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Antimicrobial properties of silver-containing wound dressings: a microcalorimetric study

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Abstract

The studies reported here have been undertaken to assess the potential use of isothermal microcalorimetry in studying the antimicrobial efficacy of wound dressings that contain antimicrobial agents. The microcalorimetric technique allows non-invasive and non-destructive analysis to be performed directly on a test sample, regardless of whether it is homogeneous or heterogeneous in nature. Microcalorimetry is an established procedure that offers quantitative measurements and has the distinct advantage over traditional antimicrobial test methodologies in that calorimetric measurements are made continuously over real-time, thus the dynamic response of microorganisms to an antimicrobial agent is observed in situ. The results described in this paper are for interaction of two silver-containing wound care products AQUACEL® Ag Hydrofiber® (ConvaTec, Deeside, UK) and Acticoat™ 7 with SILCRYST™ (Smith and Nephew Healthcare, UK) with the wound pathogenic organisms *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Both dressings are shown, microcalorimetrically, to have the capacity to kill these common wound pathogens within 1–2 h of contact. A dose–response study was conducted with the AQUACEL Ag dressing. Microcalorimetry is shown to be rapid, simple and effective in the study of the antimicrobial properties of gel forming wound dressings.

Keywords: Calorimetry; Wound dressings; Silver; Antibacterial

1. Introduction

The microbiology of most wound types is complex, involving both aerobic and anaerobic bacteria (Bowler et al., 2001), and these organisms can create a potential problem to both the wound in which they reside (i.e. autoinfection) and the surrounding environment (cross-contamination). One of the key approaches for minimising the likelihood of wound infection is the use of topical antimicrobial agents. The purpose of these antimicrobial agents is to reduce the microbial load (bioburden) in wounds, and hence the opportunity for infection. Typically these have involved (Bowler et al., 2001) the use of broad spectrum antiseptic agents (e.g. iodine and silver) and antibiotics (e.g. neomycin, bacitracin and polymyxin combinations).

Iodine is a potent antimicrobial agent that is frequently used in the management of wounds (primarily

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as povidone iodine or cadexomer iodine) and more recently silver has regained increasing acceptance as a broad spectrum alternative to iodine and topical antibiotics (Lansdown, 2002a,b). In particular, wound dressings have been developed that contain silver. Some dressings (e.g. Acticoat 7) are intended to provide a slow but sustained release of silver ions for up to 7 days (Lansdown, 2002b). All the dressings used in the work reported here form gels in the presence of aqueous media. This property, naturally, makes classical evaluation of their antimicrobial properties difficult.

Assessment of the relative antimicrobial efficacy of these gel-types of products can be achieved using traditional microbiological techniques, although this can be complex and time consuming. Automated techniques such as ATP bioluminescence, impedance measurements and flow cytometry have provided more rapid throughput in quantitative microbiological testing. They have gained acceptance over total viable counts and, indeed, the use of the polymerase chain reaction and immunoassays can also rapidly and reliably detect the presence or absence of specific microbial species (De Boer and Beumer, 1999; Vanne et al., 1996; Waites, 1997). However, these are all invasive methods in that they require sampling of the microbiological sample at specific time points.

An alternative to these techniques that is non-invasive and non-destructive is microcalorimetry (Beezer, 1980; James, 1987). This technique allows analysis to be performed directly on a test substance, regardless of its homogeneous or heterogeneous nature. Microcalorimetry is an established procedure that has been extensively used as a method for rapid bioassay procedures and organism enumeration (Beezer, 1980; Bunker and James, 1986; James, 1987). Microcalorimetry has been shown to permit quantitative measurements on very different and heterogeneous substrates such as soil, blood, milk, and foodstuffs (Bermudez et al., 1988). A further and distinct advantage is that calorimetric measurements are continuous over real-time, thus the dynamic response of microorganisms to an antimicrobial challenge may be seen in real-time. In view of the potential benefits of microcalorimetry in quantitative microbiology, a study was conducted to assess its value in determining the antimicrobial efficacy of silver-containing, gel-forming dressings against two common wound pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

More classically based studies of these wound dressings have concluded (Walker et al., 2003) that organisms become "trapped" within the forming gel. Thus there are practical difficulties associated with the interpretation of data to investigate quantitatively the dressings' antimicrobial activity. As noted above, microcalorimetry permits direct study of heterogeneous systems. The system for study here is aqueous medium, organisms and gelled wound dressings both with, and without, silver present. A dose–response relationship can be obtained through exploration of different wound dressing areas (masses) effects on a standard organism load/medium system. The metabolic energy of the organisms present being observed as the power–time output. Naturally, this system only records the power output from actively metabolising organisms.

2. Methods

Challenge organisms:	*S. aureus* (NCIMB 9518) *P. aeruginosa* (NCIMB 8628)
Silver-containing dressings:	Acticoat®,[1] 7 with SILCRYST™,[2] nanocrystals. A silver-coated dressing AQUACEL®,[3] Ag Hydrofiber® dressing (ConvaTec). A silver dressing with ionic silver

AQUACEL® Hydrofiber® dressing was used as the non-antimicrobial (control) dressing.

All equipment was sterilised prior to use. Glass ampoules with a nominal capacity of 3 ml were used with a crimp seal closure. Each experiment was performed at 37 °C in a Thermometric 2277 Thermal Activity Monitor (TAM—Thermometric AB, Jarfalla, Sweden; Fig. 1) that was housed in a constant temperature room maintained at 21±0.5 °C. All data were collected over

[1] Acticoat® is a trademark of Smith and Nephew Healthcare, UK.

[2] SILCRYST™ is a trademark of NUCRYST Pharmaceutical Corporation.

[3] AQUACEL® and Hydrofiber® are trademarks of ConvaTec, a division of E.R. Squibb and Sons, L.L.C., a Bristol Myers Squibb Company.

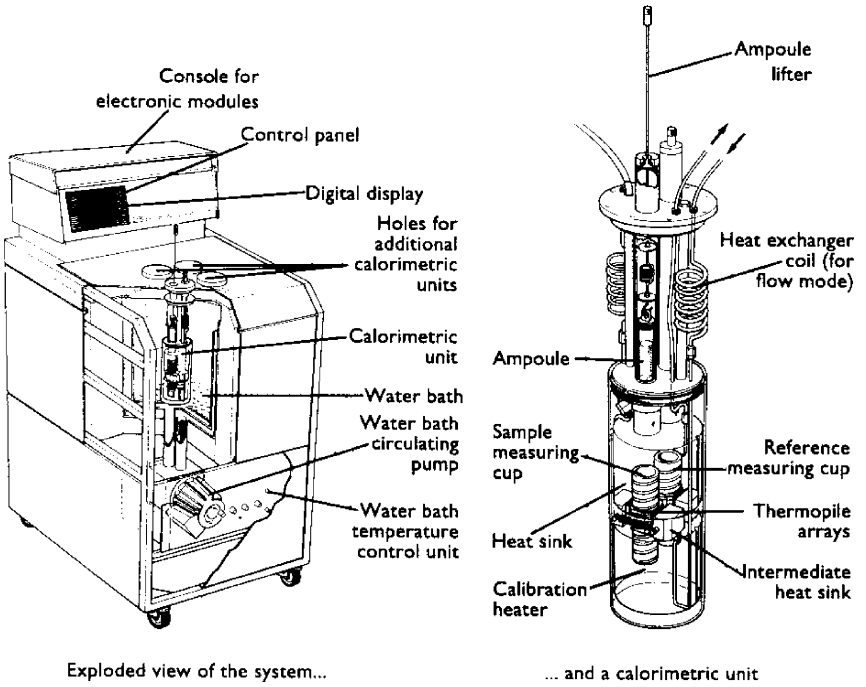

Fig. 1. Schematic of the 2277 TAM (Thermometric AB).

a minimum period of 24 h using the dedicated software package, Digitam and analysed using Microcal Origin 3.0.

Both wound pathogens (i.e. *S. aureus* and *P. aeruginosa*) were grown overnight in bacterial culture medium (Oxoid Nutrient Broth (ONB)). Dilution in ONB into a calorimetric ampoule resulted in a final cell density for each microorganism of ca. 1×10^6 colony forming units (cfu)/ml. The reference ampoule was loaded with 3 ml of growth medium and a small amount of sodium benzoate to ensure that no bacterial growth occurred during the course of the experiment (i.e. for 24 h: parallel experiments demonstrated that 0.002 g is sufficient to completely inhibit bacterial growth for 6 days). The initial, control experiments were designed to investigate the growth curves, under these experimental conditions, for the two bacterial pathogens (Fig. 2: here and throughout results are reported for a minimum of triplicate experiments under each condition). For the dressing studies, each dressing sample was aseptically cut to a known size, weighed and then rolled into a cylinder in order to fit the sample through the neck of the ampoule. 2.5 ml of ONB containing one of the pathogens at a cell density of $\sim 1 \times 10^6$ cfu/ml was then added to the ampoule containing the dressing sample. Individual vials were then crimp sealed and placed into the TAM. After allowance for equilibration recording of the power–time output was commenced a total of ca. 1 h after loading the ampoules.

In the next series of experiments, *P. aeruginosa* was used to assess the growth in the presence of the control dressing (i.e. without antimicrobial agent; here and unless noted dressings were 1 cm × 5 cm in size). Studies were also carried out to compare the AQUACEL Ag dressing with Acticoat 7, over a 24-h period, using the same procedure as outlined above. Various areas/masses of AQUACEL Ag dressing were examined to establish the nature of any resulting dose–response curve; again using the above protocol.

In further experiments ampoules containing Ag dressings (AQUACEL Ag) were inoculated with *S. aureus* and placed into the TAM for 5 days. These ampoules were then removed from the TAM opened

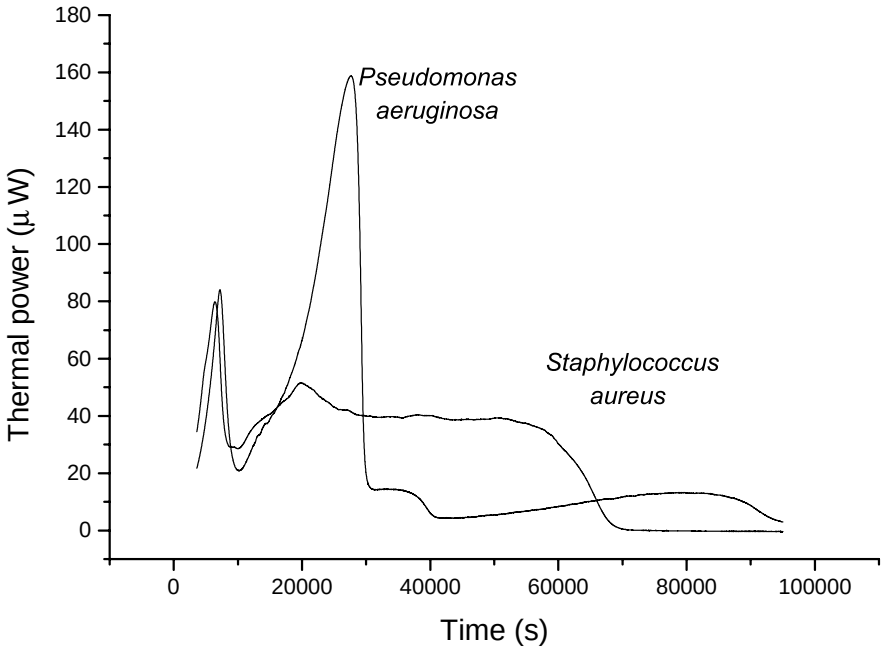

Fig. 2. Control experiments for *P. aeruginosa* and *S. aureus*.

and re-inoculated at the same organism density and the ampoule returned to the TAM (this operation took, including a repeat equilibration time, a total of ca. 1 h). Microcalorimetric recording took place for a further 24 h.

To explore longer contact with the Ag bearing dressing samples were inoculated separately with *S. aureus* and with *P. aeruginosa* and subjected to a TAM study for 40 h. After this period of time, the ampoules were removed from the TAM and stored at 37 °C before re-introduction into the TAM 14 days post-inoculation. The samples were microcalorimetrically analysed for a further 24 h to detect residual bacterial activity, and, following removal from the TAM, the ampoule suspension was cultured (agar plate) for bacterial growth over 24, 48 and 72 h.

3. Results and discussion

Both *S. aureus* and *P. aeruginosa* yield exponential power–time curves indicating almost identical growth rates over the first few hours (Fig. 2: the areas under the control curves are reproducible ($n = 3$) to 2%). The peaks and troughs are characteristic of organism growth on complex medium and with restricted (only the head space within the ampoule) oxygen availability. It is plausible that the first, exponential phase seen represents aerobic metabolism that is then followed by a change to anaerobic metabolism. Subsequent peaks and troughs are assumed to be due to the sequential utilisation of major carbohydrate sources present in the complex culture medium (i.e. ONB) (for more detailed discussion of microbial calorimetry, see Beezer, 1980; James, 1987). Following the initial trough, it is apparent that *P. aeruginosa* appeared to metabolise the medium more energetically. Both organisms were shown to be capable of producing metabolic heat for periods in excess 20 h (i.e. ∼70,000 s). Thereafter minimal growth was evident for both microorganisms and this can be attributed to, for example, pH drift, medium constituent exhaustion or other factors as yet undetermined.

Fig. 3 indicates that in the presence of *P. aeruginosa* the non-antimicrobial control dressing reduced both the power intensity and the pattern of metabolic growth of the microorganism although similarities with the control *P–t* curve are evident. This dressing

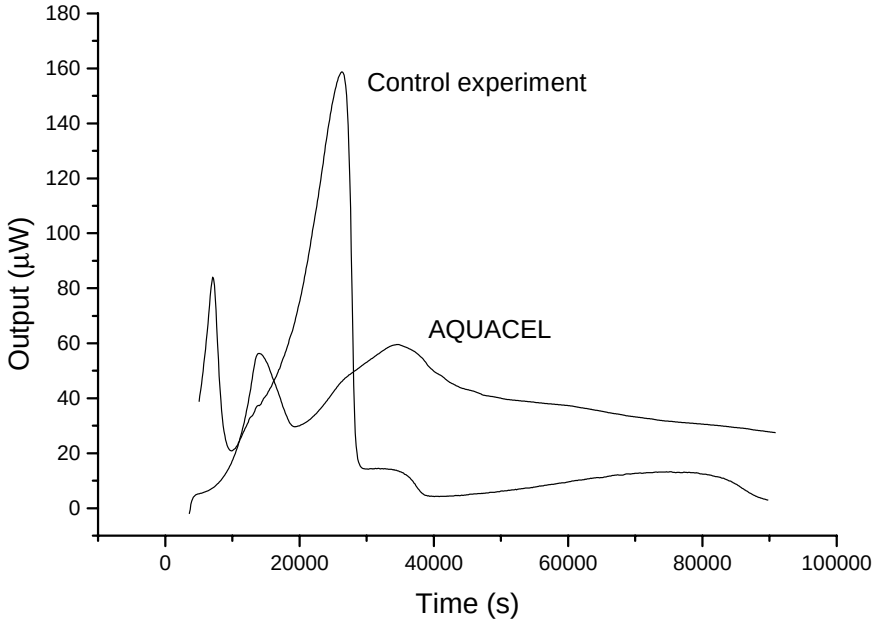

Fig. 3. Calorimetric response of *P. aeruginosa* in the presence of AQUACEL dressing (non-medicated) compared with calorimetric response for a control experiment.

markedly reduced the intensity of the initial exponential period of the *P–t* curve. One possible explanation is that some of the microorganisms become rapidly entrapped in the hydrating fibres of the non-anti-microbial dressing (Bowler et al., 1999; Walker et al., 2003). Thus, diffusion of medium to, and metabolites from, these trapped organisms will be different from those present in the suspending medium only. Further evidence in support of this explanation has been recently demonstrated by the use of scanning electron microscopy techniques (Demling and DeSanti, 2001).

Introduction of AQUACEL Ag and Acticoat 7 into the culture medium showed that there was no observable power output for either dressing (Fig. 4). From these results, it is suggested that there is a sufficient concentration of silver in the medium presented by both dressings to kill rapidly pathogenic bacteria. Evidence for a continued availability of silver (from AQUACEL Ag) is presented in Fig. 5. This shows that following 5 days of no microcalorimetrically measurable bacterial activity in the AQUACEL Ag/*S. aureus* system, the ampoule was re-inoculated with the same organism to the same nominal density (i.e. $\sim 1 \times 10^6$ cfu/ml). Now however, following re-equilibration in the TAM, there was some evidence of a small endothermic signal, which decayed towards zero over the following 48 h. That is, there appears to be an adequate reservoir of silver in the dressing to cope with the additional bacterial load.

The 14-day study performed to explore continuous release of silver ions from AQUACEL Ag shows (both microcalorimetrically and in agar plate growth studies) that no outgrowth from surviving organisms occurred over the 14-day study period. That is, all inoculated organisms are killed in the presence of the silver.

The dose–response study showed that at all added areas/masses of AQUACEL Ag dressings (range from 2.0 to 0.5 cm^3) there was no detectable power output 1 h after ampoule loading.

The sustained release of silver ions from a dressing into a wound environment is important for continued antimicrobial activity. Release of an excess of silver ions into a wound will possibly result, in the main, in the formation of silver chloride, which has a solubility of ~ 1 μg/ml in water at 37 °C (Lide, 1992). It is unlikely that any silver-containing dressing will be able to exceed this concentration of

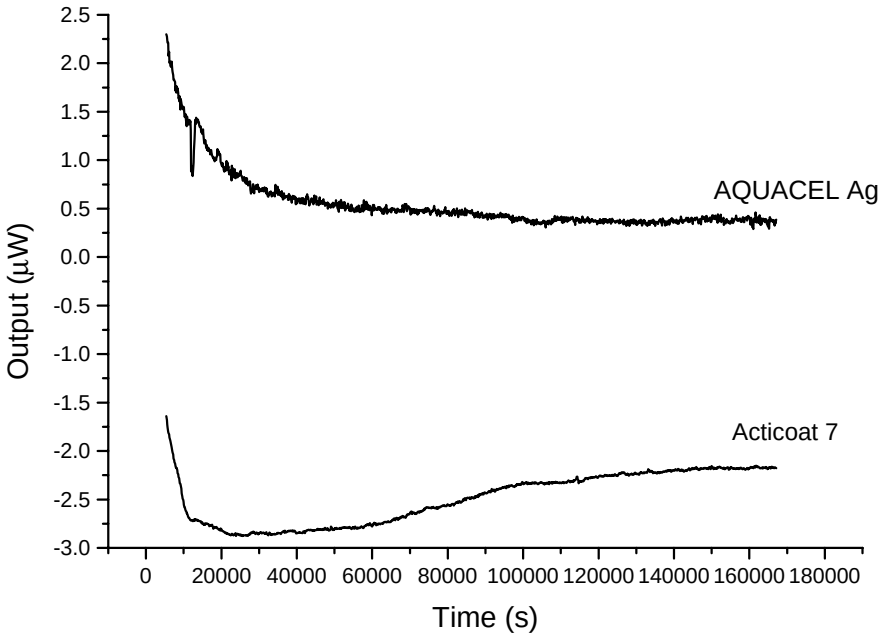

Fig. 4. Calorimetric response for *S. aureus* in the presence of AQUACEL Ag dressing and Acticoat 7 dressing.

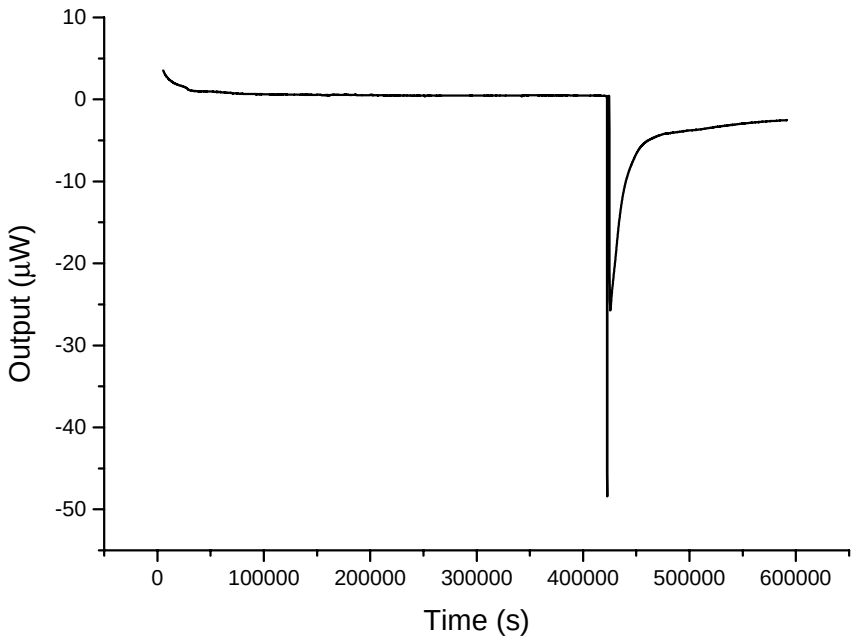

Fig. 5. Seven-day study with re-inoculation of *S. aureus* (shown by spike) after 5 days.

silver ions in the wound environment (i.e. 1 μg/ml of available ionic silver). Thus, any silver-containing dressing that can continuously release sufficient silver ions will maintain a relatively fixed concentration of silver ions in wound fluid that is independent of contact time. Precipitation of silver chloride into the wound environment is thought to be undesirable as it may lead to irritation, cytotoxicity and possible skin staining.

Acticoat 7 has a silver concentration of approximately 13%, the silver availability is 100 μg/ml water, and is claimed (www.burnsurgery.org) to have a stable release of silver ions for at least 48 h. An AQUACEL Ag dressing has a silver concentration of approximately 1.2% and likewise is claimed (Parsons and Bowler, 2003) to deliver and maintain (through an equilibrium process) a level of silver ions that is at the silver chloride solubility limit (i.e. ~1 μg/ml in water at 37 °C). In our investigations, it is shown that both dressings generate a sufficient concentration of free silver ions rapidly, and hence rapidly kill the microorganisms in the dressing test systems.

Although it is an important consideration for an antimicrobial dressing to kill wound pathogens rapidly, clinically, many wound dressings are in place for at least 24 h. Therefore, it is perhaps more important that antimicrobial activity is maintained for the wear time of the dressing. In these studies only one dressing was examined in this context and evidence is presented to suggest that the AQUACEL Ag dressing was capable of delivering a sustained release of silver ions over at least a 5-day period (Fig. 5).

Further confirmation of this dressing's ability to release silver ions continuously was shown in the final experiments, in which, following inoculation with both wound pathogens (i.e. separate experiments with *P. aeruginosa* and *S. aureus*), no calorimetric evidence of growth was observed over an initial 40-h period nor on re-investigation after storage for 14 days at 37 °C. A long-term low signal was detected with *P. aeruginosa*, which may be due to protein/metal-ion interaction with the dressing components. This signal was not thought to be due to microbial metabolism as upon completion of the calorimetric assessment, cultures of the ampoule suspensions (both challenge organisms) on agar media failed to grow, thus confirming the calorimetric data.

4. Conclusions

This study has demonstrated the potential of the use of isothermal microcalorimetry in the study of heterogeneous, microbial cellular systems; in this case in gelled wound dressing systems. It provides a rapid simple and continuous test in which antimicrobial wound dressings can be studied in situ without the need for invasive experimentation. Both AQUACEL Ag and Acticoat 7 dressings demonstrated their ability to kill common wound pathogens (e.g. *P. aeruginosa* and *S. aureus*) within 1–2 h of contact.

Acknowledgements

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