Antimicrobial activity and skin permeation of iodine present in an iodine-impregnated surgical incise drape

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Objectives: The antimicrobial efficacy of an iodine-impregnated incise drape against MRSA was evaluated in a skin model. The permeation of iodine from this drape into the skin was also assessed.

Methods: The antimicrobial efficacy was evaluated in ex vivo studies following application of the surgical incise drape for various times on the surface of donor skin, which was inoculated with either $1 \times 10^3$ or $1 \times 10^6$ cfu MRSA/cm² skin and mounted on Franz diffusion cells. In some experiments the MRSA-inoculated skin was pre-incubated for 18 h at room temperature prior to applying the drape. Permeation of iodine into the skin using this model was also determined following application of the incise drape for 6 h.

Results: The iodine-impregnated drape demonstrated antimicrobial activity compared with the non-use of drape. This reached significance when a high inoculum of MRSA was applied with no pre-incubation period and when a low inoculum of MRSA was applied with a pre-incubation period ($P = 0.002$ and $P = 0.014$, respectively). Furthermore, in experiments wherein a high inoculum of MRSA was applied with no pre-incubation period, the iodine-impregnated drape demonstrated superior antimicrobial activity compared with the use of a non-antimicrobial drape ($P < 0.001$).MIC and MBC values of iodine were attained to 1500 µm below the skin surface.

Conclusions: The iodine-impregnated surgical incise drape had detectable antimicrobial activity. Furthermore, iodine penetrated into the deeper layers of the skin. This property should suppress microbial regrowth at and around a surgical incision site, making its use preferable to the use of a standard drape or non-use of a drape.

Keywords: antiseptics, surgical infections, infection control

Introduction

Surgical site infections (SSIs) account for 14% of all healthcare-associated infections. These infections result in increases in length of hospital stay, costs and mortality rates.¹

The source of most pathogens causative of SSIs is the endogenous flora, primarily that of the patient’s skin.² Appropriate skin antisepsis does not completely eliminate this risk, as microorganisms may persist in the lower layers of the skin, leading to microbial re-colonization of the skin surface and wound edge. Surgical incise drapes have been used for many years. These adhesive plastic films are applied to the skin at the site of incision primarily to minimize contamination of the operative field by microorganisms from the skin surrounding the operative site. However, a Cochrane Review found a higher incidence of SSIs in surgical patients in whom incise drapes had been used compared with those without drapes [relative risk (RR) 1.23; 95% CI 1.02–1.48; $P = 0.03$].³ Incise drapes impregnated with antiseptics are now available and the same Cochrane Review, interestingly, further demonstrated that there was no significant difference in rates of SSI between patients having iodine-impregnated drapes applied pre-surgery and those patients with no drape applied (RR 1.03; 95% CI 0.66–1.60; $P = 0.89$).³ These findings led the National Institute for Health and Care Excellence (NICE) to recommend that if an incise drape is required, an iodine-impregnated drape should be used.⁴ 3M Health Care manufactures a vapour-permeable polyester film coated with a clear acrylate adhesive containing a PVP complex. This complex consists of N-vinyl-pyrrolidone, iodine (0.117–0.197 mg/cm²) and sodium iodide, from which free iodine is slowly released for delivery into the skin.

The iodine-impregnated incise drape has been previously evaluated both in vitro and in human studies and was shown to have antimicrobial activity against a wide range of microorganisms.⁵,⁶ The current study evaluated the antimicrobial efficacy of the iodine-impregnated incise drape against MRSA in a skin model that simulates the normal skin environment. Permeation of iodine
into the skin was also evaluated to determine whether there was detectable antiseptic present in the lower layers of skin.

**Materials and methods**

Studies were performed as in previous ex vivo studies undertaken by our group.  

**Surgical incise drapes**

An iodine-impregnated incise drape (3M™ Ioban™ 2, Neuss, Germany) and a non-antimicrobial incise drape (3M™ Steri-drape™ 2, Neuss, Germany) were evaluated in this study. The characteristics of the two types of drape used were similar, both having an identical acrylate adhesive and backing.

**Microbial cultures**

MRSA epidemic strain EMRSA-15 was inoculated onto 5% (v/v) blood agar plates (bioMérieux, Basingstoke, UK) and incubated at 37°C in air for 18 h. Colonies were then suspended in sterile PBS (Sigma-Aldrich, St Louis, MO, USA), adjusting the number of microorganisms in the suspensions by OD determination. Drop counts were performed by dilution of the suspensions in sterile PBS and placing five 20 μL aliquots of each dilution on blood agar plates (in duplicate). The plates were incubated at 37°C in air for 24 h and the mean cfu was enumerated.

**Iodine susceptibility assay**

The BSAC guidelines for determination of MICs were followed to ascertain the susceptibility of EMRSA-15 to iodine. In brief, EMRSA-15 was grown on blood agar (bioMérieux) at 37°C for 18 h and from this a suspension with a turbidity equivalent to that of a 0.5 McFarland standard was made using sterile distilled water. The suspension was diluted with tryptone soya broth (TSB; Oxoid, Basingstoke, UK) to 10^8 cfu/mL. Iodine (Videne® 10% w/w cutaneous solution of iodinated povidone containing 1000 mg/L iodine, Ecolab, Leeds, UK) was diluted with TSB using doubling dilutions. Seventy-five microlitres of both the microbial suspension and the iodine solution was added to each relevant well on a 96-well microtitre plate. After incubation at 37°C for 24 h, turbidity was evaluated.

The MIC was defined as the lowest concentration of iodine that resulted in a clear well. In addition to determination of the MIC, the MBC was determined by transfer of samples in the range around the MIC onto blood agar plates (bioMérieux) in duplicate. The cultures were incubated at 37°C in air for 48 h and the cfu enumerated. All experiments were performed in triplicate.

**Donor skin**

Full-thickness human skin was obtained from 20 patients [all women; mean age 54 years (range 37–68 years)] who underwent apronectomy and had given written informed consent. The skin was frozen at −20°C on the day of surgery and used within 4 weeks. Ethics committee approval and had given written informed consent. The skin was frozen at

**Antiseptic neutralizing solution**

The neutralizing solution contained 3% (w/v) Tween 80 (BDH, Poole, UK), 0.3% (w/v) lecinthin (Fisher Scientific, Loughborough, UK), 0.1% (w/v) l-histidine (BDH), 0.5% (w/v) sodium thiouosphate (BDH) and 0.1% (w/v) lyophilized bovine albumin (Sigma-Aldrich, St Louis, MO, USA) in tryptic soy broth (Sigma-Aldrich) made with distilled water. It was sterilized by filtration through a 0.45 μm membrane filter (Sartorius, Goettingen, Germany) under vacuum.

**Ex vivo time–kill studies on human skin**

The receptor compartment of the Franz diffusion cells was filled with 15 mL of sterile PBS and maintained at 37°C (Figure 1). Sections (3 x 3 cm) of full-thickness human skin were thawed in sterile PBS at room temperature for 30 min and rinsed with 70% (v/v) isopropl alcohol (Spiriclen®, Adams Healthcare, Leeds, UK) before mounting onto the cells. Air between the skin and the receptor fluid was removed. The skin surface was blotted dry with sterile absorbent paper towels and left to equilibrate for 30 min. The skin surface was inoculated with 100 μL of EMRSA-15 suspension containing either 3 x 10^3 or 3 x 10^6 cfu (representing 1 x 10^3 or 1 x 10^6 cfu/cm^2 skin, respectively), and then incubated at room temperature for either 5 min or 18 h. Following the incubation, a 3 x 3 cm section of the non-antimicrobial or iodine-impregnated incise drape was then applied to the surface of the skin where the EMRSA-15 had been inoculated. After 5 min, 2 h or 6 h application time, the incise drapes were removed and placed in 5 mL of neutralizing solution and microorganisms were released by mixing with glass beads in a vortex for 2 min. Microorganisms were also recovered from the surface of the skin using the scrub cup technique. This involved placing 1 mL of neutralizing solution onto the skin and releasing the bacteria by scraping the skin surface with a polytetrafluoroethylene (PTFE) spatula (Radleyes, Essex, UK) for 1 min. The solution from the surface was then aspirated with a pipette and the sampling repeated, with a further 1 mL of neutralizing solution. Samples were diluted in sterile PBS and inoculated onto chromogenic MRSA plates (bioMérieux) in duplicate. The cultures were incubated at 37°C in air for 48 h and the cfu enumerated. All experiments were performed in triplicate. The number of microorganisms from the drape with and without iodine and skin surface were combined and compared with the number of microorganisms recovered from the skin surface without application of a drape.

**Figure 1.** Franz diffusion cell.
Antimicrobial activity of surgical incise drapes

Skin permeation of iodine from an iodine-impregnated surgical incise drape

Full-thickness human skin samples from two patients were mounted onto the Franz diffusion cells as outlined above. Sections (3 x 3 cm) of the non-antimicrobial drape were applied to the surface of two samples of skin (one from each patient) and sections of the iodine-impregnated incise drape were applied to the surface of six sections of skin (three from each patient). Following a 6 h application time, the incise drapes were removed and discarded. The skin samples were immediately frozen with a cryospray (Cellpath, Powys, UK) and punch biopsies (7 mm in diameter) were removed from each skin sample in triplicate. The biopsies were then mounted into cork discs with embedding compound (OCT Embedding Matrix, Cellpath) and sectioned horizontally with a cryotome. Samples (100 μm) were sectioned from the surface (to a depth of 1500 μm). Triplicate sections were pooled in separate sterile polypropylene centrifuge tubes (Fisher Scientific, Loughborough, UK) and stored at room temperature in a freezer until analysis. The concentration of iodine in the skin was measured using an inductively coupled plasma mass spectrometer (ICP-MS) with an octopole reaction system collision cell. Rhodium internal standard solutions (1 ppm) were prepared in 0.25% (w/v) tetramethylammonium hydroxide (TMAH) and 5% (v/v) acetonitrile in ultra-pure water. Calibration solutions were prepared between 1 and 1000 ppb and were prepared in 0.25% (w/v) TMAH and 5% (v/v) acetonitrile in ultra-pure water. Stock solutions of iodide (10 ppm and 10 ppb) were analyzed with a high sensitivity ICP-MS and identified with reference to a standard calibration constructed using an Agilent 7700 inductively coupled plasma mass spectrophotometer (ICP-MS) with an octopole reaction system collision cell. Rhodium internal standard solutions (1 ppm) were prepared in 0.25% (w/v) TMAH and 5% (v/v) acetonitrile in ultra-pure water. Calibration solutions were prepared between 1 and 1000 ppb and results were linear, with R² = 0.99995.

Ex vivo studies with a low (1 x 10³ cfu/cm²) bacterial inoculum

Figure 2 shows the mean log₁₀ cfu recovered when 1 x 10³ cfu/cm² EMRSA-15 was applied to skin and incubated at room temperature for 18 h before application (or non-application) of a drape for various timepoints. Overall, cfu counts did not vary over time (P=0.526). Application of the non-antimicrobial drape did not result in the recovery of a significantly different number of cfu compared with non-use of a drape (P=0.621) or the use of an iodine-impregnated drape (P=0.994). In comparison, application of the iodine-impregnated drape resulted in the recovery of significantly fewer cfu compared with the non-use of a drape (P=0.014). The difference in the effects of the two types of drape or non-use of a drape did not vary significantly between timepoints (P=0.280, F-test).

Ex vivo studies with a high (1 x 10⁶ cfu/cm²) bacterial inoculum

Without the addition of blood, the MICs at 6, 24 and 48 h for the EMRSA strain were all 64 mg/L and the MBCs at 6, 24 and 48 h were 128 mg/L. It was not feasible to determine accurately the MIC with the addition of non-haemolysed blood due to an inability to visualize turbidity; however, the MBCs at 6, 24 and 48 h were determined and were all 256 mg/L.

Results

Iodine susceptibility assay

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Figure 2. Mean (and SEM) log₁₀ cfu recovered when 1 x 10³ cfu/cm² EMRSA-15 was applied to skin and incubated at room temperature for 18 h before application of a non-antimicrobial drape, an iodine-impregnated drape or no drape for 5 min, 2 h or 6 h at 37°C.
two types of drape or non-use of drape did not vary significantly between timepoints ($P=0.866$).

**Ex vivo studies with a high (1 $\times$ 10$^6$ cfu/cm$^2$) bacterial inoculum without pre-incubation**

Figure 4 shows the mean log$_{10}$ cfu recovered when 1 $\times$ 10$^6$ cfu/cm$^2$ EMRSA-15 was applied to skin and incubated at room temperature for 5 min before application of a non-antimicrobial drape, an iodine-impregnated drape or no drape for 5 min, 2 h or 6 h at 37°C. Overall cfu counts were significantly higher at 5 min compared with 2 and 6 h ($P=0.015$ and $P=0.003$, respectively) (2 versus 6 h: $P=0.766$). The cfu counts were significantly lower for the iodine-impregnated drape than for the non-antimicrobial drape ($P<0.001$) and non-use of a drape ($P=0.002$). There was no significant difference in cfu count between the non-antimicrobial drape and non-use of a drape ($P=0.695$). The difference in the effects of the two types of drape or non-use of a drape, as noted above, did not vary significantly between timepoints ($P=0.095$).

**Skin permeation of iodine from an iodine-impregnated surgical incise drape**

Figure 5 shows iodine concentration in excised human skin following application of a non-antimicrobial drape or an iodine-impregnated drape for 6 h. Concentrations of iodine in the skin were highest within the top 100 $\mu$m section (3.11 ± 0.84 $\mu$g/mg). The three horizontal lines in Figure 5 represent the MIC and MBC values for...
EMRSA-15 at 6, 24 and 48 h if we assume that 1 g of tissue is equivalent to 1 mL. The total concentration of iodine recovered from the full-thickness skin was $0.13 \pm 0.05$ mg/cm$^2$.

Discussion

This study demonstrated the antimicrobial activity of the iodine-impregnated surgical incise drape, tested in a number of scenarios: firstly, with a low bacterial inoculum and an 18 h pre-incubation period to both simulate the likely clinical scenario and level of microbial numbers on healthy human skin, and secondly with higher bacterial numbers to provide an extra challenge to the iodine.

In the high-inoculum experiments without pre-incubation, use of the iodine-impregnated surgical incise drape demonstrated superior antimicrobial activity compared with use of a non-antimicrobial drape or non-use of a drape. Similarly, in the low-inoculum experiments, the iodine-impregnated drape demonstrated superior antimicrobial activity compared with the non-use of a drape. The superiority of the iodine-impregnated drape compared with other drapes has been previously demonstrated in vitro.8 Whilst there are no direct clinical comparisons of rates of SSI associated with non-antimicrobial and antimicrobial incise drapes, it can be concluded from a Cochrane Review that iodine-impregnated drapes are associated with a lower rate of SSIs than non-antimicrobial drapes.9 The Cochrane Review also states that there is no difference in SSIs when either the iodine-impregnated or non-use of drape is used. However, studies that were included in the analysis involved contaminated and dirty surgery, for which this product is not indicated. Furthermore, the iodine-impregnated incise drape evaluated in our current study was the same product used in only two studies included in the Cochrane Review. Indeed, the review included studies that evaluated a variety of different brands of incise drapes. It cannot, however, be assumed that each type of incise drape offers the same level of antimicrobial activity when applied to microorganisms present on the skin or that adhesion to the skin and wound edge is identical, thereby possibly influencing delivery of the iodine and overall efficacy.

In our studies, there was no significant difference in the number of cfu recovered when either a non-antimicrobial drape or no drape was used when the samples were incubated for 18 h with either high or low initial inocula, suggesting that the non-antimicrobial drape does not demonstrate any antimicrobial activity or promote microbial growth. Indeed, our results are contrary to previous reports that have demonstrated that the use of non-antimicrobial incise drapes may actually increase rates of bacterial re-colonization. Indeed, Falk-Brynhildsen et al.10 recently demonstrated in a study of patients undergoing cardiac surgery that bacterial re-colonization of the skin occurred more frequently in patients with a non-antimicrobial drape compared with those with no drape.

Figure 5. Iodine concentration in excised human skin following application of a non-antimicrobial drape or an iodine-impregnated drape for 6 h (mean ± SEM; for the non-antimicrobial drape $n=2$ samples from two patients and for the iodine-impregnated drape $n=6$ samples from two patients). MIC and MBC values for 6, 24 and 48 h are given for EMRSA-15 with and without the addition of blood.
In our experiments with a high initial bacterial inoculum, the bacterial count at 5 min was similar between all the experiments with or without pre-incubation, possibly reflecting the lack of nutrients present to support microbial growth, which may in turn encourage biofilm formation.

When using a high inoculum and a pre-incubation period there was no statistical difference in the mean CFU observed between any of the three study groups at the three timepoints. Indeed, the superior efficacy of the iodine-impregnated drape in comparison with the non-antimicrobial drape was only observed in the absence of the extended pre-incubation scenario. This suggests that when the EMRSA was inoculated onto the skin and then incubated for 18 h, the microorganisms protected themselves from the subsequent challenge of the iodine by possible formation of a biofilm or colonizing the lower skin layers.

In experiments with a low inoculum and a pre-incubation period, the efficacy of iodine compared with the non-use of a drape was significant; however, the log reduction was relatively low. Again, this may be in part due to the extended incubation period and the presence of protein to which the iodine may bind, thereby losing some antimicrobial activity.

Skin permeation studies were undertaken following 6 h application of the iodine-impregnated drape, which encompasses the duration of most surgical procedures. As reported for chlorhexidine gluconate, iodine permeation through full-thickness skin was not linear. Indeed, it is well documented that structure variation within the top 100 μm of skin (including the stratum corneum) is the main permeation barrier. Despite this, MIC and MBC values of iodine (in the absence of blood) were attained for sections 1500 μm below the skin surface. Since MIC and MBC values determined in vitro may be affected in vivo by organic compounds, we determined the MBC of iodine for EMRSA-15 in the presence of blood. This MBC was attained in the skin down to a depth of 1000 μm. However, this was assuming that 1 g of tissue is equivalent to 1 mL, which may not be the case for the different cells located in the skin. We have, however, previously demonstrated, using a fluid replacement technique, that 1 g of full-thickness human skin is approximately equivalent to 1 mL of water (T. J. Karpanen, unpublished data). In addition, we have assumed that the proportions of iodine present in the intracellular and extracellular tissue components of the skin are similar; however, this may not be the case.

The manufacturer of the iodine-impregnated drape states that the maximum release of iodine is 0.098 mg/cm² when the drape is in contact with human skin. This suggests that after a 6 h application of the drape all iodine was released, suggesting an effective delivery modality.

In conclusion, the use of the iodine-impregnated surgical incise drape may reduce the numbers of bacteria present on the skin and reduce the likelihood of bacterial re-colonization at the surgical site, thereby decreasing the risk of subsequent SSI. These studies were undertaken in the absence of antiseptic decontamination, which may enhance the observed antimicrobial protection potentially offered by the iodine-impregnated drape. The present data also raise the question of whether new large-scale clinical studies are now required to re-evaluate the role of iodine-impregnated surgical incise drapes in the prevention of SSIs in patients undergoing surgical procedures.