In vitro antimicrobial efficacy of a silver alginate dressing on burn wound isolates

- **Objective:** To test the antimicrobial effectiveness of a silver alginate dressing on opportunistic pathogens, namely meticillin-sensitive *Staphylococcus aureus* (MSSA) and meticillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella* spp., *Enterococcus faecalis*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter sakazakii*, *Enterobacter cloacae*, *Serratia marcescens*, *Chryseobacterium indologenes*, *Proteus vulgaris* and *Acinetobacter baumannii*.

- **Method:** In total, 40 microorganisms were isolated from patients attending three burn centres in the US and evaluated for their susceptibility to a silver alginate wound dressing, employing a corrected zone of inhibition assay, conducted on Mueller Hinton agar (MHA).

- **Results:** The sizes of the corrected zones of inhibition varied between and within genera. For example, all *Acinetobacter baumannii* strains were found to be sensitive to ionic silver at pH 7, with a mean of 2.8 mm, compared with 3.5 mm at pH 5.5. The silver alginate dressing also demonstrated activity on all strains of *Enterobacter* and *Escherichia coli*, with susceptibility to the silver alginate dressing enhanced at pH 5.5. For *Enterococcus* spp. the average corrected zone of inhibition at pH 7 was 3.6 mm, versus 4.9 mm at pH 5.5. All strains of *Pseudomonas aeruginosa* were found to be sensitive to the silver alginate dressing. The average corrected zone of inhibition was 6.9 mm at pH 7, compared with 8 mm at pH 5.5. For MRSA and *Staphylococcus aureus*, it ranged from 4.5 mm to 7.5 mm at pH 7. When the pH was decreased to 5.5, the corrected zone of inhibition increased.

- **Conclusion:** This study demonstrates the activity of a silver alginate dressing on a wide range of burn isolates, including antibiotic-resistant bacteria, isolated from three different burn centres in the US. It also highlights the possible importance of pH and its potential effects on antimicrobial performance and microbial susceptibility. However, more extensive testing is required to substantiate this.

- **Conflict of interest:** SLP is employed by Advanced Medical Solutions Ltd.

---

Burn wounds are colonised with an array of different microorganisms that contribute to local and systemic infections and, therefore, delayed wound healing. In the past, particularly in the early to mid-1900s, common microorganisms most frequently isolated from burn wound sepsis included *Streptococcus* spp., most specifically *Streptococcus pyogenes*. However, with the development of advanced microbiological techniques and sample collection procedures, a greater diversity of microorganisms has been identified and linked to the delayed healing of burn wounds, localised infection and cross-infection. The two most recognised burn wound opportunistic pathogens, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and other microorganisms including *Acinetobacter baumannii*, *Staphylococcus epidermidis*, *Aeromonas hydrophila* and anaerobic bacteria, are becoming more prevalent colonisers in burn wounds.

To reduce the risk of a localised and systemic infection in patients with burn injuries, advanced wound dressings containing topical antimicrobials such as ionic silver have been developed and are being widely used, with positive clinical outcomes. Silver has been incorporated into advanced wound dressings because of its long history of safe use and its broad spectrum of activity at low concentrations. This has been demonstrated both in vitro and clinically.

It is important that silver dressings demonstrate activity against a diverse range of microorganisms found in burn wounds. They also need to demonstrate sustained antimicrobial activity over a broad pH range. In both healing acute and chronic wounds, the pH moves from an alkali state, to a neutral and then to an acidic state. As pH is purported to play a significant role in wound healing, an antimicrobial dressing will need to demonstrate that it remains efficacious at the pH range found in acute and chronic wounds. Many metals such as silver are affected by pH, and so this, along with other factors, will affect its bioavailability and thus activity.
The diversity of microorganisms isolated from burn wounds and their ability, either inherently or acquired, to develop tolerance to antimicrobials vary both in time and place. Consequently, antimicrobials that are used routinely need to be evaluated periodically in order to ensure they aid wound healing. Accordingly, in this study we used the disc diffusion antimicrobial sensitivity testing method to evaluate the antimicrobial activity of a silver alginate dressing on 40 burn isolates routinely cultured from burn wounds from three burn centres in the US. The aims of the study were to determine:

- The antimicrobial efficacy of a silver alginate dressing against microorganisms isolated from burn wounds
- The effect of pH on this antimicrobial efficacy.

### Methods and materials

#### Test microorganism

Forty different strains of microorganisms were used in this study and included:

- **Meticillin-resistant Staphylococcus aureus (MRSA)** (3)
- **Staphylococcus aureus** (1)
- **Enterococcus faecalis** (5)
- **Enterococcus faecium** (2)
- **Pseudomonas aeruginosa** (5)
- **Acinetobacter baumannii** (4)
- **Escherichia coli** (6)
- **Klebsiella oxytoca** (1)
- **Klebsiella pneumoniae** (3)
- **Proteus mirabilis** (3)
- **Chryseobacterium indologenes** (1)
- **Serratia marcescens** (1)
- **Enterobacter cloacae** (3)
- **Enterobacter sakazukii** (1)
- **Enterobacter aerogenes** (1)

The number of isolates are given in brackets. All isolates were kindly donated from three burn centres in the US:

- Shriner’s Burn Center, Cincinnati, Ohio
- Cabel-Huntington Burn Unit, Huntington, West Virginia
- Pittsburgh Burn Center, Pittsburgh.

**Streptococcus pyogenes** was not isolated from any patients at these centres and so was not included in this study.

#### Test dressings

Wound dressings evaluated in this study included a silver alginate (AMS, Winsford, UK) and a gauze dressing. (Gauze was selected as it is a widely used control.) Squares of 1cm of each dressing were prepared in an aseptic manner prior to evaluation. Each dressing was placed into a sterile vial and subjected to pre-treatment with 800µl of saline for 30 seconds. Each pre-soaked wound dressing was drip dried and placed onto inoculated Mueller Hinton Agar (MHA) plates.

### Test methods

Each purified test isolate was inoculated in 70ml of tryptic soy broth (TSB). An overnight culture was then added to 5ml of saline to give a final inoculum of 1x10⁶ colony-forming units (CFU) per ml, and inoculated onto MHA plates. Clinical Laboratory Standards Institute (CLSI) techniques were applied throughout. Both the test and control dressings were placed onto the confluent microbial lawns. All MHA plates were then incubated at 37°C for 24 hours. Following incubation, the corrected zone of inhibition was determined. All testing was done in triplicate. To investigate the effects of pH on the sensitivity of microorganisms to the silver alginate dressing, all MHA plates were made up to a pH of either 7 or 5.5 (by the addition of hydrochloric acid or sodium hydroxide).

### Measurement of the corrected zone of inhibition

The corrected zone of inhibition was determined by measuring the zone of clearing across one direction of the dressing and then subtracting this from the width of the dressing. This measurement was done in two different perpendicular directions (vertically and horizontally) across the dressing. The resulting measurement was averaged to yield the corrected zone of inhibition. In this way, it reflected only the width of the zone of clearing surrounding the dressing and was corrected for variances in dressing size. All corrected zones of inhibition were done in triplicate and an average was taken.

### Statistical analysis

A Student’s t-test was used to compare the corrected zones of inhibition between pH ranges. All data were analysed using Microsoft Excel.

### Results

This study evaluated the ability of the silver alginate dressing to inhibit microbial growth in the area immediately surrounding the dressing. The results are given in Tables 1 and 2, which indicate that the silver alginate dressing was able to produce a zone of inhibition around itself. All 40 microorganisms tested demonstrated zones of growth inhibition, with the exception of two strains of *Proteus mirabilis*, and therefore susceptibility to ionic silver.

The sizes of the corrected zone of inhibition varied between genera and within genera. For example, all *Acinetobacter baumannii* strains were found to be sensitive to ionic silver. The corrected zone of inhibition ranged from 0.5 to 4.5mm at pH 7, with a mean of 2.8mm, compared with a mean of 3.5mm at pH 5.5. The silver alginate also demonstrated activity against all of the *Enterobacter* strains evaluated. The mean corrected zone of inhibition was recorded at 2.9mm at pH 7, compared with 3.9mm at pH 5.5. The average corrected zone of inhibition...
The effect of pH on the antimicrobial efficacy of a silver alginate dressing on Gram-negative microorganisms isolated from burn patients

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>No. of isolates</th>
<th>pH 7.0</th>
<th>Mean corrected zone of inhibition (mm)</th>
<th>± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii</td>
<td>4</td>
<td>7.0</td>
<td>2.8</td>
<td>±1.7</td>
<td>0.5–4.5</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>3.5</td>
<td></td>
<td>±1.0</td>
<td>0.5–5.5</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>5</td>
<td>7.0</td>
<td>2.9</td>
<td>±0.7</td>
<td>0.5–5.5</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>3.9</td>
<td></td>
<td>±0.6</td>
<td>0.5–6.5</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>6</td>
<td>7.0</td>
<td>2.5</td>
<td>±0.3</td>
<td>0.5–3.5</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>3.2</td>
<td></td>
<td>±1.0</td>
<td>0.5–5.5</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>4</td>
<td>7.0</td>
<td>5.8</td>
<td>±2.5</td>
<td>2.5–14</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>5.3</td>
<td></td>
<td>±1.0</td>
<td>2.5–8.5</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>3</td>
<td>7.0</td>
<td>All strains were tolerant</td>
<td>±1.1</td>
<td>0–5.0</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>1.6*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>5</td>
<td>7.0</td>
<td>6.9</td>
<td>±1.5</td>
<td>1.0–10.5</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>8.0</td>
<td></td>
<td>±0.9</td>
<td>4.5–11.5</td>
</tr>
<tr>
<td>Chryseobacterium indologenes</td>
<td>1</td>
<td>7.0</td>
<td>2.3</td>
<td>±0.4</td>
<td>1.5–3.0</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>12.8</td>
<td></td>
<td>±0.4</td>
<td>12.0–13.5</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>1</td>
<td>7.0</td>
<td>3.3</td>
<td>0.3</td>
<td>3.0–4.0</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>3.3</td>
<td></td>
<td>0.2</td>
<td>3.0–3.5</td>
</tr>
</tbody>
</table>

* Two stains were tolerant

Table 2. The effect of pH on the antimicrobial efficacy of a silver alginate dressing on Gram-positive microorganisms isolated from burn patients

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>No. of isolates</th>
<th>pH 7.0</th>
<th>Mean corrected zone of inhibition (mm)</th>
<th>± SE</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus spp.</td>
<td>7</td>
<td>7.0</td>
<td>3.6</td>
<td>±0.5</td>
<td>2.0–6.5</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>4.9</td>
<td></td>
<td>±0.9</td>
<td>1.0–9.5</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>4</td>
<td>7.0</td>
<td>5.8</td>
<td>±0.4</td>
<td>4.5–7.5</td>
</tr>
<tr>
<td></td>
<td>5.5</td>
<td>6.4</td>
<td></td>
<td>±0.5</td>
<td>5.0–8.0</td>
</tr>
</tbody>
</table>

Discussion

Burn wounds are colonised by a mixed culture of microorganisms which are responsible for causing infections. A number of these microorganisms are able to penetrate the burn eschar, which could disseminate the infection. Topical antimicrobials used on burn wounds reduce the levels of colonising microorganisms, with the overall aim being to prevent or manage infection. A wide selection of silver dressings is available on the market, and as such this study was designed to provide further evidence to
support the efficacy of a silver alginate dressing on burn wounds.

Disc diffusion antimicrobial sensitivity testing is an easy, inexpensive and reliable method for the rapid screening of microorganisms.\textsuperscript{21-24} Despite its limitations, which include the growth of bacteria in a ‘planktonic/quasi-sessile’ state (as opposed to the ‘true’ biofilm phenotype) and the possibility that silver can interact with the test media,\textsuperscript{13,21,22} many researchers have used it to test the efficacy of silver dressings.\textsuperscript{12,14,15,27} We considered it appropriate for our objective of producing preliminary data to assist with future \textit{in vitro} and \textit{in vivo} investigations.

Our results demonstrate that the silver alginate dressing has powerful antimicrobial activity on the tested burn wound isolates \textit{in vitro}. The only exceptions were two strains of \textit{Proteus mirabilis}, which demonstrated tolerance to ionic silver. The activity of the silver alginate dressing demonstrated a trend towards being more effective against Gram-negative organisms. However, the results against the Gram-positive organisms were also favourable. All MRSA and vancomycin-resistant \textit{Enterococcus} (VRE) exhibited sensitivity to the dressing. This could be due to a number of factors including the composition of the bacterial cell wall.

The spectrum of activity of the silver alginate varied between and within genera. This has been documented in other studies\textsuperscript{14,15} and substantiates the need to use a large sample of microorganisms located from different geographical locations. This will help obtain more meaningful data, which would be more relevant to the \textit{in vivo} environment.

The fact that the zone of inhibition ranged to a high degree between isolates demonstrates that there is a good diffusion of the ionic silver activity from the dressing. This is an important characteristic, particular in burn wounds with thick eschar.

The pH affected the sensitivity of microorganisms to the silver alginate dressing and, despite pH changes, it effectively inhibited the growth of all 40 burn isolates, apart from the tolerant \textit{Proteus mirabilis} strains. Interesting, one of the \textit{P. mirabilis} strains, which exhibited tolerance to ionic silver at pH 7, was shown to be sensitive to silver at pH 5.5. These isolates are being screened for genetic resistance to silver using published methods.\textsuperscript{28} However, it is important to report that tolerance to silver is not a new phenomenon and some bacteria often have inherent resistance.\textsuperscript{29}

**Conclusion**

This study has demonstrated the broad antimicrobial activity and sensitivity of burn isolates to a silver alginate dressing. It has also verified the importance of pH and the role it plays in affecting the antimicrobial performance of ionic silver on microorganisms isolated from burn wounds.

Consequently, this study indicates that more robust testing and evaluation of antimicrobial dressings and their impact on wound healing is required, and that this must consider the variables known to affect efficacy. In particular, pH should be considered when assessing the antimicrobial activity of ionic silver within both \textit{in vitro} and \textit{in vivo} models on burn isolates.