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Antimicrobial activity of a chlorhexidine intravascular catheter site gel dressing

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Objectives: The antimicrobial efficacy of a chlorhexidine gluconate (CHG) intravascular catheter gel dressing was evaluated against methicillin-resistant *Staphylococcus aureus* (MRSA) and an extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli*. Chlorhexidine deposition on the skin surface and release from the gel were determined.

Methods: The antimicrobial efficacy was evaluated in *in vitro* studies following microbial inoculation of the dressing and application of the dressing on the inoculated surface of a silicone membrane and donor skin [with and without a catheter segment and/or 10% (v/v) serum] on diffusion cells. Antimicrobial activity was evaluated for up to 7 days. Chlorhexidine skin surface deposition and release were also determined.

Results: MRSA and *E. coli* were not detectable within 5 min following direct inoculation onto the CHG gel dressing. On the silicone membrane, 3 log and 6 log inocula of MRSA were eradicated within 5 min and 1 h, respectively. Time to kill was prolonged in the presence of serum and a catheter segment. Following inoculation of donor skin with 6 log cfu of MRSA, none was detected after 24 h. Chlorhexidine was released from the gel after a lag time of 30 min and increasing amounts were detected on the donor skin surface over the 48 h test period. The CHG gel dressing retained its antimicrobial activity on the artificial skin for 7 days.

Conclusions: The CHG intravascular catheter site gel dressing had detectable antimicrobial activity for up to 7 days, which should suppress bacterial growth on the skin at the catheter insertion site, thereby reducing the risk of infection.

Keywords: catheter-related infections, antisepsis, *ex vivo*, Franz diffusion cell

Introduction

Intravascular catheters are associated with a high risk of infection, which includes both superficial skin infection at the site of insertion and bloodstream infection. Recent approaches to reduce the risk of catheter-related infections (CRIs) include improved skin antisepsis, use of antimicrobial catheters and innovative catheter site dressings.[1,2] Some of these approaches have been incorporated into guidelines. These include the UK EPIC2 guidelines (Evidence-based Guidelines for Preventing Healthcare Associated Infections),[2] care bundles (Department of Health Central Venous Catheter Care Bundle, High Impact Intervention No. 1)[3] and, more recently, the Matching Michigan strategy.[4,5] However, despite these initiatives, CRI still occurs and further innovations to reduce CRI are required.

Appropriate skin antisepsis to reduce the number of microorganisms on the skin prior to carrying out an invasive procedure is critical, as it decreases the risk of subsequent infection. However, skin antisepsis does not eradicate all the microorganisms associated with the skin. This is probably related to the limited skin permeation of antiseptics and the presence of microorganisms residing in the deeper layers of the skin.[6–10] The persisting microorganisms in the skin layers may act as a nidus to contaminate the catheter, particularly at the time of insertion, and could result in either localized or systemic infection.[11,12] The interaction between antiseptics and skin flora is complex and involves other confounding factors. For example, microorganisms may also reside in the skin in aggregates or microbial biofilms,[13,14] and low concentrations of antiseptics, such as chlorhexidine and alcohol, may encourage biofilm formation.[15–17] It is therefore evident that adequate concentrations of antiseptics are required at a central venous catheter (CVC) insertion site to maximize the antiseptic activity, which in turn may prevent catheter colonization and reduce the risk of CRI.

A transparent aqueous-based gel, containing 2% (w/w) chlorhexidine gluconate (CHG), incorporated into a semi-permeable transparent polyurethane dressing (3M Health Care, St Paul, MN, USA) has been developed for securing of intravascular catheters and to deliver the antiseptic in an innovative manner. The semi-permeable dressing prevents fluid accumulation and is transparent, which allows inspection of the skin at the insertion site.

This CHG gel dressing has been previously evaluated *in vitro* and in human studies, which demonstrated its antimicrobial efficacy against a broad range of microorganisms and persistent antimicrobial activity on healthy human skin for up to 10 days.[18,19] The current study evaluated the antimicrobial efficacy of the CHG gel dressing against a CRI-causing pathogen in a skin model that simulates the normal human skin environment and 'in use' conditions.[20–22] The availability of CHG in the CHG gel dressing was also evaluated by determining the release of CHG from the gel and CHG deposition on the skin surface.

Materials and methods

Dressings

The 2% (w/w) CHG gel pad from the study dressing (3M Tegaderm CHG IV dressing; 3M Health Care) and control polyurethane film dressing (Tegaderm Film dressing; 3M Health Care) were cut aseptically into 2 cm×2 cm sections.

Microbial cultures

Methicillin-resistant *Staphylococcus aureus* (MRSA) European strain EMRSA-15 and CTX-M-15 extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* were stored on MicroBank beads (Pro-Lab Diagnostics, Cheshire, UK) at −20°C until required. The bacteria were inoculated onto 5% (v/v) blood agar (BA) plates (bioMérieux, Basingstoke, UK) that were incubated at 37°C in air for 18 h. Five colonies from the overnight culture were then suspended in sterile PBS (Sigma-Aldrich, Dorset, UK). The number of microorganisms in the suspensions was adjusted by optical density determination and confirmed by the drop count method; the suspensions were diluted in sterile PBS and five 20 μL aliquots of each dilution were inoculated (in duplicate) onto BA plates. The plates were incubated at 37°C in air for 24 h and the mean number of cfu determined.

Chlorhexidine susceptibility assay

The susceptibility of EMRSA-15 and *E. coli* to aqueous CHG was assessed by a broth microdilution assay in line with CLSI guidelines.[23] Aqueous 20% (w/v) CHG (Sigma-Aldrich) was diluted in sterile distilled water to obtain a stock solution of 0.64 g/L. Further serial dilutions of CHG were prepared in sterile Mueller-Hinton broth (Oxoid, Basingstoke, UK) and added to microtitre plates. The wells containing 0.03–32 mg/L of CHG were each inoculated with 1×10^5 cfu of EMRSA or *E. coli* (prepared as described above). The microtitre plates were incubated in air at 37°C for 24 h. The MBC was determined by inoculating the suspension from the wells onto BA plates which were incubated in air at 37°C for 24 h. The microdilution assay was repeated with 10% (v/v) horse serum (Oxoid). The assays were performed in triplicate.

In vitro time–kill studies

Dressings were placed, skin-facing side upwards, in sterile Petri dishes and inoculated with 20 μL of microbial suspension containing 1×10^6 cfu of MRSA or *E. coli*. The inoculum was then spread with the pipette tip across the dressing surface. The inoculated dressings were incubated in air at 32°C at 65% relative humidity [the humidity was controlled with a saturated solution of potassium iodide (Sigma-Aldrich)]. Following incubation for 15 s, 30 s, 1 min, 2 min, 5 min, 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 8 h and 12 h, the dressings were placed into a neutralizing solution and the viable microorganisms were released by agitation with a stomacher for 5 min followed by mixing with glass beads in a vortex for 2 min. Serial dilutions were performed in PBS and 1 mL of neat and each dilution was mixed with molten Mueller-Hinton agar (Oxoid) and was allowed to set at room temperature. The agar plates were then incubated at 37°C in air for 48 h. The number of viable microorganisms was determined and compared with the control and with the original inoculum. All the experiments were performed in triplicate.

Chlorhexidine neutralizing solution

The neutralizing solution contained 2% (v/v) Tween 80 (BDH, Poole, UK), 1.17% (w/v) lecithin (Fisher Scientific, Loughborough, UK), 0.5% (w/v) sodium thiosulphate (BDH) and 0.1% (v/v) Triton X-100 (Sigma-Aldrich) in distilled water.[24] It was sterilized by autoclaving at 121°C for 15 min.

Time–kill studies on silicone membrane

A sterile medical grade silicone membrane [0.010″ (0.25 mm) MED82-5010-10, Polymer Systems Technology Ltd, High Wycombe, UK) was used for the artificial skin model studies.[25] The sterile membranes were mounted onto Franz diffusion cells and the receptor compartment was filled with 29 mL of PBS maintained at 37°C (Figure 1). All entrapped air between the membrane and the receptor fluid was removed.

The membrane was inoculated with 20 μL of EMRSA-15 suspension containing either 1×10^3 or 1×10^6 cfu and allowed to dry for 3 min. The sections of CHG gel pad or control dressing were applied onto the surface of the membrane inoculated with MRSA. After 30 s, 1 min, 2 min, 5 min, 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h and 48 h, application time, the dressings were removed and the number of viable microorganisms on the dressing and on the surface of the membrane were determined separately. The viable microorganisms on the surface of the membrane were recovered by placing 1 mL of neutralizing solution on the surface and releasing the bacteria by scraping the membrane surface with a polytetrafluoroethylene (PTFE) spatula (Radleys, Essex, UK) for 1 min (scrub cup technique).[26] The solution from the surface was aspirated with a pipette and the sampling was repeated, with a total sample volume of 2 mL. The bacteria on the dressings were enumerated as described previously. All the samples were diluted in sterile PBS and the neat sample and 200 μL of each dilution were inoculated onto chromogenic MRSA culture plates (bioMérieux) in

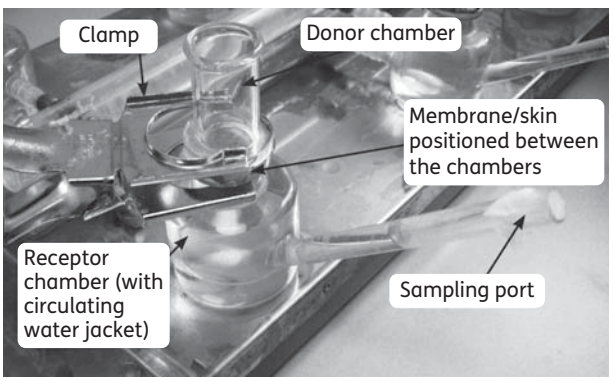

Figure 1. Franz diffusion cell.

duplicate. The cultures were incubated at 37°C in air for 48 h and the viable microorganisms enumerated. The experiment was repeated with the EMRSA-15 inoculum containing 10% (v/v) serum and a 1 cm segment of sterile catheter (polyurethane single-lumen central venous catheter, Cook Medical, Limerick, Ireland) that was placed under the dressing. The number of bacteria on the catheter segment was subsequently determined by placing the catheter in 2 mL of neutralizing solution and mixing with glass beads using a vortex mixer for 2 min and culturing the sample as described previously. All the experiments were performed in triplicate.

Time–kill studies on donor human skin

The time–kill studies on the Franz diffusion cell were repeated with donor human skin. The skin was thawed in sterile PBS at room temperature for 30 min and rinsed with 70% (v/v) ethanol (Fisher Scientific) prior to mounting on the diffusion cell. The surface of the skin was blotted dry with a sterile absorbent paper and left to equilibrate for 30 min. The donor skin was inoculated with 1×10^6 cfu of EMRSA-15 and the time–kill studies performed as described above. The experiment was repeated with MRSA in the presence of 10% (v/v) serum and a 1 cm segment of sterile catheter.

Donor skin

Full thickness human skin samples were obtained from six patients [all females; mean age 45.2 years (range 26–57 years)] who underwent apronectomy and had given written consent. The full thickness human skin was frozen on the day of excision, stored at −70°C until required, and used within 4 weeks of freezing. Full ethics committee approval was obtained from the North Staffordshire Research Ethics Committee (REC 09/H1204/92).

Evaluation of the persistence of antimicrobial activity following a single microbial challenge

The persistence of antimicrobial activity was evaluated by two methods. First, the sections of CHG gel pad and control dressing were applied onto the surface of a sterile silicone membrane in the artificial skin model. The dressings were removed after 24 h, 3 days, 6 days and 7 days and immediately inoculated with 20 μL of EMRSA-15 (containing 1×10^6 cfu) as described previously and incubated in air at 32°C at 65% relative humidity for 30 min. The number of viable bacteria was enumerated as described previously. The test was performed in triplicate.

Evaluation of the persistence of antimicrobial activity with repeated microbial challenges

The persistence of antimicrobial activity of the CHG gel dressing was also evaluated following repeated challenge with EMRSA-15. The sections of CHG gel pad and control dressing were applied onto the surface of the silicone membrane in the artificial skin model, which was inoculated with 20 μL of EMRSA-15 (containing 1×10^6 cfu). The inoculation was applied daily under the dressing for 7 days following partial removal and immediate replacement of the dressing to facilitate the manipulation. The number of viable microorganisms on the dressing and on the surface of the membrane was enumerated 24 h after the last inoculum, as described previously. The test was performed in triplicate.

Evaluation of CHG deposition from the CHG gel dressing onto the skin surface

The skin surface deposition of CHG from the 2% (w/w) CHG gel pad was evaluated on excised human skin. The excised skin samples were mounted onto Franz diffusion cells as described previously (without rinsing with alcohol) and the sections of the CHG gel dressing were placed onto the skin surface. Following durations of application of 2 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h and 48 h on the skin, the gel pad was removed and the CHG deposited onto the skin surface was determined by placing 1 mL of mobile phase solution onto the surface (3.14 cm^2) and releasing the CHG with the scrub cup technique described above and quantified by HPLC. Control skin that had no CHG gel dressing applied was analysed simultaneously to determine whether any residual CHG applied to the donor skin as part of the patients' preoperative preparation remained. (The efficacy of CHG extraction from the skin surface was validated prior to the study and the extraction achieved was 65.8%.[27]) The assay was performed in triplicate.

HPLC

HPLC analysis was performed using an Agilent 1200 series high-performance liquid instrument (Agilent Technologies UK, Edinburgh, UK) through a CPS-2 Hypersil reverse phase chromatography column (150 mm×4.6 mm, 5 μm particle size; Thermo Electron Corporation, Altrincham, UK) at a flow rate of 1.2 mL/min and with ultraviolet detection at 254 nm. The HPLC mobile phase solution for CHG analysis consisted of 75% (v/v) methanol (Fisher Scientific), 0.1% (v/v) diethylamine (Sigma-Aldrich) and 0.005 M sodium heptane sulphonate (Sigma-Aldrich) in double-distilled water. The pH was adjusted to 4.0 with glacial acetic acid (Fisher Scientific).

Evaluation of the release of CHG from the CHG gel dressing

The release of CHG from the 2% (w/w) CHG gel pad was evaluated with a cellulose dialysis membrane (BioDesign dialysis membrane; BioDesign Inc., Carmel, New York, USA) placed on a Franz diffusion cell. The pre-moistened dialysis membrane was mounted onto the Franz diffusion cell and sections of the CHG gel pad were placed onto the membrane. The amount of CHG released through the membrane into the receptor fluid was evaluated as described previously.[27] In brief, 1 mL of receptor fluid was removed after 2 min, 5 min, 10 min, 20 min, 30 min, 1 h, 2 h, 4 h, 8 h and 12 h, and immediately replaced with fresh PBS. The amount of CHG in the receptor fluid was quantified by HPLC. The study was performed over 12 h with six dressings. The assay was repeated with a Franz diffusion cell with a receptor compartment capacity of 7 mL ($n=5$).

Statistical analysis

The *in vitro* time–kill results were analysed with the Mann–Whitney test and the time–kill assay on the membrane and skin was analysed with the Kruskal-Wallis test, with pair-wise comparison with Dunn's test. Chlorhexidine skin surface deposition was analysed with the repeated measures analysis Huynh–Feldt test and the CHG release was analysed by estimating the area under the curve using the trapezium rule and the areas were compared with the Mann–Whitney test. The level of significance was 0.05.

Results

Chlorhexidine susceptibility assay

The MIC and MBC of aqueous CHG for EMRSA-15 were 0.06 and 0.25 mg/L, respectively, and for *E. coli* both were 1.0 mg/L. In the presence of 10% (v/v) serum, the MIC and MBC of CHG were 0.5 and 2.0 mg/L, respectively, for EMRSA-15, and both were 4.0 mg/L for *E. coli*.

In vitro time–kill studies

There was a significant difference at 30 s in the survival of MRSA and *E. coli* on the CHG gel dressing and control Tegaderm dressings ($P=0.002$, Mann–Whitney test) and this difference persisted for all the subsequent timepoints. Both EMRSA-15 and *E. coli* were killed within 5 min following direct inoculation onto the CHG gel dressing, with mean ± SEM $\log_{10}$ cfu reduction of 6.11 ± 0.02 and 6.34 ± 0.04 for EMRSA-15 and *E. coli*, respectively. Both strains survived up to 12 h on the control Tegaderm dressing.

Time–kill studies on silicone membrane

There were significant differences between the eradication times of EMRSA-15, when CHG gel dressing was applied onto the membrane with and without serum and/or a catheter segment ($P<0.001$, Kruskal–Wallis test). A 6.47 ± 0.06 log reduction in EMRSA-15 was achieved on the silicone membrane model within 1 h following application of the CHG gel dressing (Table 1). In the presence of 10% (v/v) serum or a catheter segment under the dressing, total kill was achieved within 4 h and 12 h, respectively. In the presence of both serum and a segment of a catheter, the eradication time was significantly longer compared with CHG gel dressing on membrane alone ($P=0.023$, Dunn's test), with EMRSA-15 killed on the surface of the membrane and catheter within 48 h.

The time–kill studies on silicone membranes were repeated with a lower inoculum. The difference in the kill time between different test scenarios of the CHG gel dressing on the membrane was also significant with lower inoculum ($P<0.001$, Kruskal–Wallis test). The time to eradication of MRSA on the membrane in the presence of a catheter segment and serum was significantly longer compared with the membrane without interfering components ($P=0.019$, Dunn's test). A 3.29 ± 0.01 $\log_{10}$ cfu inoculum of EMRSA-15 was killed within 5 min on the silicone membrane following application of the CHG gel dressing (Table 2). When the inoculum was applied in the presence of a catheter segment and serum under the dressing, EMRSA-15 was killed within 4 h. In the presence of 10% (v/v) serum or a catheter segment, total kill was achieved within 10 min and 30 min, respectively.

Time–kill studies on donor human skin

The presence of a catheter segment or serum or both, on the skin surface, significantly changed the time for bacterial eradication after application of the CHG gel dressing ($P=0.006$, Kruskal–Wallis test). On the surface of human skin, a 6.27 ± 0.11 and 6.34 ± 0.16 log cfu inoculum of EMRSA-15 with

Table 1. Time to eradication of EMRSA-15 (and the mean $\log_{10}$ cfu reduction from the control dressing) when applied to the surface of an artificial membrane, which subsequently had a CHG gel dressing added ($n=3$)

		Dressing		Membrane surface		CVC surface	
Experiment	Mean inoculum ± SEM	eradication time	$\log_{10}$ cfu reduction	eradication time	$\log_{10}$ cfu reduction	eradication time	$\log_{10}$ cfu reduction
Membrane	7.06 ± 0.004	5 min	6.81	1 h	5.79	—	—
Membrane with serum	6.30 ± 0.09	4 h	6.45	4 h	6.33	—	—
Membrane with CVC	6.72 ± 0.20	8 h	4.43	12 h	5.00	12 h	5.69
Membrane with serum and CVC	6.44 ± 0.23	8 h	5.22	48 h	6.08	48 h	5.04

The time zero sample was taken when the dressing was applied. CVC, central venous catheter segment.

Table 2. Time to eradication of EMRSA-15 (and the mean $\log_{10}$ cfu reduction from the control dressing) when applied to the surface of an artificial membrane that subsequently had a CHG gel dressing added ($n=3$)

		Dressing		Membrane surface		CVC surface	
Experiment	Mean inoculum ± SEM	eradication time	$\log_{10}$ cfu reduction	eradication time	$\log_{10}$ cfu reduction	eradication time	$\log_{10}$ cfu reduction
Membrane	3.29 ± 0.01	2 min	3.00	5 min	2.56	—	—
Membrane with serum	3.17 ± 0.01	2 min	3.09	10 min	3.16	—	—
Membrane with CVC	3.01 ± 0.02	30 min	2.88	30 min	2.58	30 min	1.64
Membrane with serum and CVC	3.44 ± 0.02	2 min	3.72	4 h	3.58	4 h	3.57

The time zero sample was taken when the dressing was applied. CVC, central venous catheter segment.

Table 3. Time to eradication of EMRSA-15 (and the mean $\log_{10}$ cfu reduction from the control dressing) when applied to the surface of a donor skin that subsequently had a CHG gel dressing added ($n=3$)

		Dressing		Skin surface		CVC surface	
Experiment	Mean inoculum ± SEM	eradication time	$\log_{10}$ cfu reduction	eradication time	$\log_{10}$ cfu reduction	eradication time	$\log_{10}$ cfu reduction
Skin	6.34 ± 0.16	24 h	5.83	24 h	5.39	—	—
Skin with serum	6.27 ± 0.11	12 h	6.37	24 h	6.91	—	—
Skin with CVC	6.00 ± 0.19	48 h	7.94	1.58 $\log_{10}$ reduction within 48 h		1.45 $\log_{10}$ reduction within 48 h)	
Skin with serum and CVC	6.63 ± 0.07	48 h	7.42	1.69 $\log_{10}$ reduction within 48 h		3.53 $\log_{10}$ reduction within 48 h)	

The time zero sample was taken when the dressing was applied. CVC, central venous catheter segment.

or without 10% (v/v) serum, respectively, was killed within 24 h (Table 3). In the presence of a catheter segment, the number of EMRSA-15 after the 48 h test period was reduced by 3.0 and 2.2 $\log_{10}$ cfu (with or without serum, respectively) compared with the control dressing (MRSA were not detected on uninoculated donor skin).

Evaluation of the persistence of antimicrobial activity following a single microbial challenge

The CHG gel dressing, which had been placed onto the artificial membrane on the Franz diffusion cell (to mimic the application of the dressing on the skin) for up to 6 days and then removed, totally eradicated within 30 min 6.43 $\log_{10}$ cfu of EMRSA-15 when subsequently applied to its surface. Chlorhexidine gel dressings that were applied onto the artificial skin model for 7 days achieved a 3.04 ± 0.11 $\log_{10}$ cfu reduction from the inoculum (2.52 reduction compared with the control dressing) within 30 min of inoculation of EMRSA-15 (inoculum 6.38 $\log_{10}$ cfu).

Evaluation of the persistence of antimicrobial activity with repeated microbial challenges

Chlorhexidine gel dressings eradicated EMRSA-15 when the microorganism was repeatedly inoculated onto the silicone membrane under the dressing (a mean daily inoculation of 6.18 ± 0.03 $\log_{10}$ cfu) for up to 7 days. In comparison, the control dressing and membrane surface contained a mean total of 5.78 ± 0.15 $\log_{10}$ cfu after the 7 day repeated inoculation with EMRSA-15.

Evaluation of CHG deposition from the CHG gel dressing onto the skin surface

Chlorhexidine was detected on the donor skin surface, reflecting the preoperative skin preparation used on the donor incision site (0.42 ± 0.11 μg/cm^2). The amount of CHG deposited on the skin surface significantly increased over time ($P=0.009$, repeated measures analysis, Huynh-Feldt test), with significantly higher levels detected at 4 h compared with the control skin ($P=0.008$). A total of 6.60 ± 2.21 μg of CHG/cm^2 was recovered from the skin surface following a 48 h application of CHG gel onto the skin (Figure 2).

Evaluation of the release of CHG from the CHG gel dressing

Chlorhexidine was detected in the receiver fluid within 30 min in the small capacity diffusion cells (7 mL; 2.55 ± 1.76 μg) and within 2 h in the large capacity diffusion cells (29 mL; 14.82 ± 6.96 μg) (Figure 3). During the 12 h study, a total of 1079 ± 73 μg and 1288 ± 235 μg of CHG were detected in the receptor fluid in the small and large capacity diffusion cells, respectively. There was no significant difference in the CHG release after 12 h between the two assays ($P=0.855$, Mann–Whitney test), however, there was a significant difference in time to detection ($P=0.004$, Mann–Whitney test), and at 2 h the level of CHG detected was higher in the smaller diffusion cells ($P=0.004$; Mann–Whitney test).

Discussion

The current study demonstrated that the CHG gel dressing has rapid antimicrobial activity against MRSA and *E. coli*. The MBC levels of both EMRSA-15 and *E. coli* used in this study were similar to previous published levels for antibiotic-resistant and non-resistant strains of these microorganisms.[28] Although 10% (v/v) serum increased the MIC and MBC of aqueous CHG against EMRSA-15 and *E. coli*, this is in line with previous studies that have demonstrated that blood or protein can reduce the antimicrobial activity of CHG.[29] The CHG gel dressing, however, maintained its efficacy in the presence of serum. This suggests that the amount of CHG available on the dressing was sufficient to overcome any neutralizing effect of the serum protein.

The CHG gel dressing also exhibited antimicrobial activity when assessed on human skin. Determination of antimicrobial

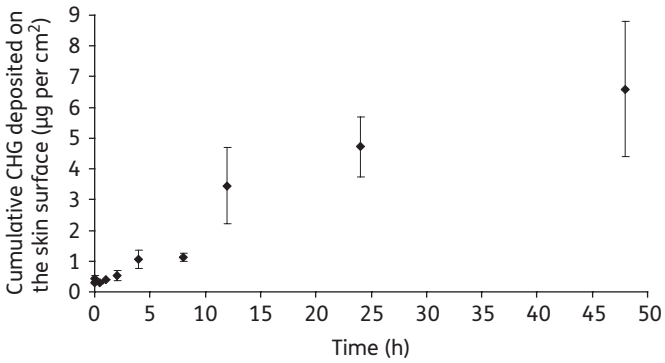

Figure 2. Mean ± SEM cumulative CHG deposited onto the surface of donor skin from the CHG Tegaderm gel dressing ($n=3$).

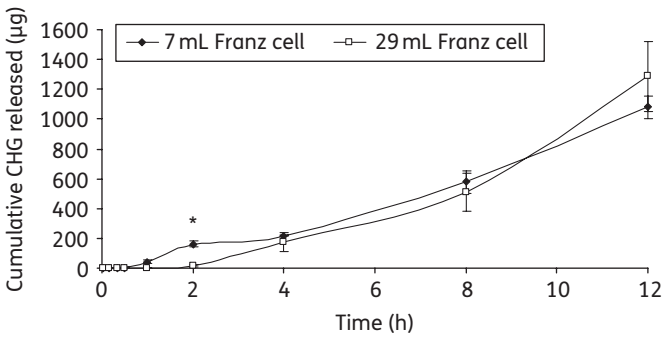

Figure 3. Mean ± SEM cumulative CHG released from the CHG Tegaderm gel dressing through a dialysis membrane in a Franz diffusion cell [small cells ($n=5$), 7 mL receptor volume; and large cells ($n=6$), 29 mL receptor volume] (*$P=0.004$; Mann–Whitney test).

activity of antiseptics, performed on donor skin in a diffusion cell, has been shown to closely mirror *in vivo* testing.[20,22] In addition, *in vitro* and *ex vivo* studies allow further control over the test environment than studies on human volunteers. The application of pathogens is also possible with such *in vitro* models.[22] On donor skin, the onset of bactericidal activity was delayed compared with the artificial silicone membrane. This may have reflected the final location of the inoculated bacteria within the skin layers. Indeed, in a previous study, *S. aureus* inoculated onto skin survived and proliferated in a three-dimensional matrix on the skin surface, and also within the skin layers, which may protect bacteria from contact with the antiseptic.[14] Other factors that may have influenced the bactericidal activity include the rate of diffusion of CHG into the skin and the presence of lipids and other organic compounds on the skin.[20] Chlorhexidine is a cationic compound and its antimicrobial activity is greatly reduced by interaction with organic matter, phospholipids and anionic emulsifying agents often present in skin creams.[30,31]

Interestingly, there was a delay in eradication of EMRSA-15 on the membrane surface following application of a higher inoculum. This may be due to an initial slow release of CHG from the dressing onto the applied surface. This highlights the importance of appropriate skin cleansing prior to the application of a dressing at the intravascular catheter site to achieve the optimum efficacy of the CHG gel dressing as soon as possible following application onto the skin. The 3 log inoculum, however, better reflects the number of bacteria present on the human skin.[10,19] The killing rates observed when this more clinically relevant inoculum was used suggest that the concentration of CHG in the dressing should achieve rapid bactericidal activity *in vivo*.

The presence of a catheter segment, with or without serum, under the dressing simulated the dressing use at the patients' catheter site. The results demonstrated that in the presence of a catheter segment and serum, the CHG in the gel was still able to eradicate the EMRSA-15 on the membrane and catheter surface as well as on the dressing itself, even with a high inoculum of bacteria. Furthermore, the results suggest that the performance of the gel allowed delivery of CHG under the catheter. The diffusion of CHG from the gel dressing has also been studied using an agar plate model (moist environment), where antimicrobial activity was detected under a catheter segment after applying the CHG gel for 24 h.[32] As the inoculum was applied under and around the catheter segment before application of the dressing, the bacteria may have been initially shielded from contact with the CHG in the gel by the catheter. In the presence of serum, the onset of antimicrobial efficacy of the CHG in the dressing on the catheter model was delayed, which may indicate that the CHG levels released from the dressing immediately after application onto the skin surface were low. However, after a lag phase, the diffusion of CHG achieved a bactericidal level also under the catheter segment/catheter surface.

The gradual release and diffusion onto the skin observed in this current study concurs with a previous investigation demonstrating that CHG migration under the catheter segment on skin of healthy human volunteers gradually increased during a 7 day application.[33] The results from the donor skin study also suggest that the CHG deposited onto the skin surface increased with time, after an initial time lag of 4 h. Low levels of CHG may have been released on the skin at the earlier stage, which could not be reliably discriminated from low levels of residual CHG ($<0.42\ \mu g/cm^2$) from the preoperative skin preparation used on the donor incision site. From the CHG release studies on the Franz diffusion cell, CHG was released from the CHG gel dressing within 30 min (level of quantification 0.052 mg/L).[27] Moreover, the antimicrobial efficacy of the dressing on the skin model also indicates that bactericidal concentrations of CHG were released onto the skin surface at an earlier stage. The estimated CHG concentration on the skin (assuming that the thickness of stratum corneum was recovered with the scrub cup technique and that CHG was recovered from that volume of skin) after 4 h application of the CHG gel on the skin surface equals approximately 0.53 g/L, which increased to an estimated 3.3 g/L after 12 h. These levels are significantly higher than the MBC level of most pathogens or skin microorganisms *in vitro*.[28]

Although the release rate was not studied over extended periods in this study, if the CHG release remained the same over the whole intended application period, it would take 125–150 h for all CHG to be released, and in our artificial model the CHG gel dressing retained its antimicrobial activity for up to 7 days. When the CHG gel applied onto a membrane was repeatedly challenged with bacteria under the dressing, which simulated reseeding of the skin surface with bacteria from the

deeper layers of skin, the dressing continued to eradicate the bacteria on both the membrane surface and on the gel dressing. The CHG gel dressing may therefore help to prevent bacterial regrowth on the skin at the catheter insertion site. These findings support the results from previous studies undertaken on the skin of healthy human volunteers, which demonstrated reduced microbial regrowth on the skin after antisepsis following application of the CHG Tegaderm dressing (compared with the control, Tegaderm dressings) for up to 7 and 10 days.[10,19] In addition, microbial counts were reduced on the unprepared skin for up to 10 days.[19]

In conclusion, the CHG gel dressing demonstrated rapid and sustained antimicrobial activity in a skin model. The release of CHG from the dressing increased with time, but the CHG gel dressing retained its antimicrobial activity for at least 7 days. The sustained release of CHG may reduce the microbial load at the catheter insertion site, thereby reducing the risk of CRI.

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Transparency declarations

T. S. J. E. is a member of a 3M advisory board. All other authors: none to declare.

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