The Development of an *In Vitro* Colonised Skin Model and its Potential Applications

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Introduction

Human skin provides a formidable barrier to pathogens, however patients with chronic wounds or burn injuries present with impaired barrier function that can lead to severe infection. There is a plethora of academically designed *in vitro* skin and wound models but a shortage of models that incorporate mammalian and bacterial cells within a reproducible assay. A lack of commercially-focused models means that the effective screening of topical wound care products *in vitro* is more challenging.

Aim

To develop an *in vitro* colonised skin model using reconstructed human epidermis and *Staphylococcus aureus*.

Method

Staphylococcus aureus was grown on Tryptone Soya Agar (TSA) overnight at 37 ± 2°C. Following incubation, *S. aureus* was harvested and diluted in Phosphate Buffered Saline (PBS) to 1 x 10^6 , 1 x 10^7 and 1 x 10^8 $CFUmL^{-1}$. *In vitro* reconstructed human epidermis (IVRHE) was prepared according to manufacturer's instructions and held at 37 ± 2°C with 5% CO_2 prior to treatment.

Aliquots of the *S. aureus* inocula were applied to the epidermis and incubated for 60, 120 and 240 minutes at 37 ± 2°C with 5% CO_2. Following incubation, the epidermis was washed to remove non-adherent *S. aureus*. The reconstructed epidermis was removed from the culture insert, suspended in recovery medium and sonicated. Following sonication, the suspension was serially diluted, plated onto TSA and incubated at 37 ± 2°C for 24 hours. Colonies of *S. aureus* were counted and expressed as $Log_{10}CFUmL^{-1}$. All conditions were tested in triplicate. Analysis of an appropriate *S. aureus* concentration and incubation time was determined. Prior to visualisation on the scanning electron microscope (SEM), samples were fixed in glutaraldehyde and dehydrated in increasing concentrations of ethanol for 10 minutes per concentration. Samples were sputter coated prior to imaging.

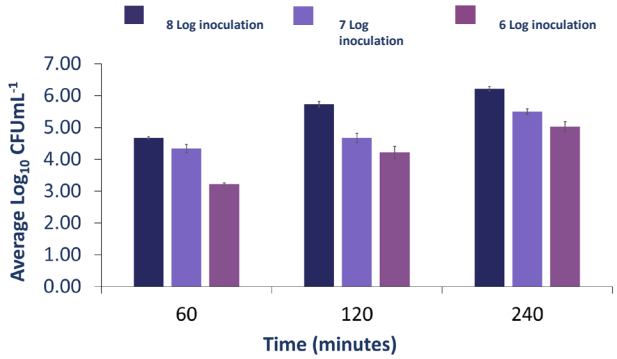

Figure 1. Quantity of *Staphylococcus aureus* attachment to an *in vitro* reconstructed human epidermis model (IVRHE), at a range of time points after inoculation and with different initial inoculum concentrations.

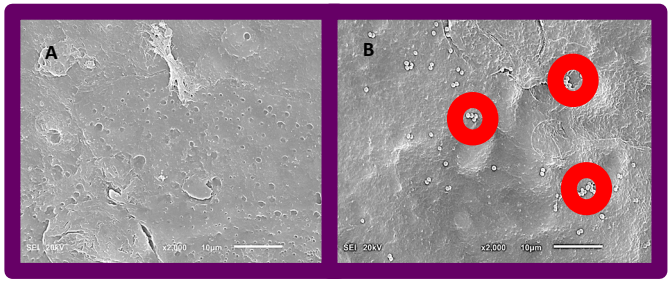

Figure 2. Scanning electron microscopy of *in vitro* reconstructed human epidermis (IVRHE). **A** = uninoculated IVRHE **B** = *Staphylococcus aureus* inoculated IVRHE. IVRHE was inoculated 1 x 10^6 $CFUmL^{-1}$ and incubated for 120 minutes. Red circle = *S. aureus* microcolonies.

Results

For each *S. aureus* concentration tested, *S. aureus* was found to be attached to the IVRHE after 60 minutes. Figure 1 showed an increase in *S. aureus* attachment in a time-dependant manner. Greater *S. aureus* seeding concentrations over the later time periods facilitated greater *S .aureus* attachment to the IVRHE.

Scanning electron images (Figure 2) revealed early microcolony formation of adhered *S. aureus* to the epidermal layer.

Discussion & Conclusions

The aim of this study was to determine a suitable seeding concentration of *S. aureus* and a suitable incubation time to enable maximum *S. aureus* attachment to the IVRHE model. Maximum adhesion was recorded at 240 minutes with the 1 x 10^8 $CFUmL^{-1}$ starting inoculum.

The significant adhesion recorded at shorter time points and lower seed inoculation levels suggests that this model could be tailored to reflect various *in vivo* scenarios.

The tissue is made from human keratinocytes on a collagen matrix and is histologically similar to the human epidermis. The model has the potential to be used for the assessment of skin irritation, skin corrosion, UV exposure, permeability, bacterial adhesion and the efficacy of antimicrobial wound dressings and solutions.

The identification of early microcolony formation was indicative of biofilm formation. The use of IVRHE to model skin biofilm provides consistency. The results of this study will allow researchers to screen topical antimicrobial products in models that represent low, medium and high-level contamination situations.

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