

Dressing conformability and silver-containing wound dressings

Phil Bowler, Samantha Jones, Victoria Towers, Rebecca Booth, David Parsons, Mike Walker

Abstract

Background: All wounds have unique and irregular topographies, including cavities where fluids and bacteria may collect and increase the risk of wound infection. **Aims:** To qualitatively and quantitatively investigate the relationship between the physical structure of antiseptic wound dressings and their ability to manage bioburden in models that simulate clinical conditions. **Methods:** *In vitro* models were utilised to enable both visualisation of the conformability of silver-containing dressings with a simulated wound tissue surface, and measure the corresponding antimicrobial effect that these dressings had on a shallow wound microbial model. **Results:** Tissue contact and antimicrobial activity was shown with a silver-containing Hydrofiber® dressing (HF-Ag) over a 48-hour contact period. In contrast, the silver-containing foam dressings tested demonstrated areas of non-conformability which were associated with reduced antimicrobial activity. **Conclusions:** These *in vitro* studies confirm that both dressing conformability and silver availability to bacteria at the wound surface are critical to the optimum functioning of silver-containing dressings. **Conflict of interest:** The study was sponsored by ConvaTec.

KEY WORDS

Hydrofiber® Technology
Foam dressing
Conformability
Silver
Antimicrobial efficacy

Chronic wounds are polymicrobial, with bacterial colonisation originating from external sources such as surrounding skin, the gut and the mouth. As a consequence, an often complex bacterial burden exists, predominantly in superficial wound tissue, which has the ability to compromise wound progression (Bowler, 2003). To minimise the opportunity for infection in chronic wounds, it is important that the bioburden is controlled and maintained in a state that

is not problematic to the host, as increasing bioburden can lead to chronic inflammation and increased risk of infection. In this respect, wound management practices such as cleansing, sharp debridement and the use of antimicrobial dressings are important within a protocol of care.

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When considering the use of antimicrobial dressings, many factors will influence the likely potency of the dressing. Wound-related factors include wound depth and size, amount of devitalised tissue, wound bioburden and the presence of biofilm. Dressing-related factors include the type and concentration of antimicrobial agent, availability of the agent from the vehicle in which it is contained, and the ability of the dressing to closely contact the surface of a wound and hence maximise exposure of the bioburden to the antimicrobial agent. This latter factor is challenged by the

fact that chronic wounds are anatomically highly variable; they may vary in depth from sinuses and penetrating pressure ulcers to relatively shallow leg ulcers. All wounds have unique and irregular topographies, including cavities where fluids may collect, and result in the creation of 'dead spaces' (Edberg, 1981; Snyder, 2005). This fluid may contain harmful components (e.g. bacteria and cellular debris) which may increase the risk of wound infection (Cutting et al, 2009).

In the *in vitro* studies described in this paper, models were developed and utilised that enabled both visualisation of the conformability of silver-containing dressings with a simulated wound tissue surface, and the corresponding antimicrobial effect that these dressings had on a shallow wound microbial model.

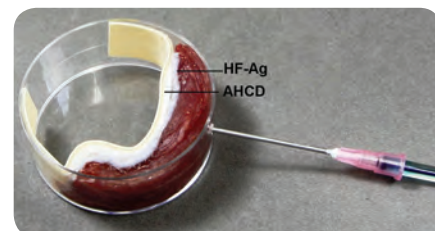


Figure 1. Porcine muscle tissue fixed to the inside wall of a 50mm Petri dish to provide an irregular tissue surface.

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Materials

The silver-containing wound dressings used are listed in *Table 1*.

Tissue conformability model:

Pork muscle tissue (purchased at a local supermarket); 50mm Petri dish; PVC tubing (1.65mm; Watson Marlow); dye solution (5–10mg of methylene blue dissolved in 50ml of physiological saline); Cane Crono PCA Pump (Applied Medical Technology, UK); an Olympus SZ61 light microscope with QImaging camera (Device 3564); Image Pro Plus 5.0 image analysis software (MediaCybernetics, UK).

Shallow wound microbial model:

Microbiological culture media: Tryptone Soy Agar (TSA), Tryptone Soy Broth (TSB); gauze dressing (Topper 8, Johnson & Johnson); physiological saline (0.85%); Challenge organisms: *Pseudomonas aeruginosa* (NCIMB 8626) and *Staphylococcus aureus* (NCIMB 9518).

Methods

Tissue conformability model

Porcine muscle tissue sections (~3x1x1 cm) were fixed to the inside wall of a Petri dish (50mm diameter) with Loctite™ glue to provide an irregular tissue surface. Before fixing the tissue, a small hole was made in the side of the Petri dish using a hot needle to allow the insertion of a syringe needle (18 gauge) into the simulated wound tissue, such that its tip was just visible at the upper surface of the tissue. The dressings investigated were HF-Ag and representative adhesive foam dressings (foams A, B and E). A small strip of each dressing was applied over the tissue surface and, where appropriate (i.e. HF-Ag dressing), a moisture retentive adhesive Hydrofiber® cover dressing (AHCD)] (*Figure 1*).

The Cane Crono PCA pump was set to provide a flow rate for the dye solution of 2ml/hour. Microscopic images were collected every 45 seconds up to a maximum of 60 images.

Shallow wound microbial model

Evaluation of antimicrobial performance was made using an indented agar plate assay (simulating a shallow wound ~2–3mm deep).

Table 1

List of silver-containing dressings used

Silver-containing dressing	Dressing type
HF-Ag (AQUACEL® Ag, ConvaTec)*	Hydrofiber® (HF) with 1.2% w/w ionic silver*
Foam A (Allevyn™ Ag Adhesive, Smith & Nephew)	Adhesive foam containing silver sulfadiazine
Foam B (Allevyn™ Ag Non-adhesive, Smith & Nephew)	Non-adhesive foam containing silver sulfadiazine
Foam C (Allevyn™ Ag Gentle, Smith & Nephew)	Soft gel adhesive foam containing silver sulfadiazine
Foam D (Allevyn™ Ag Gentle Border, Smith & Nephew)	Silicone gel adhesive foam containing silver sulfadiazine
Foam E (Mepilex® Ag, Mölnlycke Healthcare)	Silicone adhesive foam containing silver sulphate

*Note: this dressing was covered with an adhesive Hydrofiber® cover dressing (AHCD)

Preparation of indented agar plates

Within a laminar flow cabinet, molten TSA (80ml), which had been pre-cooled to ~45°C, was dispensed into 140mm Petri dishes and allowed to solidify. Pieces of gauze (2-ply, 4x4cm) were aseptically transferred to the centre of each pre-poured TSA plate and gently pressed down onto the agar surface. A second piece of gauze (2-ply, 5x5cm) was applied over the original gauze, after which a 45ml volume of molten TSA was aseptically poured over the upper gauze layer and allowed to solidify overnight. The gauzes were aseptically removed from each agar plate to create a graduated indentation (5x5cm x ~2–3mm depth), with an

irregular gauze imprinted surface (*Figures 2 and 3*).

A representative colony of each challenge bacterium was separately inoculated into TSB and incubated for four hours on a roller mixer at 35°C (±3°C) to achieve an actively growing population. Each suspension was diluted in TSB to achieve a concentration of approximately 1x10⁸cfu/ml, which was further serially diluted in physiological saline to provide a final inoculum concentration of approximately 1x10³cfu/ml (stock suspension). A microbial count was performed on each stock suspension to confirm the inoculum level.



Figure 2. Preparation of indented agar model. A: gauze fixed within agar; B: gauze being removed; C: graduated indentation, showing an irregular agar surface.



Figure 3. Cross-section of the graduated indentation (~2–3mm deep) and irregular surface.

Four millilitres of the stock suspension was then inoculated directly into the centre of the indented agar plate; this volume filled the indentation to mimic an exuding wound. A silver-containing dressing (~10x10cm, n=3 for each dressing) was placed centrally over the indent in the inoculated agar plate. For the HF-Ag dressing, an AHCD was applied as the secondary dressing, as indicated in the manufacturer's instructions for use. An inoculated indented agar plate containing no dressing was also included as a positive control (n=1 for each challenge organism) to confirm the extent of bacterial growth in the absence of silver-containing dressings.

All agar plates were incubated aerobically at 35°C (± 3°C) for 48 hours, after which time dressings were aseptically removed. Photographs of the agar plates were taken to record any immediately obvious bacterial growth beneath each dressing. All agar plates were then reincubated for a further 24 hours to allow any remaining viable bacterial cells to form complete colonies. This additional incubation step was necessary to enable image analysis to be performed and subsequent quantification of bacterial growth. The total surface area (mm²) of each indented agar surface, and proportion of the area showing bacterial growth was measured using ImageTool for Windows, version 3.0 (The University of Texas Health Science Centre). Results for each silver-containing dressing were reported as the average value from the three replicates.

Results

Tissue conformability model

The HF-Ag dressing was shown to conform well to the irregular surface of the simulated wound tissue (Figure 4a). In contrast, the adhesive foam dressings (foams A, B and E) showed intermittent contact with the tissue surface and associated areas of free fluid collection were evident (i.e. Figures 4b, 4c and 4d respectively).

Shallow wound microbial model

The positive controls of both challenge organisms showed confluent growth at the end of the study period. Figure 5 shows an example for *S. aureus*.

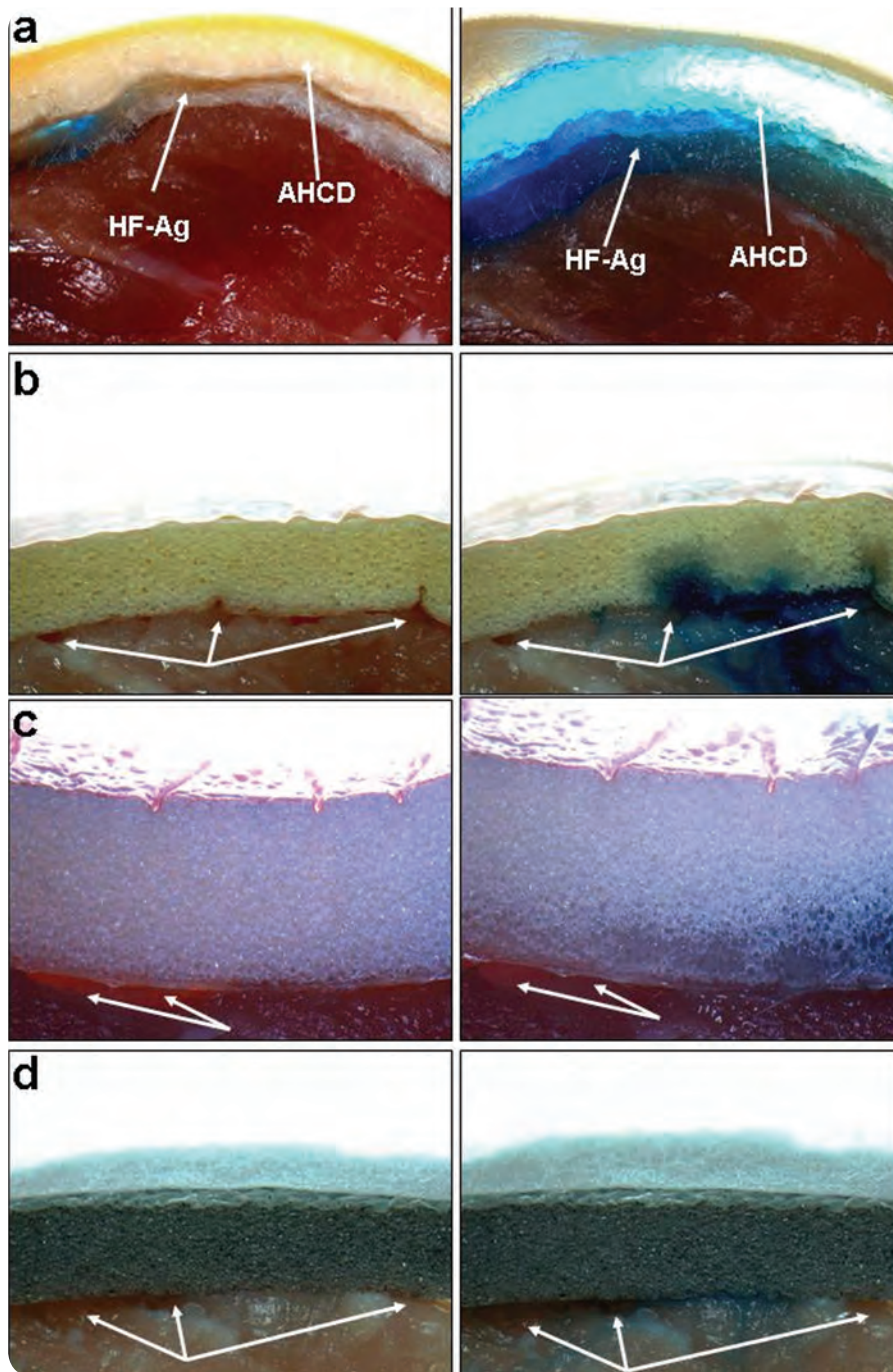


Figure 4. Dressing conformability with simulated porcine muscle tissue surface. a: HF-Ag with AHCD; b: foam A; c: foam B; d: foam E.

The HF-Ag dressing showed widespread antimicrobial activity against both *P. aeruginosa* and *S. aureus* beneath the dressing (Figure 6). The image analysis data indicated that growth of *P. aeruginosa* represented 9.1% of the indented agar surface area (Figure 7), and the corresponding growth of *S. aureus* was negligible (0.4%) (Figure 8). In contrast, all of the silver-containing foam dressings failed to inhibit *P. aeruginosa* (Figure 7). Growth of *S. aureus* beneath the silver-containing foam

dressings ranged from 16.8% (foam B) to 73.2% (foam E) of the total indented agar surface area (Figure 8).

Additionally, extended growth of both *P. aeruginosa* and *S. aureus* beyond the edges of the indented agar surface was observed in association with all of the adhesive silver-containing foam dressings (Figure 9), and growth was mirrored on the wound contact surface, in particular with foam E (Figure 10).

Discussion

Antimicrobial dressings are widely used in the care of chronic wounds, with their primary functions being to control wound bioburden (White et al, 2006), thus preventing infection and facilitating wound progression. Many host and dressing-related factors will influence the efficacy of a topical antimicrobial agent within a wound environment. In this respect, the relationship between the dressing and the antimicrobial agent contained within it is extremely important in ensuring optimal antimicrobial activity. If the dressing cannot maximise the availability of an antimicrobial agent, its efficacy is likely to be compromised. Similarly, if a dressing cannot fill the wound space and conform closely to an irregular wound surface, exposure of superficial wound bacteria to the antimicrobial agent in the dressing is likely to be sub-optimal.

Bearing these factors in mind, *in vitro* models were developed to visualise dressing conformability (i.e. porcine muscle tissue) and a contaminated simulated shallow wound to visualise bacterial growth. While it is accepted that *in vitro* models may have limitations (for example, it is not possible to mimic *in vivo* human biological support systems such as blood supply), these studies were specifically designed to evaluate the antimicrobial and tissue conformability properties of silver-containing wound dressings over a 48-hour contact period, and are an extension of previously published *in vitro* work (Jones et al, 2005).

The tissue conformability studies demonstrated that following hydration,

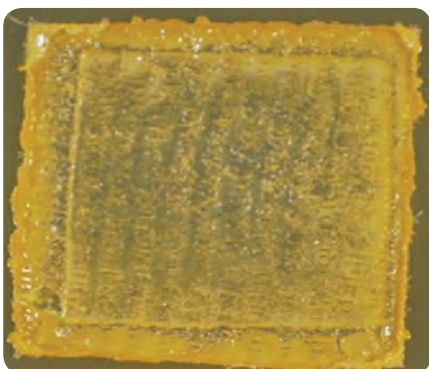


Figure 5. Example of growth of *S. aureus* without dressing application.

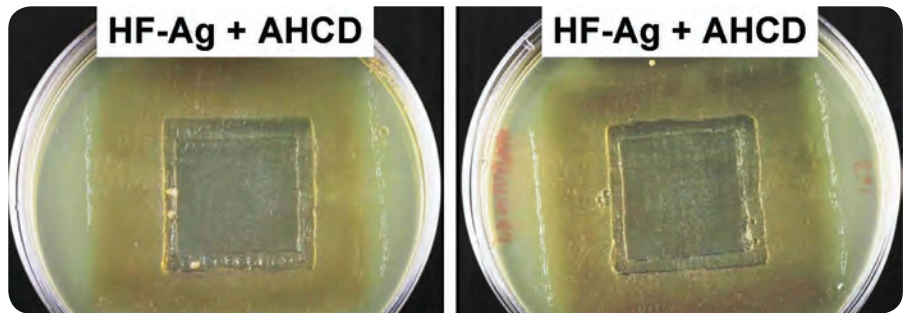


Figure 6. Example of growth of *P. aeruginosa* (left) and *S. aureus* (right) beneath HF-Ag.

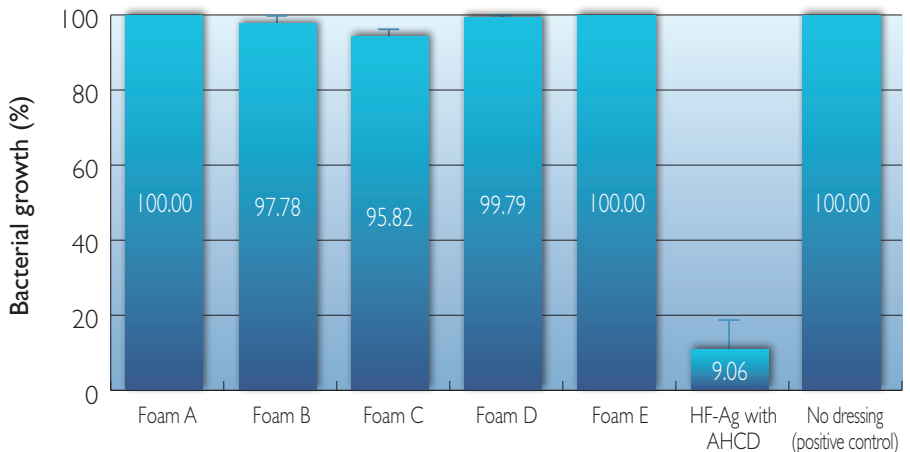


Figure 7. Percentage surface area of *P. aeruginosa* growth beneath the dressings within the indented agar surface area.

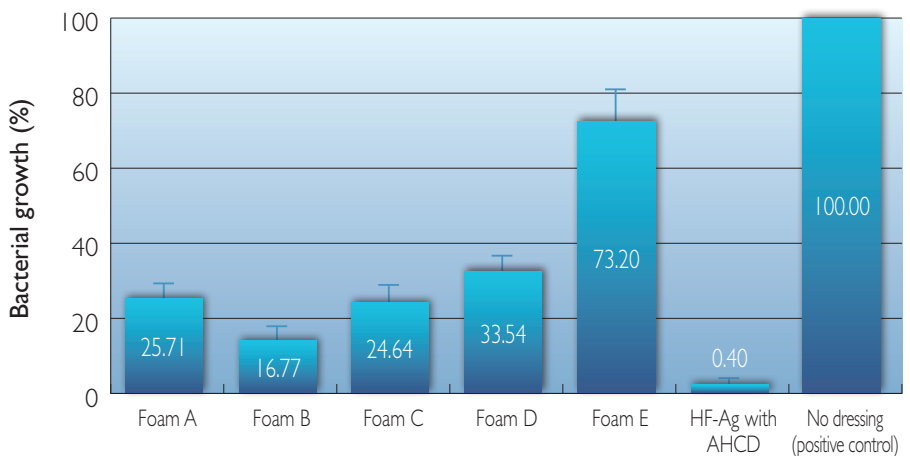


Figure 8. Percentage surface area of *S. aureus* growth beneath the dressings within the indented agar surface area.

the rapid gelling action of HF-Ag led to intimate contact between the dressing and the simulated wound tissue surface. The importance of such dressing conformability was demonstrated in the subsequent antimicrobial model where minimal growth of both *P. aeruginosa* and *S. aureus* was detected on the indented agar surface beneath this dressing.

Growth of *S. aureus* beneath the silver-containing foam dressings was evident, but

less extensive than *P. aeruginosa*. This may be explained by the fact that *S. aureus* is a non-motile bacterium and therefore has the tendency to form more discrete colonies on an agar surface, whereas *P. aeruginosa* is a motile bacterium and is more likely to swarm in a moist environment (Köhler et al, 2000).

The limited antimicrobial performance of the adhesive silver-containing foam dressings was likely to be a consequence

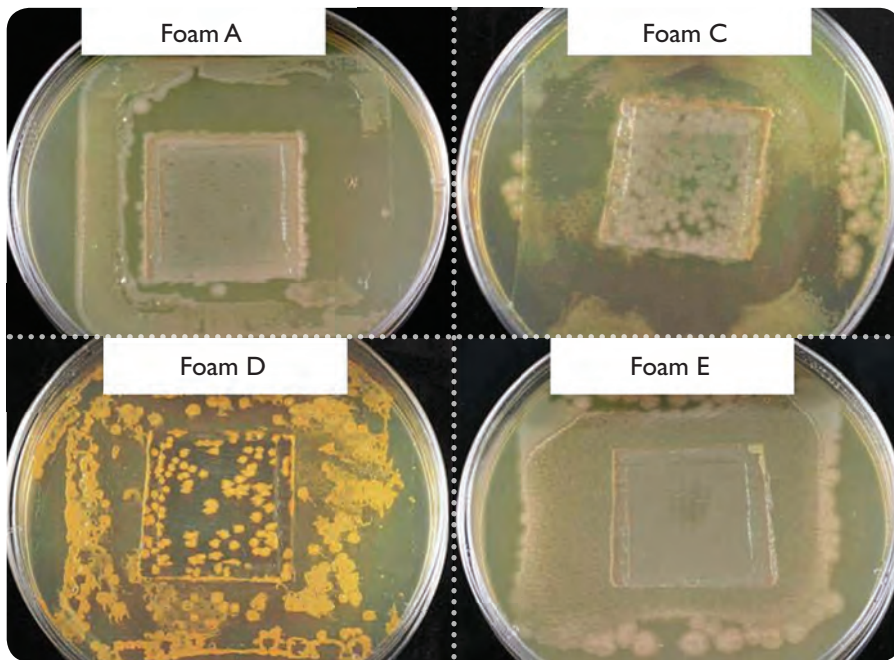


Figure 9. Examples of bacterial growth on the prominent agar surface surrounding the indentation for each adhesive silver-containing foam dressing (after an additional 24-hour incubation period following dressing contact). Foams A, C and E, *P. aeruginosa*; Foam D, *S. aureus*.



Figure 10: Example showing growth of *S. aureus* upon removal of foam E.

of their less conformable nature, as observed in the tissue conformability model where areas of non-contact were evident (Figure 4). HF-Ag (Figure 6) and the non-adhesive foam B (Figure 11) showed areas surrounding the indented area that remained clear of bacteria, indicating good conformability with the perfectly flat prominent agar surface. However, despite containing an antimicrobial agent, all the adhesive silver-containing foam dressings showed lateral bacterial spread beyond the indentation and onto the surrounding prominent agar surface.

These observations are supported by *in vitro* work undertaken by Buchholtz, who reported that, 'The soft silicone layer dressing [i.e. foam E as used in these studies] had no noteworthy release of silver

and no antimicrobial activity suggesting that the soft silicone layer may be encapsulating the foam keeping silver from reaching the wound' (Buchholtz, 2009).

This reported observation may explain the growth of *S. aureus* on foam E (Figure 10). Subsequent investigations (unpublished data by the authors of this paper) using light microscopy, scanning electron microscopy and energy dispersive X-ray analysis showed no evidence of silver existing on the wound contact surface of foam E.

Conclusion

Two *in vitro* models have been used to investigate the conformability and activity of a range of silver-containing antimicrobial dressings in simulated shallow wounds. Tissue contact and antimicrobial activity was shown with HF-Ag over a 48-hour contact period. In contrast, the silver-containing foam dressings tested demonstrated areas of non-conformability which were associated with reduced antimicrobial activity. These *in vitro* studies confirm that both dressing conformability and silver availability to bacteria at the wound surface are critical to the optimum functioning of silver-containing dressings.

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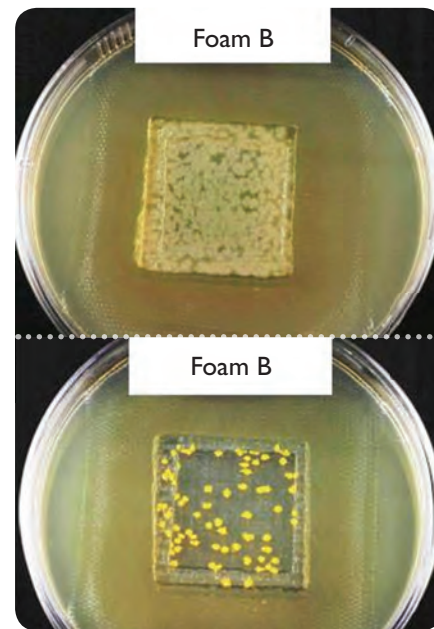


Figure 11. Examples of no spreading bacterial growth on the prominent agar surface area with the non-adhesive silver-containing dressing (foam B). Top right, *P. aeruginosa* and bottom right, *S. aureus*.

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