Comparison of the speed of kill of pathogenic bacteria using ACTICOAT® and AQUACEL Ag™ analysed using Confocal Laser Scanning Microscopy

Emma Woodmansey
Research Microbiologist
Research Centre, York
Introduction

Traditional methods for demonstrating antimicrobial efficacy rely on culture techniques following exposure to the test material, however results can be difficult to interpret for the non-scientific community. Visualisation of microbial kill by antimicrobials, using fluorescent stains and confocal laser scanning microscopy (CLSM), may complement these test results. Confocal microscopy offers several advantages over conventional microscopy, including the ability to control depth of field, elimination or reduction of background information, and the capability to collect visual sections from thick specimens. The BacLight™ LIVE/DEAD® stain allows the visualisation of live and dead bacteria by staining them green and red respectively.

In this experiment, the speed of kill was visualised for ACTICOAT and AQUACEL Ag against a variety of important pathogenic wound bacteria. Uncoated polyethylene mesh (used in the production of ACTICOAT) and AQUACEL were used as controls.

Test materials

<table>
<thead>
<tr>
<th>Dressing</th>
<th>Manufacturer</th>
<th>Lot number</th>
<th>Expiry date</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTICOAT</td>
<td>Smith &amp; Nephew</td>
<td>040305A</td>
<td>09/2006</td>
</tr>
<tr>
<td>Uncoated polyethylene net (sterile)</td>
<td>Smith &amp; Nephew</td>
<td>No batch number available</td>
<td>N/A</td>
</tr>
<tr>
<td>AQUACEL Ag</td>
<td>ConvaTec</td>
<td>4K83720</td>
<td>10/2006</td>
</tr>
<tr>
<td>AQUACEL</td>
<td>ConvaTec</td>
<td>4F77806</td>
<td>06/2009</td>
</tr>
</tbody>
</table>

Test organisms

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>NCIMB 8626</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>NCTC 10788</td>
</tr>
<tr>
<td>Epidemic Methicillin Resistant Staphylococcus aureus (EMRSA) strain-15</td>
<td>NCTC 13142</td>
</tr>
<tr>
<td>Epidemic Methicillin Resistant Staphylococcus aureus (EMRSA) strain-16</td>
<td>NCTC 13143</td>
</tr>
</tbody>
</table>

Methods

1 cm x 1 cm pieces of dressing were inoculated with bacterial suspension (over 10^7 cfu/dressing). Due to the different absorption capacities of the test materials, different volumes of inoculum were used, however the bacterial concentration was adjusted accordingly to maintain a standard total challenge for all materials tested. The dressings were incubated for 0 mins, 15 mins, 30 mins, 2hrs and 4hrs at 32°C. At the appropriate times following incubation, BacLight™ LIVE/DEAD® stain was added to each sample, with each test material
subsequently analysed using confocal microscopy. Images were taken of new dressings at each time point so that bacterial viability was not affected by prolonged contact with nucleic acid stains. Image capture took approximately 5 min; hence all time points are X+5 min. An overlay of the green/red fluorescence was captured to determine the extent of kill from each test sample, in some cases where both green and red signals were observed in the same area, the resultant fluorescent signal is seen to be yellow.

Results

In preliminary tests the absorbent layer of ACTICOAT was found to show green autofluorescence in the absence of bacteria and fluorescent stain. This autofluorescence is demonstrated in Figure 1 a, and can be visualised in relation to the dressing structure using the transmitted light images shown in Figure 1 b. In contrast, the active nanocrystalline-silver coated layer of ACTICOAT (dark net-like structure which can be seen in Figure 1 a & b) and the silver coated fibres of AQUACEL Ag (Figure 2 a & b) showed minimal background fluorescence, thus any fluorescence observed in later tests following incubation with bacteria and subsequent staining, on the nanocrystalline silver-coated part of the ACTICOAT dressing (Figure 3-6) and the AQUACEL Ag dressing (Figure 7-10), was due to the presence and viability of the test organisms.

![ACTICOAT: autofluorescence of rayon/polyester middle layer a) green/red fluorescence overlay, b) Transmitted light only](image1.png)

**Figure 1.** ACTICOAT: autofluorescence of rayon/polyester middle layer a) green/red fluorescence overlay, b) Transmitted light only

![AQUACEL Ag autofluorescence: a) green/red fluorescence overlay, b) Transmitted light only](image2.png)

**Figure 2.** AQUACEL Ag autofluorescence: a) green/red fluorescence overlay, b) Transmitted light only

Trade Mark of Smith & Nephew
Images of the inoculated test materials showed a marked difference in the extent and speed of kill between the different dressings. Following incubation with *P. aeruginosa* and *S. aureus* (Figure 3a and 4a), extensive kill was observed with ACTICOAT at all time points including the baseline sample. The autofluorescence previously demonstrated can be visualised in the background of the images of Figure 3a and 4a. Corresponding images with the uncoated polyethylene net control (Figure 3b and 4b) show bacteria can survive on this material for up to 4 h in the absence of silver.

![Images of the inoculated test materials showed a marked difference in the extent and speed of kill between the different dressings.](image)

**Figure 3.** Images to show extent of kill against *P. aeruginosa* over a time course of 0, 15, 30 min, 2 and 4 h with a) ACTICOAT, b) uncoated polyethylene net

![Images of the inoculated test materials showed a marked difference in the extent and speed of kill between the different dressings.](image)

**Figure 4.** Images to show extent of kill against *S. aureus* over a time course of 0, 15, 30 min, 2 and 4 h with a) ACTICOAT, b) uncoated polyethylene net

ACTICOAT and uncoated polyethylene net were challenged with EMRSA-15 & 16 over the same time course with images shown in Figures 5 and 6 respectively. ACTICOAT was shown to have considerable effectiveness in as little as 15 min, with no viable bacteria observed from 2 h onwards.

° Trade Mark of Smith & Nephew
In contrast both strains of EMRSA remained viable on the uncoated polyethylene net control throughout the time course, with the exception of a few clusters of dead bacteria observed by 4 h.

![Figure 5. Images to show extent of kill against EMRSA-15 over a time course of 0, 15, 30 min, 2 and 4 h with a) ACTICOAT, b) uncoated polyethylene net](image)

Dead bacteria were observed on the Aquacel Ag dressing (Figure 7 and 8), particularly inside the fibres, however, aggregates of live bacteria were found on the outside of some. A faster speed of kill of *P. aeruginosa* was observed up to the 30 minute time point when compared to *S. aureus* (figure 7). However, by the 2 hour time point, a greater population of dead *S. aureus* were observed when compared to *P. aeruginosa*. The majority of bacteria on the Aquacel control dressing were live, with limited cell death on some of the fibres.
The extent of kill using Aquacel Ag against EMRSA-15 and 16 was limited (Figure 9 and 10), with extensive survival of these persistent organisms throughout the dressing, particularly for EMRSA-16 (Figure 10). Large clusters of live bacteria were observed with minimal cell death within the fibres. Images of the Aquacel control, show live bacteria within the dressing.
Conclusions

Microorganisms are known to influence the healing process\textsuperscript{1,2} for this reason rapid speed of kill is essential for antimicrobial management to optimise wound healing.

CLSM in combination with the BacLight\textsuperscript{™} LIVE/DEAD\textsuperscript{®} stain provides a visual tool to observe microbial kill within antimicrobial dressings.

In addition to the effective kill reported with the common wound pathogens \textit{P. aeruginosa} and \textit{S. aureus} using ACTICOAT, rapid speed of kill was observed with the persistent organisms EMRSA 15 and 16.
These strains in particular, are emerging as the dominant MRSA isolates identified from hospitals in the UK. Furthermore, MRSA populations are known to undergo rapid evolution, highlighting the importance of speed of kill in wound management.

References