The Impact of Protein-Based Substrates on Biofilm Development and Disruption

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Introduction

Most chronic wounds that are colonised by microorganisms, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, also contain high levels of protein-rich biological fluids such as wound exudate and plasma proteins. In the presence of high levels of protein-rich substrates, both bacterial species have demonstrated higher levels of pathogenicity, increased attachment, and more robust biofilms, all of which require increasingly more complex treatment strategies [1, 2, 3].

Several systems are available for examining the development of biofilms and investigating biofilm disruption therapies. In this study, we utilised the high throughput Minimum Biofilm Eradication Concentration (MBEC) assay to assess the impact of a preconditioning protocol on the MBEC of four commercially available wound care products against single species *P. aeruginosa* and *S. aureus* 72 hour biofilms.

Aims

1.To assess the effect of using a protein-rich substrate to preconditioning the attachment surface of the MBEC assay on biofilm development.

2.To assess the efficacy of wound care therapies on the subsequently formed biofilms.

Methods

1. Biofilm Development

The MBEC assay pegs were pre-conditioned via the adsorption of Simulated Wound Exudate (SWE) with incubation for 24h at 37 ° C. A control plate was not pre-conditioned. After 24 hours, 150 µL *P. aeruginosa* NCIMB 10434 and *S. aureus* NCTC 8325 bacterial suspension was added to the test wells of separate 96-well plates and the pre-conditioned or control group pegs introduced. Assays were then incubated for 72 hours at 37 °C with agitation set at 110 rpm to encourage biofilm development.

2. Determination of Minimum Biofilm Eradication Concentration

The four wound care therapies were diluted (100, 75, 50, 25, 10, 5, 1, 0.5, 0.1, 0.01%) and 200 µL of each dilution added to six separate wells for both control and pre-conditioned groups.

The pegs from the biofilm development stage were then rinsed three times in PBS to remove planktonic bacteria, introduced to the challenge plates for 5 minutes, transferred to new plates containing neutraliser for 5 minutes. Plates were then sonicated for 5 minutes to quantify remaining bacteria. The MBEC for each product was identified as the lowest concentration with which no growth was observed for all replicates (Figure 1).

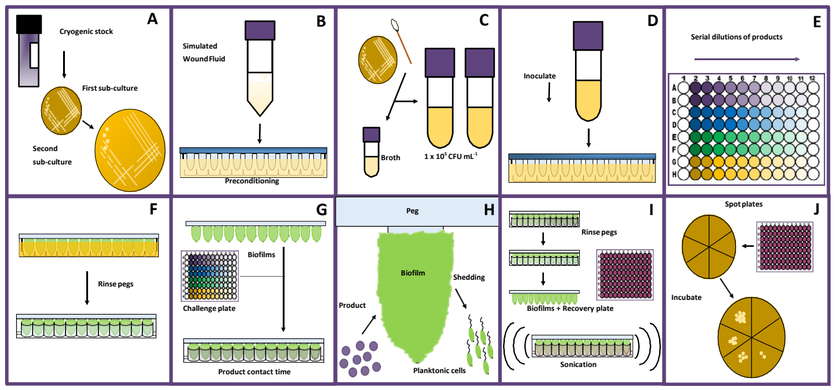

Figure 1. Schematic diagram illustrating the process for preparation of control and preconditioned pegs, growth of biofilms, treatment, and recovery.

Results

1. Biofilm Development

Following a 72 hour growth phase, a viable *P. aeruginosa* recoveries of 7.09 ± 0.04 and 7.49 ± 0.15 $Log_{10}CFUmL^{-1}$ were observed for the control and preconditioning groups, respectively (Figure 1a).

Following a 72 hour growth phase, a viable *S. aureus* recoveries of 6.27 ± 0.22 and 6.62 ± 0.24 $Log_{10}CFUmL^{-1}$ were observed for the control and preconditioning groups, respectively (Figure 1b).

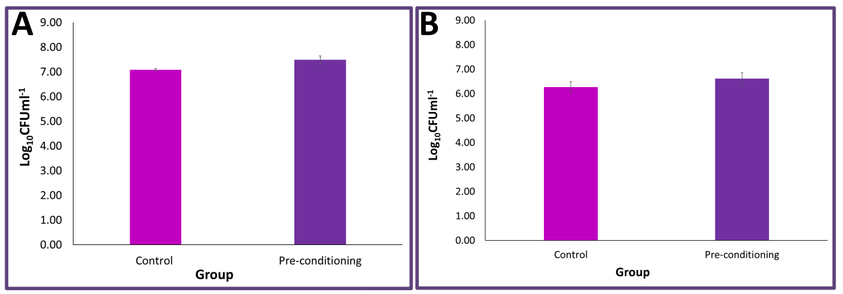

Figure 2. Average Log recovery data for control and preconditioning groups with *Pseudomonas aeruginosa* (a) and *Staphylococcus aureus* (b).

2. Minimum Biofilm Eradication Concentration

The MBECs of Product A, B, C, and D against *P. aeruginosa* biofilms for the control group were 10%, 75%, 75%, and 50%, respectively. The MBECs of Product A, B, C, and D against *P. aeruginosa* biofilms for the preconditioned group were 50%, 100%, 100%, and 100%, respectively (Figure 2a).

The MBECs of Product A, B, C, and D against *S. aureus* biofilms for the control group were 25%, 75%, 75%, and 75%, respectively. The MBECs of Product A, B, C, and D against *S. aureus* biofilms for the preconditioned group were 75%, 100%, 100%, and 100%, respectively (Figure 2b).

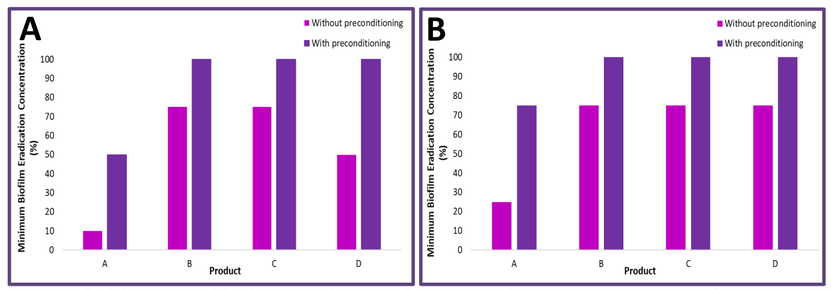

Figure 3. Minimum Biofilm Eradication Concentration for Product A, B, C, and D for control and preconditioning groups challenged with *Pseudomonas aeruginosa* (a) and *Staphylococcus aureus* (b)

Discussion

Surface preconditioning with SWE significantly increased the Log recovery of both bacterial species. Bacterial adhesion and subsequent biofilm development starts with up-regulation of attachment surface proteins that have been demonstrated to be elevated in the presence of higher protein concentrations[3, 4, 5]. This project provides preliminary data to support real-world applications, a more customised approach to biofilm testing, and provides insight that may challenge previous findings deemed efficacious by the MBEC assay.

References:

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Biofilm testing was carried out by Perfectus Biomed Ltd. Perfectus Biomed is an independent testing laboratory. Techspace one, Keckwick Lane, Cheshire, WA4 4AB. Tel +441925 737 237.
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