Efficacy of Poloxamer-Based Wound Dressings on *Acinetobacter baumannii* Biofilms

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Objective: This study evaluated the antimicrobial and antibiofilm activity of a concentrated surfactant gel preserved with antimicrobials and a concentrated surfactant gel with 1% silver sulfadiazine (SSD) against 12 clinical strains of *Acinetobacter baumannii* and the type strain *A. baumannii* ATCC 19606.

Approach: The biofilm-forming potential of the *A. baumannii* isolates was investigated using a crystal violet assay and classifying the isolates as “non-adherent,” “weak,” “moderate,” or “strong” biofilm formers. The antimicrobial activity was determined using the zone of inhibition (ZOI) method. The antibiofilm activity was evaluated against *A. baumannii* ATCC 19606 using the Center for Disease Control bioreactor model.

Results: *A. baumannii* readily forms biofilms with 8 out of the 12 clinical isolates being classified as strong biofilm formers ($OD_{570} > 0.4$). The concentrated surfactant gel with 1% SSD demonstrated antimicrobial activity against all isolates with a ZOI of 7.2–14 mm. Antibiofilm activity against a 24 h biofilm of *A. baumannii* ATCC 19606 was found, with a ≥ 7 log decrease in bacterial cell density following 24 h treatment with the concentrated surfactant gel with 1% SSD. The concentrated surfactant gel preserved with antimicrobials also showed some biofilm disruption with ~ 3 log decrease in bacterial cell density being found.

Innovation: The concentrated surfactant gel with 1% SSD used in this study showed antimicrobial and antibiofilm activity against *A. baumannii*.

Conclusion: The concentrated surfactant gel with 1% SSD used in this study showed efficacy against *A. baumannii*, a common cause of wound infections, and should be considered for treatment of wounds infected with *A. baumannii* in health care settings.

Keywords: *Acinetobacter baumannii*, wound care, poloxamer, biofilm, antimicrobial, silver sulfadiazine

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INTRODUCTION

ACUTE AND CHRONIC WOUNDS are a large burden on health care, with the annual cost estimated at £5.3 billion in 2012–2013 in the United Kingdom alone.¹ In recent years, an increasing amount of research has been published showing the importance of biofilms in the development of chronic wounds.^{2–4} Although biofilms can

consist of a single species, often they comprise of *multiple species*, including aerobic bacteria, anaerobic bacteria, and fungal species.³ Microorganisms within a biofilm can share genetic material through horizontal gene transfer at a higher rate than planktonic cells.⁵ Genetic exchange can occur through different mechanisms, including plasmid conjugation and

also via DNA uptake and transformation following secretion as part of the extracellular polymeric substance production.^{5–7} Biofilms are a reservoir of antimicrobial resistance genes; therefore, genetic exchange of resistance genes can also occur.^{8–10} Additionally, biofilms show an increased tolerance to antimicrobial treatment through multiple mechanisms, making them difficult to treat once they are established.^{3,11}

Acinetobacter baumannii is an opportunistic pathogen and is included on the list of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* (ESKAPE) pathogens as one of the most common multi-drug resistant (MDR) bacterial species causing nosocomial infections.¹² *A. baumannii* is associated with a wide range of infections, including ventilator-associated pneumonia, skin and soft tissue infections (SSIs), and chronic wounds, including burns and deep wound infections.^{13–15} Additionally, occurrence of MDR *A. baumannii* infections is on the rise, and the need for new antibiotics to tackle carbapenem-resistant *A. baumannii* is now classed as critical by the World Health Organization.^{12,16}

This aim of this study was to assess the antimicrobial and antibiofilm activity of a concentrated surfactant gel preserved with antimicrobials and a concentrated surfactant gel with 1% silver sulfadiazine (SSD) against 12 clinical isolates of *A. baumannii* and a type strain of *A. baumannii* to identify antimicrobials active against this species.

CLINICAL PROBLEM ADDRESSED

A. baumannii can readily form biofilms and cause a wide range of infections, including those contributing to chronic wounds. Biofilms show recalcitrance to antimicrobials and antibiotics and are therefore difficult to treat clinically. Additionally, occurrence of *A. baumannii* MDR infections is on the rise. In this study, a concentrated surfactant-based wound dressing, with and without 1% SSD, was evaluated for efficacy against *A. baumannii*, as a potential treatment for these difficult-to-treat infections.

MATERIALS AND METHODS

Test dressings

Two non-ionic surfactant-based wound dressings were included in this study: a concentrated surfactant gel preserved with antimicrobials and a concentrated surfactant gel with 1% SSD. A polyhexamethylene biguanide (PHMB) surfactant gel was included as a comparison.

Bacterial strains

Twelve clinical isolates of *A. baumannii* were obtained from the NHS Royal Liverpool University hospital and used in this study. All strains were isolated from blood samples. The type strain *A. baumannii* ATCC 19606 was also included. All isolates were plated onto Tryptone soy agar (TSA; Oxoid, UK) and grown at 37°C. Overnight broth cultures were set up by inoculating 10 mL Tryptone soy broth (TSB; Oxoid) with a single colony and incubating overnight at 37°C and 125 rpm.

Zone of inhibition

The zone of inhibition (ZOI) of test articles against the 11 clinical *A. baumannii* isolates and *A. baumannii* ATCC 19606 was determined. *A. baumannii* 141733 was not included in the study as it showed poor growth on the non-antimicrobial control. Each overnight inoculum was adjusted to 0.5 McFarland ($\sim 1 \times 10^8$ CFU/mL; Pro-Lab Diagnostics, UK) and further diluted to $\sim 1 \times 10^6$ CFU/mL. TSA was inoculated with each adjusted culture and plates were incubated at 37°C for 4 h. Following incubation, 10 mm sterile membrane discs (Sigma-Aldrich, UK) were coated with each test article and placed in the center of individual agar plates. Each treatment was tested in duplicate per strain. All plates were incubated overnight at 37°C. The following day the corrected ZOI was calculated by measuring the diameter of the inhibited zone and subtracting the diameter of the membrane disc. Filter discs were removed and the plates were re-incubated for a further 24 h to see if the microorganisms exhibited re-growth. The samples were tested in duplicate and the experiment was repeated.

Biofilm-forming potential

The biofilm-forming potential of 12 clinical *A. baumannii* isolates and *A. baumannii* ATCC 19606 was determined using a previously described method with some modifications.¹⁷ Overnight cultures were adjusted to 0.5 McFarland ($\sim 1 \times 10^8$ CFU/mL) and added to a 96-well plate at a final concentration of $\sim 1 \times 10^6$ CFU/mL in TSB. Each strain was added to the plate in triplicate ($n = 3$). A negative control was set up containing TSB only. The plate was incubated at 37°C and 125 rpm for 24 h. Biofilms were washed with 10 mM phosphate buffered saline (PBS) three times and allowed to air-dry before the addition of 99% methanol (Sigma-Aldrich) to each well. The plate was incubated at room temperature for 20 min. Biofilms were washed with 10 mM PBS three times and left to air-dry before adding 0.5% crystal violet (Sigma-

Aldrich) to each well. The plate was incubated for 15 min at room temperature. Biofilms were washed with 10 mM PBS three times and left to air-dry before adding 30% acetic acid (Sigma-Aldrich) to each well. The plate was incubated for 5 min at room temperature and 125 rpm. Absorbance was read straight after at 570 nm. The absorbance of the negative control containing TSB only was subtracted and an average absorbance reading calculated for each isolate. Each isolate was tested in triplicate, in at least two separate experiments. The biofilm-forming potential of isolates was classified in accordance with Stepanovic (2000). Isolates were classified based on optical density (OD) readings as strong (>0.4), moderate (>0.2 – 0.4), weak (≤ 0.2) or non-adherent (0) biofilm formers.¹⁸

Center for Disease Control bioreactor

The Center for Disease Control (CDC) bioreactor assay was performed following an adapted version of ASTM 2871–13. Antibiofilm activity of test articles was determined against *A. baumannii* ATCC 19606. Overnight cultures were adjusted to 0.5 McFarland ($\sim 1 \times 10^8$ CFU/mL) and added to a CDC bioreactor at a final concentration of $\sim 3.3 \times 10^5$ CFU/mL. The CDC bioreactor was incubated at room temperature and 125 rpm for 24 h. Coupons were washed twice in 0.85% NaCl before adding them individually to 4 g of each test article in triplicate ($n=3$). A control of the coupons only was also set up in triplicate. The coupons were incubated for 24 h at room temperature. The following day, coupons were removed from gels, washed twice in 0.85% NaCl, and added to 10 mL Dey-Engley neutralizing broth (Sigma-Aldrich). Coupons were sonicated at full power for 30 min. All tubes were vortexed briefly and samples were serial-diluted in PBS. Dilutions were plated onto TSA in duplicate and plates were incubated at 37°C overnight. The following day colony counts were enumerated to determine CFU/mL.

Statistical analysis

Statistical analysis was performed using Prism GraphPad software. Statistical comparison of the CFU/mL obtained from the CDC bioreactor was carried out using two-way analysis of variance.

RESULTS

Zone of inhibition

The concentrated surfactant containing 1% SSD and the PHMB-based surfactant gel demonstrated antimicrobial activity against all isolates with a ZOI ranging from 7.2–14 to 6.0–12.25 mm, respectively (Fig. 1). No growth inhibition was observed

Figure 1. Comparison of the antimicrobial activity of a concentrated surfactant containing 1% SSD and a PHMB-based surfactant gel on 11 clinical strains of *A. baumannii* and *A. baumannii* ATCC 19606 (gray) using a zone of inhibition model. A concentrated surfactant gel preserved with antimicrobials was also included. Data are an average of two separate experiments with each isolate tested in duplicate ($n=4$). Error bars represent the standard error of the mean. PHMB, polyhexamethylene biguanide; SSD, silver sulfadiazine.

with the concentrated surfactant gel preserved with antimicrobials. Following ZOI determination, the coated discs were removed and plates were re-incubated overnight to determine if re-growth of isolates occurred. Re-growth was observed with one and seven isolates of *A. baumannii* following removal of the discs coated with the concentrated surfactant containing 1% SSD and the PHMB-based surfactant gel, respectively.

Biofilm-forming potential

Of the 12 clinical isolates tested, 8 isolates were strong biofilm formers, with 3 isolates classed as weak biofilm formers and 1 isolate being non-adherent (Fig. 2). *A. baumannii* ATCC 19606 was classed as a moderate biofilm former with an absorbance reading of 0.3. Within the group classified as strong biofilm formers, a range of absorbance readings were found, with *A. baumannii* 152584 being the strongest biofilm former with an OD of 2.5.

CDC bioreactor

The antibiofilm activity of a concentrated surfactant gel preserved with antimicrobials, a concentrated surfactant containing 1% SSD, and a PHMB-based surfactant gel was determined against *A. baumannii* ATCC 19606 (Fig. 3). No bacterial colonies could be detected from samples treated with the concentrated surfactant containing 1% SSD or the PHMB-based surfactant gel. In comparison, the untreated control had a cell density of 2.56×10^7 CFU/mL, showing a >7 log reduction in the treated groups ($p < 0.0001$). A 3 log reduction in bacterial cell density was also

Figure 2. Biofilm-forming potential of 12 clinical isolates of *A. baumannii* and *A. baumannii* ATCC 19606. Each isolate was tested in triplicate per experiment and tested in at least two experiments. The error bars represent the standard error of the mean. OD, Optical density.

found following treatment with the concentrated surfactant gel preserved with antimicrobials, with a bacterial count of 2.44×10^4 CFU/mL ($p < 0.0001$).

DISCUSSION

In this study, a concentrated surfactant gel preserved with antimicrobials, a concentrated surfactant gel with 1% SSD, and a PHMB surfactant gel were evaluated for antimicrobial and antibiofilm properties against *A. baumannii*.

A. baumannii resistance to antimicrobials is well documented, and occurrence of MDR isolates

Figure 3. Log₁₀ bacterial cell density of *A. baumannii* ATCC 19606 following 24 h treatment with a concentrated surfactant gel preserved with antimicrobials, a concentrated surfactant containing 1% SSD, and a PHMB-based surfactant gel. Error bars represent the standard error of the mean. A significant reduction in bacterial cell density in comparison to the untreated control is displayed on the graph (*) ($p < 0.0001$).

continues to increase.¹⁹ One case study reviewed war wound infections at a medical center facility in Texas and found that of the 38 cultures of *Acinetobacter* species isolated, 29 were MDR.¹³ Of the MDR isolates, all but four were susceptible to imipenem, an antibiotic of last resort. *A. baumannii* resistance to carbapenems, such as imipenem, has been increasingly reported, with an occurrence of over 80% being found in some European countries in 2015.¹⁹ In addition, carbapenem resistance is often combined with resistance to fluoroquinolones and aminoglycosides.¹⁹ Another study showed that of the 21 *A. baumannii* isolates obtained from a hospital setting, all were MDR, only showing susceptibility to colistin and tigecycline.²⁰

Due to the increasing MDR found with *A. baumannii* infections, it is essential to identify agents with antimicrobial efficacy against it. Silver is a commonly used antimicrobial in wound care and has shown efficacy against a broad range of bacterial and fungal species.^{21,22} Silver has been shown to bind to several distinct bacterial sites, although it is mainly thought to exert antimicrobial efficacy through disrupting the cell membrane and cell wall.^{23–25} PHMB is also used as an antimicrobial to prevent wound infection and medical device-related infection.²⁶ It is also thought to have antimicrobial activity through binding to and disrupting the bacterial cell wall; however, other research has shown inhibition of cell replication, indicating it may bind to bacterial DNA.²⁷

In this study, the antimicrobial activity of the test articles was assessed using the ZOI method. Both the concentrated surfactant gel with 1% SSD and the PHMB surfactant gel demonstrated antimicrobial activity against all of the *A. baumannii* isolates. Interestingly, the gels showed greater inhibition of the clinical isolates than the type strain. The concentrated surfactant gel preserved with antimicrobials showed no growth inhibition of any of the isolates tested.

Although *A. baumannii* is associated with a wide range of infection types, it is commonly associated with wound infections and surgical site infections.^{13,14} *A. baumannii* infection in wounds is highly concerning due to the increased occurrence of MDR strains and the ability of bacterial species to co-exist in a biofilm and share genetic material.^{9,10} In this study, the ability of the *A. baumannii* isolates to form biofilms was assessed. Of the 12 clinical isolates included in this study, 8 were classified as strong biofilm formers, demonstrating the isolates' readiness to form biofilms. The ability of *A. baumannii* to form biofilms has also been reported elsewhere.²⁸

The antibiofilm activity of the test articles was evaluated against *A. baumannii* ATCC 19606 as a representative strain using the CDC bioreactor biofilm model. *A. baumannii* ATCC 19606 was selected as it demonstrated the ability to form biofilms and was the least susceptible isolate to the gels in the ZOI assay. The concentrated surfactant gel with 1% SSD and the PHMB surfactant gel showed potent antibiofilm activity against *A. baumannii* with a ≥ 7 log decrease in bacterial cell density over 24 h. The concentrated surfactant gel preserved with antimicrobials also reduced the bacterial cell density of *A. baumannii* by 3 log.

Antibiofilm activity of the concentrated surfactant gel with 1% SSD and the concentrated surfactant gel preserved with antimicrobials has been documented by the authors previously against *S. aureus*, *P. aeruginosa*, *Staphylococcus epidermidis*, and *Enterococcus* species.²⁹ In this study, the concentrated surfactant gel preserved with antimicrobials was shown to cause biofilm detachment and dispersion, which may explain the reduction in *A. baumannii* bacterial cell density observed here. Another study evaluated a surfactant-based dressing in the treatment of *A. baumannii* biofilms.³⁰ This study showed the surfactant-based dressing could prevent biofilm formation, but relevant antibiotics were needed to treat the biofilm.

INNOVATION

The study here demonstrated the antimicrobial and antibiofilm properties of a concentrated surfactant gel with 1% SSD against *A. baumannii* isolates. The findings showed that the concentrated surfactant gel with 1% SSD may be useful in health care settings to treat *A. baumannii* infections, which often exhibit MDR to clinically used antibiotics.

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KEY FINDINGS

- Of the 12 *A. baumannii* clinical isolates included in this study, 8 were classed as strong biofilm formers showing the strain's readiness to form biofilms.
- The concentrated surfactant-based wound gel containing 1% SSD demonstrated antimicrobial activity against *A. baumannii*.
- The concentrated surfactant-based wound gel, with and without 1% SSD, were efficacious against *A. baumannii* biofilm showing a >7 log and 3 log drop in bacterial cell density, respectively.

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AUTHOR DISCLOSURE AND GHOSTWRITING

No competing financial interests exist. The content of this article was expressly written by the authors listed. No ghostwriters were used to write this article.

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Abbreviations and Acronyms

CDC = Center for Disease Control
MDR = multi-drug resistant
OD = optical density
PBS = phosphate buffered saline
PHMB = polyhexamethylene biguanide
SSD = silver sulfadiazine
SSI = skin and soft tissue infection
TSA = Tryptone soy agar
TSB = Tryptone soy broth
ZOI = zone of inhibition