An *in vitro* study of antimicrobial activity and efficacy of iodine-generating hydrogel dressings

- **Objective:** To determine the antimicrobial activity and efficacy of different formulations of novel biooxygenating hydrogel dressings (which deliver both iodine and oxygen into the wound) against various target organisms by means of an *in vitro* test system that more effectively mimics the conditions encountered when dressings are in contact with wounds.
- **Method:** Three biooxygenating hydrogels were tested: Oxyzyme, which releases low levels of iodine into the wound, and Iodozyme 402 and Iodozyme 401, which release higher levels of iodine, with Iodozyme 402 releasing twice the amount of 401. Cellulose filter disks (n=32) were inoculated with indicator species and placed equidistant from each other as a matrix onto agar test beds. Cut squares of control or test dressings were placed on top of each disk. Kill curves were constructed from determinations of the numbers of survivors (log cfu per disk) over time by removing disk samples at various time points.
- **Results:** Significant differences (p<0.05) were observed between the controls and test samples. The order of sensitivity for Oxyzyme was *Fusobacterium nucleatum*, *Bacteroides fragilis*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Candida albicans* and *Pseudomonas aeruginosa*. The order of efficacy of the three hydrogel dressings (Iodozyme 402, followed by Iodozyme 401 and then Oxyzyme) was the same regardless of the target species.
- **Conclusion:** The novel hydrogel skin surface wound dressings are broad-spectrum in activity, encompassing antibiotic-resistant organisms, anaerobes and yeasts; their antimicrobial function appears to be rapidly effective.
- **Declaration of interest:** The authors wish to thank Insense for supplying various hydrogel test dressings and for summer studentship financial support (for RMS Thorn).

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**Microbial colonisation occurs in virtually all acute, traumatic, surgical and chronic wounds.** Colonisation often involves potentially pathogenic organisms that can cause wound infection, delaying healing, ‘traumatising’ the patient and increasing treatment costs. Burns, diabetic foot ulcers, leg ulcers and pressure ulcers are particularly prone to complications from infection — for example, it is estimated that 75% of deaths following burn injuries are related to infection. There is thus growing demand for effective wound management and therapeutic options to limit the risk of infection.

Practitioners and other decision-makers need to make rational choices between different supposedly antimicrobial dressings, but have little objective evidence on which to base them. These choices require an understanding of the complex underlying nature of wound infection, and the interaction between the wound, the microbial flora and the dressing.

Simple laboratory evaluations of the antimicrobial potency of dressings can be misleading, particularly with modern composite dressings, as the tests do not take account of this complex interaction. There is a need for *in vitro* laboratory tests that take into account at least the most basic aspects of this interplay, especially the way in which wound microbes are bathed in fluid rich in organic, nutritional substances, most of which are drawn into the dressing where they can inactivate antimicrobial agents.

Although systemic antibiotics are regarded as the ultimate treatment against wound infection, they are inappropriate in some cases, such as in burn injuries, and can result in wound colonisation by resistant organisms. Antiseptic skin-surface dressings, such as those containing iodine and silver, have long been used to control wound microflora and, in clinical practice, to combat infection. The efficacy of dressings in combating wound infection is variable. Those that promote moist wound healing limit the spread of bacteria by aerosol formation on removal. *In vitro* some can sequester and retain microorganisms, reducing the microbial load in the wound and surrounding environment.

**References**

Although the use of antiseptics in wound care has long been debated, a recent wide-ranging review concluded that antiseptics need not be omitted from the therapeutic armamentarium of wound care, quoting limited toxicity data, the broader antimicrobial spectrum and lower sensitisation rates. As antiseptics incorporated into wound dressings stay in contact with the wound for much longer than when in solution form, they can be more dilute, less toxic and exert a prolonged antimicrobial effect.

As wound-dressing technology becomes more advanced, new developments are emerging in which antimicrobial agents are delivered in a controlled manner from composite dressings that interact with the wound. For example, the prototype wound dressings Oxyzyme and Iodozyme utilise an enzyme (glucose oxidase) to help drive oxygen into the wound and synthesise a predetermined dose of iodine in the wound bed (Fig 1). The integration of antiseptic iodine with oxygenation of the wound bed provides an effect we describe as wound 'bioxygenation'.

The steady release of iodine from both dressings exerts a gentle, surface antimicrobial effect at the interface between wound and dressing, which is ideal for wounds where the potential for infection must be considered. The level of iodine generated is determined by the concentrations of the enzyme and iodide in the dressing, as well as the pH, surfactant concentration and the dimensions of the gel layers. Iodozyme contains higher levels of iodide, and thus generates more iodine, than Oxyzyme. Iodozyme is thus designed for wounds with clinical signs of contamination; Oxyzyme is designed for clean wounds without obvious signs of contamination.

Currently, these biochemically active wound dressings are built into a hydrogel matrix consisting of three-dimensional networks of hydrophilic polymers that are flexible, non-antigenic and capable of absorbing large amounts of wound fluid; the dressings also provide a barrier to external infection. A moist wound-healing environment is created by the occlusive nature of the hydrogel sheets and their ability to donate moisture. Both dressings are still in development and are not available commercially.

The antimicrobial activity of such composite, multifunctional dressings cannot easily be evaluated by established in vitro methods as this would need to take account of their controlled mechanism of action and potential to interact with the wound. The key features of Oxyzyme and Iodozyme hydrogel systems that need to be considered are the:

- Mechanism and dynamics of iodine generation
- Associated oxygen production
- Steady take-up of wound-fluid (which causes an associated swelling and dilution of the biochemical constituents).

Under aerobic, in vitro conditions it is difficult to measure the full impact of the oxygenation effect (which depends on the involvement of leucocytes), even though the oxygen-delivery mechanism will be active. However, the new test system enables the antimicrobial efficacy of the gradual synthesis and release of iodine from the dressings to be determined.

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1. Oxygen from the air is enzymically captured into the hydrogel by the action of glucose oxidase in the enzyme surface layer as oxygen is only sparingly soluble in the hydrogel.
2. Glucose in the wound contact layer is oxidised to form gluconic acid. This leads to the production of hydrogen peroxide, which is used to transport oxygen through the hydrogel layers.
3. Within the hydrogel matrix, the hydrogen peroxide is consumed in a complex reaction pathway involving iodide ions, resulting in oxygen being released from the wound surface.
4. Iodine is also generated in this reaction pathway, and is gradually released from the wound contact hydrogel matrix.
Moreover, it allows evaluation against anaerobes.

The specific aim of this in vitro study was to determine the antimicrobial activity and efficacy of three variants of this novel bioxygenating hydrogel system. However, the general approach can be used to compare and evaluate any dressing claimed to have antimicrobial effects. The general test system utilised here can therefore be used to help practitioners make rational decisions as to which active/advanced dressing to use in wound management, especially as it is important to objectively determine antimicrobial activity against a broad spectrum of target species, including methicillin-resistant Staphylococcus aureus (MRSA), anaerobes and yeasts. The efficacy of dressings against anaerobes is often overlooked, despite the high correlation between the incidence of anaerobic bacteria and wound infection.1

Materials and method

Growth and maintenance of microorganisms

In this study a mixture of clinical and laboratory-typed strains were used to enable both comparisons with existing data for other novel antimicrobial dressings and determination of antimicrobial efficacy against clinically relevant pathogens.

Staphylococcus epidermidis (UWE laboratory typed strain isolated from human skin), MRSA Llewelyn clinical strain, Pseudomonas aeruginosa PAO 1161, Candida albicans NCTC 10288, Propionibacterium acnes (UWE laboratory typed strain isolated from human skin), Bacteroides fragilis ATCC 25285 and Fusobacterium nucleatum (subspecies nucleatum) ATCC 10953 were all maintained and stored as laboratory stocks.

Staphylococci and Pseudomonas aeruginosa were maintained on nutrient agar (Oxoid, Basingstoke, UK), Candida albicans on potato dextrose agar (Oxoid), and Propionibacterium acnes and the strict anaerobes on fastidious anaerobe agar (Oxoid) supplemented with 5% defibrinated horse blood (TCS Biosciences, Buckingham, UK).

Broth cultures of Staphylococcus epidermidis, MRSA, Pseudomonas aeruginosa, Candida albicans and Propionibacterium acnes were grown in 1% tryptone-0.5% yeast extract, and Bacteroides fragilis and Fusobacterium nucleatum in brain-heart infusion broth.
The staphylococci *Pseudomonas aeruginosa* and *Candida albicans* were incubated at 37°C aerobically (Genlab M1005L incubator, Cheshire, UK), and *Propionibacterium acnes* and strict anaerobes were incubated at 37°C anaerobically (MK3 Anaerobic work station, Don Whitley Scientific, Shipley, UK). All cultures were subcultured weekly.

### Experimental wound dressings

The wound dressings tested were prototype Oxyzyme and Iodozyme hydrogels.

The Oxyzyme hydrogel comprised a 100 x 100mm glucose plus iodide wound-contact layer and a 60 x 60mm enzyme surface layer. The latter is responsible for the hydrogen peroxide-driven oxygen transport and iodine generation from the wound-contact layer (Fig 1).

The essentially similar Iodozyme hydrogels were supplied at two iodide concentrations (test hydrogels 401 and 402), again comprising a 100 x 100mm glucose plus iodide wound-contact layer but with a 100 x 100mm enzyme surface layer. These generated and released higher concentrations of iodine. The concentration of iodide in hydrogel 402 was twice that of hydrogel 401.

All wound dressings were supplied in their standard packaging, and cut into 16 sections of required size just before use.

### Controls

The control wound dressing (control 1) comprised only the glucose plus iodide wound-contact layer and not the surface enzyme layer, thus preventing hydrogen peroxide and iodine generation (Fig 2). Uncovered disks (n=16) were also placed onto the test plates containing equivalent numbers of target species as an additional control (control 2).

### Test method

Test cell suspensions were prepared from neat 24-hour broth cultures of Staphylococci and *Pseudomonas aeruginosa*, 48-hour broth cultures of *Candida albicans* and 72-hour broth cultures of *Propionibacterium acnes* and strict anaerobes, diluted if necessary with sterile broth to give optical density readings of 0.5–0.7 using a 1cm light path CE-373 linear readout grating spectrophotometer (Cecil Instruments, Cambridge, UK) at wavelength 540nm.

Ultraviolet-sterilised 1cm² cellulose disks (n=32) were then immersed in vortex-mixed test-cell suspensions for five minutes and placed into a sterile plastic Petri dish; excess fluid was removed from each disk by sterile blotting. All disks then contained approximately the same numbers of viable cells.14

### Analysis of results

For each test species and each condition, plots were made of log numbers of survivors over reaction time, and kill rates determined by linear regression, enabling direct comparison of the results over time for the test and two controls for each target species.

Table 1. Kill rates and equivalent D values for target species treated with the Oxyzyme hydrogel dressing

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Kill rate (K = slope ± SD) (log cfu reduction per hour)</th>
<th>D value (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus epidermidis</td>
<td>-0.346 ± 0.040</td>
<td>2.887</td>
</tr>
<tr>
<td>MRSA</td>
<td>-0.312 ± 0.084</td>
<td>3.201</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>-0.160 ± 0.030</td>
<td>6.262</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>-0.223 ± 0.010</td>
<td>4.466</td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>-0.485 ± 0.034</td>
<td>2.061</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>-1.322 ± 0.297</td>
<td>0.756</td>
</tr>
<tr>
<td>Fusobacterium nucleatum</td>
<td>-3.657 ± 0.299</td>
<td>0.273</td>
</tr>
</tbody>
</table>

D value = the time taken to reduce the microbial population by 1 log (ie, 90% kill) SD = standard deviation

Graph construction, statistical analyses and modelling were conducted using GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego, USA). Analysis of covariance (ANCOVA) was used to ascertain whether there was a significant difference between rates. A p value of <0.05 was regarded as significant. In order to take into account any
change in colony-forming units (cfu) per cm² within control samples, and to allow direct comparison of all test organisms and conditions, log plots were also constructed using the following formula:\[15\]

\[ Y = (\log N_t/N_0)^\text{test} - (\log N_t/N_0)^\text{control} \]

Where: \( N_0 = \text{cfu/cm}² \) at zero time and \( N_t = \text{cfu/cm}² \) at later time (t).

**Results**

Both Oxyzyme and Iodozyme test dressings exhibited a significant antimicrobial effect against all test organisms. Plotting log numbers of survivors over the reaction time enabled the determination of kill rates. Those for Oxyzyme are given in Table 1.

To compare with other species and conditions, an average kill rate value was estimated between the maximum and minimal rates of the acceleration. All test kill rates were significantly different (\( p<0.0001 \)) to no change (flat line) or either of the two corresponding control conditions, and to each other.

The death kinetics for *Staphylococcus epidermidis*\[14\] and MRSA treated with Oxyzyme exhibited an accelerating kill rate. When Oxyzyme was tested against *Pseudomonas aeruginosa* there was a significant antimicrobial effect for the first 6½ hours of treatment. After this period significant regrowth occurred, eventually reaching levels close to that of both controls, even though both sets of control samples exhibited significant growth rates.

No such regrowth was seen during treatment with the Iodozyme hydrogels. Significant growth on the control samples (control 2) was observed for *Candida albicans*. However, all test hydrogels exhibited a significant kill rate throughout the treatment duration for Candida.

For all anaerobes (*Propionibacterium acnes, Bacteroides fragilis* and *Fusobacterium nucleatum*) treated with the Oxyzyme hydrogel, significant kill rates were observed for both test and control samples. However, test conditions (in the presence of active hydrogels) gave the highest kill rates and were significantly different to the control kill rates (\( p<0.0001 \)).

Using the formula stated in the methodology\[15\] a comparative graph of Oxyzyme kill rates for all target species was constructed (Fig 3). This method takes into account and corrects for any population changes that occur within the control samples. All kill rates were significantly different from each other (\( p<0.0001 \)). Hence, the order of sensitivity to the test Oxyzyme hydrogel was:

- *Fusobacterium nucleatum*
- *Bacteroides fragilis*
- *Propionibacterium acnes*
- MRSA
- *Staphylococcus epidermidis*
- *Candida albicans*
- *Pseudomonas aeruginosa*.

*Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Candida albicans* were treated with all three hydrogel wound dressing formulations (Oxyzyme, Iodozyme 401 and Iodozyme 402). All wound dressings gave kill rates significantly different (\( p<0.0001 \)) to no change (flat line) or either of the two control

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conditions, and all kill rates were significantly different from each other (p<0.0001).

The order of efficacy of the hydrogel dressings was the same regardless of target species: Iodozyme 402, followed by Iodozyme 401, and then Oxyzyme.

**Discussion**

These findings point the way to the further development of a realistic *in vitro* wound-bed model, and will help to refine the mode of use and optimisation of the wound dressings themselves.

It should be noted that the low-iodine Oxyzyme hydrogel is mainly intended as a prophylactic to minimise the risk of infection; the higher-iodine lodozyme variants are designed for infected wounds. Although all test dressings exerted a significant antimicrobial effect against all target species, they did so, as expected, with different potency.

Oxyzyme hydrogel was most effective against the strict anaerobes *Bacteroides fragilis* and *Fusobacterium nucleatum*, followed by the aerotolerant anaerobe *Propionibacterium acnes*.

As might be expected with anaerobes, aerobic conditions alone (controls 1 and 2) produced a killing effect. However, in all cases these rates were considerably less than those induced by the active test dressing, so the difference between the control and test rates can be ascribed to the antimicrobial efficacy of the dressing.

Control 1 (glucose plus iodide wound-contact layer without the surface enzyme layer) produced a higher kill rate than control 2, possibly because the inactive iodide in the test dressing was slowly oxidised by redox activity of the test microbial cells, yielding traces of iodine.

The marked efficacy of Oxyzyme against anaerobes is of particular clinical significance in the light of increasing clinical publications correlating prevalence of anaerobic bacteria with wound infection.1

Oxyzyme was significantly effective against *Staphylococcus epidermidis* and MRSA, which is important in view of the emergence of antibiotic-resistant organisms, particularly MRSA, within wounds. This has heightened the need for an effective prophylactic that can reduce the colonisation and/or the bacterial load of wounds by antibiotic-resistant organisms.

Although fungal infections of skin wounds are less common than those associated with bacteria,16 the increase in antifungal resistance shown by *Candida albicans*17 makes this an interesting target species to test against Oxyzyme. The killing of the eukaryotic *Candida albicans* was less effective than that measured against the prokaryotic anaerobes and Staphylococci, although it was still significant.

The more resistant nature of *Candida albicans* (compared with Staphylococci) has also been observed previously in photodynamic killing using methylene blue and light.18

The lower efficacy of Oxyzyme against *Pseudomonas aeruginosa*, with regrowth after a killing phase of 6½ hours, is consistent with the known robustness of this organism. *Pseudomonas aeruginosa* is a persistent and versatile opportunistic pathogen that can adapt to and utilise numerous environmental conditions — a capability that strengthens its pathogenic potential in wounds (particularly burns) often associated with transmissible virulence factors.19

Although Oxyzyme had limited antimicrobial efficacy against *Pseudomonas aeruginosa*, both lodozyme hydrogels (401 and 402) exerted significant and prolonged antimicrobial activity, with no regrowth. This suggests the increased generation of iodine within these two hydrogel dressings increases their antimicrobial power.

This association between increased iodine generation capacity and antimicrobial power was also evident in the experiments with the lodozyme hydrogels and *Staphylococcus epidermidis*14 and *Candida albicans*. In all cases a significant difference was seen between all three hydrogel dressings, with increasing antimicrobial power relating to iodide content.

**Conclusion**

Many skin-surface dressings incorporate antiseptics including iodine, chlorhexidine or silver compounds. Oxyzyme and lodozyme hydrogels use antimicrobial systems that integrate active oxygenation with controlled synthesis and release of iodine. The correction of wound hypoxia by delivery of oxygen promotes natural antimicrobial effects (especially via enhanced leucocyte activity) and biochemical healing processes, all within a moist wound environment, resulting in many antimicrobial effects.

In view of the demonstrated effective antimicrobial properties of these novel hydrogel dressings, their use in the clinical environment could be significant in reducing the microbial load of a wound, thereby limiting or inhibiting infection. They are broad-spectrum in activity, including antibiotic-resistant organisms, anaerobes and yeasts, and were found to be rapidly effective. These dressings could significantly enhance the treatment of wounds prone to complications of infection. This offers potential benefits to the patient through decreased trauma, and could significantly reduce treatment costs.

There are limitations with the *in vitro* model used in that the host response is not taken into account, and so will not determine wound events or healing but merely the propensity of the dressings to kill potentially pathogenic wound-infecting microbes.

Further testing is needed to determine the effectiveness of the dressings in limiting colonisation of potentially pathogenic organisms in the wound environment *in vivo*. Further developments of the *in vitro* model will be undertaken, especially as a means through which to optimise dressing performance.