Research article

An *in vitro* comparison of the antimicrobial activity of honey, iodine and silver wound dressings

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The main line of treatment for chronic wounds is the application of an appropriate dressing. Dressings can be used to reduce odour and pain, maintain a moist healing environment, remove excessive exudate and prevent clinical infection. Antimicrobial compounds such as silver, honey and iodine have been in use for millennia. The discovery of antibiotics in the early 20th century greatly reduced the routine usage of such compounds. More recently, there has been renewed interest in these compounds, with manufacturers adding these to dressings to provide greater antimicrobial action and aid the healing process. Much of the published literature on the antimicrobial properties of silver, honey and iodine-containing dressings is contradictory, with varying degrees of efficacy reported. This study aimed to independently compare the *in vitro* antimicrobial activity of a wide variety of dressings against common wound pathogens; *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, in order to provide further evidence and aid dressing selection.

Although no significant differences were reported between honey, iodine and silver; a significant difference was observed between the individual dressings, indicating that determination of bacterial species present within a wound can aid clinical staff in the selection of the most appropriate dressing.

**Key words:** antimicrobial, wound dressing, silver, honey, iodine, chronic wound.

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Introduction

Wound classification

A wound may be defined as a breach in the epidermis or dermis, due to trauma or physiological change, activating the repair process.1 Wounds can be classified as either acute or chronic. An acute wound will heal as expected, usually within a relatively short time with few complications, for example, a surgical wound. In comparison, chronic wounds fail to heal as expected or become fixed at one phase of wound healing for a period greater than 6 weeks.1 This characteristic prolonged healing period is accompanied by inflammation, increased wound exudate and complications such as infection.2 Examples of chronic wounds include: pressure ulcers, venous, arterial and diabetic ulcers. Such wounds involve patient-specific factors that predispose individuals to develop poorly healing wounds, for example, venous insufficiency, diabetes mellitus, arterial occlusion or high external pressure, all resulting in local tissue hypoxia, cell dysfunction and death.3 Many patients develop predispositions to delayed healing with increasing age and in correlation chronic wounds are frequently seen in the elderly.1

Acute wounds usually require little intervention and heal rapidly. Therefore, the following article details the management of chronic wounds and some cases of burns, specifically those which have become or are at risk of becoming infected. Management of these highly complex wounds is labour intensive and costly to treat.1 Appropriate wound care from day 1 can improve the outcome and quality of life of the patient and also potentially reduce the financial implications for the National Health Service (NHS).

Wound infection

The moist environment of chronic wounds is an ideal growth medium for bacteria4 and infection is the prominent cause of
delayed healing. This has become an increasing problem with the recent expansion of antibiotic-resistant bacteria. 5 Burns and chronic wounds are particularly prone to infection, with ~75% of deaths following burns involving infection. 6

The majority of open wounds will become contaminated by bacteria; however, this does not necessarily impair the healing process. Bacteria can delay wound healing by competing with host cells for nutrients and oxygen, cause damage via cytotoxic enzymes and waste products, and interfere with the host’s immune responses. High levels of bacteria, multi-resistant organisms and bacterial biofilms can significantly affect wound healing. Infection of a wound may result in tissue death, local hypoxia, vessel occlusion and an increase in wound size, ultimately slowing the healing process. 7

Infection of chronic wounds is often polymicrobial, with high numbers of multiple species of bacteria preventing efficient healing. 8 Gram-positive cocci (Streptococci and Staphylococci) are most commonly responsible, but wounds may display greater microbial diversity with Enterococci, Enterobacteriaceae anaerobes and Pseudomonas species. This makes it difficult to determine the most appropriate treatment. 9 The recent rise in resistant pathogens is a major concern, in particular methicillin-resistant S. aureus (MRSA) where exposure can lead to bacteraemia, local epidemics and potential fatalities. 5 MRSA may remain as reservoirs on hospital equipment, for example, beds, which are a particular issue in wards where pressure ulcers are common. 5

Antibiotics or surgery may be required for systemic, deep or progressive infections, such as necrotizing fasciitis. 10 Although generally regarded as the ultimate treatment for infection, antibiotics are inappropriate in cases such as burns and may actually encourage colonization by resistant microorganisms. 5 It is important to remember that chronic wounds often develop due to an alteration in blood flow to the affected area and therefore the use of systemic antibiotics in these cases has little or no effect on the wound site itself as the reduction in blood flow prevents access of the antibiotic to the affected area. 5

Wound dressing
Traditionally, it was believed that the best way to promote healing was through encouraging drying and scab formation. However, in the 1960s, the theory of moist wound healing developed, following experimental evidence that healing time was reduced in a moist environment. 1 However, there was also clear evidence that moist environments increased the risk of infection, which ultimately led to the development of modern dressings. These maintain a moist environment while providing a physical barrier against infection. 1 Alginate, foam, hydrogel and hydrocolloid dressings control the level of wound hydration, the choice of which generally depends on: wound type and size, the presence of infection and patient and clinician preferences. 11

There is increasing interest in the use of topical antimicrobials for wound care. Compounds such as honey, iodine and silver have been incorporated into dressings to provide antimicrobial action and aid the healing process. 5 Currently, many antimicrobial dressings are available. Some of which are new materials specifically developed to carry an antimicrobial compound. Other dressings are simply the addition of an antimicrobial to a pre-existing product. 12 Advances in dressing technology have led to the development of controlled antimicrobial delivery; dressings may be active (releasing the antimicrobial to exert effects within the wound), or passive (exudate is absorbed and interacts with the antimicrobial within the dressing structure). 6 Many in vitro investigations into the efficacy of such products have been performed; however, there is a confusing mixture of evidence and differences in research methodologies making interpretation and comparison of results difficult. In addition, there are very few randomized controlled trials comparing wound care products in clinical practice. 13

Honey
Originally used by the ancient Egyptians and Greeks, honey is a viscous, saturated sugar solution now widely used in wound care. 14 High osmolarity prevents the growth of bacteria and encourages healing; this can be utilized for wound management through the application of sugar paste or honey. In addition, honey is believed to have specific antimicrobial properties, for example, preventing the growth of S. aureus even when diluted beyond the point at which osmolarity is no longer inhibitory. 15 Studies have reported that it may modestly decrease wound-healing time, act as an anti-inflammatory, deodorize wounds, enhance cell proliferation and expansion in vitro. 14 However, a large-scale randomized trial showed no significant advantage for the use of honey dressings over ‘standard wound care’ in the treatment of chronic wounds. 17 Studies in support of its use indicate that honey-based treatment is preferential to silver or iodine, due to its comparative lack of toxicity. 16

Although honey is believed to have a broad-spectrum antimicrobial action, different honeys, for example, Manuka (New Zealand), Heather (UK) and Khadikraft (India) vary substantially in activity. 18 The precise mechanisms of action are still not fully understood; however, the antimicrobial activity of most honeys is linked to the production of hydrogen peroxide by the enzyme glucose oxidase which, combined with high acidity, exerts an antimicrobial effect. 19 In addition, unidentified phytochemical factors (non-peroxide factors) exert a high antimicrobial effect in some honeys (e.g. Manuka honey) that do not breakdown when treated with heat or light and are still effective even when diluted. 20
Iodine

Iodine is used in aqueous and alcoholic preparations for hand washing and skin preparation prior to surgery. Molecular iodine (I₂) is considered to be active against bacteria, fungi and viruses. It rapidly penetrates microorganisms, damaging proteins, nucleotides and fatty acids, leading to rapid cell death. Iodine denatures proteins and enzymes by binding to thiol and sulphydryl groups, and alters phospholipid membrane structures by blocking hydrogen bonding.

There has been a decline in the use of iodine-based dressings due to the concerns over possible toxic effects and the limited timescale for their use. Iodine may be absorbed through large wounds or during prolonged usage and there is the potential risk of interactions with patients taking lithium, resulting in an increased risk of hypothyroidism.

Silver

Silver has been used medicinally for thousands of years. Dressings containing silver have recently been strongly marketed and have increased in usage by ~200% since 1996. Silver-impregnated dressings are now used extensively in the care of chronic wounds. However, some studies have demonstrated significant cytotoxicity towards fibroblasts and keratinocytes—essential components involved in wound repair.

The antimicrobial properties of silver are believed to be due to the ability to form ionic salts (Ag⁺) in the presence of acids. Positively charged silver ions are attracted to negatively charged structures of the cell membrane, allowing silver to bind and enter the bacterial cell. It is believed that the interaction of Ag⁺ with bacterial thiol (–SH) groups leads to inactivation, the blocking of key pathways such as cellular respiration structural changes in the bacterial membrane and the blocking of enzyme and transport systems. They may also act denaturing bacterial RNA or DNA; inhibiting transcription and replication. Unlike antibiotics which are generally specific, silver is toxic to multiple components of bacterial cells. This multi-system affect means it is less likely that bacteria will develop resistance to silver, as multiple random mutations would be required. However, it is possible that continued low level exposure may aid the development of resistance. Research has shown silver to be effective against some antibiotic-resistant bacteria, including MRSA and vancomycin-resistant Enterococci. Loh et al. tested MRSA isolated from wounds for known silver resistance genes and although these were identified in some of the isolates, all strains were found to be susceptible to the silver dressing used.

The present study

As infection is common in chronic wounds, there is increasing demand for effective wound care in order to limit infection risk. Clinical staff have access to a wide range of antimicrobial dressings, but little objective evidence to base their decisions on.

The aim of this study was to compare the in vitro antimicrobial activity of a range of antimicrobial dressings containing honey, iodine and silver, thus providing further evidence for their usage.

The study’s objectives included: to identify appropriate in vitro testing methods that suitably replicate the wound environment using simulated wound fluid (SWF) and to ensure that testing methods are able to assess the antimicrobial activity of a range of dressings and products and to determine a suitable range of test microorganisms.

Materials and methods

Test dressings

This study assessed the in vitro antimicrobial activity of the honey, iodine and silver-containing dressings shown in Table 1. These dressings were kindly donated by the Sheffield Wound Care Group. Squares of each dressing measuring 10 × 10 mm were prepared in an aseptic manner. An aseptic area and equipment was produced for their usage.

The wound dressings and products under investigation grouped according to antimicrobial compound. *UrgoCell TLC used as a comparison for UrgoCell Silver.
Test organisms
Three organisms were used in this study; *S. aureus (SH1000)*, *E. coli (JM109)* and *P. aeruginosa (H085180216)*. These were maintained on Mueller-Hinton agar and broth (Oxoid, Basingstoke, UK).

Test medium
*In vitro* investigations were performed using either Mueller-Hinton broth (Oxoid) or SWF. SWF was prepared using 50% v/v foetal calf serum (Hallam Biosciences dept.), 50% v/v maximum recovery diluent (0.1% w/v peptone (Oxoid) 0.9% w/v sodium chloride (Fisher Scientific, Loughborough, UK)) as described by Parsons *et al.* Both broth and SWF were initially tested to ensure that bacterial growth could be sustained by comparing colony counts (cfu/ml) following an incubation period at 37°C for 24 h.

*In vitro* growth inhibition assay
Square Petri dishes containing a 5 mm layer of Mueller-Hinton agar were inoculated with 1 ml of a 1 x 10^6 cfu/ml broth culture of each test organism. The suspension was distributed uniformly over the surface of the plate and allowed to dry. Four squares of the same dressing (10 mm x 10 mm) were placed towards the corners of an agar plate (wound contact surface downwards) at least 30 mm from the edges of the plate to allow for development of a zone of inhibition (ZOI). The test was performed on at least two separate occasions for each dressing, giving a total of eight replicates and the average ZOI for each dressing recorded. Dressings were moistened using 0.9% w/v sodium chloride (Fisher Scientific) if recommended by the manufacturer. All plates were incubated at 37°C for 24 h.

Following incubation, the plates were examined for bacterial growth and the presence of a ZOI, both under and around the dressing. If detected, the width of the ZOI was measured in millimetres from the edge of the dressing.

In order to better reproduce the conditions of the clinical setting, each dressing was also tested using SWF. The above method was repeated using 1 ml of a 1 x 10^6 cfu/ml SWF culture of each organism.

Statistical analysis
The average ZOI for each dressing against each bacterium was calculated and recorded. Analysis of variance (ANOVA) was used to determine whether there was a significant difference between the dressings, the effect of the two medium used and the effect against the different bacteria. ANOVA demonstrated either a significant difference (*p* < 0.05) or no significant difference (*p* > 0.05) for the results obtained. Where a significant difference was observed, the least significant difference (LSD) test was used to determine where the differences occurred.

Results
The aim of this study was to compare the *in vitro* antimicrobial activity of a range of antimicrobial dressings containing: honey, iodine and silver. This was performed using both Muller-Hinton Broth and SWF.

Honey
Although Actilite showed antimicrobial action against *E. coli* and *S. aureus*, a degree of bacterial growth was observed within the ZOI. It was clear that the honey contained within the dressing had been able to diffuse around 12 mm ([± 0.64, *n* = 8]) with *E. coli* and 16 mm ([± 1.07, *n* = 8]) with *S. aureus* as recorded in Tables 2 and 3, and shown by the zone clearly visible on the agar in Fig. 1. However, there appeared to have been some bacterial growth within this zone, this growth was less dense than the lawn of bacteria outside the zone, indicating that the honey has some but not complete antibacterial activity. No ZOI was observed for *P. aeruginosa*.

Iodine
Iodoflex showed complete inhibition of all *E. coli* and *S. aureus*, and the largest ZOI of all the dressings tested observed against *P. aeruginosa* (Table 2).

Iodozyme and Oxyzyme dressings are supplied with a primary wound contact gel and a secondary hydrogel. The results in Tables 2 and 3 show that without the secondary hydrogel no antimicrobial activity is observed. When the secondary hydrogel is applied, a degree of antimicrobial activity is observed for both dressings, where Iodozyme showed a larger ZOI than Oxyzyme for all bacteria. It was demonstrated that the difference in the antimicrobial action between Iodozyme and Oxyzyme was significant (*p* = 0.019).

Table 3 shows the results when tested using SWF, there is a reduction in the size of the ZOI produced by Iodozyme and Oxyzyme (plus secondary hydrogel) compared with the ZOI when using Muller-Hinton broth. The decrease in ZOI equates to a reduction of 52%, 21% and 100% for Iodozyme and 100%, 9%, 100% for Oxyzyme with *E. coli*, *S. aureus* and *P. aeruginosa*, respectively. Analysis indicated that there was a significant reduction in antimicrobial action in the presence of SWF (*p* = 0.023).

Silver
The silver dressings showed a wide range of activity, where all except Mepilex Ag displayed some degree of antimicrobial activity (Table 2). Actisorb Silver 220 only inhibited bacterial growth in areas of direct contact. The other silver-containing dressings showed significant differences in the size of ZOI surrounding the dressing (*p* = 7.19 x 10^-4). Analysis of these dressings using ANOVA and LSD enabled grouping by level of activity from highest to lowest; Group 1(Sorbsan Silver Flat), Group 2 (Aquacel...
Table 2. Comparison of the zones of inhibition observed for antimicrobial dressings

<table>
<thead>
<tr>
<th>Dressing</th>
<th>Escherichia coli</th>
<th>Staphylococcus aureus</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inhibition under patch</td>
<td>Zone of inhibition</td>
<td>Inhibition under patch</td>
</tr>
<tr>
<td>Actilite</td>
<td>Yes</td>
<td>12.13 mm*</td>
<td>Yes</td>
</tr>
<tr>
<td>Iodoflex</td>
<td>Yes</td>
<td>Complete</td>
<td>Yes</td>
</tr>
<tr>
<td>Iodozyme</td>
<td>No</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Iodozyme + second gel</td>
<td>Yes</td>
<td>5.25 mm</td>
<td>Yes</td>
</tr>
<tr>
<td>Oxyzyme</td>
<td>No</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Oxyzyme + second gel</td>
<td>Yes</td>
<td>4.25 mm</td>
<td>Yes</td>
</tr>
<tr>
<td>Actisorb Silver 220</td>
<td>Yes</td>
<td>Complete</td>
<td>Yes</td>
</tr>
<tr>
<td>Aquacel Ag</td>
<td>Yes</td>
<td>3.75 mm</td>
<td>Yes</td>
</tr>
<tr>
<td>Mepilex Ag</td>
<td>No</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Seasorb Ag</td>
<td>Yes</td>
<td>1.88 mm</td>
<td>Yes</td>
</tr>
<tr>
<td>Sorbsan Silver Flat</td>
<td>Yes</td>
<td>4.38 mm</td>
<td>Yes</td>
</tr>
<tr>
<td>UrgoCell Silver</td>
<td>Yes</td>
<td>0.81 mm</td>
<td>Yes</td>
</tr>
<tr>
<td>UrgoCell TLC</td>
<td>No</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Urgosorb Silver</td>
<td>Yes</td>
<td>2.00 mm</td>
<td>Yes</td>
</tr>
<tr>
<td>Urgotul SSD</td>
<td>Yes</td>
<td>6.29 mm</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Average measurement of the ZOI (mm) surrounding antimicrobial dressings against E. coli, S. aureus and P. aeruginosa using Mueller-Hinton broth. *Some growth was observed within the ZOI.

Table 3. Comparison of the SWF zones of inhibition observed for antimicrobial dressings

<table>
<thead>
<tr>
<th>Dressing</th>
<th>Escherichia coli</th>
<th>Staphylococcus aureus</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inhibition under patch</td>
<td>Zone of inhibition</td>
<td>Inhibition under patch</td>
</tr>
<tr>
<td>Actilite</td>
<td>Yes</td>
<td>12.5 mm*</td>
<td>Yes</td>
</tr>
<tr>
<td>Iodoflex</td>
<td>Yes</td>
<td>Complete</td>
<td>Yes</td>
</tr>
<tr>
<td>Iodozyme</td>
<td>No</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Iodozyme + second gel</td>
<td>Yes</td>
<td>2.25 mm</td>
<td>Yes</td>
</tr>
<tr>
<td>Oxyzyme</td>
<td>No</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Oxyzyme + second gel</td>
<td>Yes</td>
<td>None</td>
<td>Yes</td>
</tr>
<tr>
<td>Actisorb Silver 220</td>
<td>Yes</td>
<td>None</td>
<td>Yes</td>
</tr>
<tr>
<td>Aquacel Ag</td>
<td>Yes</td>
<td>4.25</td>
<td>Yes</td>
</tr>
<tr>
<td>Mepilex Ag</td>
<td>No</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Seasorb Ag</td>
<td>Yes</td>
<td>2.13</td>
<td>Yes</td>
</tr>
<tr>
<td>Sorbsan Silver Flat</td>
<td>Yes</td>
<td>4.50</td>
<td>Yes</td>
</tr>
<tr>
<td>UrgoCell Silver</td>
<td>Yes</td>
<td>0.38</td>
<td>Yes</td>
</tr>
<tr>
<td>UrgoCell TLC</td>
<td>No</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>Urgosorb Silver</td>
<td>Yes</td>
<td>2.50</td>
<td>Yes</td>
</tr>
<tr>
<td>Urgotul SSD</td>
<td>Yes</td>
<td>3.50</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Average measurement of the ZOI (mm) surrounding antimicrobial dressings against E. coli, S. aureus and P. aeruginosa using SWF. *Some growth was observed within the ZOI.
Ag, Urgotul SSD), Group 3 (Urgosorb Silver, Seasorb Ag) and Group 4 (UrgoCell Silver).

Grouping the dressings according to the type allowed comparison of the different dressing structures. Dressings were grouped into Alginate (Seasorb Ag, Sorbsan Silver Flat and Urgosorb Silver), Foam (Mepilex Ag and UrgoCell Silver) and Hydrocolloid (Aquacel Ag and Urgotul SSD). Dressings with other structures were not included in this analysis. A significant difference was recorded, with hydrocolloid and Alginate dressings significantly more effective than foam ($p = 0.041$).

UrgoCell TLC was included in this study as a comparison for the silver-containing dressing UrgoCell Silver. Tables 2 and 3 show UrgoCell TLC had no antimicrobial affect, whereas UrgoCell Silver showed antimicrobial activity against all species.

**Discussion**

The methods used were designed to compare the antimicrobial performance of honey, iodine and silver dressings in vitro. The second assay aimed to more accurately replicate the clinical setting. Analysis of data from both investigations indicated that there was no significant difference in the size of ZOI using broth compared with SWF for honey or silver ($p = 0.981, 0.567$); however, a significant difference was observed for iodine.

**Honey**

Although Actilite showed antimicrobial action against *E. coli* and *S. aureus*, a degree of bacterial growth was observed within the ZOI which was less dense than the lawn of bacteria outside the zone. Possible explanations for this observation include: Manuka honey may be bacteriostatic (inhibiting the growth or reproduction of bacteria), depleted over time (within $<24$ h) or the bacteria may have developed a resistance; however, this is unlikely within the time period. A literature search found no record of similar results described previously. From the results obtained, it is not possible to state an explanation for the observations and further research is necessary to determine the likely cause.

Actilite showed antimicrobial activity against *E. coli* and *S. aureus*, but not *P. aeruginosa*. Previous studies have shown Manuka honey to be effective against *Staphylococci* species particularly *S. aureus* which would collaborate this study's results. However, other studies have shown Manuka honey to also be effective against *P. aeruginosa*. Large discrepancies have been reported by hospitals using honey, most likely due to the variability in potency of different honeys used. It is believed that the antimicrobial activity of honey varies between and also within source species, making determination of activity difficult.

A review of published literature by Oliatan et al. reported that many people were sceptical about the medicinal use of honey, as it may be considered both antimicrobial and a reservoir for microbes. Although many bacteria have been shown to be susceptible to the effects of honey, others are able to withstand concentrated sugar, acidity and the antimicrobial action of honey. Combination of results from multiple studies indicated that sources of microbial contamination may include honeybees, soil, flowers and air, with species including *Streptococcus, Clostridium, Enterobacter, Klebsiella* and *Pseudomonas* identified as possible contaminants. More research is necessary to determine the risk of infection developing as a result of topical honey usage.

**Iodine**

Iodoflex was effective against all three bacteria investigated, showing complete clearance of all *E. coli* and *S. aureus* on the plates, and a significant ZOI against *P. aeruginosa* ($p = 1.93 \times 10^{-15}$). Iodozyme and Oxyzyme also contain

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**Figure 1.** Actilite dressing on *E. coli* (left) and *S. aureus* (right). On both *E. coli* and *S. aureus*, the Actilite dressing (a) produced an obvious zone of inhibition (b). However, some bacterial growth (c) could be observed within the zone, which was less dense than the bacterial growth (d) outside the zone.
iodine; however, these dressings showed significantly less inhibition. Both are hydrogel dressings of similar structure and the difference in activity observed between Iodoflex and the hydrogel dressings is likely to be due to variation in structure and content of the dressing. Iodoflex consists of a starch matrix of spherical absorbent micro-heads containing 0.9% iodine. As wound fluid is absorbed, these beads swell to form a gel and release iodine slowly to the wound until the equilibrium is reached. The maintained release of iodine is toxic to bacteria and has also been associated with toxicity in the patient; resulting in a decline in usage. The release of iodine from a dressing placed over a patient’s open wound could lead to iodine absorption, transportation and concentration in the thyroid. A case study by Prager and Gardener showed development of hypothyroidism with a slight goitre caused by long term (2 years) usage of two topical iodine-containing medications. Additionally, reports have shown degrees of cytotoxicity of topical iodine towards human cell lines of granulocytes, monocytes, keratinocytes and fibroblasts. However, it is believed that the development of products such as cadexomer iodine (e.g. Iodoflex) has reduced the number of iodine-associated toxicity cases. In addition, it has been suggested that the overall risk of toxicity to normal patients is minimal, but caution should be taken with children, pregnant or lactating women, patients with previously existing thyroid dysfunction or extensive burns.

As mentioned, Iodozyme and Oxyzyme are provided with a secondary hydrogel; the first gel is applied directly to the patients wound and the second on top of the first. The activity of both dressings was investigated with and without the secondary gel. Only with the application of the secondary gel was a significant antimicrobial effect observed against E. coli and S. aureus. Both dressings rely on an enzymatic reaction involving glucose oxidase to synthesize a pre-determined amount of iodine and deliver oxygen to the wound. The glucose oxidase enzyme is contained within the secondary hydrogel, making it essential for the synthesis of iodine and therefore key to the antimicrobial activity of the dressing as a whole. Iodozyme is recommended for already infected wounds, while Oxyzyme is recommended for use as prophylaxis in order to prevent wound infection and so as would be expected, the results showed a significant difference between the antimicrobial action of the dressings ($p = 0.019$). Similar results were reported by Thorn et al., however, their results reported higher efficacy for Iodozyme against P. aeruginosa, while this study reported equally low activity for both dressings against this species.

Analysis confirmed that there was a significant difference between the results observed with broth and those with SWF ($p = 0.023$), which is consistent with the previous reports by Simon et al. and Michaels et al. who suggested that the activity of iodine products is reduced by interaction with the protein content of wound fluid. However, there appears to be little published literature to explain this observation. There is a large amount of literature relating to the interaction of serum proteins, namely albumin with antibiotics; leading to sequestration of the molecule and a reduction in antibacterial activity. However, it is unclear whether the mechanisms that reduce antibiotic activity can be applied to iodine.

Silver

Mepilex Ag showed no antimicrobial activity in the first investigation; however, the silver component of Mepilex Ag is said to be inert, becoming ionized in the presence of wound exudate. Therefore, it would be expected to show no antimicrobial effect in the absence of wound fluid. However, Mepilex Ag also showed no in vitro antimicrobial activity when exposed SWF in the second part of this investigation, this does not support a review by Barrett which claimed a wide antibacterial effect against 19 different bacteria including S. aureus and P. aeruginosa. However, it is possible that the SWF used in this study does not completely reflect the properties of patients wound fluid.

Actisorb Silver 220 only showed inhibition of bacterial growth in areas of direct contact between dressing and bacteria. The absence of a ZOI can be explained as this dressing is designed to be passive in action. No silver is expected to be released and so the dressing should only exert a surface antimicrobial effect at the border between wound and dressing; therefore, the method used may have underestimated the antimicrobial performance of this dressing. These findings are consistent with those of Thomas and McCubbins.

Where inhibition was observed, the dressings exhibited differences in action against each bacteria, most likely due to differences in susceptibility of the particular bacteria to silver. ANOVA for each bacteria showed that Urgotul SSD, Sorbsan silver flat and Aquacel Ag had the most significant antimicrobial effect on E. coli. Sorbsan Silver and Aquacel also showed a significant antimicrobial effect against S. aureus and Sorbsan silver again showed significant effect against P. aeruginosa. As all these dressings contain silver, it would suggest that another factor must also affect bacterial susceptibility. It is possible that the structure and mechanisms of the dressing account for the observed variation in action. Due to the variation in structure, composition and silver content of the current range of dressings available, Thomas and McCubbins suggested that there may be a significant variation in the dressing’s ability to exert significant antimicrobial effect. This study contained Alginate, Foam and Hydrocolloid dressings, and there was significant evidence to indicate that silver-containing Alginate and hydrocolloid dressings were both more effective than foam ($p = 0.006$). Both Alginate and hydrocolloid absorb wound exudate to form a non-adherent gel. When wound exudate is absorbed by alginate fibres, initially there is ion exchange between silver and the sodium/calcium within wound.
exudate releasing silver ions to the wound. Additionally, silver ions may be chelated by proteins within wound fluid; releasing them from the alginate. This suggests that alginate dressings are able to more readily release silver ions to the wound due to the physical change they undergo and therefore exert greater antimicrobial effect than foams. Silver dressings may contain different forms of silver such as: silver nitrate, silver ions or silver-based crystalline nanoparticles. A selection of different forms of silver were used in this study; for example, silver sulphadiazine (Urgotul SSD) and metallic silver impregnated fabrics (Urgosorb Silver, Aquacel Ag). The exact composition of many of the dressings was difficult to determine because many of the manufacturers would not disclose detailed information about their products. It has been suggested that elemental silver (Ag0) has little or no antibacterial activity; however, in its ionic cation form (Ag+), it is highly active. In the presence of wound exudate, silver readily ionizes; the variation in the form of silver between dressings may therefore affect the ability to release ions.

This study included one parent dressing which did not contain an antimicrobial compound. UrgoCell TLC was used as a comparison for UrgoCell Silver, which is an adaptation of the original UrgoCell product. UrgoCell Silver showed a degree of antimicrobial activity, while UrgoCell TLC showed no antimicrobial effect. This supports the idea that silver-containing dressings are beneficial; reducing the amount of bacteria in an infected wound. The dressings investigated in this study were limited to those provided by the Sheffield wound care group. It is possible that parent dressings without the addition of an antimicrobial agent still have intrinsic antimicrobial activity. As many of the products currently on the market are simply the addition of silver to a pre-existing product, further research could aim to directly compare the activity of these dressings with the parent dressing.

**Bacterial species**

The organisms used in this study were carefully selected to ensure the dressings under investigation were able to exert antimicrobial effect against several common wound pathogens. *Staphylococcus aureus* is a common gram-positive bacteria associated with wound infections and is part of normal skin flora, while *E. coli* is a common gram-negative bacteria. *Pseudomonas aeruginosa* is an opportunistic pathogen commonly infecting hospitalised patients, particularly those in burns units where the organism can readily gain access through damaged skin.

The molecular structure of gram-negative bacteria is generally less complicated than gram positives, for example, the membrane of *E. coli* contains a higher proportion of negatively charged phosphate groups, possibly making it more susceptible to silver. It has been suggested that these negatively charged groups attract positively charged Ag⁺ making silver more effective against gram-negative bacteria. Although in general the dressings appear to be more effective against gram-negative bacteria, *E. coli* and *P. aeruginosa*, analysis confirmed that there was no significant differences in the action of honey, iodine or silver against the bacteria investigated (p = 0.342).

Further analysis showed that the individual dressings did vary significantly against each bacterial species. Iodoflex showed a significant degree of activity against all three bacteria, while the other dressings varied in action towards each species. Urgotul SSD, Sorbsan silver Flat, Aquacel Ag and Iodozyme showed the most significant effect against *E. coli* (p = 1.69 x 10⁻³). Iodozyme showed significant activity against *S. aureus*, closely followed by Oxyzyme (p = 1.63 x 10⁻⁸). Finally, Sorbsan silver, followed by Urgosorb silver and Aquacel Ag were significantly effective against *P. aeruginosa*. These results suggest that careful selection of the dressing based on bacterial species present in the wound could more effectively reduce bacterial infection and potentially improve healing. This could be particularly important with bacteria such as *P. aeruginosa* which contributes substantially to wound-related morbidity and mortality.

Cutting et al. suggested that dressing selection varies depending on the characteristics of the wound and the ability of the dressing to manage pain, control the level of fluid within the wound, sustain a release of antimicrobial compound over time and modulate inflammation. In addition to these properties, this study shows that the species of bacteria present is also an important consideration when choosing an antimicrobial dressing.

**Conclusion**

The aim of this study was to compare the *in vitro* antimicrobial activity of a range of antimicrobial dressings and ultimately provide clinical staff with more information on which to base their treatment decisions. Although no overall difference was observed between the antimicrobial compounds, a significant difference was observed between the dressings against each bacterium. This is most likely due to the structural properties of the individual dressing affecting delivery of the compound. These results suggest that determining the species of bacteria present can allow more effective choice of dressing and reduce bacterial colonization.

Further research could involve a wider range of bacteria to determine which dressings are more/less effective against particular species and aid the choice of dressing in a clinical setting based on laboratory diagnosis of wound swabs. Additional research could include comparison; of antimicrobial dressings with parent dressings to confirm the usefulness of the addition of such compounds and longer testing methods, with measurement of antibacterial effect over a period of days to confirm frequency of change
stated by the manufacturers. The inclusion of a greater range of dressings may allow determination of any significant differences in performance of each antimicrobial compound.

Finally, care should be taken when applying the results of an in vitro study to the clinical situation. Although this study aimed to replicate the conditions in a wound, using SWF, other variables cannot be accounted for. Further independent research is necessary in order to fully understand the varied effects of antimicrobial dressings. Randomized clinical trials would be able to provide greater evidence for the activities of antimicrobial dressings.

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