Objective: To compare the antimicrobial effectiveness of silver- and iodine-containing wound dressings against preformed mature biofilms of pathogenic wound bacteria grown in vitro.

Method: Biofilms of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were grown within an in vitro flat bed perfusion biofilm model. Mature biofilms were removed and exposed to wound dressings containing either silver or iodine (Aquacel Ag and Iodozyme) within a static diffusion method, for up to 24 hours. This method was designed to reflect certain key features that determine antimicrobial activity within the wound. The numbers of viable bacteria surviving in the biofilms were determined at set time intervals over the test period.

Results: Both test dressings exerted an antimicrobial effect against the target species biofilms, although the iodine dressing was more efficacious under the experimental conditions employed.

Conclusion: There are large and potentially significant differences (as measured in vitro) in the effectiveness of wound dressings containing broad-spectrum antimicrobial agents such as silver and iodine against specific types of bacterial biofilms.

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against pre-formed biofilms of two microorganisms commonly found in wounds (Pseudomonas aeruginosa and Staphylococcus aureus).

**Method**

**Growth and maintenance of microorganisms**

P. aeruginosa PAO-1 (ATCC 15692) and S. aureus (NTCC 8325) were maintained on nutrient agar (Oxoid, Basingstoke, UK) and stored on frozen beads (-80°C; Pro-Lab Diagnostics, Neston, UK).

**Test dressings**

Iodozyme (10 x 10cm) and Aquacel Ag (10 x 10cm) wound dressings were obtained from the manufacturer or a local pharmacy.

**Biofilm growth and development**

Mature biofilms were grown within 1cm² cellulose matrices using a flat bed perfusion biofilm model. Cellulose matrices were inoculated with 10⁵ colony forming units (cfu) of target cells, incubated for two hours, and then perfused with either sterile 0.1% foetal calf serum (FCS, heat inactivated at 57°C) in phosphate-buffered saline (PBSa) for P. aeruginosa or 2% FCS + 0.1% glucose in PBSa for S. aureus, at a rate of 1ml per hour. After 24 hours of media perfusion, reproducible quasi-steady state biofilm communities formed at a density of around 10⁷cfu/cm². These were removed and used immediately as the target biofilm samples for subsequent assessment of wound-dressing antimicrobial activity within the in vitro static diffusion method.

**In vitro static diffusion method (Figs 1 and 2)**

Preformed mature biofilm samples were distributed across the surface of 10 x 10 x 10cm² hydrogel polymer test bed (HPT) containing a reservoir of pre-absorbed serum; test dressings can then be applied to the biofilm surface.

Biofilms are removed at defined time points, serially diluted and plated onto recovery media to determine the number of viable survivors.

**Statistical analysis**

The kill rate (k) of test treatments against biofilms of target species was determined by linear regression analysis. Analysis of covariance (ANCOVA) was used.
to determine if there was a significant difference between rates. A two-tailed unpaired t-test was used to determine significant differences between individual data points. A p value of <0.05 was regarded as significant. Graph construction, statistical analyses, and modelling were conducted with the use of GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, CA, USA).

**Results**

The two wound dressings exhibited differing abilities to reduce the total viable biofilm population density of the two microorganisms tested. The antimicrobial kinetics (the speed of kill) for *S. aureus* biofilms treated with either the iodine or the silver dressing are shown in Fig 3. Both dressings exerted significant antimicrobial effects, causing kill rates exceeding those observed with bacteria in the control samples over the first eight hours of treatment (p<0.0001). The iodine dressing exerted a significantly greater antimicrobial effect (k = -0.468) than the silver dressing (k = -0.081), producing a >3-log fold reduction in viable cell numbers within eight hours of treatment. Moreover, no microbial survivors were recovered after 24 hours of treatment with the iodine dressing (according to the MDL of these experiments). In contrast, a viable biofilm population density that was not significantly different from the control samples was recovered from beneath the silver dressing after the same treatment period. The antimicrobial kinetics for *P. aeruginosa* biofilms treated with either the iodine or silver dressings are shown in Fig 4. Both dressings exerted significant antimicrobial effects, causing kill rates exceeding those observed with bacteria in the control samples (p<0.0001). During the first eight hours of treatment, there was no significant difference in the kill kinetics recorded for the iodine dressing (k = -0.514) and the silver dressing Ag (k = -0.504). After 24 hours of treatment, there was a significant increase (p<0.05) in the total viable counts of biofilm bacteria recovered from beneath the silver dressing (approximately 10^4 cfu) compared with after eight hours of treatment, although counts were still significantly lower than the control values (p<0.0001). In contrast, no viable cells were recovered from beneath the iodine dressing-treated samples after 24 hours of treatment (according to the MDL of these experiments).

**Discussion**

Mature monospecies biofilms were preformed in a flat bed perfusion biofilm system which has been previously shown to produce reproducible quasi-steady state communities, whereby surface associated cells are enclosed in a self-produced polymeric mix.20 Biofilms were extracted for use as defined target samples within a static diffusion method,21 enabling quantifiable kill kinetics to be determined. Both dressings tested exerted significant antimicrobial effects against *P. aeruginosa*, but the initial activity of the silver dressing appeared to wane by 24 hours. This is perhaps surprising as, while this dressing has a ‘relatively modest silver content’,21 it has previously demonstrated prolonged antimicrobial activity *in vitro* against this microorganism.21 The use of preformed mature biofilms as the target sample presumably accounted for the differences in the antimicrobial effects observed in the two studies. Although the silver dressing was significantly less effective than the iodine dressing against *P. aeruginosa* biofilms at 24 hours, overall the silver dressing still exerted a substantial antimicrobial effect,
with a >3-log fold reduction in viable biofilm cells, leaving only a low-level, residual population.

When the dressings were tested against S. aureus biofilms, the difference in antimicrobial activities of the two dressings was greater. The iodine dressing exerted a sustained antimicrobial effect throughout the treatment period, reducing the total viable biofilm population to below the MDL by 24 hours. In contrast, the silver dressing exerted a weak but significant activity during the first eight hours of treatment but, by 24 hours, the total biofilm population had recovered to levels not distinguishable from the control. The limited antimicrobial activity of the silver dressing against S. aureus has previously been documented in studies using a dressing suspension test that also produced kill kinetic data.24

The kill rates recorded for the iodine dressing against target species biofilms in this study were significantly lower than those observed in tests against a similar density (10^5cfu) of planktonic immobilised target organisms of the same species, within a static diffusion method.21

The physiological state of the target biofilm samples, therefore, appears to confer a higher level of resistance against the topical broad-spectrum antiseptic agents tested. It should be noted that the biofilm cells would have been growing at a much lower growth rate, and it is likely that the difference in underlying growth rates is a major factor in the observed difference in relative susceptibility. Nonetheless, the iodine dressing caused a >3-log fold reduction in viable counts by eight hours against both S. aureus and P. aeruginosa biofilms, which is indicative of a bactericidal effect, and by 24 hours no viable cells of either organism were recovered.

When tested against preformed mature biofilm populations within an in vitro static diffusion method, the iodine dressing exerted a greater antimicrobial activity against biofilms than did the silver dressing. It should, of course, be expected that different wound dressings containing different broad-spectrum antimicrobial agents will have different antibacterial profiles when tested against different microorganisms. Such differences are likely to be more pronounced when the microorganisms are present as biofilms, which confer some protection to the bacteria present.

Given the various and disparate modes of antimicrobial action of silver and iodine,22 it would be surprising if these agents produced similar antimicrobial effects. Nevertheless, the apparent superiority of a ‘traditional’ agent (iodine) over a second-generation, ‘modern’ embodiment of silver in this in vitro study is significant. Further clinical study is required to determine the extent to which these in vitro findings should influence practitioners’ choice of antimicrobial dressing when the wound pathogen is known.

An ideal in vitro experimental system for assessing the antimicrobial efficacy of wound treatments would incorporate the same (or very similar) physicochemical conditions that occur in a real wound bed. All in vitro methods have their limitations and in the present study only two types of monospecies biofilms were assessed, whereas a complex mixed-species biofilm is likely to be present in a real wound bed. Moreover, although certain physicochemical parameters were controlled for, this model in no way replicates all of the factors involved in a real wound bed in vivo, particularly the host response.

Further study should involve more challenging experimental conditions and a greater selection of wound dressings. It will be important to investigate whether the pattern of activity against biofilms observed in this study is replicated when these and other dressings are tested within even more challenging experimental conditions, in conjunction with appropriate controlled clinical trials.

References

5 Thomson, P.D. Immunology, microbiology, and the recalcitrant wound.

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