Staphylococcal Biofilm Attachment – An *in vitro* Model

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BACKGROUND

Total joint replacement (TJR) has revolutionised the way chronic joint pain is treated. In 2017, alone Victorians had over 25,000 hip and knee joints replaced[1]. A devastating complication of TJR is infection, which effects 2% of patients[2]. This difficult to treat infection is exacerbated by the presence of biofilm forming bacteria. Biofilms are bacteria embedded within an extracellular matrix that protects them from traditional therapies. A severe PJI can leave a patient with a poorly functioning joint and on long term suppressive antimicrobials[3]. Existing diagnostic protocols do not test biofilm formation of causative organisms due to the difficulty of bacterial isolation from a biofilm.

The aim of this project was to develop a high throughput *in vitro* model of biofilm attachment

BIOFILM ATTACHMENT ASSAY

TSA plates were inoculated with bacteria and incubated overnight at 37°C. 5 ml TSB was inoculated with a single colony from each bacterial strain. Greiner 96 well plates were set up with 200µl of bacterial culture at 0.1 OD_{600nm}, with sterile TSB acting as the control. OD_{600nm} was measured for the planktonic bacteria at 0, 3, 6, 9, 12 and 24 hr. Microtitre plates were incubated at 37°C under rocking at 12rpm and a 10° tilt. Wells were washed twice and stained with Periodic Acid-Schiff stain (PAS). Visualised using inverted microscopy. 30% Acetic Acid was used to leach the stain and the absorbance was measured at 570nm. All results were normalised against the control wells.

BACTERIA STRAINS

Bacterial Reference Strain	Biofilm Forming Ability
Staphylococcus aureus (NBSA01)	No
Staphylococcus epidermidis (ATCC® 12228™)	No
Staphylococcus aureus (ATCC® 35556™)	Yes

FINDINGS

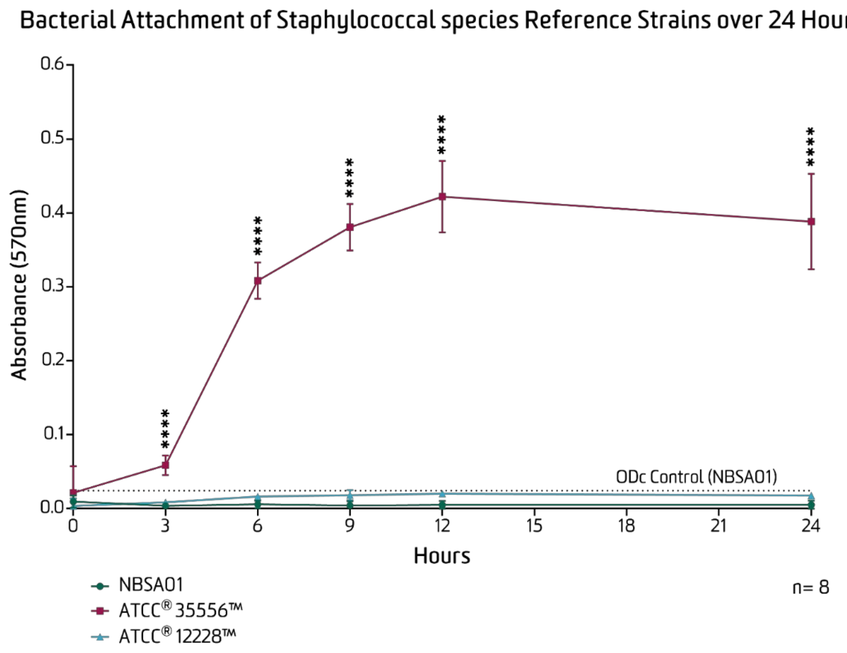

Figure 1 Attachment of Staphylococcal bacteria across a 24 hour period. **** = p <0.01

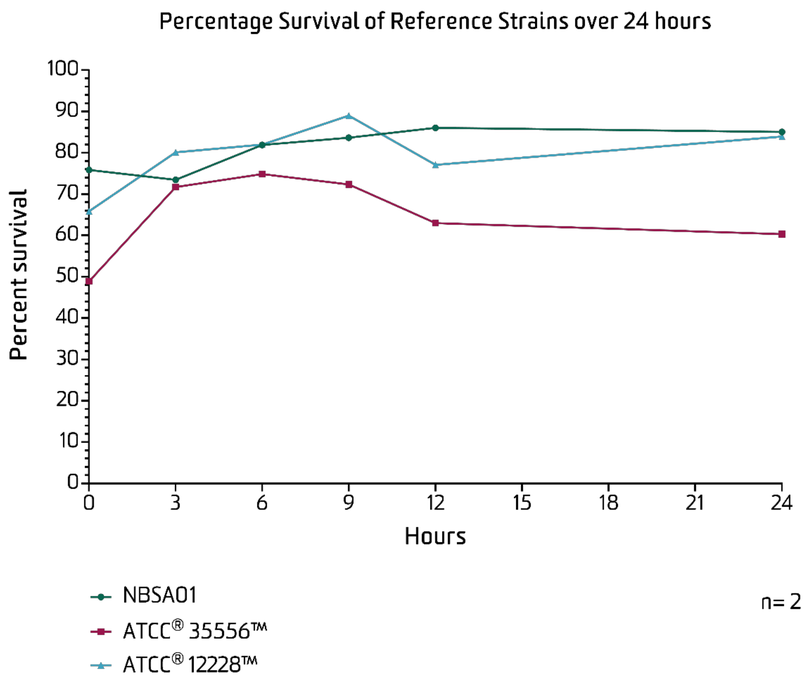

Figure 2 Percentage of Dead Bacteria in Planktonic Culture measured by Flow Cytometry across 24 hours

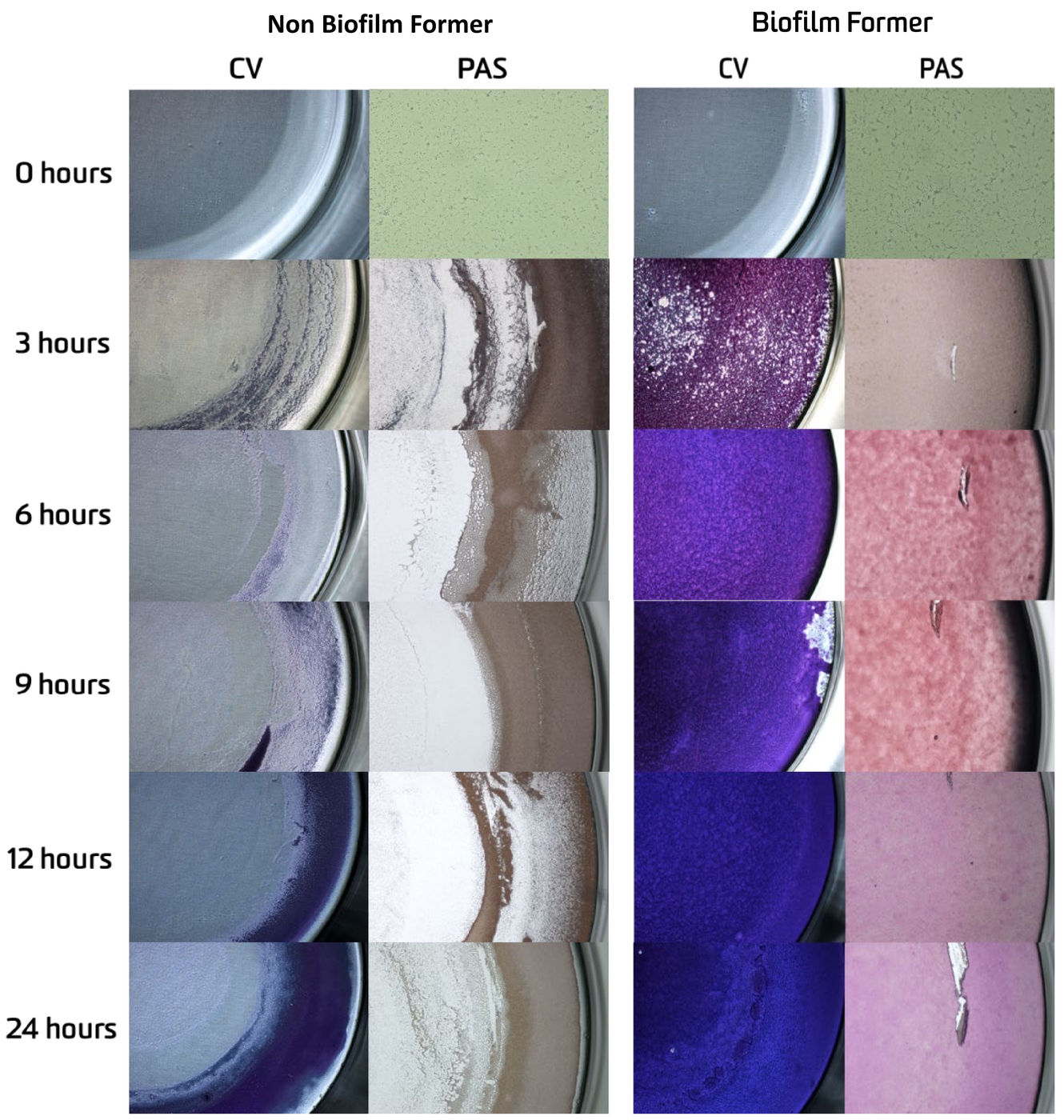

Figure 3 Inverted Microscope images of non-biofilm and biofilm forming bacteria at set time points over 24 hours.

CONCLUSIONS

- Reference strains attach within 3 hours of being introduced into the system
- The non biofilm former appears to have more alive bacteria at 24 hours
- Does this imply that viable bacteria are present within biofilm?
- Validation required!
- Clinical strains are highly variable and do not show the same level of attachment as reference strains

References

1. Australian Orthopaedics Association National Joint Replacement Registry. Annual Report - Hip, Knee and Shoulder
2. A. J. Tande and R. Patel, *Clin Microbiol Rev*, 2014, 27, 302-345.
3. R. Trebse, V. Pisot and A. Trampuz, *Journal of Bone & Joint Surgery, British Volume*, 2005, 87, 249-256.