Comparison of a continuous flow system and immersion in suspension to develop biofilm on contact lenses

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Introduction

Within the environment bacteria typically attach to a surface and exist within a self-produced matrix known as a biofilm that offers protection from antimicrobial agents. Bacteria that reside within a biofilm are typically between 10 and 1000-fold less susceptible to antimicrobial treatments than planktonic bacteria.

Micro-organisms in ocular infections, consistent with other infections, take the biofilm mode of growth and therefore testing of therapeutics against *in vitro* biofilms, in place of planktonic organisms, is useful to establish unique claims and product support data.

Current options for modelling biofilms on contact lenses, such as immersing the lens into inoculated media, are not highly representative of real world scenarios and do not incorporate features such as appropriate flow rate.

The drip flow biofilm model generates a substantial biofilm *in vitro* and provides a robust challenge for test products. This continuous flow model can run at low flow rates, comparable to tear flow rates, and can be intentionally inoculated with micro-organisms. The flow is allowed to travel over the contact lens surface and establish a biofilm. The media can be adapted in terms of salt and protein content to simulated tears and the biofilms generated are reproducible allowing statistical analyses of test interventions. This study compares recovery of *Staphylococcus aureus* from the surface of contact lenses grown in either the drip flow reactor or suspension.

Methodology

Contact lenses were removed from packaging and rinsed once in Phosphate Buffered Saline (PBS) to remove any storage solution. Contact lenses were then incubated in a 1 x 10^7 $CFUmL^{-1}$ suspension of *S. aureus* at 37°C at 100 rpm for 24 hours. Following 24 hours incubation, inoculated contact lenses were placed onto glass microscope slides with absorbent pads attached (Figure 1). Artificial tear solution, containing saline and a protein source, was prepared. Using a peristaltic pump, the artificial tear solution was applied to the contact lens at a flow rate of 5 mL/hour (Figure 2). The drip flow reactor was incubated under flow at 37°C for 72 hours.

Following incubation, triplicate contact lenses were removed from the drip flow reactor and washed once in PBS to remove planktonic bacteria. Viable bacteria were removed by sonication and enumerated by serial dilution. Single contact lenses did not go through the sonication step and were instead stained using Filmtracer™ LIVE/DEAD™ Biofilm Viability stain and imagined using a Fluoview FVIOi confocal laser scanning microscope.

Figure 1. Photograph of a contact lens in the drip flow reactor following inoculation with *Staphylococcus aureus* and prior to incubation.

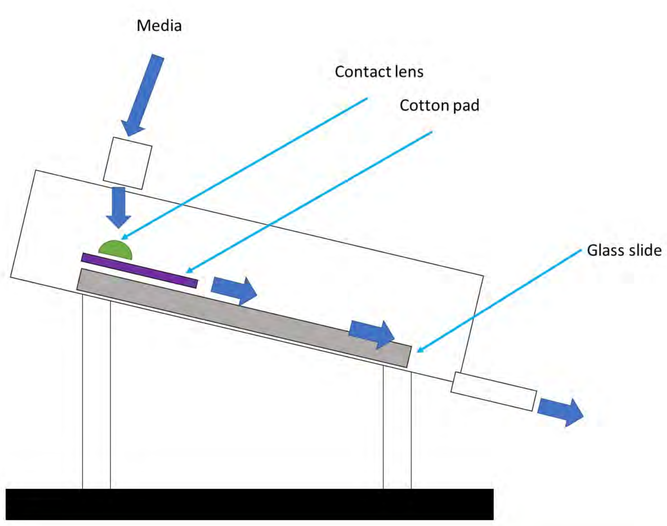

Figure 2. Schematic diagram of the drip flow reactor.

For lenses grown in suspension only, contact lenses were incubated in a 1 x 107 CFUmL-1 bacterial suspension at 37°C at 100 rpm for 72 hours. Following incubation, contact lenses were removed from the suspension and washed once in phosphate buffered saline to remove planktonic bacteria. Viable bacteria were removed by sonication and enumerated by serial dilution.

Results

Biofilm established in the biofilm reactor resulted in an average of 7.60 ± 1.10 $Log_{10}CFUmL^{-1}$ *S. aureus being* recovered from the contact lenses. Following 72 hours incubation in suspension, an average of 6.89 ± 0.23 $Log_{10}CFUmL^{-1}$ *S. aureus* were recovered from contact lenses (Figure 3). Confocal microscopy of inoculated contact lenses incubated in the drip flow reactor showed a large number of individual cells and small clusters of *S. aureus*. The majority of *S. aureus* cells were live (green) (Figure 4).

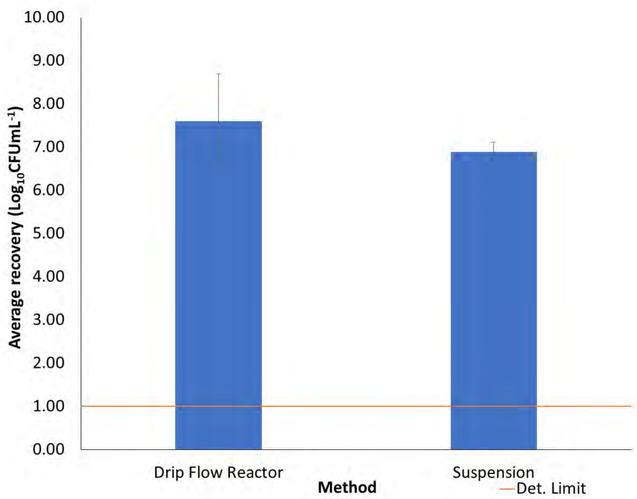

Figure 3. Average quantities of *Staphylococcus aureus* recovered from contact lenses incubated for 24 hours in suspension and 72 hours in the drip flow reactor or for 72 hours in suspension only.

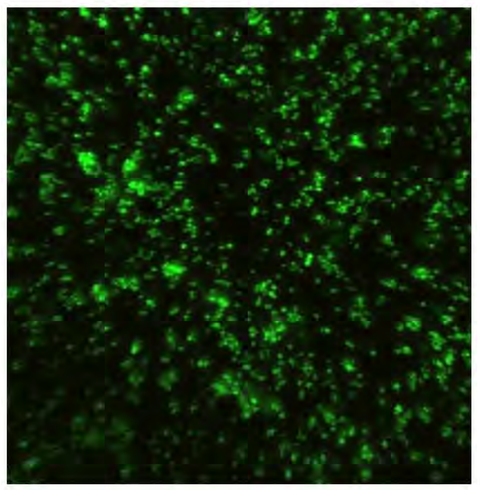

Figure 4. Confocal microscopy images of *Staphylococcus aureus* following 24 hours incubation in suspension, 72 hours incubation in the drip flow reactor and staining with Filmtracer™ LIVE/DEAD™ Biofilm Viability stain.

Conclusion and Discussion

This study has demonstrated that bacterial biofilms can be established on the surface of contact lenses. This method can be utilised to demonstrate the efficacy of cleaning solutions or to investigate contact lens materials, coatings and topographies that may demonstrate varying affinities for bacterial binding. Comparable quantities of bacteria were recovered from contact lenses incubated in the drip flow reactor and in suspension.

Biofilms formed using the shaking suspension method are likely to be more representative of the real world. The drip flow reactor method represents a useful model for the assessment and differentiation of contact lens products. It is highly reproducible and suitable for scenarios that would fit both single use and long term wear of contact lenses.

This project was carried out by Perfectus Biomed Group
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