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(54) Title: ANTIMICROBIAL COMPOSITION

(57) Abstract: An antiseptic composition suitable for use on skin and wounds comprising a source of an antimicrobial agent and an agent which disrupts biofilms.



WO 2007/068938 A2

Antimicrobial Composition

This invention relates to an antimicrobial composition which can be applied to skin, wounds, cuts, abrasions or burns for the prevention or treatment of infections. More particularly the invention relates to a composition capable of providing effective antimicrobial activity while at the same time avoiding wound and skin irritation and retardation of wound healing.

Overuse of antibiotics and the associated increase in bacterial resistance is impacting the efficacy of antibiotics in the treatment of wound infection. Effective alternatives to antibiotics are thus desirable.

Topical antimicrobial materials and preparations containing them have long been recognised as playing an important part in minimising the opportunity for skin and wound infections. Antiseptics are non-selective chemical agents that can be safe to use on living tissue. Molecular iodine, ionic silver and oxidising agents such as sodium hypochlorite and chlorine dioxide have been recognised as antiseptic agents with effectiveness against a wide range of micro-organisms. There are however several barriers to making an effective antimicrobial composition for application to wounds based on such agents. One problem is that these antiseptic agents tend to react with organic materials found in the wound other than the intended microbial targets. This means that to be effective, antiseptic agents need to be included in treatment compositions at high levels, which may cause undesirable side effects with prolonged use such as cell toxicity, hypersensitivity reactions, skin staining and systemic effects. Such side effects are further described in "In vitro cytotoxicity of silver: implication for clinical wound care". Poon VK, Burd A. Burns. 2004 Mar;30(2):140-7, "A review of iodine toxicity reports". Pennington JA. J Am Diet Assoc. 1990 Nov;90(11):1571-81 and "Topical antimicrobial toxicity".

Lineaweaver W, Howard R, Soucy D, McMorris S, Freeman J, Crain C, Robertson J, Rumley T. Arch Surg. 1985 Mar;120(3):267-70.

Wounds are often colonised by a variety of micro-organisms, some of
5 which may cause infection. It is increasingly recognised that microbial
populations living within a biofilm environment contribute to delayed
healing and infection. Biofilms are comprised of exopolymeric substances
that are produced by bacteria once they attach to a surface, and this helps
to protect micro-organisms from immune cells and antimicrobial agents.
10 Since efficacy of antimicrobial agents (e.g. antibiotics and antiseptics) is
compromised by the biofilm matrix, strategies to disrupt the biofilm and
expose micro-organisms within can be helpful in increasing the activity
level of antimicrobial agents and thus reducing the concentration of such
agents needed to make an effective composition.

15

Ethylenediaminetetra-acetic acid (EDTA) added as the di-sodium or
calcium di-sodium salts has been used to treat topical infections or to treat
hard surfaces such as catheters. WO03/047341 describes the use of
EDTA for example as an additive for a toothpaste. EDTA is also used as
20 a formulation agent to reduce the effects of water hardness and generally
as a chelating agent.

EDTA has been described in combination with other antibiotic agents for
example in US 5998488 it is used in combination with an antimicrobial
25 preservative in a solution for ophthalmic use. Formulations suitable for
topical use on wounds have not been proposed.

There is thus a need for a composition which has the benefits of an
antimicrobial agent but which reduces the potential of adverse reactions.

Suprisingly we have found that EDTA is capable of disrupting biofilms by chelating the metal ions, calcium and magnesium, that maintain the integrity of the biofilm matrix.

5 Accordingly a first aspect of the invention provides an antimicrobial composition suitable for use on skin and wounds comprising a source of antiseptic agent and an agent which disrupts biofilms such as EDTA.

The presence of EDTA enhances the effect of the antiseptic so that the
10 concentration of antiseptic agent may be reduced and yet still achieve effective antiseptis. By increasing the effectiveness of the antiseptic agent, its concentration in the composition can be reduced thereby reducing the potential for adverse reactions.

15 This aspect of the invention allows the preparation of compositions able to reduce the bacterial bioburden to a level manageable by the host within 24 hours using at least 50% less of the antiseptic agent, more preferably 50-60% less and ideally 65-85% less. This then allows the wound to progress towards healing and can be evidenced by a visual improvement
20 in the wound.

We have also found that it is possible to prepare a composition which includes EDTA which is effective under the conditions of pH normally found in a wound.

25

Accordingly a second aspect of the invention provides a composition suitable for use on wounds comprising di-, tri- and tetra-basic salts of EDTA at a pH of 4 to 8.

30 The compositions according to a first aspect of the invention comprise an antiseptic agent, preferably molecular iodine, ionic silver, chlorhexidine,

or hydrochloric acid or an oxidising agent such as sodium hypochlorite, chlorine dioxide, hydrogen peroxide or peroxy acid salts . The antiseptic agent is preferably included in the composition at a level of from 0.01% to 10% by weight, more preferably 0.1% to 5% by weight. Iodine is preferably included in the composition at a level of from 0.01% to 10% by weight and more preferably from 0.1% to 1.0% by weight. Preferably the iodine source is an iodide and the composition further comprises an oxidant and a buffer, the oxidant being held separately from the iodide until the point of use. The buffer is preferably capable of maintaining the pH of the composition at between pH4.5 and pH 6 so that iodine is generated at a physiologically acceptable and efficacious rate. Compositions comprising iodide and an oxidant held separately from the iodide are described further in EP1158859B.

Where iodide is present, the amount of oxidant in the composition is tailored to provide a stoichiometric match with iodide. Preferably the oxidant is iodate and is provided in a molar ratio of from 1:4 to 1:10 with iodide. In this way the iodide where present in the composition fully reacts with the oxidant. Iodide and Iodate are preferably present as sodium salts although other usual counter ions may be present.

Where the antiseptic agent is ionic silver it is preferably included in the composition at a level of from 0.1% to 10% by weight and more preferably 0.5% to 1.5%.

The pH of the composition is generally below 8 and preferably between 4 and 8, more preferably between 4 and 6 and most preferably between 4.5 and 5.5. The desired pH may be achieved by incorporating buffering agents in the composition. Examples of buffering agents which may be included are citric acid/di-sodium hydrogen phosphate, citric acid/sodium citrate, acetic acid/sodium acetate. The buffering agent may conveniently

be present in an amount of about 1% to 20% by weight of the composition preferably about 4% to 6% by weight and particularly about 5% by weight so as to provide an isotonic composition

5 EDTA is preferably present as the di-, tri- or tetra-basic salts of EDTA. We have found that these salts are effective for eradicating microorganisms in the free floating or planktonic state and biofilm state alone or in the presence of an antiseptic agent. For example we have found that EDTA at concentrations of 0.1-40% weight by volume was
10 effective in killing a range of microorganisms both in the planktonic and biofilm state. Microorganisms that were effectively killed by EDTA included *Pseudomonas aeruginosa*, *Serratia marcescens*, vancomycin resistant Enterococcus (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA).

15 EDTA is preferably present in the compositions of either aspect of the present invention at a level of 0.5% to 10% by weight of the composition, more preferably 1% to 3% by weight.

20 The compositions of the present invention may be in the form of a water based gel which maintains a moist wound healing environment and promotes healing. A gel gives the advantage of flow into the wound to form an intimate contact with the wound bed and provide antimicrobial effects to the whole wound. Preferably the gel has a high enough
25 viscosity that it does not flow out of wounds on areas of the body that are or become non-horizontal. Preferably the pH of the gel is buffered at around 5.5 as this does not alter the pH balance of the peri-wound tissue and therefore protects it.

30 The following examples are illustrative of the present invention.

Example 1Effect of EDTA on Iodine

- 5 Compositions containing iodine and EDTA were prepared by making a pair of aqueous gels which were intimately mixed at the point of use. Each gel was made by preparing an aqueous solution containing all of the appropriate water-soluble parts according to the formulations below and then adding a slurry of a non-ionic cellulosic viscosifier
- 10 (hydroxyethylcellulose) in propylene glycol.

Gel A

Component	% w/w
Water	To 100
Propan-1,2-diol	10
Hydroxyethylcellulose	3.86
Sodium iodate	0.16
Citric acid	3.99
di-sodium phosphate	15.06

15 Gel B

Component	% w/w
Water	To 100
Propan-1,2-diol	10
Hydroxyethylcellulose	4.14
Sodium iodide	0.59
Ethylenediaminetetraacetic acid tetra-sodium salt tetrahydrate	0.1-4.0
Phosphoric acid	As necessary to adjust aqueous

	phase to ph 5.5
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A commercially available cadexomer iodine ointment was present as a positive control as it contains 0.9% iodine. Intrasite is an amorphous hydrogel and was present as a negative control as it contains 0% iodine.

5

Method

9 ml of simulated wound fluid was added to a 17 ml volume cell well. A 1 ml culture of *Staphylococcus aureus* was added to each well to give a final culture concentration of 10⁶ cfu/ml. Controls for this experiment involved adding 2g of cadexomor iodine ointment (positive control) and hydrogel (negative control) separately to three cell wells each. 1g of gels A and B, with different concentrations of EDTA, were then added to separate cell wells (in triplicate). The cell wells containing the culture and gels were then shaken at 600 rpm at 35°C. After time intervals of 4, 24, 48, 72 and 96 hours a 0.1 ml test sample was taken from each well and placed into 9.9 ml MRD (maximum recovery diluent) containing 1% sodium thiosulphate. A 1 ml sample was then transferred to Tryptone Soy Agar plates and incubated for 48 hours. Bacterial counts were then recorded.

20

Results

Test composition	4 hours	24 hours	48 hours	72 hours	96 hours
Iodosorb(positive control)	10	10	10	10	10
Intrasite(negative control)	7600000	310000000	530000000	1000000000	14000000
0%I ₂ 0%EDTA confirm data?	7600000	310000000	530000000	1000000000	14000000

0.3%I ₂ 0%EDTA	1240000	1000000	1000000	10000000	100000000
0.3%I ₂ 0%EDTA	1010000	1000000	1000000	10000000	100000000
0.3%I ₂ 0%EDTA	1670000	1000000	1000000	10000000	100000000
0.3%I ₂ 0.5%EDTA	1500000	1000000	1000000	1000000	1000000
0.3%I ₂ 0.5%EDTA	92000	10	10	10	10
0.3%I ₂ 0.5%EDTA	58000	10	10	10	10
Test composition	4 hours	24 hours	48 hours	72 hours	96 hours
0.3%I ₂ 1%EDTA	8100	10	10	10	10
0.3%I ₂ 1%EDTA	130000	10	10	10	10
0.3%I ₂ 1%EDTA	110000	10	10	10	10
0.3%I ₂ 1.75%EDTA	710	10	10	10	10
0.3%I ₂ 1.75%EDTA	4400	10	10	10	10
0.3%I ₂ 1.75%EDTA	31000	10	10	10	10
0.3%I ₂ 2.5%EDTA	8500	10	10	10	10
0.3%I ₂ 2.5%EDTA	10600	10	10	10	10
0.3%I ₂ 2.5%EDTA	6200	16000	10	10	10

0.3%I ₂ 4%EDTA	93000	10	10	10	10
0.3%I ₂ 4%EDTA	8500	10	10	10	10
0.3%I ₂ 4%EDTA		1400	10	10	10

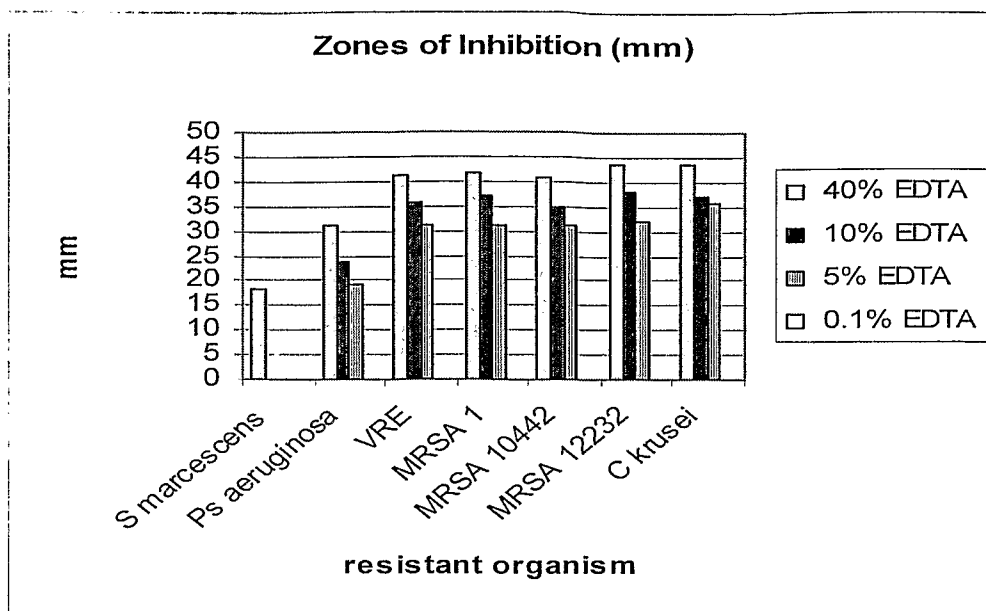
These results are shown graphically in Figure 1.

5 These results show by adding as little as 0.5% EDTA (calculated as the di-sodium salt) to iodine the efficacy of iodine, at 0.3%, is enhanced when compared to the control of 0.3% iodine with no EDTA. Clearly from the results EDTA enhances the effects of iodine within 24 hours to the same efficacy of 0.9% iodine (positive control).

10 Example 2

Zones of inhibition with tetra sodium EDTA

15 Seven antibiotic resistant microorganisms were used to evaluate the efficacy of EDTA in killing bacteria and yeasts grown on agar. For this experiment filter paper discs were soaked in EDTA at concentrations ranging from 0.1-40%. EDTA was made up by dissolving it in an appropriate amount of sterile double distilled water. The filters were then added onto Muller Hinton agar which had been inoculated with a
 20 microorganism under study for 24 hours at 35°C. All microorganisms were tested twice.



These results show that the “zones of inhibition” ranged from 20 to 43mm (including disc 13mm). Zones were higher with increasing levels of EDTA indicating that EDTA alone is an effective antiseptic agent.

Example 3

Zones of inhibition with EDTA

10

Poloxamer F127 hydrogels (Univar, Basildon, Essex, UK) are di-block co-polymers of polyoxyethylene and polyoxypropylene that demonstrate thermo-reversible gelation properties. At temperatures below 15°C, poloxamer is liquid and fully miscible with water but changes to a firm gel at temperatures in excess of 15°C. Poloxamer encourages bacteria to exhibit a more clinically relevant biofilm phenotype. Gilbert *et al* determined that *P. aeruginosa* cells grown on poloxamer hydrogel (biofilm form) express outer membrane proteins between 78 and 87 kDa, which are not evident in cells grown on standard nutrient agar (‘planktonic’) (Gilbert et al., 1998). Consequently poloxamer gel cultures

20

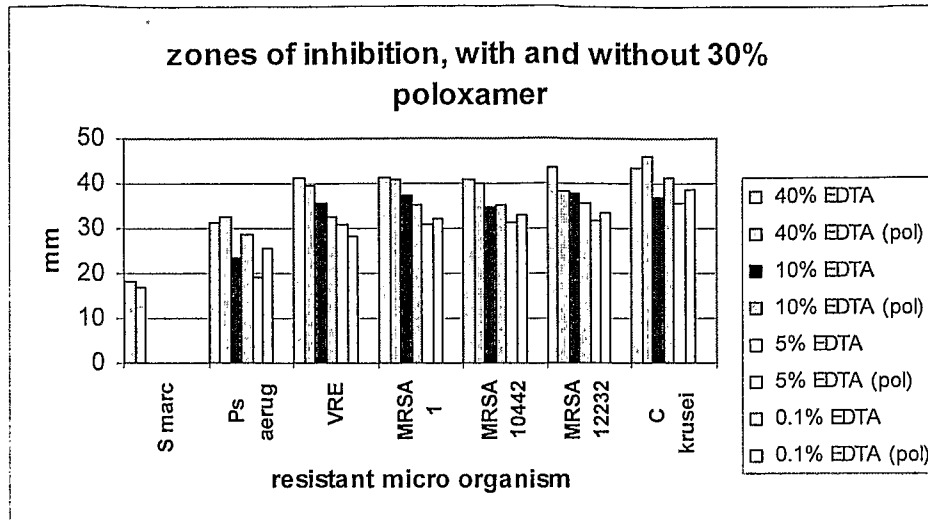
mimic many of the properties of biofilm-grown *P. aeruginosa* (Gilbert et al., 1998). This indicates that there is a phenotypic difference between *P. aeruginosa* cells grown on poloxamer hydrogel and nutrient agar, with only poloxamer grown cells resembling biofilm cells. It was found from
5 Wirtanen's study (1998) that bacteria which are grown in poloxamer have biofilm properties and associated enhanced biocide resistance. Gilbert and colleagues suggested that bacteria grown in poloxamer hydrogels could be exposed to biocides to provide a reproducible method for testing the antimicrobial efficacy of biocides against biofilm bacteria (Gilbert et al.,
10 1998).

Gilbert P, Jones MV, Allison DG, Heys S, Maira T, Wood P. The use of poloxamer hydrogels for the assessment of biofilm susceptibility towards biocide treatments. *Journal of Applied Microbiology* 1998;85:985-990.

15 Wirtanen G, Salo S, Allison DG, Mattila-Sandholm T, Gilbert P. Performance evaluation of disinfectant formulations using poloxamer-hydrogel biofilm-constructs. *Journal of Applied Microbiology* 1998; 85:965-971.

20 Seven antibiotic resistant microorganisms were used to evaluate the efficacy of EDTA in killing bacteria and yeasts grown on poloxamer gel (biofilm state). For this experiment filter paper discs were soaked in EDTA at concentrations ranging from 0.1-40%. In this present study poloxamer F127, a di-block copolymer of polyoxyethylene and
25 polyoxypropylene, was used as a medium on which bacteria could be grown as a biofilm phenotype and express the characteristics more appropriate to the 'real world'. The filters were then added onto Muller Hinton agar which had been inoculated with the microorganism under study for 24 hours at 35°C. All plates were done in duplicate.

30



These results show that the zones of inhibition were slightly smaller in the presence of poloxamer indicating that bacteria/yeasts growing as biofilms are physically more resistant to EDTA when compared to their planktonic counterparts. *C. krusei* and *Ps aeruginosa* were the exception with larger zones with poloxamer. Zones of inhibition were evident on all organisms tested at 40% EDTA. At 10% and 5% EDTA there were no zones with *Serratia marcescens*. At 0.1% EDTA no zones of inhibition were evident with any organism studied indicating that EDTA is not effective at this level.

Example 4

15 Minimum inhibitory concentrations (MIC's) and Minimum Bactericidal Concentrations (MBC's) with Tetra, Tri and Di basic salts of EDTA

Method: A microtitre plate and optical density readings and visual inspection were performed to obtain the MIC's for a variety of microorganisms (see Figure 2). Concentrations of EDTA included in this study were 40mg/ml pH 10.00 as tetra-Na, 40mg/ml as tri-Na pH 6.84, 40 mg/ml as di-Na pH 5.50. Into each microtitre plate, 100 µl of

inoculum and EDTA was added. The plate was then incubated for 24 hours at $35^{\circ}\text{C} \pm 3^{\circ}\text{C}$. Following incubation all microtitre plates were inspected visually for growth.

5 The results are presented in Figure 2.

In general all MIC's recorded were equivalent for all the salts of EDTA studied. This suggests that the pH of both solutions is equivalent. Therefore, the activity of EDTA is not affected by the salt form added to
10 the microtitre plate. These results show that low concentrations of EDTA are very effective on bacteria.

Example 5The effect of EDTA on the antimicrobial efficacy of silver containing wound dressings

5

The antimicrobial dressings used in this study were Acticoat™ (Smith and Nephew) and AQUACEL® Ag Hydrofiber® (ConvaTec). Acticoat™ is a nanocrystalline silver antimicrobial barrier dressing which consists of a rayon/polyester non-woven inner core laminated between two layers of silver-coated high density polyethylene mesh (HDPE). The layers are held together with ultrasound welds. AQUACEL® Ag is comprised of sodium carboxymethylcellulose Hydrofiber® and ionic silver. The silver cations in AQUACEL® Ag are associated with the individual highly absorbent anionic carboxymethylcellulose fibres of the Hydrofiber® dressing. AQUACEL® Hydrofiber® dressing (without silver) was also used as a control.

Method: All dressings (AQUACEL Hydrofiber [control – without silver], AQUACEL Ag Hydrofiber and Acticoat (nanocrystalline dressing) were hydrated with 20 mg/ml of tetra-Na EDTA. All tests were performed against *Ps aeruginosa* and tested on Mueller Hinton agar (MHA) and Poloxamer gel (incorporating Mueller Hinton broth (MHB). This involved inoculating either a MH agar plate or poloxamer gel plate with a specific isolate and then adding an appropriate hydrated (to saturation point) wound dressing (360 μ l (MRD) for AQUACEL and AQUACEL Ag and 150 μ l (sterile distilled water- as per manufacturers instructions) for Acticoat). The plates were then incubated at 35°C \pm 3°C for 24 hours after which the zone of clearance (no growth) around the dressing was measured. Zones of inhibition were measured horizontally and vertically (inclusive of the dressing sample) and a mean value was calculated from the duplicate set of results. The mean dressing size was then subtracted

from the mean zone of inhibition to determine the corrected zone of inhibition (CZOI). A CZOI test allows for any inherent variability in the shape and size of zones created by the silver dressings which may change in dimension following hydration.

5

Table to show the size of corrected zone of inhibition (CZOI) in mm on MH agar and poloxamer gel

Hydrating Fluid	MHA (Non-biofilm bacteria)			Poloxamer gel with MHB (biofilm bacteria)		
	AQUACEL	AQUACEL Ag	Acticoat	AQUACEL	AQUACEL Ag	Acticoat
EDTA	0	16	10	6	6	7
MRD	0	18	N/A	0	3	N/A
Water	N/A	N/A	7	N/A	N/A	3

10 **Conclusion:** When EDTA was added to AQUACEL no zones of inhibition were observed on MHA (non-biofilm state). However, ZOI's were observed around AQUACEL Ag on MHA (non-biofilm bacteria) when EDTA or (maximal recovery diluent) MRD was added indicating the antimicrobial activity of silver. Larger ZOI's were observed around
 15 Acticoat following the addition of EDTA when compared to hydration with water. In the presence of poloxamer gel an increase in the CZOI was observed following hydration of AQUACEL Ag with MRD when compared to EDTA indicating an additive effect with the use of EDTA. Overall the results showed that EDTA enhances the effects of ionic silver
 20 on bacteria grown in the biofilm state (poloxamer gel). Overall these results suggest that by using an EDTA dressing it is effective against both planktonic and biofilm microorganisms because AQUACEL alone is not effective when the microorganisms are in the planktonic state.

Table to show the size of corrected zone of inhibition in mm on MH agar and poloxamer gel

Hydrating fluid	MHA (non-biofilm bacteria)			Aquacel	Poloxamer gel with MHB (biofilm bacteria)	
	Aquacel	Aquacel Ag	Nanocrystalline silver dressing		Aquacel Ag	Nanocrystalline silver dressing
EDTA	0	15.99	9.57	6.28	5.96	6.73
MRD	0	18.04	N/A	0	3.42	N/A
Water	N/A	N/A	6.74	N/A	N/A	2.82

5

Example 6

Minimum Inhibitory Concentrations (MIC) for a two component gel comprising iodine and a two component gel as detailed in Example 1 containing EDTA (diNa EDTA, triNa EDTA and tetraNa EDTA)

10

This experiment shows the effect of pH on the activity of a range of EDTA forms.

15 For this experiment a two component gel as detailed in Example 1 was made without the HEC component (gel). This experiment was therefore performed using only liquid compositions in order for MIC's to be calculated. The pH of the TCG/EDTA solutions was approx 5.5. The organisms tested included: *Staphylococcus aureus*, *Pseudomonas*
20 *aeruginosa*, *Escherichia coli* (repeated twice) and *C albicans*.

TCG Solution (A)

Component	% w/w
Water	To 100
Sodium iodate	0.1
Citric acid	3.99
di-sodium phosphate	15.06

TCG Solution (B)

Component	% w/w
Water	To 100
Sodium iodide	0.4
Ethylenediaminetetraacetic acid tetra-sodium salt tetrahydrate	As shown in Table 1
Phosphoric acid	As necessary to adjust aqueous phase to pH 5.5

5

MIC's for TCG solution and TCG solution combined with EDTA (values in brackets are EDTA concentrations in mg/ml)

Bacterium	TCG solution*	TCG EDTA	TCG & tri Na EDTA	TCG& tetra Na EDTA
<i>S aureus</i>	0.25 (0)	0.008 (0.31)	0.008(0.31)	0.008 (0.31)
<i>Ps aeruginosa</i>	0.5 (0)	0.062 (2.5)	0.062 (2.5)	0.062 (2.5)
<i>E coli</i>	0.25 (0)	0.031 (1.25)	0.031 (1.25)	0.031 (1.25)

10 * (working concentration was 0.2% iodine)

Conclusion

MIC's were reduced considerably in the presence of EDTA (di, tri and tetra Na). It can be seen that the MICs, expressed as Iodine concentration, are lower in the presence of di, tri and tetra sodium
5 EDTA.

The antimicrobial benefit of having EDTA present is achieved at concentrations of 0.31 mg/ml. Despite different forms of EDTA being known to provide differential antimicrobial efficacy, at a constant pH (5.5
10 in this case) all EDTA forms were equally effective in significantly reducing the MIC's for all three organisms compared to be iodine generating solution without EDTA.

Claims

1. An antiseptic composition suitable for use on skin and wounds comprising a source of an antimicrobial agent and an agent which disrupts
5 biofilms.
2. An antiseptic composition as claimed in claim 1 characterised in that the agent which disrupts biofilms is EDTA.
- 10 3. An antiseptic composition suitable for use on skin and wounds as claimed in claim 1 or claim 2 characterised in that the antimicrobial agent is selected from the group of iodine, ionic silver or an oxidising agent such as sodium hypochlorite or chlorine dioxide.
- 15 4. A composition suitable for use on wounds comprising di-, tri- and tetra-basic salts of EDTA at a pH of 4 to 8.
5. A composition as claimed in claims 1 to 3 characterised in that the composition has a pH of between 4 and 8.
20
6. A composition as claimed in claims 1 to 4 characterised in that the composition has a pH of between 4.5 and 5.5.
7. A composition as claimed in any preceding claim in the form of a
25 gel.
8. A composition as claimed in any preceding claim characterised in that the composition comprises iodine.
- 30 9. A composition as claimed in any preceding claim characterised in that the composition comprises an iodine source, an oxidant and a buffer

wherein the iodine is held separately from the oxidant until the point of use.

10. A composition as claimed in claim 9 characterised in that the
5 composition is capable of generating from 5microg of iodine per g of composition per hour to 1500microg of iodine per g of composition per hour.

11. A composition as claimed in claims 1 to 6 characterised in that the
10 composition comprises ionic silver.

12. A composition as claimed in claim 11 characterised in that the composition comprises from 0.1% to 10% by weight and more preferably 0.5% to 1.5% by weight of ionic silver.

15

13. A method of making an antiseptic composition as claimed in claim 4 comprising the steps of adding EDTA as a tetrasodium salt in aqueous solution and then adjusting the pH of the composition to pH 4 to 8.

20 14. Use of an antiseptic composition in the manufacture of a medicament suit comprising an antimicrobial agent and an agent which disrupts biofilms for use in the treatment of chronic wounds and burns.

25 15. Use of an antiseptic composition as claimed in claim 14 characterised in that the agent which disrupts biofilms is EDTA.

16. Use of an antiseptic composition as claimed in claim 14 or claim
15 characterised in that the antimicrobial agent is selected from the group of iodine, ionic silver or an oxidising agent such as sodium
30 hypochlorite or chlorine dioxide.

Figure 1
Antimicrobial activity of a two component iodine generating solution with and without EDTA against *Staphylococcus aureus*

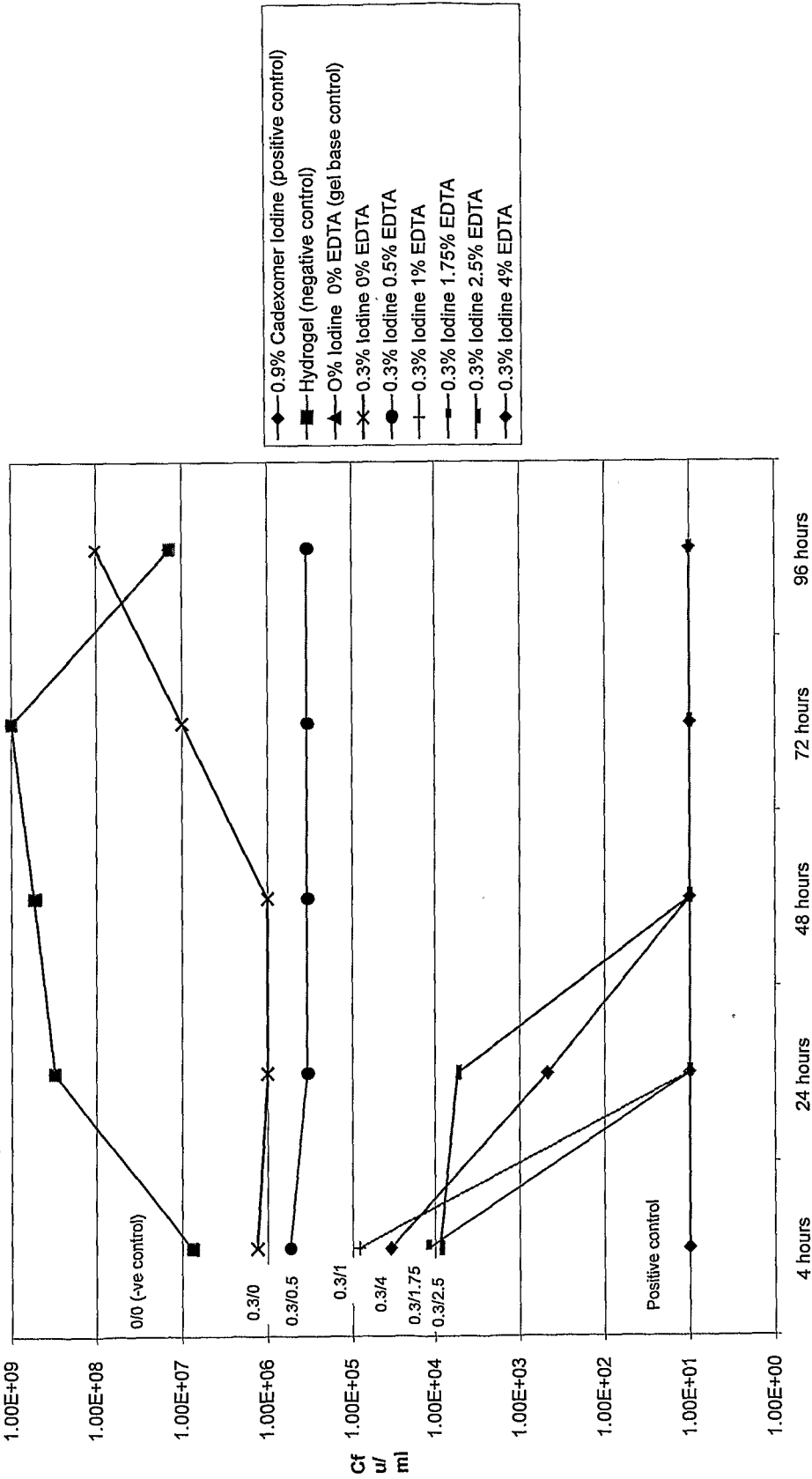


Figure 2
Appendix A: MICs* for di, tri and tetra EDTA

Micro organism	identification	inoculum, cfu	MICs		
			di Na	tri Na	tetra Na
<i>S marcescens</i>	multi res	5.00E+04	0.938	1.875	0.938
<i>Ps aeruginosa</i>	NCTC 8506 (res)	6.75E+04	0.234	0.234	0.234
<i>Ps aeruginosa</i>	NCIMB 8626	7.00E+04	0.938	0.938	0.938
<i>E coli</i>	NCIMB 8545	5.50E+04	0.469	0.469	0.469
<i>E coli</i>	NCIMB 10544	<5.0+04	0.469	0.938	0.469
<i>Kl pneumoniae</i>	033 clinical isolate	<5.0+E4	0.938	0.938	1.875
<i>Ent cloacae</i>	166 clinical isolate	5.00E+04	1.875	0.938	0.938
<i>Pr mirabilis</i>	NCTC 9559	1.50E+05	0.469	0.469	0.469
<i>A baumannii</i>	NCIMB 9214	4.40E+04	0.234	0.234	0.469
<i>S aureus</i>	NCIMB 9518	4.00E+04	0.234	0.234	0.234
MRSA	1 Cardiff PHL	5.50E+04	0.117	0.469	0.234
MRSA	2 Cardiff PHL	5.75E+04	0.234	0.469	0.234
MRSA	26	1.25E+04	0.234	0.234	0.234
MRSA	NCTC 12232	5.00E+04	0.234	0.234	0.469
MRSA	NCTC 10442	6.50E+04	0.234	0.234	0.234
MRSA	103731 Chester PHL	6.00E+04	0.234	0.117	0.234
<i>Ent faecalis</i>	141 clinical isolate	2.50E+04	0.234	0.234	0.234
VRE	1 Cardiff PHL	2.75E+04	0.234	0.469	0.234
VRE	2 Cardiff PHL	3.25E+04	0.234	0.234	0.234
VRE	NCTC 12201	6.00E+04	0.234	0.234	0.234
<i>Strep pyogenes</i>	NCTC 8198	7.00E+04	0.234	0.469	0.469
<i>B subtilis</i>	NCTC 3610	3.00E+04	0.234	0.234	0.234
<i>C krusei</i>	NCPF 3876 (res)	1.50E+04	0.938	0.938	0.938
<i>B fragilis</i>	NCIMB 9343	1.90E+05	0.117	0.234	0.234
<i>Cl perfringens</i>	362 clinical isolate	9.00E+03	0.117	<0.117	<0.117
<i>Pep anaerobius</i>	NCTC 11460	1.30E+06	0.234	0.469	0.469