Report (Adtec Healthcare 001)

1.0 Aim

To demonstrate the anti-biofilm activity of gas plasma using a CDC reactor biofilm model.

2.0 Materials and Methods

2.1 Test microorganisms

Staphylococcus aureus NCTC 8325

2.2 Test agents

Agent	Agent Format
Phosphate Buffered Saline	Solution
Gas plasma treatment	Hand held device

Table 1. Test agents used throughout the study.

2.3 Method

2.3.1 Preparation of bacterial inoculum

A twenty-four hour culture of *Staphylococcus aureus* was harvested from a Tryptone Soya Agar (TSA) plate using a sterile swab and re-suspended in 20ml of Tryptone Soya Broth (TSB). The suspension was diluted in TSB to give an overall concentration of 10^7 cfuml^{-1} and used as the initial inoculum for the CDC reactor. The CDC reactor was placed into an orbital incubator for 24 and 72 hours at 37°C and 50rpm in order to encourage biofilm growth.

2.3.2 Treatment

Following each incubation period coupons were removed from the reactor and washed three times in sterile PBS. Washed coupons were treated with gas plasma for 2 or 5 minutes. Coupons were treated on a platform 2cm away from the gas plasma device. The gas plasma treatment was set to 80W, 7.1V and 41°C. The coupon treatment protocol involved treating both sides of the coupon for the allotted time. Control coupons were treated with PBS for 5 minutes.

2.3.3 Recovery and quantification

Following treatment coupons were transferred into 2ml of TSB and sonicated for 5 minutes in order to recover remaining viable microorganisms. Recovered microorganisms were quantified using serial dilutions and drop plates.

3.0 Results

No viable organisms were recovered from coupons that had been treated for 5 minutes with gas plasma. When gas plasma treatment was reduced to 2 minutes a 1.74 log reduction in the number of viable bacteria was recorded compared to the 24 hour PBS control and a 1.40 log reduction was reported compared to the 72 hour controls (Figure 1).

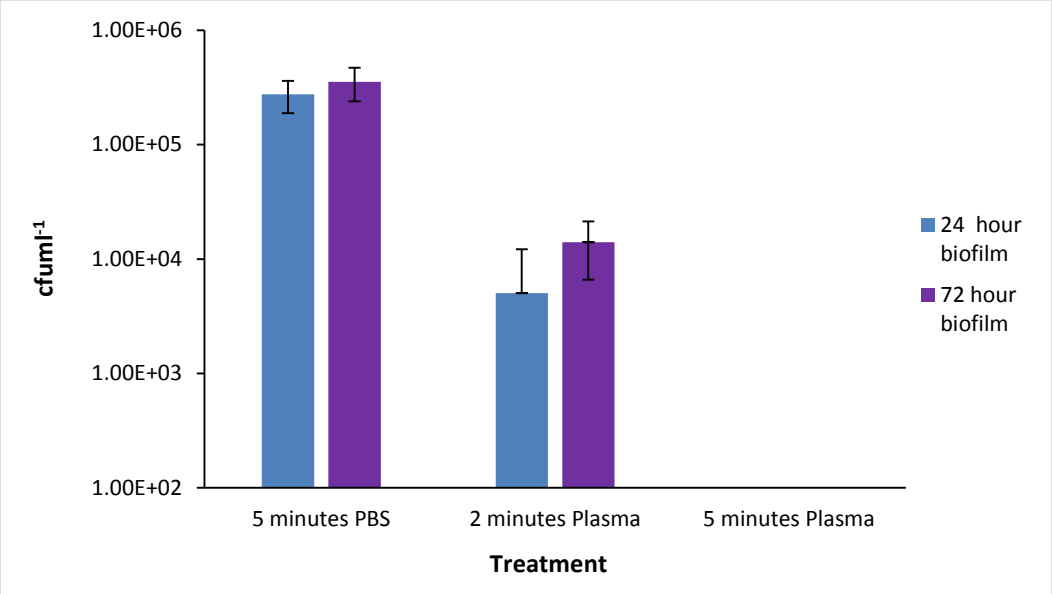

Figure 1. Quantity of viable *Staphylococcus aureus* recovered from 24 and 72 hour pre-formed biofilms following 2 or 5 minutes treatment with gas plasma or PBS.

4.0 Discussion

Five minute treatment with gas plasma at the settings used in this study effectively reduced the number of recoverable viable organisms to zero. Further testing would be required in order to determine whether the treatment had a bactericidal or bacteriostatic treatment effect and it should be noted that 'no recoverable organisms' does not mean that no viable organisms remained, only that if present they were present at levels below the detectable levels.

The variation in efficacy of treatment times suggests that although a reduction in microorganisms is seen after 2 minutes of treatment, either repeated treatments, or a longer duration of treatment would be required in order to effectively treat/disrupt a biofilm. This study does not provide data on the efficacy of gas plasma treatment against multispecies biofilms or biofilms within tissue models. Further studies would be required in order to investigate these applications.

The CDC reactor model provides a reliable and repeatable hard surface model for the investigation of biofilms however it uses surface attachment as a measure of biofilm

formation and does not attempt to address the complexities of the wound scenario. These complexities include, but are not limited to, the attachment of biofilm forming bacteria to mammalian cells, the impact of the host's immune system and the production of growth factors, MMP's and other wound metabolites. Further work would be required to address these limitations.

5.0 Future work

- Repetition of this study with other wound-relevant bacterial species such as *Pseudomonas aeruginosa.*
- Repetition of this study with a mixed species biofilm.
- A study that demonstrates repeated treatments (daily) for a prolonged period of time (weekly) would help to inform on the efficacy of repeated short (2 minute) treatments.
- Studies investigating 3 and 4 minute treatment times would help to determine the minimum treatment time required to treat a single or multispecies established biofilm. Collectively this *in vitro* data could help inform patient treatment protocols.
- Fluorescent microscopy images would demonstrate the viability of remaining attached bacteria.
- Models such as flow cells and the colony drip flow reactors incorporate a continuous flow system and an upwards wicking of nutrients which can be used to more closely mimic a wound situation.
- *Ex vivo* tissue models involve biofilm growth on mammalian cells and therefore more closely mimic wound biofilms.

Project Start Date: 20/07/15

Project Completion Date: 12/08/15

End of report.