Evaluation of low-adherent antimicrobial dressings

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Abstract

Aims: To investigate the physical and antimicrobial properties of low-adherent, silver-containing gelling fibre and certain adhesive foam dressings *in vitro*. *Methods:* An *in vitro* model was used to quantitatively measure fibroblast adhesion to dressings in their dry and hydrated states. Microscopy techniques were used to visualise and quantify aspects of dressing composition and structure. Finally, a flat bacteria-seeded-agar *in vitro* model was used to semi-quantitatively assess the antimicrobial activity of each dressing and to investigate dressing structure related effects. *Results: In vitro* cell adhesion to the gelling fibre dressings was significantly less than for the adhesive foam dressings tested (p<0.001), particularly when hydrated. The adhesive foam dressings tested did not prevent bacterial proliferation in this model. In the authors' opinion, this suggests the adhesive layer of the selected dressings may be a physical barrier to the availability of the antimicrobial agent. *Conclusions:* Dressing technology and construction may be important factors in determining the adherence properties and antimicrobial activity of dressings. The simple addition of silver to a non-antimicrobial dressing may not necessarily be sufficient to ensure antimicrobial activity. *Conflict of interest:* The authors (with the exception of Christine Cochrane) are employees of ConvaTec Ltd.

KEY WORDS
Hydrofiber® technology
Alginate and foam dressings
Bioadhesion
Conformability
Silver

Through careful design of their physical characteristics, many modern wound dressings aim to provide a moist environment that is conducive to healing. Also, and perhaps equally as important, is the aim to ensure that there is minimal tissue trauma upon their removal. Several different approaches have been used to meet these design challenges, most notably the use of a low-tack or soft adhesive wound contact layer, such

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as those based on silicones, which claim to minimise wound adherence (Thomas, 2003); and those based on materials that gel on contact with exudate, for example, Hydrofiber® dressings, which have clinically been associated with reduced pain upon dressing removal (Caruso et al, 2004; Jurczak et al, 2007).

The terms 'adherent' and 'adhesive' are often inappropriately used as interchangeable descriptive words relating to dressing properties. Thomas (2003) suggested that 'adhesive' should describe the interaction between the dressing and the peri-wound tissue, whereas 'adherent' describes the interaction of the dressing and the wound. While the former may be desirable, the latter should be avoided in a chronic wound. Dressing removal without causing tissue damage or pain to the patient is an important factor in helping to achieve the best possible clinical outcome within an appropriate protocol of care.

In addition to low-adherence, a dressing may also be designed to reduce the risk of infection. This is most commonly achieved by the addition of an antimicrobial agent.

A recent *in vitro* study on silver-containing dressings, including gelling fibre dressings and certain adhesive foam dressings, suggested that both dressing conformability and silver availability at the wound surface may be important in optimising the antimicrobial function of the dressing (Bowler et al, 2010).

This paper presents further *in vitro* studies which investigate the challenges of combining low-adherence with antimicrobial protection in a modern wound dressing. This was undertaken by comparing commercially available examples of silver-containing gelling fibre dressings with certain silver-containing foam dressings that have an adhesive wound contact layer.

Materials
The silver-containing dressings and secondary adhesive cover dressing (ACD) used are listed in *Table 1*.

Bioadhesion studies
Cell culture grade 6-well plates (Griener Ltd, UK); primary equine chronic wound fibroblasts isolated from tissue debrided during surgery; 0.5% Trypsin-EDTA solution; fetal calf serum (FCS) (Sigma, UK);

Table 1
Materials used in the studies

Silver-containing dressings	Dressing type
HF-Ag (AQUACEL® Ag, ConvaTec)*	Hydrofiber® dressing with 1.2% w/w ionic silver*
AL-Ag (SILVERCEL™ Non-adherent, Systagenix Wound Management)*	Non-adherent Hydro-Alginate dressing containing silver-coated nylon fibres
Foam A (Mepilex™ Ag, Mölnlycke Healthcare)	Silicone adhesive foam containing silver sulphate
Foam B (Allevyn™ Ag Gentle, Smith and Nephew)	Soft gel adhesive foam containing silver sulfadiazine
Foam C (Allevyn™ Ag Gentle Border, Smith and Nephew)	Silicone gel adhesive foam containing silver sulfadiazine

*Note: these dressings were covered with the adhesive cover dressing (ACD) (Versiva® XC® Adhesive (10x10cm), an adhesive gelling foam dressing with Hydrofiber® Technology: ConvaTec)

Hank's balanced salt solution (HBSS) (Invitrogen, UK), Neubauer counting chamber; a liquid growth medium comprised of: Dulbecco's modified Eagle medium (DMEM), supplemented with 10% fetal calf serum, 20mm Hepes buffer, 100µg/ml penicillin/streptomycin and 0.5µg/ml amphotericin (Invitrogen Ltd, UK).

Microscopy
Light microscope (Olympus SZ61) with QImaging camera (Device 3564); Image Pro Plus 7.0 image analysis software (MediaCybernetics, UK); a QUANTA 200 scanning electron microscope (SEM) (FEI Electron Optics, Holland) with Oxford INCA Energy Dispersive X-Ray Micro-Analysis (EDX) (Oxford Instruments, UK); K550X Sputter Coater (EMITECH, UK).

Seeded Agar Microbial Model
Microbiological Media: pre-dried Tryptone Soy Agar Plates (TSA); molten Tryptone Soy Agar (TSA) (pre-cooled to ≈45°C); Maximal Recovery Diluent (MRD); DE Neutralising Agar (DEA); Challenge organisms: *Staphylococcus aureus* (NCIMB 9518) and *Pseudomonas aeruginosa* (NCIMB 8626).

Methods
Simulated bioadhesion studies
These studies used a quantitative *in vitro* model that was developed for the measurement of cellular adhesion to wound dressings (Cochrane et al, 1999). Briefly, equine fibroblasts were harvested from stock dishes, and plated out at 2×10^5 cells per well in six-well cell culture grade plates. The plates were incubated at 37°C for 24 hours to allow the cells to attach to the dish. Test dressings were cut into 1cm^2 pieces and either applied dry or pre-hydrated in isotonic saline solution, placed firmly but carefully onto the surface of the cell layer and incubated for a further 24 hours. After incubation, the test dressings were carefully removed to avoid cell damage or disruption and washed with HBSS to remove any non-adherent cells. After washing, trypsin-EDTA was applied to the dressings to release adhered cells and the reaction was stopped by adding DMEM with FCS. The released cells were centrifuged at 1400rpm for four minutes and the supernatant was discarded. The cell pellets were re-suspended in DMEM, the number of released cells was determined manually using a Neubauer counting chamber, and the number of cells that had adhered to each dressing was calculated. This experiment was repeated six times for each test dressing both in the dry and pre-hydrated states.

Microscopy
Topographical features of the wound contact surface of each dressing were studied using a light microscope at low magnification (x15 to x50), and a scanning electron microscope (SEM) for higher magnification. For SEM, samples were gold-coated before examination (approximately 2–3 minutes in the sputter coater).

Image analysis
Quantitative measurements of the visible open liquid absorption channels (i.e. pores) on the wound contact surface of each dressing were made from images produced under the light microscope. Image analysis software (Image-Pro Plus Version 7.0, MediaCybernetics®, UK) was used to measure and calculate the maximum dimensions, surface area and percentage surface area of these channels from images each with a field of view of approximately 2x1cm.

Seeded agar microbial model
Preparation
Two bacterial species (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) were studied separately. Overnight-colonies were cultured on TSA plates, from which bacterial suspensions in MRD were prepared, such that they had an optical density equivalent to approximately 1×10^8cfu/ml. These suspensions were further diluted in MRD to give a population of approximately 1×10^7cfu/ml. A quantitative count of this solution was performed to accurately confirm the inoculum concentration.

Molten TSA (80ml) was dispensed into 140mm Petri-dishes and allowed to solidify. 2ml of the 10^7 bacterial suspension was inoculated into 200ml of molten TSA (pre-cooled to approximately 45°C) to give a final concentration in the agar of approximately 1×10^5cfu/ml. A 45ml volume of this bacteria-containing molten TSA was aseptically poured over the solidified 80ml agar layer already in the dish and allowed to cool. The result was a seeded-agar layer, approximately 2–3mm in depth overlaying a 6mm deep sterile agar layer. All seeded agar plates were incubated at 35°C (±3°C) for four hours to initiate growth.

Table 2

Adherence of fibroblasts to the silver-containing dressings

Dressing	Number of cells adhered, average (n=3)		Cell adherence expressed as a % of highest result		% reduction of cell adherence caused by pre-hydration
	Dry	Pre-hydrated	Dry	Pre-hydrated	
HF-Ag	27167	17333	34	25	36
AL-Ag	46333	21167	58	31	54
Foam A	75000	69000	95	100	8
Foam B	69000	55000	87	80	20
Foam C	79333	68667	100	100	13

Dressing application

Immediately following the four-hour incubation period, a silver-containing dressing (≥10cm×10cm) was aseptically applied to the centre of a seeded-agar plate (n=3 for each challenge organism), and gently pressed down to ensure good contact with the surface. For HF-Ag and AL-Ag dressings, an adhesive absorbent cover dressing (ACD) was applied as a secondary dressing, as indicated in the manufacturers' instructions for use. A seeded-agar plate without a dressing was also included as a negative control (n=1 for each challenge organism) to confirm the extent of uninhibited bacterial growth. All plates (including negative controls) were incubated aerobically at 35°C (±3°C) for 48 hours, after which time dressings were aseptically removed and the plate and the wound contact surface of the dressing were photographed. All seeded-agar plates were re-incubated at 35°C (±3°C) for an additional 24

hours to allow any inhibited but still viable bacteria to mature into visible colonies. After incubation the plates were visualised and photographed. To assess whether the silver-containing dressings were bactericidal (i.e. killed bacteria) or bacteriostatic (i.e. inhibited bacterial growth), a stab culture (i.e. a sterile loop inserted into the bacteria seeded agar) was taken from the centre of each seeded-agar plate and sub-cultured onto DEA plates. DEA was used because of its ability to neutralise any residual silver present. The negative controls were processed in an identical manner to confirm the viability of each challenge organism. All DEA plates were incubated at 35°C (±3°C) for at least 24 hours before visual observation for bacterial growth or no growth.

In addition to testing whole dressings in a simulated wound model, the effect of dressing orientation and cutting was also investigated.

Three sections of each dressing were aseptically cut using sterile scissors (minimum 5mm wide across the full width of the dressing). Each section was applied to a seeded-agar plate with a different face in contact, i.e. the first with the wound contact surface exposed to the seeded-agar, the second with the upper or dressing-backing surface exposed, and the third was with a cut edge of the dressing. Plates were then incubated as above, removing the dressing to make observations and take photographs after 48 hours.

Statistical analyses

In the *in vitro* bioadhesion studies, a two-sample significance test (i.e. t-test) was carried out using the statistical package Minitab Release 15 for Windows® 2007.

Results

Simulated bioadhesion

There were marked differences in the adherence of fibroblasts to the silver-containing dressings tested. This was dependent on the dressing type and level of hydration or gelling effect (*Figure 1* and *Table 2*).

Cell adherence to the tested adhesive foam dressings was significantly greater in this *in vitro* model than that for gelling fibre dressings (p<0.001) in both the dry and pre-hydrated states. The AL-Ag dressing exhibited significantly less cell adherence (p<0.001) when compared to the adhesive foam dressings tested in both states. The HF-Ag dressing showed significantly less cell adherence
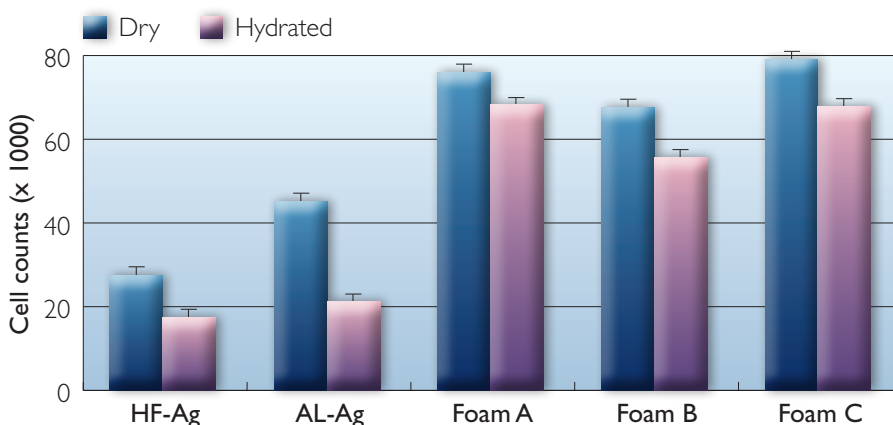

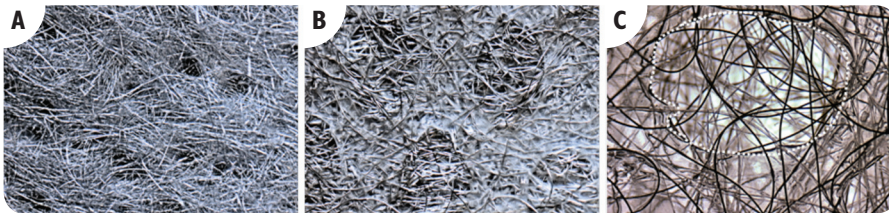Figure 1. Simulated bioadhesion of dry and pre-hydrated silver-containing dressings.

Figure 2. Light microscopy images of the WSCL of the silver-containing fibrous dressings visualising HF-Ag (A); AL-Ag (B). Higher magnification observations of both light and dark fibres visualised within the AL-Ag dressing (C).
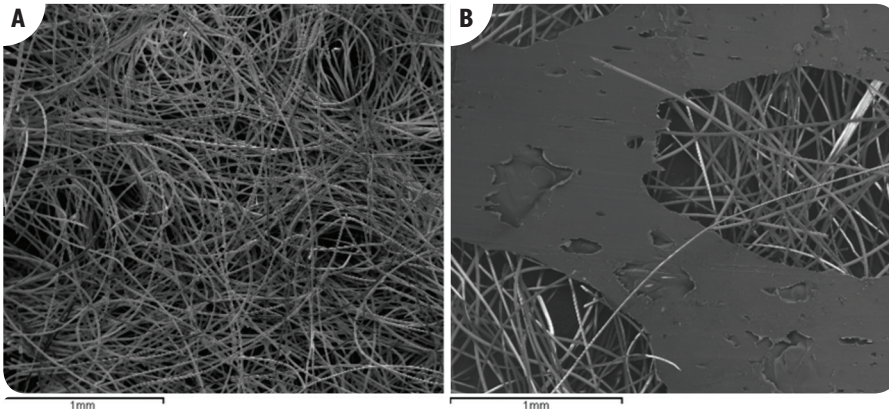

Figure 3. SEM observations of the WSCL of the two silver-containing fibrous dressings, HF-Ag (A); AL-Ag (B).

Figure 4. Light microscopy observations of the WSCL of the three silver-containing foam dressings. Foam A (A); foam B (B); foam C with higher magnification observation of raised rims (C).
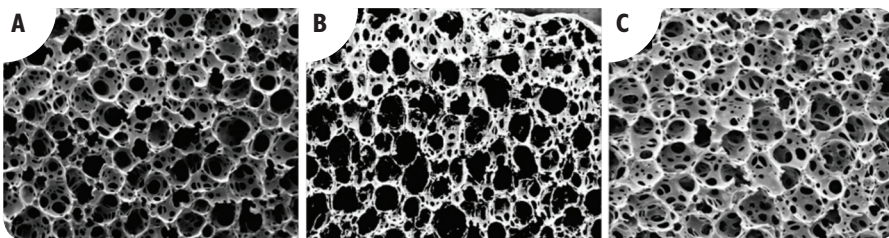

Figure 5. SEM observations of the overall structure of the three foam dressings. Foam A (A); foam B (B) and foam C (C).
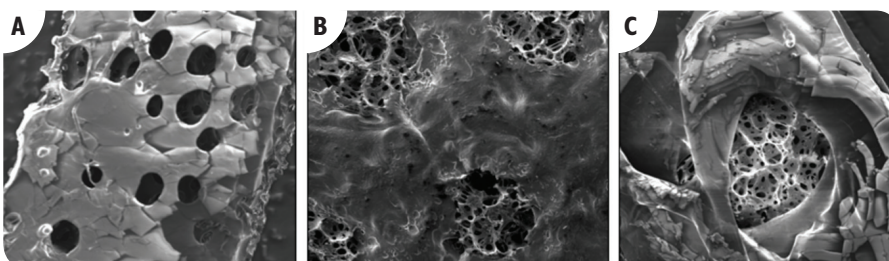

Figure 6. SEM observations of the WSCL of the three silver-containing foam dressings. Foam A (A); foam B (B); and foam C (C).

($p<0.001$) when compared to the AL-Ag in the dry state, and the foam dressings in both dry and pre-hydrated states. No statistical differences were observed between the foam dressings in either state.

All the dressings were observed to have lower adherence when the dressing was pre-hydrated compared to the corresponding dry dressing, but this was greater both in numerical and percentage terms for the gelling dressings.

The greatest relative (54%) and absolute reduction (25,166 cells/cm^2) in cell adherence was observed for AL-Ag. However, the HF-Ag had the lowest adherence in both dry and hydrated states in this *in vitro* model.

Microscopy

Light microscopy revealed that the HF-Ag dressing was composed of randomly oriented uniform fibres as previously described (Hoekstra et al, 2002). The surface of this dressing was difficult to define as it was not flat, smooth or regular. There were no observed differences between the sides (or faces). None of the dressing fibres were occluded, therefore all of the surface area (100%) would be available for absorption of fluids from a wound (*Figures 2a* and *3a*).

The AL-Ag dressing had a similar physical fibre-pad structure (*Figure 2b*). However, at a higher magnification (x50) two different fibre types could be identified (*Figure 2c*). One fibre was observed to be dark in colour (possibly the metallic silver-coated nylon fibres described by the manufacturer), while the other fibre was observed to be more translucent (possibly the gelling alginate fibre). The fibre pad of the AL-Ag dressing was wrapped in a thin 'plastic sleeve' perforated with regular and approximately oval holes. These holes were visibly observed in *Figure 2b*, are highlighted in *Figure 2c*, and were most clearly visible in the SEM image (*Figure 3b*). The size (approximately 2.0mm maximum x 1.3mm minimum) and area of these perforations was measured by image analysis and it was estimated that they constituted
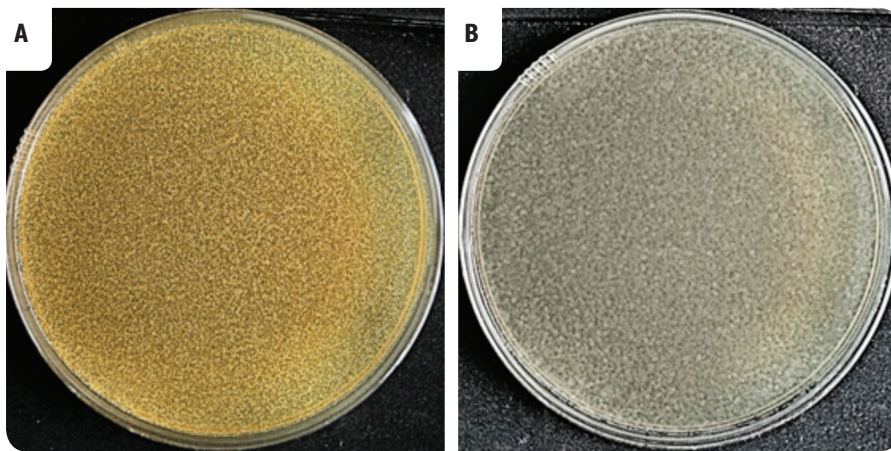
Figure 7. Negative control seeded agar plates (containing no dressing). Confluent growth of *S. aureus* (A) and *P. aeruginosa* (B) is observed.
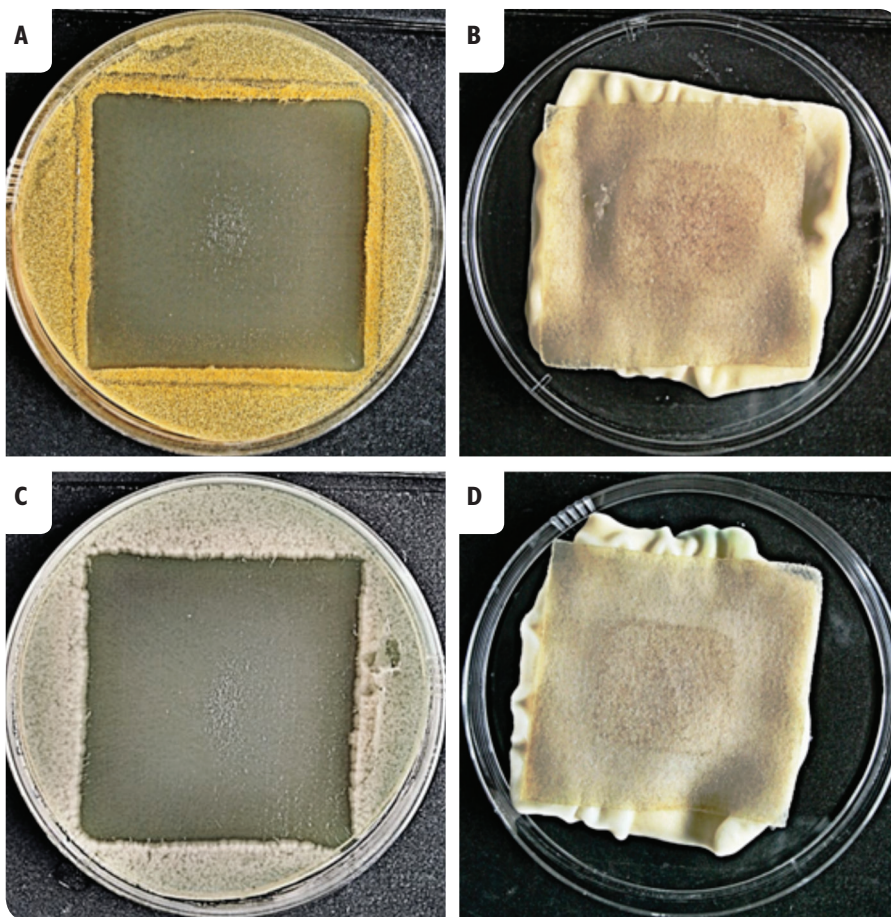

Figure 8. Observed effect of HF-Ag/ACD dressing on a *S. aureus* (A) and *P. aeruginosa* (C) simulated colonised wound surface after a 48-hour contact period. No bacterial growth was visually observed beneath the position where the dressing had been applied and similarly there was no observable evidence of *S. aureus* (B) or *P. aeruginosa* (D) growth upon the respectively removed HF-Ag/ACD dressings after the 48-hour contact period.

different and more random in structure. Similarly, the foam components of foams B and C appeared visually to be similar but different from foam A. However, scanning electron micrographs of the three foams demonstrated that they shared many structural similarities and were observed to be composed of a series of interconnecting chambers (*Figures 5a, b and c*). The WSCL of foam A was observed to have random and irregular shaped surface openings (*Figure 6a*). In comparison, foams B and C had regular, uniformly distributed, equally sized and approximately 1mm diameter circular perforations in the adhesive (*Figures 6b and c*). An estimation of exposed accessible pores in foam A was <7%, whereas foams B and C gave areas of approximately 10%. The WSCL surfaces of foams A and B were observed to be relatively flat (*Figures 6a and 6b* respectively), whereas foam C was observed to have a more undulating surface, with visible raised rims around each perforation (*Figures 4c and 6c* respectively). The adhesives all appeared to have some hydrophobic properties and were also observed to be a barrier to liquid water (data not shown).

Elemental analysis (EDX elemental maps and spectra not shown) of the SEM samples indicated that silver was associated with all of the fibres in the HF-Ag dressing and to be evenly distributed. Silver was also detected on a significant proportion of the fibres within the AL-Ag dressing, but there was no observable evidence of silver associated with the non-adherent sleeve areas (*Figure 3b*). For the foam dressings, no silver was detected or associated with the WSCL, it was only observed in the foam portion of the dressing. The WSCL adhesive components of foams A and C were observed to contain significant amounts of silicone; this element was not observed to be present in the adhesive layer of foam B, suggesting this was a different type of adhesive.

Seeded-agar microbial model

The seeded-agar layer of the negative control plates (no dressing) appeared opaque and contained confluent bacterial

approximately 40% of the surface area of the dressing. The thin film of the sleeve was visually observed to be a barrier to liquid water (data not shown).

The wound surface contact layer (WSCL) of each foam dressing was observed to be a transparent and perforated layer which appeared to be well attached to the translucent bulk foam structure behind (*Figures 4a, b and c*). The WSCLs of foams B and C were visually similar and regular, but that of foam A appeared to be somewhat
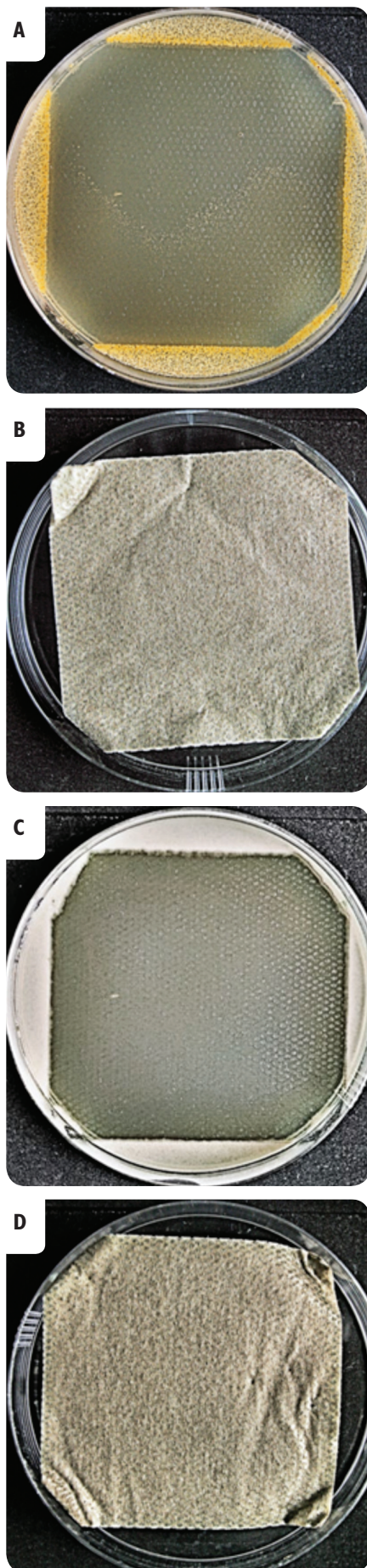
Figure 9. Observed effect of AL-Ag dressing on a *S. aureus* (A) and *P. aeruginosa* (C) simulated colonised wound surface after a 48-hour contact period. Negligible growth of *S. aureus* was visually observed beneath the position where the dressing had been applied (A); no growth of *P. aeruginosa* was visually observed beneath the position where the dressing had been applied (C). There was no observable evidence of *S. aureus* (B) or *P. aeruginosa* (D) growth upon the respectively removed AL-Ag dressings after the 48-hour contact period.

growth (*Figure 7*). Stab cultures taken from this seeded layer yielded heavy growth, thus confirming the viability of both *S. aureus and P. aeruginosa* over the test period. *Table 3* summarises the bacteriostatic and bactericidal properties of the dressings tested.

At the end of the initial 48-hour dressing contact period and after a further 24 hours incubation of the plate with the dressing removed, the area

that had been in contact with the HF-Ag dressing remained transparent and no bacterial colonies were observed (*Figures 8a* and *8c*). Negligible growth of *S. aureus* was observed with AL-Ag (*Figures 9a and 9c*). Stab cultures of the HF-Ag plates produced no growth on DEA (indicating bactericidal activity), whereas growth was observed from both organisms for stab cultures taken from AL-Ag plates (indicating only bacteriostatic activity). Both the HF-Ag and AL-Ag dressings prevented bacterial proliferation when presented with either face and cut edge, thereby showing no orientation effects (*Figure 15*).

Growth of both *S. aureus* and *P. aeruginosa* in the seeded-agar beneath all of the foam dressings was observed after 48 hours contact (*Figures 9, 10* and *11a* and *11c*). *S. aureus* growth was also observed on the WSCL of foams A and C (*Figures 10b* and *11b*).
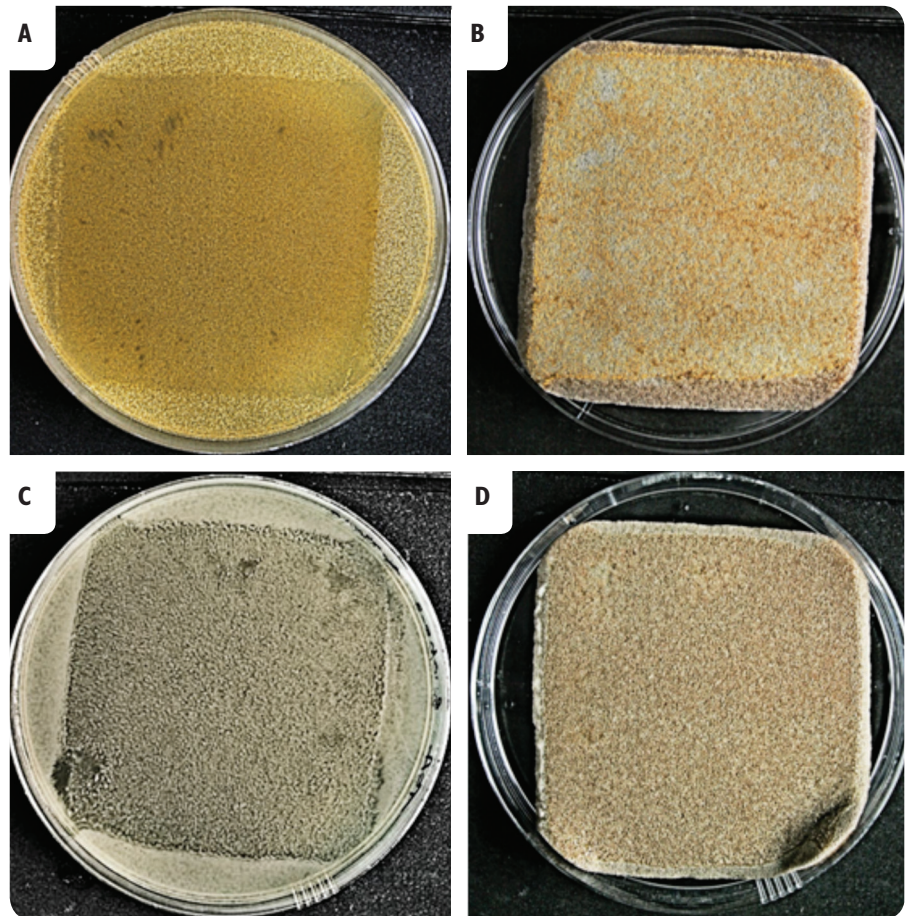

Figure 10. Observed effect of foam A dressing on a *S. aureus* (A) and *P. aeruginosa* (C) simulated colonised wound surface after a 48-hour contact period. Heavy bacterial growth was visually observed beneath the position where the dressing had been applied (C). Similarly, heavy growth was observed of *S. aureus* (B) and *P. aeruginosa* (D) growth was observed upon the respectively removed AL-Ag dressings after the 48-hour contact period.
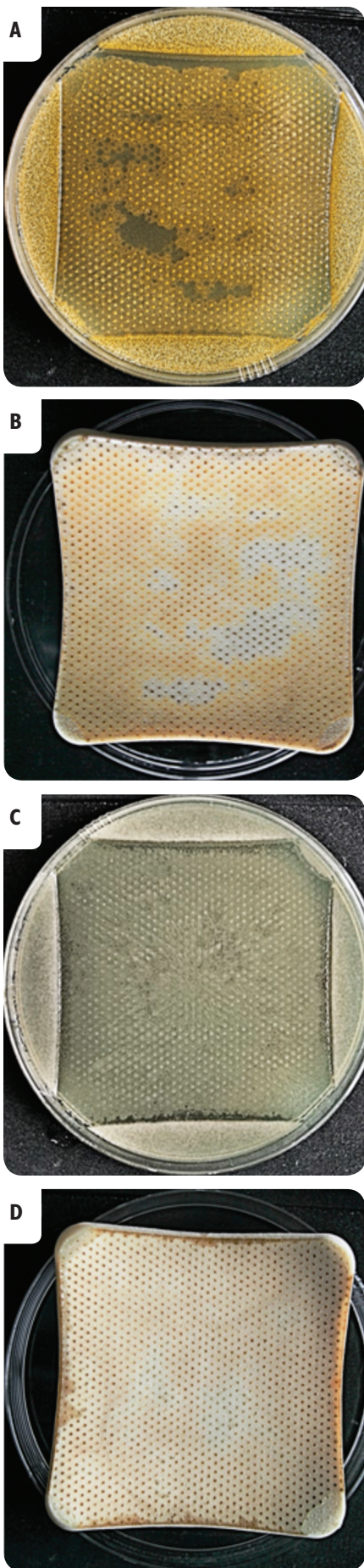
A

B

C

D

Figure 11. Observed effect of foam B dressing on a *S. aureus* (A) and *P. aeruginosa* (C) simulated colonised wound surface after a 48-hour contact period. Bacterial growth was visually observed beneath the position where the dressing had been applied (A and C). *S. aureus* growth was observable on the removed dressing after the 48-hour contact period (B), but no visible growth was observed with *P. aeruginosa* (D).

The appearance of the seeded-agar was unchanged following a further period of 24 hours' incubation. Stab cultures were positive for both microorganisms for all three foam dressings. Distinctive patterns of bacterial growth were observed beneath foams B and C (*Figures 11* and *12*), consistent with the size and spacing of the pores in the adhesive layer.

When dressing orientation was tested, similar results were observed for the three foam dressings. *Figure 13* demonstrates how this test was performed. The results indicated that

visualisation of bacterial growth was independent of the challenge organism, but may be dependent on a dressing's ability to make the antimicrobial agent available at the dressing/seeded-agar interface. When the upper surface of the dressing was presented to the seeded-agar surface, there was a strong tendency for foams B and C to curl away from the surface (*Figures 15c* and *15d*). However, even where it remained in contact, there was no observed inhibition of bacterial growth. The visualised pattern of growth was also different because of the absence of pores (*Figures 15c and 15d*). Although foam A remained in contact with the seeded-agar surface it was observed that both the WSCL and the upper surface did not inhibit growth (*Figure 14*). When a cut edge of each foam dressing was placed onto the seeded-agar surface, no growth was observed (*Figures 14* and *15c* and *15d*).
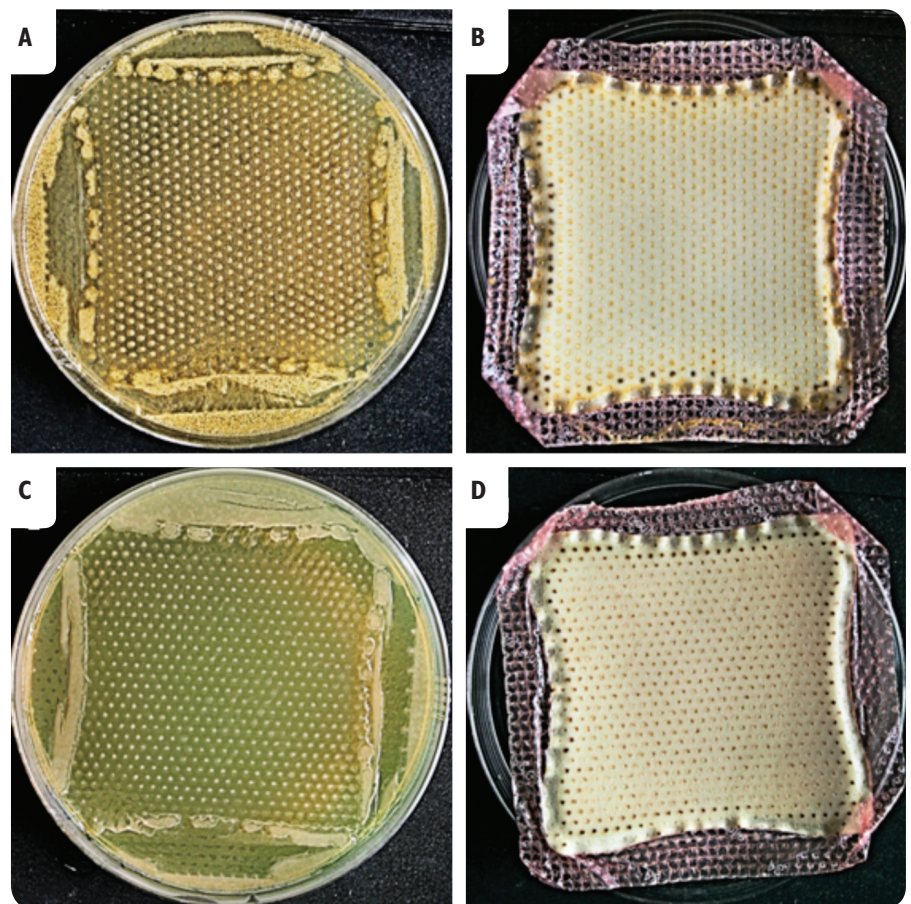

A

B

C

D

Figure 12. Observed effect of foam C dressing on a *S. aureus* (A) and *P. aeruginosa* (C) simulated colonised wound surface after a 48-hour contact period. Bacterial growth was visually observed beneath the position where the dressing had been applied. *S. aureus* growth was observable on the removed dressing after the 48-hour contact period (B), but no visible growth was observed with *P. aeruginosa* (D).
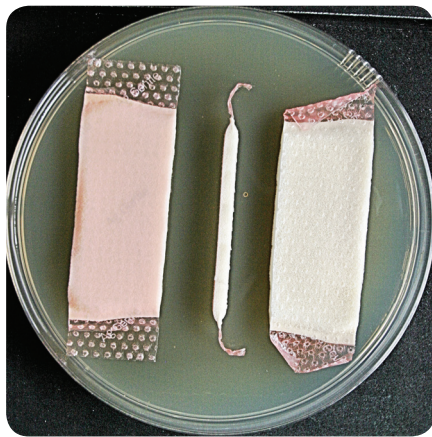
Figure 13. Example showing foam B dressing strip orientation on the seeded agar plate.
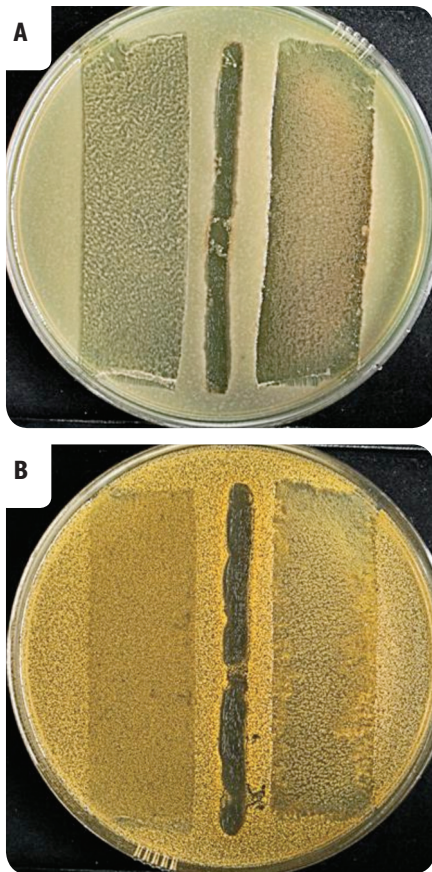

Figure 14. Observed effect of different foam A dressing faces on a *P. aeruginosa* (A) and (B) *S. aureus* seeded agar surface after a 48-hour contact period. Bacterial growth is visually observed beneath the position where the wound contact and the outer surface dressing strip had been applied.

For the AL-Ag dressing there is no specific WSCL and both faces of the dressing could be placed onto the seeded-agar surface. Consequently, both sides were observed to show slight growth. However, when this

dressing was placed on its exposed cut edge, no growth was visible (*Figure 15b*). For the HF-Ag dressing, no growth was observed regardless of the orientation of the dressing (*Figure 15a*).

Discussion

One of the key issues for patients, regardless of wound aetiology, is the prevention of pain and trauma associated with dressing removal through adherence to the wound bed (Moffatt et al, 2002; World Union of Wound Healing Societies [WUWHS], 2007; Woo et al, 2008).

Equally important to clinicians is the need to limit the risk of infection in wounds due to the presence of invading wound pathogens (Cutting and Harding, 2004; WUWHS, 2008; Best Practice Statement, 2011). Both aspects should be considered as part of a protocol of care for 'at-risk' wounds. However, wound care product awareness and an ability to select an appropriate wound dressing is still thought to be highly variable (Moffatt et al, 2002).

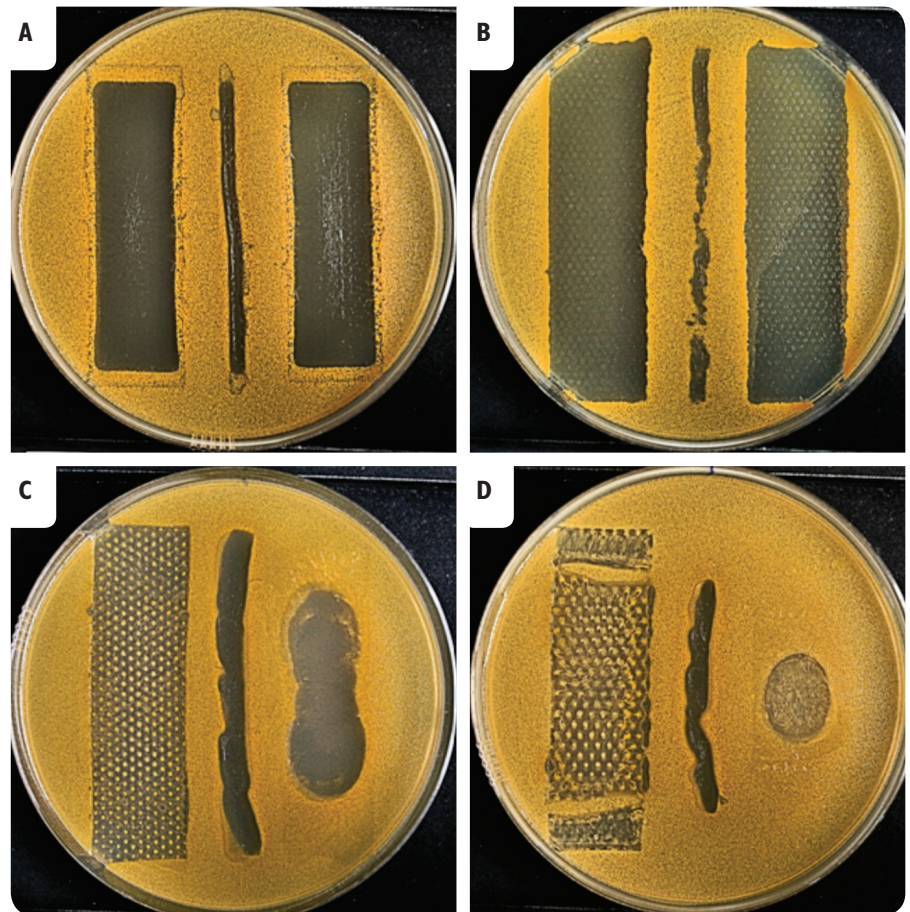In an *in vitro* fibroblast bioadhesion model, it was observed that all the

Figure 15. (A) Observed effect of different HF-Ag dressing faces on a *S. aureus* seeded agar surface after a 48-hour contact period. No bacterial growth is visually observed beneath the position of each dressing strip.
(B): Observed effect of different AL-Ag dressing faces on a *S. aureus* seeded agar surface after a 48-hour contact period. Negligible growth of *S. aureus* is visually observed beneath the position where the wound contact and the outer surface dressing strip had been applied.
(C): Observed effect of different foam B dressing faces on a *S. aureus* seeded agar surface after a 48-hour contact period. Bacterial growth is visually observed beneath the position where the wound contact strip had been applied; negligible growth of *S. aureus* is visually observed beneath the outer dressing surface strip.
(D): Observed effect of different foam C dressing faces on a *S. aureus* seeded agar surface after a 48-hour contact period. Bacterial growth is visually observed beneath the position where the wound contact and the outer surface dressing strip had been applied.

Table 3
Bacteriostatic and bactericidal properties of the dressings tested

Dressing	P. aeruginosa			S. aureus		
	Bactericidal	Bacteriostatic	No effect detected	Bactericidal	Bacteriostatic	No effect detected
HF-Ag	■			■		
AL-Ag		■			■	
Foam A			■			■
Foam B			■			■
Foam C			■			■

Notes:
*The comments regarding bacteriostatic activity (e.g. stab culture of the seeded agar plate) are the result of visual observations of DEA plates (n=3 unless stated)

+The comments regarding bactericidal activity (e.g. stab culture of simulated colonised wound surface) are the result of visual observations of DEA plates (n=3 unless stated)

tested dressings had a higher cellular adhesion when applied in a dry state than when hydrated, and that the two examples of gelling fibre dressings were significantly less adherent than the three examples of adhesive foam dressings tested (p<0.001). Although cellular adhesion was reduced by product hydration, this reduction was only slight for the adhesive foams, suggesting that the presence of fluid may have had little effect on their adhesive properties. For the gelling dressings, the reduction in adherence was greater and more significant (*Figure 1*). This suggests that the fibres' ability to absorb fluid and swell considerably to form a soft cohesive gelled structure (as discussed for HF-Ag by Newman et al, 2006) may be an important factor in reducing cell adhesion, and may explain the reported significantly reduced pain levels observed both during wear time (reduction from baseline at three days [p=0.0006], Chen et al, 2005) and during dressing changes (Caruso et al, 2006 [p=0.009]; Saba et al, 2009 [p<0.01]; and Muangman et al, 2010 [p<0.02]).

Recent *in vitro* studies suggest a possible correlation between achieving close proximity of silver-containing dressings to the wound bacterial bioburden and antimicrobial activity

(Bowler et al, 2010; Cavanagh et al, 2010). These studies investigated the relative abilities of certain adhesive foam dressings and gelling fibre dressings to contour closely to a simulated shallow wound bed (Bowler et al, 2010; Cavanagh et al, 2010).

In these latest *in vitro* studies, a flat agar surface was used so that close contact between the WSCL of the dressing and the contaminated surface was guaranteed. Under these conditions the ability of each dressing to make available sufficient antimicrobial agent to exert an effect at the wound-dressing interface was investigated. The gelling fibre dressing (HF-Ag) killed bacteria on the flat agar surface. The tested foam dressings with an adhesive WSCL did not appear to prevent the growth of bacteria on the flat agar surface. While AL-Ag showed bacteriostatic activity (i.e. prevented bacterial proliferation but did not kill all bacteria), HF-Ag produced a bactericidal action (killed all viable bacteria) (*Table 3*). The three silver-containing foam dressings tested were neither bacteriostatic nor bactericidal in this *in vitro* model, and bacterial growth was observed directly beneath these intact foam dressings. However, when a cut edge of each dressing was placed directly onto a bacteria-seeded agar surface (i.e. exposing the inner

foam matrix), greater antimicrobial activity was visually observed.

Elemental analysis revealed that the silver was exclusively contained within the foam structure and was not associated with the adhesive. These observations suggested that the adhesive WSCL of the foam dressings may have been acting as a barrier to the availability of the antimicrobial agent to the underlying bioburden. Similar conclusions were drawn in an independent study, which reported hydrophobicity and antimicrobial activity only from the exposed foam (Cavanagh et al, 2010). HF-Ag was observed to contain only gelling fibres with silver evenly distributed throughout. Contact with the high water content agar caused the surface to gel, and close contact was observed between the dressing and agar over the whole interface between the dressing and the bacteria-seeded agar. AL-Ag was observed to be a mixture of gelling fibres and non-gelling fibres (e.g. silver-coated nylon fibres). The fibrous pad was contained within a perforated outer sleeve, which limited the area of direct contact between the pad and the bacteria-seeded surface to approximately 40%. It was possible to suggest that a combination of a limited direct exposure, the nature of the source of silver and the presence of

non-gelling fibres may be contributory factors to the greater cell adhesion and less effective antimicrobial performance of AL-Ag compared to HF-Ag.

Conclusions

In these *in vitro* studies, dressings constructed from gelling fibres produced a lower cell adherence than certain foam dressings with an adhesive layer across the wound-contact surface, particularly in the presence of hydration. The lowest adherence was observed for HF-Ag, which was the only dressing tested to be entirely constructed from gelling fibres.

Observed antimicrobial activity was highly variable in this *in vitro* model, despite all the dressings containing silver and the test model being designed to eliminate the possible effect of dressing conformability and ensure direct contact between the dressing and the underlying bioburden. The observations from this simple *in vitro* model suggest that the presence of an adhesive layer may be a physical barrier to silver contained

within certain dressings. The dressing constructed entirely from gelling fibres with silver distributed homogeneously throughout and without a containing outer sleeve (HF-Ag) exhibited bactericidal activity in this *in vitro* model.

These studies, in conjunction with other published data (Parsons et al, 2005; Bowler et al, 2010; Walker and Parsons, 2010) suggest that dressing technology and construction may be important factors in determining the antimicrobial activity of dressings. Clinical studies and a variety of *in vitro* models should be used to obtain additional relevant data to assist with product selection. **W**ᴜᴋ

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