Standardizing an *in vitro* procedure for the evaluation of the antimicrobial activity of wound dressings and the assessment of three wound dressings

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**Objectives:** A wide selection of wound dressings is available on the market with varying claims of antimicrobial efficacy. A valid standard method for evaluation of their antimicrobial activity *in vitro* has not been established. In this study we suggest a standardized time–kill assay procedure for antimicrobial activity evaluation of wound dressings in order to make studies more comparable and reproducible. We also tested two silver-containing dressings and one propolis-containing dressing against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* using our proposed procedure.

**Methods:** The following dressings were tested: the ionic silver-containing dressing (ISCD), nanocrystalline silver coated dressing (NSCD) and a propolis-containing dressing (PCD) that is in development. A time–kill assay and the zone of inhibition test were used in the study.

**Results:** NSCD exhibited the most potent antibacterial activity against all organisms. ISDC also demonstrated good antibacterial activity although inferior to NSCD. PCD did not show any bactericidal effect.

**Conclusions:** Based on our findings we suggest that a time–kill assay with a 1 cm² dressing is used for evaluation of the antimicrobial activity of wound dressings, and that the dressings should be exposed to a standardized inoculum of 1–1.5 × 10⁶ cfu/mL with Mueller–Hinton broth as the most appropriate medium. PCD requires further research to establish its clinical value.

**Keywords:** ionic silver, nanocrystalline silver, propolis

**Introduction**

The procedures for the antimicrobial activity evaluation of wound dressings vary significantly in terms of medium, inoculum and sample size.¹⁻⁵ This may result in confusing differences in evaluating wound dressings.⁶ We proposed to develop a standard method for testing the antimicrobial activity of wound dressings and tested three dressings by this method. We utilized the method of Parsons et al.,² with modifications, to improve reproducibility and quality.

**Materials and methods**

**Isolates**

We used *Staphylococcus aureus* NCTC 6571, *Streptococcus pyogenes* NCTC 8198-15, *Escherichia coli* NCTC 106118 and *Pseudomonas aeruginosa* NCTC 10662. These represent the most common wound pathogens and are widely used in standard antimicrobial susceptibility testing.

**Dressings**

The following dressings were tested: ionic silver-containing dressing (ISCD, Aquacel Ag (ConvaTec, Uxbridge, UK)), nanocrystalline silver coated dressing (NSCD, Acticoat (Smith & Nephew, Hull, UK)) and a propolis-containing dressing (PCD) that is in development. Sterile gauze was used as a negative control.

**Time–kill assay**

A suspension of each organism in peptone water was prepared from fresh colonies on blood agar plates after overnight incubation, and the turbidity was adjusted so that it was equivalent to that of a 0.5 McFarland standard (1–1.5 × 10⁸ cfu/mL) on a spectrophotometer. The suspension was then diluted to a concentration of 1–1.5 × 10⁷ cfu/mL. Subsequently, an aliquot of that suspension was added to the broth with dressings to give a final turbidity of 1–1.5 × 10⁶ cfu/mL. Squares of 1 cm² of each dressing were prepared in an aseptic manner. Each square was placed in a sterile vial, and 2.7 mL of either brain heart infusion (BHI) broth (Oxoid Ltd, Basingstoke, UK) or...
Mueller–Hinton (MH) broth (Oxoid Ltd, Basingstoke, UK) was added to each dressing. Within 15 min of bacterial culture preparation, 300 μL of the 1×10^7 cfu/mL bacterial suspension was then added to each vial to make up to a total volume of 3 mL, and 1 cm² of each dressing was immersed in 3 mL of the resulting 1×10^6 cfu/mL bacterial suspension.

After adding the dressings, the vials were incubated at 37°C for 48 h. Aliquots of broth were taken from each vial at specific time intervals (0, 2, 4, 6, 24 and 48 h), diluted and spread onto plates with Iso-Sensitest agar (Oxoid Ltd, Basingstoke, UK), or Iso-Sensitest agar with 5% horse blood for *S. pyogenes*. The plates were incubated overnight at 37°C, and bacterial counts (cfu/mL) were measured. The mean log_{10} cfu/mL for each aliquot was calculated. Bactericidal activity was recorded as change in bacterial count in log_{10} cfu/mL over time. For graphical representation, a too numerous to count (‘TNTC’) colony count was shown as 10^10 cfu/mL.

**Zone of inhibition test**

A suspension representing a 0.5 McFarland standard (1–1.5×10^8 cfu/mL) was prepared and inoculated onto MH and Iso-Sensitest agar plates (Oxoid Ltd, Basingstoke, UK). Dressing squares of 1 cm² were placed in the centre of the plates and incubated for 24 h at 37°C. A metric ruler was used to measure the zone of inhibition across each square.

**Results**

**Time–kill assay**

Detailed results are shown in Figures 1 and 2.

For sterile gauze and PCD all four organisms reached the maximum growth within 24 h, and the results for MH and BHI broths were similar. NSCD suppressed the growth of all four microorganisms with no regrowth at 24 h in MH broth, apart from *S. aureus*. In BHI broth with NSCD the number of *E. coli* organisms never reached zero, with regrowth at 4 h. ISCD killed all four organisms by 24 h in MH broth with no regrowth. In BHI broth with ISCD *P. aeruginosa* started to regrow at 6 h, while the growth of *S. aureus* was minimally suppressed by 24 h and then regrowth occurred.

**Zone of inhibition test**

Sterile gauze: all organisms grew up to the edge of dressings, and no zone of inhibition was noted. PCD: a zone of clearance of irregular shape was observed on *S. aureus* and *S. pyogenes* plates as the dressing absorbed into the agar. No inhibition of *P. aeruginosa* or *E. coli* was detected. NSCD: a zone of inhibition
was observed for all organisms (18 mm for *P. aeruginosa* and *E. coli* and 16 mm for *S. aureus* and *S. pyogenes*). ISCD: a zone of inhibition was observed for all organisms (15 mm for *P. aeruginosa* and *S. pyogenes*, 16 mm for *E. coli* and 14 mm for *S. aureus*).

**Discussion**

In this study we tested three dressings using a proposed standard *in vitro* method for evaluating the antimicrobial activity of wound dressings. We found NSCD to have the highest antimicrobial activity. Both NSCD and ISCD demonstrated bactericidal activity against all four organisms, killing all of them by 24 h in MH broth. Despite the fact that the growth of *S. aureus* was suppressed to zero by 24 h for NSCD, the organism regrew after that time, reaching high levels by 48 h. Therefore, NSCD was the most active against *P. aeruginosa*, *E. coli* and *S. pyogenes*. The limited antimicrobial activity of silver dressings against *S. aureus* has been shown in other studies. In our study, the zone of inhibition test showed bactericidal activity of both silver-containing dressings against all organisms and some antibacterial activity of PCD against *S. pyogenes* and *S. aureus*. However, PCD dissolved into the agar, producing a zone of clearance of a very irregular shape that was impossible to measure and compare with those of the other dressings. The zone of inhibition test is therefore not suitable for all kinds of dressings. The questionable efficacy of the zone of inhibition test has been previously discussed.9

PCD exhibited some antimicrobial activity against Gram-positive organisms, marginally slowing down growth compared with the gauze. The effect appears to be bacteriostatic and of limited duration. PCD delayed the growth of *S. pyogenes* up to 6 h; however, the organism multiplied quickly after that time. The growth of *S. aureus* up to the maximum level was deferred by 2 h in comparison with sterile gauze. PCD did not show any activity against the Gram-negative organisms as their growth profiles were similar to those with sterile gauze.

Propolis is a natural product from a honey bee and is highly allergenic. This fact may limit the use of this substance. In contrast, the allergenic potential of silver is quite limited. Other antimicrobial agents widely used in wound management that should be tested in future include polyhexanide and taurolidine. The first interim results of a controlled, randomized, prospective, multicentre study that compared polyhexanide with silver in the

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**Figure 2.** Effect of four different dressings on (a) *S. aureus*, (b) *S. pyogenes*, (c) *P. aeruginosa* and (d) *E. coli* over a 48 h period using BHI broth.
treatment of wound infection showed no difference, but the dressings with polyhexanide reduced pain faster compared with those with silver. A bacterial suspension between $1 - 1.5 \times 10^6$ and $1 - 1.5 \times 10^8$ cfu/mL is probably the best for in vitro assessment of antibacterial activity as it is an established concentration of organisms by the BSAC, allowing comparison with the results of other studies. The size of the dressings of 1 cm$^2$ is optimal for in vitro assessment as a smaller size could be physically difficult to cut out and a bigger size might require a larger volume of broth for immersion.

A less potent antimicrobial effect exhibited by dressings in the BHI broth compared with MH broth can be explained by the fact that BHI contains more nutritious substances and because BHI contains sodium chloride, which inhibits the activity of ionic silver. MH broth is more appropriate for the in vitro evaluation of the antimicrobial activity of wound dressings as it is a long-established medium for antibacterial susceptibility testing, it supports the growth of most non-fastidious bacteria and it does not contain inhibitors of silver.

The limitations of our study are that we did not test a more extensive range of dressings; this is currently planned.

**Conclusions**

NSCD exhibited the most potent antibacterial activity against all organisms. ISCD had good antibacterial activity, but PCD did not show any bactericidal effect. PCD might have other positive effects on wounds such as promotion of reparative processes in wound healing, but further research is required to establish its clinical value.

Based on our findings we suggest that a time–kill assay with a 1 cm$^2$ dressing is used for evaluation of the antimicrobial activity of wound dressings, and that dressings should be exposed to a standardized inoculum of $1 - 1.5 \times 10^6$ cfu/mL with MH broth as the most appropriate medium.

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**Transparency declarations**

The Skin Science Consultancy (Cambridge, UK) provided all the dressings for the study and contributed towards some consumable costs; they are not the manufacturers of any of the products tested.

**References**