluent. The Octenidine conc. should be 0,05 mg/ml (to be within detection area) if 100% was recovered after incubation and filtration.

v) Controls were prepared by diluting the formulation solution in eluent (50% McIlvaine buffer/50% Methanol) to conc. 0,05 mg/ml (dilution x20).

vi) The samples and controls were analysed using HPLC.

The results were as follows.

Solution no.	Surfactant:	Octenidine	Recovery in SWF (%)	Recovery in water (%)
A	1% Tween 20	1 mg/ml	99	100
B	1% Tween 80	1 mg/ml	100	100
C	1% Plantacare 810 UP	1 mg/ml	71	100
D	1% Plantacare 2000 UP	1 mg/ml	69	100
E	1% Benzalkonium chloride	1 mg/ml	47	100
F	1% Empigen BB	1 mg/ml	97	100
G	1% Decanesulfonate	1 mg/ml	7	n.a.
H	1% Plantacare 1200 UP	1 mg/ml	54	99
I	Water	1 mg/ml	26	100

The results show that Octenidine is precipitated by mixing with protein and salt containing solutions as well as when formulated with anionic surfactants, such as decanosulfonate. However, when co-formulated with nonionic (plantacare, Tween), cationic (Benzalkonium chloride) or zwitterionic (Empigen) surfactants, Octenidine is protected against precipitation, most probably by hydrophobic-hydrophobic interaction between octenidine and detergents, scavenging the octenidine molecule from interacting with salts and/or proteins.

5. Release of Octenidine from fibrous wound dressings

This example was carried out to gain an understanding of how to introduce an amphiphilic antiseptic, such as octenidine, into a fiber-based wound dressing based, e.g., on alginates or hydrofibers, such as carboxymethylcellulose (CMC). As a hydrophobic molecule, octenidine has shown to associate to surfaces and thereby reduce mobility in a matrix. To overcome this, it was investigate whether formulations with increased mobility of octenidine could be achieved by co-formulating with a surfactant.

The experiment was conducted Coloplast Biatain alginate wound dressings and a generic CMC based wound dressing and on free fibers of CMC. Fibers and dressings were prepared according to the below table.

Sample	Substrate	Octenidine	Tween 80
1	CMC dressing	2 mg/ml	0 %
2	CMC dressing	2 mg/ml	2 %
3	Alginate dressing	2 mg/ml	0 %
4	Alginate dressing	2 mg/ml	2 %
5	CMC fiber	0.1 %	0 %
6	CMC fiber	0.1 %	2 %

For the dressings, a sample is punched out with Ø 20 mm, which sample is then impregnated with solutions of 2 mg/ml OCT in ethanol, 2 mg/ml OCT in ethanol with 2 % Tween 80, or 2 % Tween 80 in ethanol (without OCT). The samples were placed in Petri dishes and added 2x 500 microliters impregnation solution for the CMC and 1x 500 microliters for alginate. The samples were left to dry in the fume hood over the night.

For the fibers, 30 g of fiber was dipped into a solution containing either 0.1 % octenidine (OCT) in EtOH or 0.1 % OCT in a solution of 2% Tween 80 in EtOH. The samples were dipped for 5 minutes and thereafter dried.

The extraction was carried out as follows:

- For the dressing samples: The impregnated disk of the dressings is placed in 50 ml centrifuge tubes.
- 0.5 g of fibers where weighed off and put into a polypropylene tea bag which was following heat sealed. The fiber containing teabag was following placed in a 50 mL centrifuge tube.
- Each tube is added 10 ml of PBS buffer pH 7,4
- The tubes are placed at a shaking table at 100 rpm and after 24 hours the pieces of fiber sample are carefully transferred to a new 50 ml centrifuge tube containing 10 ml PBS.
- After 24, 48 and 72 hours the same procedure is repeated, but after 72 hours the samples are instead thrown out.
- The test tubes containing the release media at time point 24, 48 and 72 hours are analysed by UV measurement for determination of the concentration of Octenidine. Samples where filtered at a 0.45 µm to filter out any solid material that could interfere with UV measurement.
- For Alginate dressings, measurements were only carried out for 48 h, due to starting disintegration of the sample.

The results are as shown in the table below. Recovery is given as total accumulated recovery, i.e. the sum of the recovery at the indicated time point and previous time point(s), in percentage of the total impregnated octenidine.

Sample	Description	OCT	Tween-80	Recovery (24h)	Recovery (48h)	Recovery (72h)
1	CMC dressing	+	-	8 %	13 %	18 %
2	CMC dressing	+	+	82 %	88 %	90 %
3	Alginate dressing	+	-	12 %	18 %	-
4	Alginate dressing	+	+	86 %	91 %	-
5	CMC fiber	+	-	1 %	4 %	6 %
6	CMC fiber	+	+	88 %	100 %	100 %

The results show that octenidine can be co-formulated with a surfactant (Tween 80) and that this co-formulation increases its mobility in fiber-based wound dressings. Ethanol was used as a solvent and fibers could be wetted and dried without visible changes. The surfactant (Tween 80) was dissolved into ethanol, together with octenidine, and significantly increases the release of octenidine from the impregnated dressings and fibers.

Conclusions

Formulating octenidine with non-ionic or cationic or zwitterionic surfactants - preferably non-ionic surfactants – increases the mobility and stability of the octenidine. Formulating with Decyl glucoside (plantacare) resulted in the highest amount of total release octenidine with a total amount of released octenidine reaching 85% at 72 h together with an increased stability to salts. The results show that amphiphilic compounds can interact with octenidine and increase stability of octenidine. Highest mobility and stability increase was seen when using decyl glucoside (Plantacare) followed by Tween 20. Glycerol did not have any effect on octenidine mobility or stability, while NaCl caused precipitation, if octenidine had not been stabilized by amphiphiles before adding salts.

The experiments show that the nature of the surfactant must be carefully considered when providing formulations for fibrous wound dressings.

Although the invention has been illustrated with reference to a number of embodiments, aspects and examples, the skilled person can combine such embodiments, aspects and examples within the scope of the appended claims.

CLAIMS

1. A fibrous wound dressing comprising a formulation of (a) an amphiphilic antiseptic and (b) at least one separate non-ionic surfactant or (c) at least one separate cationic surfactant or (d) at least one separate zwitterionic surfactant, preferably at least one separate non-ionic surfactant.

2. The fibrous wound dressing according to claim 1, wherein said surfactant has one hydrophobic moiety and one hydrophilic moiety.

3. The fibrous wound dressing according to any one of claims 1-2, wherein said surfactant is a fatty acid monoester or fatty acid monoamide of a polyhydroxy compound.

4. The fibrous wound dressing according to claim 3, wherein said fatty acid monoester or fatty acid monoamide comprises a C2-C22 fatty acid moiety, e.g. a C4-C18 fatty acid moiety or a C6-C12 fatty acid moiety.

5. The fibrous wound dressing according to any one of claims 3-4, wherein said fatty acid moiety is saturated.

6. The fibrous wound dressing according to any one of claims 1-2, wherein said surfactant is a fatty alcohol monoether of a polyhydroxy compound.

7. The fibrous wound dressing according to claim 6, wherein said fatty alcohol monoether comprises a C2-C22 fatty alcohol moiety, e.g. a C4-C18 fatty alcohol moiety or a C6-C12 fatty alcohol moiety.

8. The fibrous wound dressing according to any one of claims 3-7, wherein said fatty alcohol moiety is saturated.

9. The fibrous wound dressing according to any one of claims 3-8, wherein said polyhydroxy compound is selected from glycerol, sorbitan, ethoxylated sorbitan, glucose, ethylene glycol, polyethylene glycol. or amine derivatives thereof.

10. The fibrous wound dressing according to any one of claims 1-2, wherein said surfactant is a di-block copolymer (A-B), wherein one block of said copolymer (A) is hydrophobic, and the other block (B) of said copolymer is hydrophilic.

11. The fibrous wound dressing according to claim 10, wherein said hydrophobic block (A) is selected from polypropylene oxide, polypropylene ethylene oxide copolymers, polysiloxanes, polystyrene, polylactide, or polycaprolactone.

12. The fibrous wound dressing according to any one of claims 10-11, wherein said hydrophilic block (B) is selected from polyethylene oxide, poly(ethylene oxide co-propylene oxide), polyoxazoline or poly(vinyl pyrolidone).

13. The fibrous wound dressing according to any one of the preceding claims, wherein said surfactant has a hydrophilic-lipophilic balance (HLB) between 6 and 20 inclusive, such as between 10 and 17 inclusive.

14. The fibrous wound dressing according to any one of the preceding claims, wherein the formulation is a solution of said components in water and/or alcohols, such as methanol or ethanol.

15. The fibrous wound dressing according to any one of the preceding claims, wherein the amphiphilic antiseptic is selected from benzalkonium chloride, benzethonium chloride, chlorhexidine, polyhexanide (PHMB), octenidine or ethyl lauroyl arginate (LAE), preferably octenidine; or salts thereof.

16. The fibrous wound dressing according to any one of the preceding claims, wherein the formulation comprises between 0.001 - 10% w/w, preferably between 0.05 - 5 wt% of said amphiphilic antiseptic.

17. The fibrous wound dressing according to any one of the preceding claims, wherein the formulation comprises between 0.05 – 10% w/w, preferably between 0.01 – 5 wt%, more preferably between 0.1 - 5 wt% of said surfactant.

18. The fibrous wound dressing according to any one of the preceding claims, wherein said formulation is free from inorganic salts.

19. The fibrous wound dressing according to any one of claims 1-18, wherein said wound dressing comprises a fibrous dressing material which comprises the formulation according to any one of the preceding claims.

20. The fibrous wound dressing according to any one of claims 1-19, wherein said formulation is coated on the surface of the fibrous wound dressing, preferably the wound-contacting surface thereof.

21. The fibrous wound dressing according to any one of claims 1-19, wherein said formulation is comprised within the fibres of said fibrous composition.

22. A method for manufacturing a fibrous wound dressing according to any one of the preceding claims, said method comprising

a. providing a formulation of (a) an amphiphilic antiseptic and (b) at least one separate non-ionic surfactant or (c) at least one separate cationic surfactant, or (d) at least one separate zwitterionic surfactant said formulation additionally including a solvent; and

b. applying the formulation to a pre-formed fibrous wound dressing, such that the formulation becomes coated on a surface of the fibrous wound dressing.

23. A method for manufacturing a fibrous wound dressing according to any one of the preceding claims, said method comprising

a. providing a formulation of (a) an amphiphilic antiseptic and (b) at least one separate non-ionic surfactant or (c) at least one separate cationic surfactant, or (d) at least one separate zwitterionic surfactant said formulation additionally including a solvent;

b. applying the formulation to fibers, such that the formulation becomes coated on said fibres; and

c. forming a fibrous wound dressing from said coated fibres.

24. A method for manufacturing a fibrous wound dressing according to any one of the preceding claims, said method comprising

a. providing a formulation of (a) an amphiphilic antiseptic and (b) at least one separate non-ionic surfactant or (c) at least one separate cationic surfactant, or (d) at least one separate zwitterionic surfactant said formulation additionally including a solvent;

b. providing a polymer composition;

c. mixing said formulation with said polymer composition and spinning the mixture to form fibers impregnated with said formulation,

d. forming a fibrous wound dressing from said impregnated fibres.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2019/050214

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61L15/42 A61L15/46 A61L15/48
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61L

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2017/019868 A1 (CURALINE INC [US]) 2 February 2017 (2017-02-02) paragraphs [0063], [0064] -----	24
X	US 2004/082925 A1 (PATEL HARISH A [US]) 29 April 2004 (2004-04-29) paragraph [0044]; claims 29,30 -----	1-6,9, 13-16, 18-20, 22,23
X	WO 2012/034032 A2 (UNBIVERSITY OF TEXAS SYSTEM BOARD OF [US]; RAAD ISSAM [US]; ABIAAD GEO) 15 March 2012 (2012-03-15) claims 8,9,30 ----- -/--	1,2,6-8, 13-17, 19,20, 22,23

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

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 September 2019	07/10/2019

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INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2019/050214

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2009/148395 A1 (FUGMANN BURKHARD [DE] ET AL) 11 June 2009 (2009-06-11) example 14	1-24

X	ALKHATIB Y ET AL: "Controlled extended octenidine release from a bacterial nanocellulose/Poloxamer hybrid system", EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS, ELSEVIER SCIENCE PUBLISHERS B.V., AMSTERDAM, NL, vol. 112, 24 November 2016 (2016-11-24), pages 164-176, XP029892985, ISSN: 0939-6411, DOI: 10.1016/J.EJPB.2016.11.025	1,2, 10-12, 14-16, 18-20,22
Y	1. Introduction , 2. Materials and Methods; abstract	24

X	EP 0 136 900 A2 (SURGIKOS INC [US]) 10 April 1985 (1985-04-10) page 4, lines 4-14 page 7, lines 10-35	1-5,19, 20,22

X	S Bradbury ET AL: "Prontosan made easy", Wound International, 1 May 2011 (2011-05-01), pages 1-6, XP055128934, Retrieved from the Internet: URL:http://www.woundsinternational.com/pdf/content_9864.pdf [retrieved on 2014-07-15] section 'How to use Prontosan': step 2 and step 3	1-5,14, 19-22

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/DK2019/050214

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2017019868 A1	02-02-2017	EP 3328460 A1	06-06-2018
		US 2019083675 A1	21-03-2019
		WO 2017019868 A1	02-02-2017
US 2004082925 A1	29-04-2004	AU 2003287080 A1	13-05-2004
		BR 0315428 A	16-08-2005
		EP 1555980 A2	27-07-2005
		EP 2371335 A2	05-10-2011
		IL 167946 A	30-12-2010
		JP 2006503647 A	02-02-2006
		KR 20050083804 A	26-08-2005
		MX PA05003802 A	08-06-2005
		TW I301068 B	21-09-2008
		US 2004082925 A1	29-04-2004
		US 2012089069 A1	12-04-2012
		US 2014178564 A1	26-06-2014
		WO 2004037115 A2	06-05-2004
WO 2012034032 A2	15-03-2012	US 2013231302 A1	05-09-2013
		WO 2012034032 A2	15-03-2012
US 2009148395 A1	11-06-2009	DE 102007048080 A1	09-04-2009
		EP 2222350 A2	01-09-2010
		TW 200932293 A	01-08-2009
		US 2009148395 A1	11-06-2009
		WO 2009043499 A2	09-04-2009
EP 0136900 A2	10-04-1985	AU 567372 B2	19-11-1987
		BR 8404894 A	20-08-1985
		CA 1223208 A	23-06-1987
		DE 3482445 D1	19-07-1990
		EP 0136900 A2	10-04-1985
		HK 21591 A	28-03-1991
		JP S6092750 A	24-05-1985
		JP S6259582 B2	11-12-1987
		MX 159125 A	20-04-1989
		NZ 209534 A	23-01-1987
		SG 90790 G	18-01-1991
		ZA 8407685 B	28-05-1986