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Staphylococcus biofilm inhibition, *in vitro,* using an antiseptic emollient containing chlorhexidine and benzalkonium chloride

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Introduction and Objective

Biofilms, comprising surface-associated, highly structured communities of microorganisms enclosed within a protective extracellular matrix, can exhibit increased antimicrobial resistance compared to their planktonic (free-floating single celled) forms.

Bacterial biofilms are associated with approximately 65% of infections, and their role in cutaneous diseases, such as atopic dermatitis, wounds and impetigo is widely recognised.

Dermol Lotion™ (DERL) is a dermatologically 'friendly' antiseptic emollient containing chlorhexidine dihydrochloride and benzalkonium chloride, each at the low concentration of 0.1%.

This *in vitro* study investigated the capability of DERL to inhibit *Staphylococcus* biofilms, as measured by a) metabolic inhibition of sessile bacteria present in the biofilm, and b) dispersal of the biofilm matrix.

Materials & Methods

- Two clinical isolates, MRSA (EMRSA 100) and MSSA (Newman), and two reference strains of *S. aureus* (NCTC 6751) and *S. epidermidis* (RP62A) were used to form biofilms *in vitro* using the methodology reported by Smith *et al* (2008).*
- These biofilms, 8 per strain, were washed in buffer (PBS) to remove any planktonic bacteria and then challenged for 24 hours with DERL serially double-diluted in sterile PBS, pH=7.
- After washing in PBS again, the metabolism of bacterial cells was assessed by Alamar Blue for visual determination of the Sessile Minimum Inhibitory Concentration (SMIC).
- At the SMIC only, the percentage dispersal of the biofilms was measured colorimetrically at 570 nm after staining in crystal violet followed by ethanol de-staining.

*Smith *et al.* Journal of Medical Microbiology (2008), **57**, 1018-1023.

Results

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after 24 hours incubation. The MIC associated with microorganisms present in the protective biofilms (sessile bacteria) is normally referred to as Sessile Minimum Inhibitory Concentration (SMIC).

The SMICs for DERL dilutions are presented in Table 1.

Table 1. Sessile Minimum Inhibitory Concentration for DERL

Microorganism	DERL SMIC
S. aureus (NCTC 6751)	1 in 16 dilution
S. epidermidis (RP62A)	1 in 8 dilution
MRSA (EMRSA 100)	1 in 8 dilution
MSSA (Newman)	1 in 16 dilution

The percentage dispersal of the biofilm, at the SMICs, is presented in Table 2.

Table 2. Percentage dispersal

Microorganism	% dispersal at SMIC
S. aureus (NCTC 6751)	67%
S. epidermidis (RP62A)	32%
MRSA (EMRSA 100)	12%
MSSA (Newman)	0%

The above results indicate that DERL, even at high dilutions, can significantly inhibit the growth of these microorganisms. This may indicate that antimicrobial active ingredients penetrate into the protective *Staphylococcus* biofilms.

Conclusion

Tested *in vitro*, dilutions of topical antiseptic emollient Dermol Lotion™ (DERL) significantly inhibited *Staphylococcus* biofilms, and this was not generally associated with high levels of biofilm dispersal, indicating that the antimicrobial agents penetrated inside the biofilm matrix.

Please turn over for poster summary.

Staphylococcus biofilm inhibition, *in vitro*, using an antiseptic emollient containing chlorhexidine and benzalkonium chloride

Biofilms are structured communities of sessile (i.e. fixed or not free-moving) microbial cells that adhere to surfaces and are enclosed in a protective, self-produced polymeric matrix. Biofilms can exhibit increased antimicrobial resistance compared to their planktonic forms, which are individual free moving cells.

Bacterial biofilms are associated with approximately 65% of infections, and their role in cutaneous diseases, such as atopic dermatitis, wounds and impetigo is widely recognised.

Dermol Lotion is a topical antimicrobial emollient and soap substitute formulated to be dermatologically 'friendly'. It contains two antiseptics, chlorhexidine dihydrochloride and benzalkonium chloride, that work synergistically and so are present at the low but effective level of 0.1% each, to minimise the risk of skin irritancy.

Summary of Poster Overleaf:

- Biofilms of MRSA and MSSA (from clinical isolates) and *S. aureus* and *S. epidermidis* (reference strains) were formed *in vitro*.
- After removal of planktonic bacteria, Dermol Lotion (at different dilutions) was added to the biofilms. The Sessile Minimum Inhibitory Concentration (SMIC) was assessed (the MIC associated with microorganisms present in the protective biofilm).
- For Dermol Lotion (as shown in Table 1):
  - The SMIC was a 1 in 8 dilution against *S. epidermidis* and MRSA
  - The SMIC was a 1 in 16 dilution against *S. aureus* and MSSA
- The percentage dispersal of the biofilm matrix at the respective SMIC ranged from 0% to 67% (Table 2).
- These results indicate that Dermol Lotion, even at high dilutions, can significantly inhibit the growth of these microorganisms without substantially dispersing the biofilms.
- This may indicate that antimicrobial active ingredients penetrate into the protective *Staphylococcus* biofilms.

Conclusion:

"Tested *in vitro*, dilutions of topical antiseptic emollient Dermol Lotion™ (DERL) significantly inhibited *Staphylococcus* biofilms, and this was not generally associated with high levels of biofilm dispersal, indicating that the antimicrobial agents penetrated inside the biofilm matrix."

Dermol® 500 Lotion

Benzalkonium chloride 0.1% w/w, chlorhexidine dihydrochloride 0.1% w/w, liquid paraffin 2.5% w/w, isopropyl myristate 2.5% w/w.

Adverse events should be reported. Reporting forms and information can be found at yellowcard.mhra.gov.uk. Adverse events should also be reported to Dermal.

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