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Antibacterial properties and reduction of MRSA biofilm with a dressing combining polyabsorbent fibres and a silver matrix

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Antibacterial properties and reduction of MRSA biofilm with a dressing combining polyabsorbent fibres and a silver matrix

Objective: This study was designed to evaluate the antibacterial activity of a wound dressing which combines polyacrylate fibres and a silver lipido-colloid matrix (UrgoClean Ag, silver polyabsorbent dressing), against biofilm of methicillin-resistant *Staphylococcus aureus* (MRSA).
Method: Samples of silver polyabsorbent dressing and the neutral form of this dressing (UrgoClean) were applied to biofilms of MRSA formed on a collagen I-coated surface, cultured for 24 hours. Different exposure times were tested (1, 2, 4 and 7 days) without dressing change. The biofilm reduction was quantified by using culture methods and by confocal laser scanning microscopy experiments.
Results: The application of the silver polyabsorbent dressing resulted in a significant decrease of the biofilm population by a log reduction of 4.6, after 24 hours of exposure. Moreover, the antibiofilm activity was maintained for 7 days with reduction values up to 4 log (reduction of biofilm superior to 99.99%). The application of the neutral dressing also induced a significant reduction of the concentration of sessile cells after 1 day (about 0.90 log). The results obtained with this neutral form of the dressing showed that the polyacrylate fibres were able to exert a mechanical disruption of the biofilm architecture.
Conclusion: These *in vitro* experiments demonstrated that silver polyabsorbent dressing was able to strongly reduce the biofilm of MRSA. The antibiofilm mechanism of this dressing can be explained by a dual action of the polyabsorbent fibres (based on ammonium polyacrylate polymer around an acrylic core) which induced a mechanical disruption of the biofilm matrix and/or a sequestration of sessile cells, and the diffusion of silver ions which produced bactericidal activity.
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antibacterial activity • biofilm • silver-containing wound dressing • mechanical effect • polyacrylate fibres • MRSA

Chronic wounds lack an orderly and predictable process of healing and are an important global public health problem. These wounds are often colonised by a community of microorganisms. Wounds will heal despite the presence of a large variety of bacteria encountered in this environment. Nonetheless, invasive bacteria that are not normal members of the human skin microflora and/or opportunistic pathogenic bacteria may cause wound infections. Among these bacteria, *Staphylococcus aureus* is often present and considered a major pathogenic bacterial species in wound infections.[1–4] More particularly, methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of colonisation and infection in chronic soft-tissue wounds and is a worldwide problem with increasing prevalence.[5,6]

Historically, bacteria in chronic infections were considered to be growing in a planktonic state,[7] however, studies have demonstrated that biofilm form (sessile state) is mainly involved in wound infections. Studies performed with animal models or in humans have confirmed the existence of bacterial biofilm in wounds.[7–11] In chronic wounds, biofilm is composed of a multispecies communities held together and attached to the wound bed. The cells are embedded in extracellular polymeric substances (EPS) produced by the bacteria.[12,13] Although this matrix shows a great heterogeneity, the major components are hydrated polysaccharides, proteins, nucleic acids and lipids.[14,15] *Staphylococcus aureus* biofilms play a significant role in the wound healing process, depending on the strain ability to form biofilm.[16–18]

The challenge faced with regard to biofilm in chronic infections lies in their tolerance to antibiotics treatments.[19] As bacterial cells in biofilm have a specific physiological state (reduced growth rate and adaptive stress responses) and the matrix acts as an antimicrobial barrier; these cells are more resistant to antimicrobial agents when compared with planktonic cells.[20,21] Furthermore, cell-to-cell communication and intercellular genetic exchange can also contribute to the higher resistance of biofilm cells to antimicrobial stress, and to the persistence of biofilm infections.[22]

Since the demonstration of an association between wound chronicity and the presence of bacterial biofilm,[7] the need to control and reduce biofilm structures has been recognised. As alternatives to systemic or topical treatment with antibiotics, a first generation of wound

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dressings incorporating antimicrobial substances, such as silver or iodine, have been developed to treat chronic wounds.[23–27] The bactericidal activities of these silver-containing dressings have been demonstrated and are well documented. The majority of these studies were performed on planktonic cells or using agar diffusion methods.[27–36] Several studies have used *in vitro* biofilm models to demonstrate the antibacterial activities of silver dressing.[37–42] However, when these dressings are applied on mature biofilms with low growth rates and a solid matrix, they can only inhibit further growth and do not resolve the infection.[19]

Successful treatment of biofilm-related infections requires novel and more effective strategies. The most promising strategies concern the degradation (mechanical, enzymatic or physical) of the biofilm matrix and the disruption of biofilm.[43,44] Dressings that combine the use of silver and agents able to destabilise the biofilm matrix, facilitating the diffusion of silver ions, have been developed. For example, a new dressing which contains two agents that are known to disrupt biofilms, ethylenediaminetetraacetic acid (EDTA) and benzethonium chloride, in addition to ionic silver as an antimicrobial agent,[45] has demonstrated *in vitro* antibiofilm activity.[45] Other technologies use dispersing agents, such as Dispersin B[46] or surfactant[44] which disseminate bacterial biofilm by targeting the extracellular polysaccharides (EPS), destabilising the biofilm framework. These strategies would be beneficial in combination with a microbicide agent for managing bacterial biofilm contamination within the wound. One other way of disrupting the biofilm concerns the use of superabsorbent and absorbent dressings, which remove bacteria from the wound by absorbing and maintaining the bacteria in the dressing matrix or by hydrophobic interactions between the wound contact layer and bacteria.[23,47] Recently, Cooper and Jenkins[48] have demonstrated that dialkyl carbamoyl chloride (DACC)-coated dressings bind MRSA biofilm *in vitro*.

In this study, we investigated the *in vitro* ability of a polyabsorbent silver dressing (UrgoCleanAg, Urgo) which combines polyacrylate fibres and silver lipido-colloid matrix (silver sulfate and carboxymethylcellulose dispersed in a lipophilic layer), to reduce and eliminate biofilm of MRSA.

Methods

Wound dressings tested

We tested three lipido-colloid non-adhesive dressings:

- An absorbent silver-containing dressing (UrgoClean Ag)
- The same absorbent dressing without silver (UrgoClean, polyabsorbent dressing)
- A non-absorbent neutral dressing (UrgoTul, non-absorbent dressing).

The dressings without silver were used as controls. UrgoTul is composed of CMC dispersed in a lipophilic layer (TLC technology), coated on a non-occlusive fine mesh. UrgoClean and UrgoCleanAg are absorbent dressings which are composed of a soft-adherent lipido-colloid matrix (TLC technology) coated on a sterile compress composed of polyacrylate polymer fibres. In UrgoCleanAg, the lipido-colloid contact layer is impregnated with silver sulfate. All the wound dressings were kindly provided by Laboratoires URGO.

Bacterial strain and culture conditions

MRSA ATCC 43300 was used to evaluate the antibiofilm activity of the wound dressings. Stock cultures of bacteria are kept, in cryovials, under glycerol stocks (15% v/v) at –70°C for long-term storage. Bacteria were cultured at 35±2°C, in Tryptic Soy Broth (TSB; BioKar Diagnostics) under agitation for 16–24 hours.

In vitro biofilm formation

The model used for the biofilm formation was similar than those described by Werthen et al.[49] Briefly, an overnight culture of MRSA strain was diluted in fresh sterile TSB to give a final concentration of 10^7 colonies forming units (CFU)/ml. An aliquot (0.5 ml) of this suspension was added to each well of a polystyrene 24-well microplate coated with a collagen type I (BD Biocoat Collagen I). The inoculated plates were incubated at 35±2 °C for 24 hours in static conditions to allow biofilm to establish on collagen I-coated surface. After the incubation, the waste medium was aspirated gently, and the wells of the plates were washed twice with 0.5 ml of saline diluent (0.9 % w/v NaCl) to remove planktonic cells.

Application of wound dressings and evaluation of antibiofilm activity

Dressing samples were cut under aseptic conditions into small squares of 1 cm^2 (10 mm x 10 mm). Absorbent dressings were pre-wetted with their saturation volume. A 0.5 ml volume of saline diluent was added to each well containing mature biofilm to mimic exudative conditions. Then, a square of each wound dressing was aseptically introduced in wells containing the biofilm and pressed down gently to ensure contact between dressing and biofilm. In each plate, a well containing only 0.5 ml of saline diluent was used as a control for untreated biofilm. All plates were incubated at 35 ± 2 °C during defined exposure times (1, 2, 4 and 7 days) without dressing change.

To study the effect of the dressing change, the samples of wound care dressing were removed using sterile forceps after 2 days of exposure and the saline diluent was aspirated gently. Fresh saline diluent (0.5 ml) was added to each well and a new square of each wound dressing was added to a corresponding well. The plates were then incubated at 35 ± 2 °C for 2 days.

After the incubation time, the wound dressings were removed using sterile forceps. The waste saline diluent was aspirated gently and the non-adherent cells were eliminated by two washing steps with 0.5 ml of saline diluent. Biofilm were thoroughly scraped with a micropipette tip and the sessile cells were resuspended into 1 ml of saline diluent. Serial 10-fold dilutions were

performed in saline diluent. It should be pointed out that serial dilution was used to neutralise the antimicrobial activity of the silver ions. Five drops (10 µL) of each dilution were plated onto Tryptic Soy Agar plates (TSA, Biokar Diagnostics). The plates were incubated at 35± 2 °C for 24–48 hours. After incubation, the number of CFU were counted. We repeated each experiment three independent times for each condition. The antibiofilm activity of the wound dressing was determined as the decrease in the log concentration of viable cells compared to the non-treated biofilm. For each dressing tested, the antibiofilm activity was calculated using the following formula:

Antibiofilm activity (A) = log10 of sessile cells concentration of the untreated biofilm – log10 of sessile cells of the biofilm treated with a wound dressing

Fluorescent labelling and confocal laser scanning microscopy image acquisition

Biofilm architecture was studied using confocal laser scanning microscopy (CLSM). After 24 hours in the presence or absence of the wound dressing, dressing samples were removed and non-adherent cells were eliminated as previously described. A volume of 1 ml of fresh TSB was added in each well and sessile cells were fluorescently tagged by adding two fluorescent markers, SYTO9 and propidium iodide (PI), in the fresh medium (1:500 dilution from Filmtracer LIVE/DEAD Biofilm Viability Kit). Plates were incubated in the dark for 30 minutes to enable the fluorescent labelling of bacteria cells. After incubation, the plate was then mounted on the motorised stage of an inverted confocal microscope (Leica TCS SP8 AOBS, LEICA Microsystems). The microtiter plates were scanned using a 40×/0.8 N.A. (LEICA) water immersion objective lens with a 488 nm argon laser set at 25% intensity. Emitted green fluorescence was recorded within 493–575 nm (gain 750V) to visualise live cells and emitted red fluorescence collected 630–750 nm (gain 750V) to visualise dead cells. Images were acquired at 600 Hz with a z-step of 1 µm between each xy image for z-stack for each biofilm in three different areas in each well. For each condition three independent experiments were performed.

Two-dimensional-projections of biofilm structure were reconstructed using IMARIS 7.1 software (Bitplane, Switzerland). Quantitative structural parameters of the biofilm architecture (biovolume, thickness and roughness) were also calculated using this software.

Statistical analysis

All data are expressed as mean standard deviation (SD) of the triplicate experimental data (independent test). Data were analysed using an unpaired t-test to determine the differences in sessile cells concentration between the control and each group. For the biofilm assay, $p<0.05$ was taken as significant. The statistical analysis was performed using XLSTAT version 2015.2.01.17502 (Addinsoft).

Results

Antibiofilm activity

Untreated biofilm remained stable around 10^8 CFU/cm^2 during 2 days and a decrease of about 1 log unit was observed after 7 days of incubation (Fig 1). This slight reduction can be explained by a spontaneous detachment of the bacteria during the biofilm development[50] or by the reduced growth rate of sessile cells. In mature biofilm, sessile cells have been show to enter a metabolically inactive, dormant state, and may be viable but noncultivable.[51] The treatment with the non-absorbent dressing had no significant effect on the viability of sessile cells.

The application of the absorbent dressing induced a significant ($p<0.05$) reduction of the concentration of sessile cells after 1 and 2 days of exposure (Fig 1). The concentration of sessile cells was reduced by about 0.9 log compared with the untreated conditions (Table 1). The disruption of MRSA biofilm can be explained by the strong absorbent property and the sequestration of bacteria on polyabsorbent fibres. Therefore, this mechanical effect was not maintained after 4 days of exposure. Loss of mechanical effect can be explained by the fact that the cell-surface properties can modulate stress conditions, such as starvation (nutrient stress conditions). These conditions occur in this *in vitro* biofilm model where there may be a paucity of nutrients due to the non-addition of nutrient broth during the incubation.

Biofilm exposed to the polyabsorbent silver dressing showed a significant decrease ($p<0.05$) in the concentration of sessile cells compared with the untreated biofilm (Fig 1). This dressing exhibited an antibacterial

Table 1. Antibiofilm activities of the three dressings. The untreated biofilm was used as control condition to calculate the antibiofilm activity

	Antibiofilm activity (average log reduction)			
	1 day	**2 days**	**4 days**	**7 days**
Non-absorbent	NR	NR	NR	NR
Polyabsorbent	0.90±0.15†	0.94±0.24*	0.27±0.32	NR
Silver polyabsorbent	4.61±0.27†	4.64±0.53*	4.15±0.28*	4.52±0.81*

*p<0.05, †p<0.01 significant difference between control (untreated biofilm) and treatments; The values represent the mean value ± standard deviation of results from three independent experiments. NR indicates no reduction of the sessile cells concentration

activity against MRSA biofilm after 1 day which was maintained throughout the 7 days of contact. After 1 day of exposure, the polyabsorbent silver dressing strongly reduced the concentration of sessile cells by a log reduction of 4.6 (reduction >99.99%) when compared with the untreated condition. The antibiofilm activity was maintained for 7 days with reduction values up to 4.0 log (Table 1). The application of the polyabsorbent silver dressing was characterised by a bactericidal activity on biofilm during the first 2 days, followed by a stabilisation of the effect after 4 days probably due to the complete diffusion of silver ions.

Effect of the dressing on MRSA biofilm architecture

CLSM is a method of choice for studying biofilms because it allows a non-destructive analysis at a cell scale of their hydrated spatial arrangement.[52]

Representative 24-hour biofilm structures are shown in Fig 2. These images are 2D-reconstructions obtained from confocal stack images. *Staphylococcus aureus* ATCC 43300 formed flat and compact structures with multilayered structures. The MRSA strain produced biofilm structures that covered the entire surface. These observations confirmed the results of the quantitative analysis of the biofilms (Fig 1). The application of non-absorbent dressing had no impact on biofilm structure (Fig 2). The untreated biofilm and the biofilm treated with non-absorbent dressing was stained green and no dead or damaged cells were detected. The treatment with the polyabsorbent dressing induced modifications of the biofilm architecture (Fig 2). We noted the presence of holes of different sizes which may be caused by the mechanical effect of this dressing. Surprisingly, areas of red-labelled cells were also observed.

The application of the polyabsorbent silver dressing had a drastic effect on the biofilm structure (Fig 2). No biofilm structures were observed in the middle of the well where the dressing was in close contact with the biofilm (data not shown). Some biofilm structures were observed at the periphery area of well. In these areas, the polyabsorbent silver dressing application significantly disrupted the structural integrity of the biofilm after 24 hours of exposure. These observations were in agreement with the decrease of the sessile cells concentration quantified previously.

Quantitative parameters such as biovolume (μm^3), mean thickness (μm) and surface area coverage (%) were extracted from confocal stack images to quantify biofilm structures with numerical data (Table 2). Analysis showed that application of the non-absorbent dressing had no significant effect on the MRSA biofilm architecture. Significant reduction of the mean thickness of biofilm was observed after exposure to polyabsorbent dressing (Table 2), application reduced the surface area coverage parameter and the biovolume compared with the untreated biofilm. These differences were not significant. It is important to underline that biofilm treated with this dressing demonstrated the highest variability in terms of surface area coverage and biovolume.

Fig 1. Sessile cells concentration of untreated biofilm and those exposed to the three test dressings

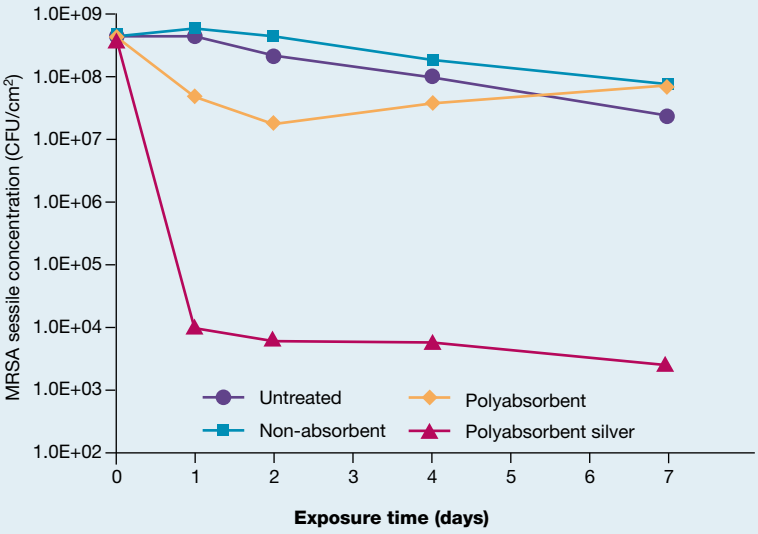


Fig 2. CLSM observations of MRSA biofilms exposed to the different lipido-colloid non-adhesive dressings tested. These images are examples of representative observations of biofilm architecture and those used to quantify the structural parameters of biofilms. Untreated biofilm (**a**), biofilm treated with non-aborsobent (**b**), biofilm treated with polyabsorbent (**c**) and biofilm treated with polyabsorbent silver (**d**). Biofilms were stained with FilmTracer LIVE/DEAD Biofilm Viability Kit. Green-labelled bacteria represent viable sessile cells and red-labelled bacteria represent dead or damage sessile cells

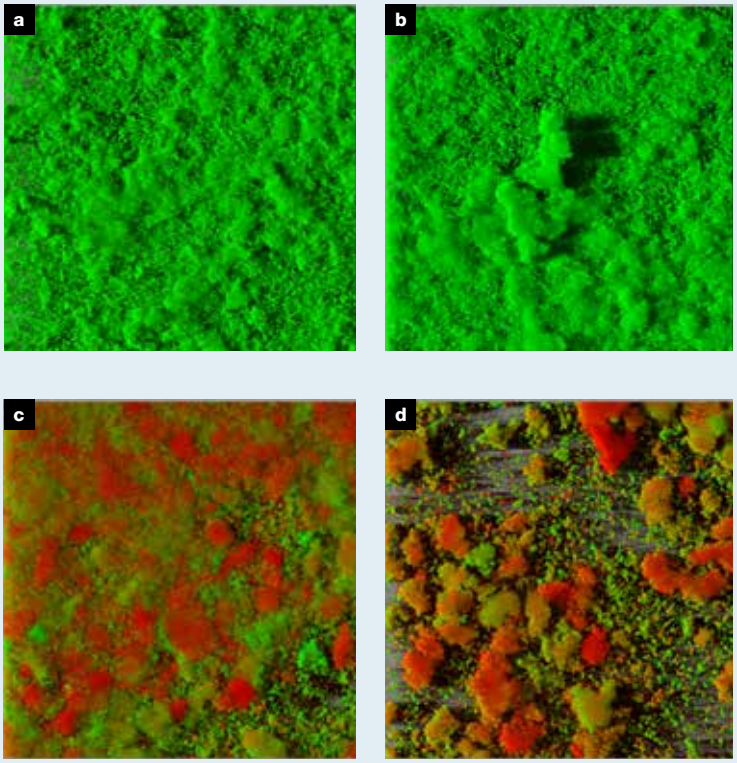
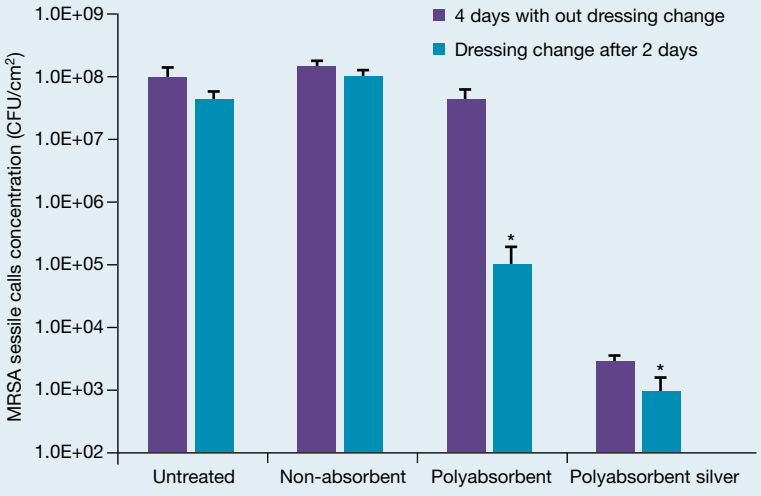


Fig 3. Sessile cells concentration of untreated biofilm and biofilms exposed to the three tested dressings



The treatment with polyabsorbent silver dressing resulted in a significant reduction for the three structural parameters of biofilms compared with the non-treated biofilm. Biofilm exposed to the polyabsorbent silver dressing demonstrated the lowest biovolumes and average thickness in accordance with the antibiofilm activity previously quantified (Table 1). A greater decrease in biovolume was observed in polyabsorbent silver dressing-treated biofilm (about 45–50%).

Effect of the wound dressing replacement on the antibiofilm activities

We found a significant effect ($p<0.05$) after the dressing replacement of the polyabsorbent and the polyabsorbent silver dressing. A decrease of the sessile cells concentration was observed after the dressing change of polyabsorbent dresising (from $3.9x10^7$ CFU/ml to $1.6x10^5$ CFU/ml) and the polyabsorbent silver dressing (from $4.4x10^3$ CFU/ml to $1.1x10^3$ CFU/ml).

Discussion

The fact that biofilms are especially tolerant to antibiotics explains why some wounds fail to respond to antimicrobial interventions.[48,53] Studies have also shown that higher silver nitrate levels are required to treat an infection caused by a biofilm-associated bacteria strain than a culture of planktonic cell of the same strain.[54] Mahami et al. have reported that the biofilm phenotype of *Staphylococcus aureus* was found to be four-times more resistant to silver nitrate than planktonic cells.[55] Moreover, the range of bacterial cells with differing physiological and functional variations within a mature biofilm suggests that multiple inhibitory assaults are likely to be more effective than a single antimicrobial intervention.[56]

In this study, we used an *in vitro* biofilm model to evaluate the antibiofilm activity of a polyabsorbent silver dressing which combines two technologies, poly-absorbent fibres and a silver lipido-colloid matrix. Contrary to other silver-containing dressings where the silver component is part of the dressing structure, such as silver alginate, silver foam or silver hydrofiber dressings, the silver sulphate is incorporated in a lipido-colloid layer coated on one side of the dressing. Preliminary studies have demonstrated the antibacterial activity of this dressing against planktonic cells of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, MRSA and vancomycin-resistant *Enterococci* using zone of inhibition assays and/or direct-contact method (unpublished data). In this model, MRSA biofilm exhibited a bacterial population about 10^8–10^9 CFU/cm^2 which was representative of those in severely infected wounds[57] and the matrix was well established and conferred a higher level of resistance towards antimicrobial agents.[58]

In these *in vitro* conditions, the neutral form of this dressing (e.g. polyabsorbent without silver) demonstrated mechanical disrupting capacities which induce the reduction of the biofilm after 1 and 2 days of exposure. We suggest two mechanisms which can explain the mechanical effect of this dressing. First, the interactions between the polyabsorbent fibres, which are based on an ammonium polyacrylate polymer around an acrylic core, and the sessile cells or the main component of the biofilm matrix. In a recent study, a DACC-coated dressing has demonstrated a capacity to bind MRSA biofilm.[48] In this case, hydrophobic interactions between the DACC-coated fibres and MRSA sessile cells were proposed to

Table 2. Quantitative analysis of confocal data for the biovolume (μm^3), average thickness and substratum coverage of the biomass

	Surface area coverage (%)	Biovolume (x 10^5 μm^3)	Mean thickness (μm)
Untreated	93.87 ± 5.69	7.96±1.49	12.89±2.23
Non-absorbent	91.71 ± 7.37	8.00±1.98	12.49±2.25
Polyabsorbent	78.54 ± 23.05	6.92±3.77	9.60±3.06*
Silver polyabsorbent	57.51 ± 14.34†	5.70±1.01†	10.35±1.68*

*$p<0.05$ and †$p<0.01$ compared with untreated condition; The results are means of datasets obtained from analysis of nine CLSM images acquired at random positions in each of the biofilm.

explain the mechanical effect. In the polyabsorbent dressing, the fibres have a high density of ionic charges.[59] In the wound environment, the polyacrylate fibres are negatively charged and attraction can occur between positively charged molecules and negatively charged polyacrylate fibres. Bacterial cells have a net negative charge on the cell wall, although the magnitude of this charge varies from strain to strain. The cellular surface of *Staphylococcus aureus* has a moderately negative net charge at neutral pH, which is probably due to the fact that the teichoic acids contain fewer positively charged D-alanine residues than negatively charged phosphate groups.[60] In particularly, MRSA strains harbour more positive surface charges.[61] For these strains, binding of sessile cells can be due to electrostatic forces that arise between their positively charged groups and the negatively charged carboxyl groups of the polyabsorbent fibres. However, the biofilm matrix of *Staphylococcus aureus* was mainly composed of poly-N-acetyl glucosamine (PNAG) polymer which is a positively charged linear homoglycan. The exo-polysaccharides play a significant role in integrity of the matrix and the interaction of water molecules with the hygroscopic components of EPS leads to hydration of the matrix and biofilm environment required for survival of biofilm bacteria.[43] Electrostatic interactions may occur between the negatively charged of polyabsorbent fibres and the major component of the matrix and lead to disruption of the biofilm architecture.

Second, the ability of this dressing to trap sessile cells also can be explained by the gelling properties of the polyabsorbent fibres. These fibres made of polyacrylate polymers are highly absorbent. On contact with the saline solution, they swell resulting in the formation of a gel. Hence, we can hypothesise that sessile cells can be trapped and immobilised in the gel. Wiegand et al. suggested that in salt solution, polyacrylate fibres react primarily with water, then, bacterial cells can be bound by the developing electrostatic interactions and are trapped in the forming gel.[62] Similar properties have been reported with Hydrofiber dressings. Newman et al. have demonstrated the ability of these dressings to sequester and immobilise planktonic cells of *Staphylococcus aureus*.[63] Similar mechanisms can occur after application of the polyabsorbent dressing used here.

The polyabsorbent silver dressing exerted an *in vitro* antibiofilm activity against mature MRSA biofilm, while the CLSM pictures of different biofilms have showed the dressing was quite effective in the destruction and the removal of MRSA biofilm. The rapid *in vitro* antibiofilm activity could be explained by the synergistic activity of the polyabsorbent fibres and the diffusion of silver ions. The disruption of the matrix caused by the polyabsorbent fibres may facilitate the diffusion of silver ions. Alhede et al. showed that induction of growth, by disrupting the biofilm mechanically, left the biofilm more sensitive to high concentrations of tobramycin when compared with the non-disrupted biofilm.[64]

It is important to highlight that polyabsorbent silver dressing was not able to completely eradicate biofilm and that some aggregates of living sessile cells persist in the well (>10^4 CFU/cm^2). The quantification of sessile cells showed a stabilisation of the viable cell population after two days of application. This result could be explained by the progressive diffusion of silver from dressing. But, in these *in vitro* conditions, the replacement of the dressing after 2 days of application allows an increase of the antibiofilm activity.

The ability of some wound dressings to sequester and immobilise bacterial cells into the wound dressing has already been described.[48,65] This property is now a recognised benefit to control the wound infection. The polyabsorbent silver dressing combines the trapping properties of the polyabsorbent fibres and the antimicrobial activity of silver. All these results suggest that polyacrylate fibres can facilitate the disorganisation of the biofilm structure and improve the antibacterial effect of silver. The combination of these two technologies improves the efficiency of this dressing to remove MRSA biofilms.

Limitations and future works

Due to the difficulties in diagnosing biofilm infections, *in vitro* biofilm studies have widely been applied in the study of antibiofilm treatment strategies as an obvious alternative to clinical trials.[19,66] This study was performed using *in vitro* biofilm with wound-like properties. Under these conditions, the biofilm generated would have behaved differently to if they had been established *in vivo*. It would be interesting to use a medium which simulates the wound bed environment with a continuous production of exudates and contains host components such as proteins and immune cells. Indeed, the level of silver released into the wound is mainly dependent on the wound environment.[67] Several *in vitro* models have been described, but none can accurately reproduce the complex conditions within a wound. This study is a first step to demonstrate the antibiofilm activity of the polyabsorbent silver dressing however, these results must be confirmed by clinical observations.

Conclusion

This study has demonstrated *in vitro* antibacterial activity of the polyabsorbent silver dressing to reduce MRSA biofilm. The ability of the polyabsorbent fibres to interact with the biofilm matrix appears to improve the silver ions diffusion and the bactericidal activity against sessile cells. The development of this novel synergistic combination with a mechanical action on biofilm matrix that enhances the efficacy of silver may have a significant therapeutic value, its should be considered as a novel therapeutic approach for the management of biofilm in wound infections. JWC

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References

1 Thomsen, T.R., Aasholm, M.S., Rudkjobing, V.B. et al. The bacteriology of chronic venous leg ulcer examined by culture-independent molecular methods. Wound Repair Regen 2010; 18: 1, 38–49.
2 Kirketerp-Moller, K., Jensen, P.O., Fazli, M. et al. Distribution, organization, and ecology of bacteria in chronic wounds. J Clin Microbiol 2008; 46: 8, 2717–2722.
3 Dowd, S.E., Sun, Y., Secor, P.R. et al. Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. BMC Microbiol 2008; 8: 43.
4 Bowler, P.G., Duerden, B.I., Armstrong, D.G. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 2001; 14: 2, 244–269.
5 Reich-Schupke, S., Warneke, K., Altmeyer, P. et al. Eradication of MRSA in chronic wounds of outpatients with leg ulcers is accelerated by antiseptic washes--results of a pilot study. Int J Hyg Environ Health 2013; 213: 2, 88–92.
6 Ammons, M.C., Tripet, B.P., Carlson, R.P. et al. Quantitative NMR metabolite profiling of methicillin-resistant and methicillin-susceptible Staphylococcus aureus discriminates between biofilm and planktonic phenotypes. J Proteome Res 2014; 13: 6, 2973–2985.
7 James, G.A., Swogger, E., Wolcott, R. et al. Biofilms in chronic wounds. Wound Repair Regen 2008; 16: 1, 37–44.
8 Davis, S.C., Ricotti, C., Cazzaniga, A. et al. Microscopic and physiologic evidence for biofilm-associated wound colonization in vivo. Wound Repair Regen 2008; 16: 1, 23–29.
9 Akiyama, H., Huh, W.K., Yamasaki, O. et al. Confocal laser scanning microscopic observation of glycocalyx production by Staphylococcus aureus in mouse skin: does S. aureus generally produce a biofilm on damaged skin? Br J Dermatol 2002; 147: 5, 879–885.
10 Akiyama, H., Oono, T., Saito, M. et al. Assessment of cadexomer iodine against Staphylococcus aureus biofilm in vivo and in vitro using confocal laser scanning microscopy. J. Dermatol 2004; 31: 7, 529–534.
11 Wolcott, R.D., Rhoads, D.D., Dowd, S.E. Biofilms and chronic wound inflammation. J Wound Care 2008; 17: 8, 333–341.
12 Bjarnsholt, T., Kirketerp-Moller, K., Jensen P.O. et al. Why chronic wounds will not heal: a novel hypothesis. Wound Repair Regen 2008; 16: 1, 2–10.
13 Flemming, H.C. The perfect slime. Colloids Surf B Biointerfaces 2011; 86: 2, 251–259.
14 Sutherland, I. Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 2001; 147: Pt 1, 3–9.
15 Flemming, H.C., Wingender, J. The biofilm matrix. Nat Rev Microbiol 2010; 8: 9, 623–633.
16 Schierle, C.F., De la Garza, M., Mustoe, T.A. et al. Staphylococcal biofilms impair wound healing by delaying reepithelialization in a murine cutaneous wound model. Wound Repair Regen 2009; 17: 3, 354–349.
17 Kirker, K.R., Secor, P.R., James, G.A. et al. Loss of viability and induction of apoptosis in human keratinocytes exposed to Staphylococcus aureus biofilms in vitro. Wound Repair Regen 2009; 17: 5, 690–699.
18 Secor, P.R., James, G.A., Fleckman, P. et al. Staphylococcus aureus Biofilm and Planktonic cultures differentially impact gene expression, mapk phosphorylation, and cytokine production in human keratinocytes. BMC Microbiol 2011; 11: 143.
19 Alhede, M., Alhede, M. The biofilm challenge. EWMA Journal 2014; 14: 1, 54–58.
20 Stewart, P.S., Franklin, M.J. Physiological heterogeneity in biofilms. Nat Rev Microbiol 2008; 6: 3, 199–210.
21 Harmsen, M., Yang, L., Pamp, S.J. et al. An update on Pseudomonas aeruginosa biofilm formation, tolerance, and dispersal. FEMS Immunol Med Microbiol 2010; 59: 3, 253–268.
22 Stewart, P.S., Costerton, J.W. Antibiotic resistance of bacteria in biofilms. Lancet 2001; 358: 9276, 135–138.
23 Larko, E., Persson, A., Blom, K. Effect of superabsorbent dressings in a 3D acellular tissue model of Pseudomonas aeruginosa biofilm. J Wound Care 2015; 24: 5, 204, 206–210.
24 Atiyeh, B.S., Costagliola, M., Hayek, S.N. et al. Effect of silver on burn wound infection control and healing: review of the literature. Burns 2007; 33: 2, 139–148.
25 Lipp, C., Kirker, K., Agostinho, A. et al. Testing wound dressings using an in vitro wound model. J Wound Care 2010; 19: 6, 220–226.
26 Silver, S., Phung, le T., Silver, G. Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds. J Ind Microbiol Biotechnol 2006; 33: 7, 627–634.
27 Castellano, J.J., Shafii, S.M., Ko, F. et al. Comparative evaluation of silver-containing antimicrobial dressings and drugs. Int Wound J 2007; 4: 2, 114–122.
28 Edwards-Jones, V. Antimicrobial and barrier effects of silver against methicillin-resistant Staphylococcus aureus. J Wound Care 2006; 15: 7, 285–290.
29 Aramwit, P., Muangman, P., Namviriyachote, N. et al. In vitro evaluation of the antimicrobial effectiveness and moisture binding properties of wound dressings. Int J Mol Sci 2010; 11: 8, 2864–2874.
30 Ip, M., Lui, S.L., Poon, V.K. et al. Antimicrobial activities of silver dressings: an in vitro comparison. J Med Microbiol 2006; 55: Pt 1, 59–63.
31 Cavanagh, M.H., Burrell, R.E., Nadworny, P.L. Evaluating antimicrobial efficacy of new commercially available silver dressings. Int Wound J. 2010; 7: 5, 394–405.
32 Hooper, S.J., Williams, D.W., Thomas, D.W. et al. An in vitro comparison of two silver-containing antimicrobial wound dressings. Ostomy Wound Manage 2012; 58: 1, 16–22.
33 Percival, S.L., Slone, W., Linton, S. et al. The antimicrobial efficacy of a silver alginate dressing against a broad spectrum of clinically relevant wound isolates. Int Wound J 2011; 8: 3, 237–243.
34 Thomas, J.G., Slone, W., Linton, S. et al. In vitro antimicrobial efficacy of a silver alginate dressing on burn wound isolates. J Wound Care 2011; 20: 3, 124–128.
35 Muller, G., Winkler, Y., Kramer, A. Antibacterial activity and endotoxin-binding capacity of Actisorb Silver 220. J Hosp Infect 2003; 53: 3, 211–214.
36 Jones, S.A., Bowler, P.G., Walker, M. et al. Controlling wound bioburden with a novel silver-containing Hydrofiber dressing. Wound Repair Regen 2004; 12: 3, 288–294.
37 Percival, S.L., Bowler, P., Woods, E.J. Assessing the effect of an antimicrobial wound dressing on biofilms. Wound Repair Regen 2008; 16: 1, 52–57.
38 Percival, S.L., Bowler, P.G., Dolman, J. Antimicrobial activity of silver-containing dressings on wound microorganisms using an in vitro biofilm model. Int Wound J 2007; 4: 2, 186–191.
39 Hammond, A.A., Miller, K.G., Kruczek, C.J. et al. An in vitro biofilm model to examine the effect of antibiotic ointments on biofilms produced by burn wound bacterial isolates. Burns. 2011; 37: 2, 312-321.
40 Thorn, R.M., Austin, A.J., Greenman, J. et al. In vitro comparison of antimicrobial activity of iodine and silver dressings against biofilms. J Wound Care 2009; 18: 8, 343–346.
41 Percival, S.L., Thomas, J.G., Slone, W. et al. The efficacy of silver dressings and antibiotics on MRSA and MSSA isolated from burn patients. Wound Repair Regen 2011; 19: 6, 767–774.
42 Thomas, J.G., Slone, W., Linton, S. et al. A comparison of the antimicrobial efficacy of two silver-containing wound dressings on burn wound isolates. J Wound Care 2011; 20: 12, 580–586.
43 Alavi, M.R., Stojadinovic, A., Izadjoo, M.J. An overview of biofilm and its detection in clinical samples. J Wound Care 2012; 21: 8, 376–383.
44 Wolcott, R. Disrupting the biofilm matrix improves wound healing outcomes. J Wound Care 2015; 24: 8, 366–371.
45 Said, J., Walker, M., Parsons D. et al. An in vitro test of the efficacy of an anti-biofilm wound dressing. Int J Pharm 2014; 474: 1-2, 177–181.
46 Gawande, P.V., Clinton, A.P., LoVetri, K. et al. Antibiofilm Efficacy of DispersinB((R)) Wound Spray Used in Combination with a Silver Wound Dressing. Microbiol Insights. 2014; 7: 9-13.
47 Ljungh, A., Yanagisawa, N., Wadstrom, T. Using the principle of hydrophobic interaction to bind and remove wound bacteria. J Wound Care 2006; 15: 4, 175–180.
48 Cooper, R., Jenkins, L. Binding of two bacterial biofilms to dialkyl carbamoyl chloride (DACC)-coated dressings in vitro. J Wound Care 2016; 25: 2, 76–82.
49 Werthen, M., Henriksson, L., Jensen, P.O. et al. An in vitro model of bacterial infections in wounds and other soft tissues. APMIS 2010; 118: 2, 156–164.
50 Rollet, C., Gal, L., Guzzo, J. Biofilm-detached cells, a transition from a sessile to a planktonic phenotype: a comparative study of adhesion and physiological characteristics in Pseudomonas aeruginosa. FEMS Microbiol Lett 2009; 290: 2, 135–142.
51 Li, L., Mendis, N., Trigui, H. et al. The importance of the viable but non-culturable state in human bacterial pathogens. Front Microbiol 2014; 5: 258, 1–20.
52 Bridier, A., Dubois-Brissonnet, F., Boubetra, A. et al. The biofilm architecture of sixty opportunistic pathogens deciphered using a high throughput CLSM method. J Microbiol Methods 2010; 82: 1, 64–70.
53 Mah, T.F., O'Toole, G.A. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 2001; 9: 1, 34–39.
54 Bjarnsholt, T., Kirketerp-Moller, K., Kristiansen, S. et al. Silver against Pseudomonas aeruginosa biofilms. APMIS 2007; 115: 8, 921–928.
55 Mahami, T., Adu-Gyamfi, A., Owulah, C. Comparative susceptibility of in vitro biofilm and planktonic cells of Staphylococcus aureus to antimicrobials. African Journal of Microbiology Research 2010; 4: 12, 1209–1214.
56 Cooper R.A., Bjarnsholt T., Alhede M. Biofilms in wounds: a review of present knowledge. J Wound Care 2014; 23: 11, 570–582.
57 Agostinho A.M., Hartman A., Lipp C. et al. An in vitro model for the growth and analysis of chronic wound MRSA biofilms. J Appl Microbiol 2011; 111: 5, 1275–1282.
58 Hoyle, B.D., Costerton, J.W. Bacterial resistance to antibiotics: the role of biofilms. Prog Drug Res 1991; 37: 91–105.
59 Wiegand, C., Abel, M., Ruth, P. et al. Superabsorbent polymer-containing wound dressings have a beneficial effect on wound healing by reducing PMN elastase concentration and inhibiting microbial growth. J Mater Sci Mater Med 2011; 22: 11, 2583–2590.
60 Gross, M., Cramton, S.E., Gotz, F., et al. Key role of teichoic acid net charge in Staphylococcus aureus colonization of artificial surfaces. Infect Immun 2001; 69: 5, 3423–3426.
61 Mishra, N.N., Bayer, A.S., Weidenmaier, C. et al. Phenotypic and genotypic characterization of daptomycin-resistant methicillin-resistant Staphylococcus aureus strains: relative roles of mprF and dlt operons. PLoS One 2014; 9: 9, e107426.
62 Wiegand, C., Abel, M., Muldoon, J. et al. SAP-containing dressings exhibit sustained antimicrobial effects over 7 days in vitro. J Wound Care 2013; 22: 3, 120–127.
63 Newman, G.R., Walker, M., Hobot, J.A. et al. Visualisation of bacterial sequestration and bactericidal activity within hydrating Hydrofiber wound dressings. Biomaterials. 2006; 27: 7, 1129–1139.
64 Alhede M., Kragh K.N., Qvortrup K. et al. Phenotypes of non-attached Pseudomonas aeruginosa aggregates resemble surface attached biofilm. PLoS One 2011; 6: 11, e27943.
65 Bowler, P.G., Jones, S.A., Davies, B.J. et al. Infection control properties of some wound dressings. J Wound Care 1999; 8: 10, 499–502.
66 Crone, S., Garde C., Bjarnsholt, T. et al. A novel in vitro wound biofilm model used to evaluate low-frequency ultrasonic-assisted wound debridement. J Wound Care 2015; 24: 2, 64–72.
67 Hill, K.E., Malic, S., McKee, R. et al. An in vitro model of chronic wound biofilms to test wound dressings and assess antimicrobial susceptibilities. J Antimicrob Chemother 2010; 65: 6, 1195–1206.