Investigations into methods to improve the antibacterial activity of Acticoat

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Multiple studies have shown that the antibacterial dressing Acticoat can inhibit growth of bacteria but is unable to completely clear a wound of infection, which could leave patients vulnerable to sepsis. Agar inoculated with four different Staphylococcus aureus strains and overlain with Acticoat showed growth inhibition beneath and within a 1 mm perimeter of the dressing after 24 h. When lifted from inoculated agar and briefly blotted onto fresh agar plates, Acticoat transferred viable bacteria. Scanning electron microscopy of the surface of Acticoat that overlaid meticillin-resistant S. aureus for 24, 48 and 72 h showed dense clusters of apparently undamaged bacteria distributed across the mesh. The number of bacteria growing on inoculated pig skin, underneath and on the surface of Acticoat, was lower than on controls for the first 8 h, but after 24 h the number of bacteria on the skin was 2.3-fold greater than the untreated controls. In contrast, after 24 h the number of bacteria surviving on the surface of the Acticoat was 11.9 % of controls. Acticoat moistened with 10 % glycerol plus antimicrobial peptides (AMPs) mel12–26 or bac8c (50 μg ml⁻¹) reduced the numbers of bacteria on the dressing and on the skin underneath to below 10 % and 0.01 % of the controls, respectively. When lysozyme (1 mg ml⁻¹) was added to Acticoat wetted with glycerol and the AMP bac8c, the dressing was able to prevent the survival of bacteria on densely inoculated pig skin and on the surface of Acticoat for up to 24 h. In effect, biocompatible solvents and AMPs significantly enhance the bactericidal efficacy of Acticoat.

INTRODUCTION

Advances in nanotechnology and materials science have allowed the development of wound dressings that absorb exudate, minimize blood loss, allow gas exchange and provide a scaffold to augment healing, whilst also containing bioactive components such as nanoparticle silver (Stashak et al., 2004; Church et al., 2006). The antimicrobial effects of silver have been known for millennia and have been used in many applications, such as drinking vessels, eating utensils and surgical equipment, to preserve the health of the user or patient (Alexander, 2009).

Silver has both intracellular and extracellular antimicrobial effects, but the exact mechanisms have not been fully elucidated (Feng et al., 2000; Anas et al., 2013). The main property of silver that confers antibacterial activity appears to be its ability to bind weak bases such as sulphur and phosphate (Prabhu & Poulose, 2012), disrupting intramolecular disulphide bonds within proteins, which are often crucial for their stability, secondary structure formation and activity (Betz, 1993, Morones-Ramirez et al., 2013). Many proteins, especially extracellular proteins with β-sheet structures, require disulphide bonds to maintain their functional conformation. Cleavage of structurally important disulphide bonds allows silver to disrupt multiple functions in bacteria, including membrane integrity and metabolic processes (Prabhu & Poulose, 2012; Morones-Ramirez et al., 2013). A similar mechanism has been demonstrated in dehydratase enzymes which use the [4Fe–4S] cluster as an active site. When these enzymes are exposed to silver, a mis-metallation reaction occurs which removes iron from the cluster and inactivates the enzyme (Xu & Imlay, 2012). Silver therapy is also believed to exploit the sensitivity of bacteria to reactive oxygen species, by disabling superoxide dismutase and catalase enzymes, leading to an increase in the concentration of reactive oxygen species in the cell (Park et al., 2009; Prabhu & Poulose, 2012). Silver has also been shown to bind DNA and, depending on the concentration of silver, cause a structural transition to one of three denatured, bio-inactive states (Arakawa et al., 2001).

The use of silver nanoparticle-loaded dressings, such as Acticoat, has now become commonplace for the prevention...
of infection in burn patients and those with open wounds (Fong & Wood, 2006; Department of Health, 2011; Wounds International, 2012). However, the effectiveness of these devices in preventing infection of the vulnerable burn eschar is contentious (Supp et al., 2005; Ukkur et al., 2005; Fong & Wood, 2006). The antibacterial activity of these dressings may be augmented by combining treatment with other topical antibacterial compounds. One possibility is application of the dressings with antimicrobial peptides (AMPs). AMPs are a structurally diverse class of proteins that are integral to the innate defence systems of a wide array of organisms (Wiesner & Vilcinskas, 2010; Pushpanathan et al., 2013). Most AMPs are multifunctional and generally perform roles linked to immunity, such as chemotraction of immune cells and wound healing (Wiesner & Vilcinskas, 2010). As host defence molecules, AMPs can protect against bacterial pathogens by forming transmembrane pores in the phospholipid membranes and killing the cell by lysis or by targeting intracellular organelles (Wiesner & Vilcinskas, 2010; Seo et al., 2012; Pushpanathan et al., 2013).

Multiple studies have challenged the therapeutic effectiveness of an AMP-based drug delivered systemically (Hamamoto et al., 2002; Rozek et al., 2003; Matuszaki, 2009). However, the rapid, potent and broad-spectrum activity of these peptides makes them attractive candidates for topical treatment of bacterial infections. It has been demonstrated that both silver and some AMPs have an influence on cutaneous wound healing, and may also protect cells against viral infection (Tian et al., 2007). The nanoparticle-silver-loaded dressing Acticoat is commonly used to protect patients with significant burns against bacterial infection, prior to eschar excision and skin grafting. Multiple studies have demonstrated that Acticoat is able to decrease the bacterial load in the wound and reduce patient discomfort (Fong & Wood, 2006). However, a recent consensus from medical practitioners has concluded that the wound-healing ability of silver-loaded dressings is negligible (Wounds International, 2012). Animal studies have also shown that Acticoat and similar dressings may inhibit bacterial growth, but are not effective at eliminating pathogenic bacteria from topical wounds (Supp et al., 2005; Ukkur et al., 2005; Fong & Wood, 2006).

Patients with deep wounds, compromised immune systems or who have recalcitrant infections may have limited benefit from Acticoat treatment, achieving reduction but not elimination of the bacterial load. This study aimed to evaluate the efficacy of Acticoat against meticillin-resistant *Staphylococcus aureus* (MRSA) and to investigate additives that might improve the antibacterial efficacy of the dressing. The non-haemolytic AMPs mel12–26 (a truncated analogue of the honeybee venom peptide melitin) (Yan et al., 2003) and bac8c (a truncated and modified bovine neutrophil peptide) (Spindler et al., 2011) were selected to test the effectiveness of Acticoat when used with AMPs.

**METHODS**

**Bacterial strains and growth conditions.** *S. aureus* strains were provided by the Australian Collaborating Centre for *Enterococcus* and *Staphylococcus* Species (ACCESS) Typing and Research, Faculty of Health Sciences, School of Biomedical Sciences, Curtin University, Perth, Western Australia, Australia. The MRSA strains used in this study were: Au53 (a mercuric acetate- and mercuric chloride-resistant clone of ST239-MRSA-III; Coombs et al., 2007), Bengal bay (ST772-MRSA-V origin; Coombs et al., 2012a) and WBG 8287 (genetic lineage ST1-Iva; Coombs et al., 2007), and the meticillin-sensitive *S. aureus* strain W175 (ST93-MSSA lineage (Coombs et al., 2012b)). Bacteria were cultured in bactopeptone medium (25 g l⁻¹; Oxoid), pH 7.4 and incubated at 37 °C with orbital shaking at 200 r.p.m.

**AMPs.** Peptides bac8c (RIWVIWRR-OH; Spindler et al., 2011) and mel12–26 (GLPALISWIKRRQ-OH; Yan et al., 2003) were synthesized in l-isomer form, 95 % purity as determined by MS (Mimotopes).

**Pig skin preparation.** Pig skin, which included the epidermis, dermis and subdermal fat layer, was purchased from a local abattoir. The fat layer was cut from the dermis and the skin was cut into 2 cm × 2 cm squares. The skin was sterilized by immersion in 70 % ethanol for 1 h, washed by 3 × 5 min immersions in high-purity H₂O and allowed to dry for 15 min under UVC bactericidal lights. The skin was placed in molten bactopeptone agar 2 mm deep, leaving the epithelial surface above the gel, to immobilize and rehydrate the sterilized tissue. The plates were stored at 4 °C.

**Analysis of bacterial growth inhibition on agar medium by Acticoat.** Acticoat (Smith & Nephew) was generously donated by the Fiona Woods Foundation, Fiona Stanley Hospital, Perth, Western Australia, Australia. The polyethylene Acticoat mesh, coated with silver nanoparticles, was separated from the inner absorbent lining and cut into rectangles (1 cm × 0.5 cm) using a scalpel and forceps. Agar plates, containing 1.5 % (w/v) bacteriological grade agar (VWR) in bactopeptone medium (25 g l⁻¹) were spread with 2.5 ml PBS in which a single overnight colony of WBG 8287 was dispersed evenly by rocking. Excess medium was removed and the surface was allowed to dry for 10–15 min. Acticoat strips were placed on the agar and the plates were incubated for 24 h at 37 °C. Grade 4 cellulose filter paper (GE Healthcare) strips 1 cm × 0.5 cm were placed on the inoculated agar as bactericidal negative controls. After incubation at 37 °C for 24 h, the zone of bacterial growth inhibition around Acticoat and paper strips was measured. In subsequent experiments, the agar was set under Textured sterile moulds to create striated, nodulated or creased surfaces.

**Scanning electron microscopy (SEM) examination of Acticoat after contact with MRSA-inoculated agar.** Bacteria (WBG 8287) were seeded on bactopeptone agar plates with flat or textured surfaces. Acticoat, cut to 1 cm × 1 cm squares, was placed on the inoculated agar and the plates were incubated at 37 °C. After 24 h the Acticoat was removed from the agar and immersed in 1 ml PBS for 1 min to remove media components. The Acticoat strips were immersed in 2.5 % glutaraldehyde (Asia Pacific Speciality Laboratory Chemicals) supplemented with 75 mM lysine (Sigma-Aldrich) and incubated at 37 °C for 10 min. Lysine was included to allow the formation of stabilizing links between carbohydrate molecules in MRSA biofilm and to preserve its structure during dehydration (Ratnayake et al., 2012). The Acticoat strips were washed with high-purity H₂O to remove lysine and glutaraldehyde, and the bacteria attached to Acticoat were fixed for a further 24 h in 3 % glutaraldehyde at room temperature. The strips were immersed briefly in high-purity H₂O, and dehydrated by immersion in 50, 70, 80, 90 and 100 % ethanol for 5 min each. The Acticoat strips were frozen by
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Measuring bacterial survival on Acticoat. Bactopeptone agar plates with flat or textured agar surfaces were inoculated with WBG 8287 and overlain with Acticoat or filter paper and incubated for 24, 48 or 72 h, as described earlier. After incubation, the strips were lifted and placed on fresh bactopeptone agar for 5 min. The strips were then removed and the plates incubated at 37 °C for 24 h. The ability of MRSA to survive contact with and be transferred to fresh surfaces by Acticoat was assessed by the growth observed on the inoculated and ‘blotted’ agar surfaces, respectively.

Similar experiments were conducted with cells inoculated onto agar-immobilized pig skin. A WBG 8287 suspension (2 μl, 10^6 c.f.u. ml^-1 in PBS) was placed on the centre of each square of skin and incubated at 37 °C for 30 min. Acticoat strips were then applied to each square of skin, and the survival of bacteria on the skin and the Acticoat was measured after 24, 48 and 72 h, as described earlier.

Coating Acticoat with agar or agarose. Molten agarose or bacteriological agar (200 μl) containing Triton X-100 (0.1 % (v/v) in high-purity H_2O; Ajax) was cast in a circular 1.9 cm^2 well. An Acticoat strip was placed on the surface of the gel, together with 20 μl fusidic acid (100 mg ml^-1) or the AMP bac8c (50 μg ml^-1). An additional 200 μl agarose or agar with Triton X-100 was added to the well. A 10 ml semi-automatic pipette with a tip cut to create an opening of ~ 1.9 cm^2 was used to withdraw the Acticoat gel discs from the plates and place them on WBG 8287-inoculated pig skin. Bacterial transfer and survival was measured after 24 h incubation, as described earlier.

Measurement of MRSA survival after Acticoat exposure. Acticoat was overlain for 10 s on pig skin, which had been inoculated with 2 μl WBG 8287 at 10^6 c.f.u. ml^-1, then removed and transferred to a 5 ml tube containing 1 ml PBS. The skin was also transferred to a tube containing 1 ml PBS. The tubes were agitated by vortexing for 30 s, and the resulting bacterial suspension was spread on bactopeptone agar plates, incubated for 24 h and colonies counted. Survival was expressed as a percentage of colonies from a control skin sample without Acticoat exposure. The process was repeated with Acticoat exposure for 0.5, 1, 4, 8 and 24 h.

Matching experiments were performed with Acticoat which had been soaked with solutions of wetting agents or AMPs. Samples of pig skin were inoculated with 2 μl WBG 8287 at 10^6 c.f.u. ml^-1. Acticoat strips were brushed with 10 % (v/v) glycerol, with or without mel12–26 or bac8c (50 μg ml^-1) and bacterial survival was measured after 24 h incubation at 37 °C. This procedure was repeated using (1) a lysozyme solution (1 mg ml^-1) with or without the AMPs and (2) a combination of 10 % (v/v) glycerol plus lysozyme (1 mg ml^-1) with or without the AMPs.

Erythrocyte isolation and haemolysis assay. Whole blood (15 ml) was drawn from the median cubital vein of a 26-year-old male volunteer and stored at 4 °C in Vacutainer vessels (BD Biosciences). Erythrocytes were separated by centrifuging at 500 g for 5 min at 4 °C. The supernatant was removed, and erythrocytes were washed with 10 ml ice-cold PBS and recentrifuged. The supernatant was removed and the erythrocytes resuspended in 10 ml PBS.

Erythrocytes were suspended as triplicate samples at 10^8 cells ml^-1 in PBS with 10 % (v/v) glycerol, lysozyme (1 mg ml^-1) and either mel12–26 or bac8c (50 μg ml^-1). Triton X-100 (1 % (v/v) in H_2O) was used as a positive control for haemolysis. The samples were incubated at 37 °C for 30 min with 150 r.p.m. orbital shaking and then centrifuged at 500 g. The A_560 of the supernatant was measured using a Victor Multilabel plate reader.

RESULTS

Exposure of MRSA to Acticoat on agar medium

The inhibitory effects of Acticoat against four different S. aureus strains were investigated by overlaying Acticoat

![Fig. 1. Acticoat treatment of MRSA on textured agar plates. (a–d) WBG 8287 populations were spread on agar plates with flat (a), striated (b), nodulated (c) and creased (d) surfaces. Strips of Acticoat (AC) and sterile filter paper (FP) were placed onto the inoculated surface for 24, 48 and 72 h (columns left to right, respectively). (e–h) After incubation the strips were lifted carefully from the plate and placed briefly onto fresh agar plates which were incubated for 24 h. Columns show triplicates of each test sample.](http://jmm.microbiologyresearch.org)
strips on inoculated agar plates. The effect of surface topography on the inhibitory activity of Acticoat was also examined by casting different textures onto the surface of agar plates. For 24 h, bacterial growth on MRSA-inoculated flat agar was suppressed beneath the Acticoat and within a zone ~1 mm wide around the periphery (data not shown). Plates with striated or creased textures (Fig. 1b, d) showed similar zones of inhibition to that of flat-textured agar (Fig. 1a). On the most heavily textured surface (nodulated agar), significant growth of bacteria was observed beneath the Acticoat, in regions that were not in direct contact with the dressing (Fig. 1c). After incubation on inoculated plates for 24, 48 and 72 h, Acticoat and filter paper strips transferred to fresh agar plates for 10 s resulted in the transfer of viable bacteria (Fig. 1e–h). Although the amount of bacteria transferred was not directly quantified, it appeared that Acticoat in contact with the inoculated plate for 3 days generally carried more bacteria than those removed at days 1 and 2. However, the relationship between exposure time and the extent of transference was not clearly defined. Despite the visible bacterial growth under Acticoat on nodulated agar surfaces, Acticoat lifted from those plates was shown to transfer fewer bacteria than from other textures (Fig. 1g). Acticoat lifted from the creased agar transferred the greatest number of bacteria (Fig. 1h).

**Examination of Acticoat and attached bacteria by SEM**

The surface of Acticoat that had been overlaid on MRSA-inoculated agar was examined by SEM to identify regions of the bandage where the bacteria were most likely to localize and whether the surface of bacteria attached to Acticoat showed any damage. Microscopically, Acticoat showed a grainy texture with dimples spaced across its surface (Fig. 2b), and its larger structure consisted of a network of raised nodes each connected to six other nodes by a lattice of connecting arms (Fig. 2a). Results from placing Acticoat on WBG 8287 on a flat surface are shown in
Fig. 2(c, d, g, h, k, l). Results from striated agar are shown in Fig. 2(e, f, i, j, m, n). Over 3 days’ incubation, the Acticoat on flat agar surfaces accumulated bacterial clusters one to five layers deep (Fig. 2f, j, i). Acticoat from both flat and striated agar surfaces showed dense clusters of cells on the nodes of the mesh (Fig. 2f, j). Although growth was sporadic across the dressing, fewer bacteria were attached to the surface of the connecting lattice than to the nodes. In some cases, accumulated bacteria were sufficiently dense to be observed at relatively low magnification (Fig. 2e, g, m). At higher magnification, bacteria showed little or no surface damage (Fig. 2d, h, j), with just a few individual cells showing disruption to their structure (Fig. 2d). The density of cells in bacterial clusters did not clearly correlate with the length of time Acticoat was in contact with the culture plate.

**Exposure of MRSA on pig skin to Acticoat**

Pig skin was used as a more clinically relevant medium to test the ability of MRSA to attach and survive on the surface of Acticoat. Acticoat strips from inoculated pig skin, blotted onto fresh agar, transferred viable bacteria (Fig. 3a) with efficiency similar to the filter paper controls (Fig. 3b). Bacterial transfer from pig skin was visibly greater than from agar plates. Acticoat was encased in agar or agarose as it was hypothesized that the semi-solid medium would allow a greater amount of silver to diffuse off the surface of the bandage. The Acticoat antimicrobial disc transferred a large number of viable bacteria from inoculated pig skin to agar plates (Fig. 4), but in most cases a rectangular region corresponding to the location of the Acticoat within the gel disc remained devoid of bacteria (Fig. 4). The addition of bac8c or fusidic acid (2.5 and 50 µg ml⁻¹, respectively) to the agar or agarose surrounding the Acticoat did not measurably increase its antibacterial efficacy. The number of c.f.u. on the surface of the pig skin and Acticoat was quantified over 24 h to more accurately assess the antibacterial activity of the bandage compared to controls. The percentage of bacteria surviving on pig skin and on Acticoat over 24 h showed high variability in results, but it appeared that growth of MRSA was largely inhibited for the first 8 h after placement of Acticoat (Fig. 5). Surprisingly, after 24 h the cells present between the Acticoat and the pig skin outnumbered those on the pig skin controls.
In contrast, the number of surviving cells on the Acticoat surface after 24 h corresponded to 11.9 % of controls.

**Efficacy of Acticoat plus antibacterial solutions**

Acticoat was soaked with a solution containing 10 % (v/v) glycerol or lysozyme (1 mg ml⁻¹) prior to being overlaid on MRSA-inoculated pig skin to test the potential of using a wetting agent or peptidoglycan-degrading enzyme with the bandage. mel12–26 or bac8c (50 µg ml⁻¹) was also added to the solution to test whether the intracellular activity of silver could be augmented with an AMP. Soaking Acticoat with 10 % (v/v) glycerol alone or in combination with mel12–26 or bac8c (50 µg ml⁻¹) reduced WBG 8287 survival on the dressing to <0.1 % of controls (Fig. 6a). The effect of glycerol on the survival of bacteria on pig skin, beneath the Acticoat, was enhanced when used in combination with AMPs, but complete elimination of viable bacteria was not observed in any of the tests. When applied to Acticoat, lysozyme (1 mg ml⁻¹) reduced the number of viable bacteria on the Acticoat surface to 62.5 % of the control (Fig. 6b). Lysozyme combined with bac8c (50 µg ml⁻¹) reduced survival to 19 %, and the addition of bac8c (50 µg ml⁻¹) reduced survival to 0.03 % (Fig. 6b). The greatest reduction in bacterial survival was observed when Acticoat was soaked with a combination of 10 % glycerol, lysozyme (1 mg ml⁻¹) and bac8c or mel12–26 (50 µg ml⁻¹) (Fig. 6c). Of the two antimicrobial peptides, addition of mel12–26 (50 µg ml⁻¹) reduced bacterial survival on pig skin and on the Acticoat itself to 1.04 and 0.01 %, respectively (Fig. 6c). Replacing mel12–26 with bac8c killed all MRSA on Acticoat and pig skin (Fig. 6c).
Haemolytic potential of antibacterial additives

The haemolytic potential of the antibacterial additives used to soak Acticoat in the earlier experiment was tested by spectrophotometrically analysing the release of haemoglobin from red blood cells exposed to the additives (Table 1). Samples exposed to glycerol plus lysozyme and mel12–26 showed 9.6 % haemolysis of fully lysed controls. Replacing mel12–26 with bac8c increased haemolysis to 35.8 %.

DISCUSSION

Diffusion of nanocrystalline silver off the Acticoat bandage and through the agar medium is believed to explain a lack of observable MRSA growth, over 72 h, beneath Acticoat and within a peripheral zone of ~ 1 mm. Nanoparticle diffusion may explain why a textured surface on the agar did not visibly reduce the inhibitory efficacy of the dressing, as might be expected where surface contact is not continuous. The exception seen with growth on a nodulated texture is believed to be a consequence of the larger gaps in contact between Acticoat and the agar. This appeared to place some bacteria outside the inhibitory range of the silver nanoparticles, which is consistent with the smaller amount of bacteria transferred by Acticoat taken from a nodulated agar surface. In clinical application, it is clear that an Acticoat dressing must make the most intimate contact possible with the area requiring protection for the successful prevention of microbial growth.

The lower density of bacteria on the connecting lattice between nodes of the Acticoat mesh appears likely to be related to the protrusion of the nodes beyond the plane of the mesh. In clinical use this feature would appear likely to decrease the effective contact between the protective dressing and the vulnerable tissue, and more effective treatment might result if the Acticoat surfaces were level throughout.

When Acticoat was embedded within agar or agarose, the area directly below the dressing strip did not transfer...
viable bacteria, which suggests that combining Acticoat with a sterile, non-toxic, semi-solid medium such as gelatin or ultrapure agarose could improve its capability to protect vulnerable tissues. However, areas of the antimicrobial disc outside this region were still able to transfer viable bacteria. This supports our previous argument that for Acticoat to effectively control a bacterial infection, the entire bandage must make intimate contact with the wound, even when encased in a semi-solid medium. Addition of fusidic acid or the AMP bac8c did not improve the antibacterial efficacy of the Acticoat gel dressing. Possibly, the anionic polymers of agar might sequester cationic peptides, preventing their diffusion from the gel. However, bac8c also proved to be ineffective in the agarose gel, which contains no major, charged components.

Smith & Nephew, the manufacturer of Acticoat, recommend leaving Acticoat on a wound for 3 days. From our results it appears that after 24 h the bacteria have overcome the antibacterial activity of the dressing. Therefore, it might reduce the risk of recurring infection if dressings were changed at intervals of 24 h.

The Western Australian Department of Health guidelines (Department of Health, 2011) and the application guidelines for Acticoat are supported by our conclusions that moistening the Acticoat provides a conduit between the dressing and the patient’s tissues. Maintaining a moist environment should allow a sustained release of silver particles into infected areas that are not in direct contact with the dressing. However, the efficacy of glycerol in preventing colonization of the Acticoat surface by WBG 8287 and the bacteria. This supports our previous argument that for Acticoat to effectively control a bacterial infection, the entire bandage must make intimate contact with the wound, even when encased in a semi-solid medium. Addition of fusidic acid or the AMP bac8c did not improve the antibacterial efficacy of the Acticoat gel dressing. Possibly, the anionic polymers of agar might sequester cationic peptides, preventing their diffusion from the gel. However, bac8c also proved to be ineffective in the agarose gel, which contains no major, charged components.

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On densely inoculated pig skin and on the surface of the overlaid Acticoat, bacterial growth was eliminated completely by Acticoat that was soaked with glycerol, lysozyme and bac8c. However, it is important to note that vortexing the Acticoat strip in PBS prior to plating on nutrient medium may have allowed more silver to diffuse off the Acticoat and decrease the amount of quantifiable bacteria. Additionally, the results observed in this study may not be representative of what would be observed on patients’ wounds. A major limitation of AMPs, that restricts their use in clinical therapy, is their sensitivity to peptidases, exogenous peptides and salts present in biological serum (Ganz, 2003; Rozek et al., 2003). Similarly, silver has been shown to be inactivated by components of human blood and bacterial cytoplasm, such as proteins which have incorporated thiol groups in reduced states (Mulley et al., 2014). Therefore, despite the encouraging results obtained in this study, the same treatment on a burn wound with biological exudate may not be as effective. Another limitation of AMPs is their potential haemolytic and cytotoxic activity (Rozek et al., 2003; Yeaman & Yount, 2003). In this study, erythrocytes exposed to a combination of glycerol, lysozyme plus mel12–26 or bac8c showed measurable haemolysis. This haemolytic activity may limit the clinical applications of Acticoat if the treatment requires contact with deep open wounds such as from punctures or lacerations. However, from our results it appears possible that biocompatible solvents and AMPs may increase the efficacy of treating topical wounds with silver-based dressings.

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