The role of media changes in the optimisation of static staphylococcal biofilm models

Mitchell Clements[1,2], Darcie Cooper[1,2] and Eugene Athan[2,3]

[1] Geelong Centre for Emerging Infectious Diseases, [2]School of Medicine, Deakin University, VIC, [3]Department of Infectious Diseases, Barwon Health Geelong Hospital, VIC

Presenting Author: mjclemen@deakin.edu.au

Introduction

Total joint arthroplasties have been increasing in Australia[1]. As the number of prosthetic joints increase, the number of prosthetic joint infections (PJI) will also increase. The infection rate of prosthetic joints is 1-3%[2]. The main cause of PJIs are bacteria that produce biofilms, a slime like layer which are resistant antimicrobial therapy[3]. Different models to study biofilm development have been investigated and although there have been advancements made in the field, greater understanding remains elusive[4]. Static biofilm models do not have a continuous supply of fresh medium and removal of waste - meaning it has to be done manually. A drawback to this model is establishing longer biofilm models due to lack of nutrients[5,6]. We aim to optimize long-term growth of a static biofilm by comparing 24 hour half media and 24 hour full media changes in both 2 day biofilm and 3 day biofilm assays.

Bacteria Lines

Staphylococcus aureus (ATCC® 35556™)
Staphylococcus aureus laboratory specific reference strain (NBSA01)
Staphylococcus epidermidis (ATCC® 35984™)
Staphylococcus epidermidis (ATCC® 12228™)

Methods

All bacterial strains mentioned above were culture on Muller Hinton Agar at 37˚C overnight to isolate single colonies. A single colony from each strain was use to inoculate 15 mL of Tryptic Soy Broth and was also incubated at 37˚ overnight.

After incubation overnight culture was diluted to an OD_{600nm} of 0.1 and 200µL was pipetted into 4 96-well plate as shown in Figure 1. Plates were incubated at 37˚C on a plate rocker with a 10˚ tilt at 12 rpm, incubation time varied for the different plates.1 plate was incubated for 24 hours, 1 plate was incubated for 48 hours which had 1 media change conducted at the 24 hour timepoint , 1 plate was incubated for 72 hours and had 2 media changes which were conducted at the 24 and 48 hour timepoints and the final plate was incubated for 72 hours with no media changes.

Figure 1 Biofilm assay optimisation: Layout of 96-well plate, also shows how media changes were performed.

After a plate had finished incubation, wells were washed twice with MilliQ H_2O and underwent Peroidic Acid-Schiff (PAS) staining. The PAS stain was then leeched from the wells by pipetting 200µL of 30% acetic acid into wells, plate absorbance was taken at 570nm using an Epoch plate reader.

Results

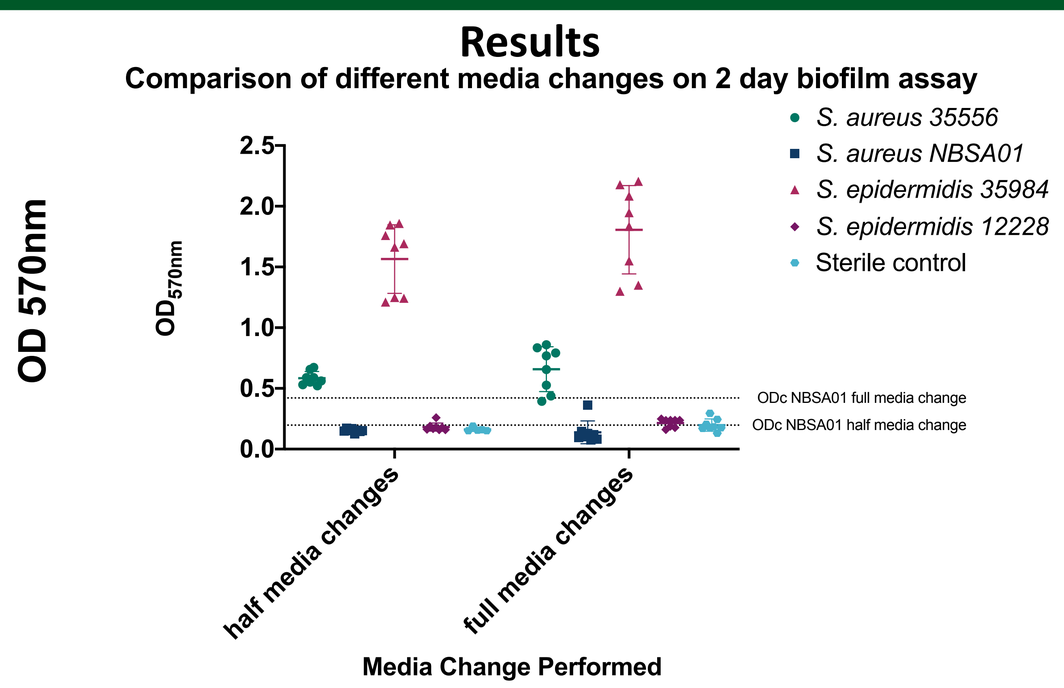

Figure 2 OD_{570nm} readings of 2 day biofilm assay: Shows the optical density at 570nm for the 2 day biofilm assay separating half media and full media changes. *S. aureus* 35556 biofilms with full media changes were 13% greater then those with half media changes whilst *S. epidermidis* 35984 biofilms with full media changes were 28% greater however these values were statistically insignificant, p=0.4873 and p=0.1304 respectively.

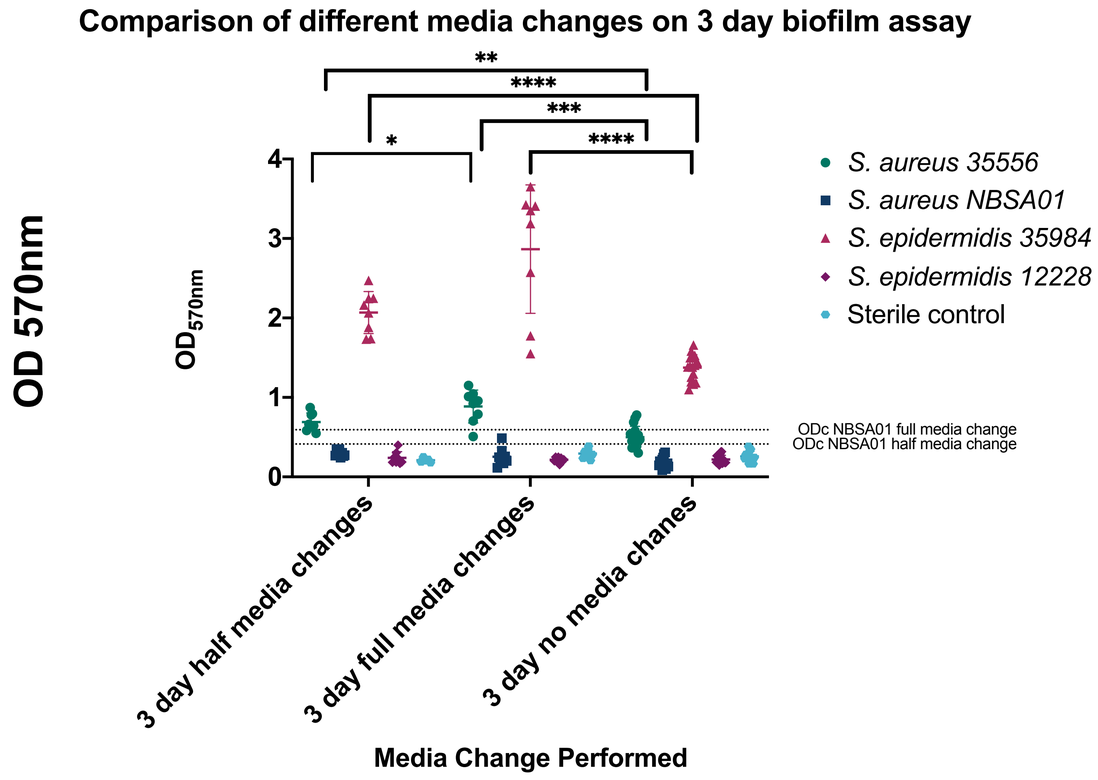

Figure 3 OD_{570nm} reading of 3 day biofilm assay: Shows the optical density at 570nm for the 3 day biofilm assay, separating half media, full media and no media changes. *S. aureus* 35556 biofilms with full media changes were 28% greater then those with half media changes, *p=0.0468 whilst *S. epidermidis* 35984 biofilms with full media changes lead to 39% greater biofilms however this value was statistically insignificant, p=0.0650. For both biofilm forming strains no media changes lead to significantly smaller biofilms then both full media and half media changes; **p= <0.01, ***p=<0.001, ****p=<0.0001.

Conclusions

- For all samples full media changes lead to greater biofilms then half media changes however this was only significant for the 3 day *S. aureus* 35556 biofilms, p=0.0468 however the 3 day *S. epidermidis* 35984 biofilms were almost significant p=0.0650
- For all the 3 day biofilms no media changes lead to smaller biofilm growth when compared to the half and full media changes
- Results of this experiment lead to full media changes being used in other experiments involving multiple day biofilm assays
- Future work on media changes with static biofilms longer then 3 days would be beneficial

References

1. Inacio, MCS, Graves, SE, Pratt, NL, Roughead, EE & Nemes, S 2017, 'Increase in Total Joint Arthroplasty Projected from 2014 to 2046 in Australia: A Conservative Local Model With International Implications', *Clinical orthopaedics and related research*, vol. 475, no. 8, pp. 2130-7.
2. Kurtz, SM, Ong, KL, Schmier, J, Mowat, F, Saleh, K, Dybvik, E, Ksrrholm, J, Garellick, G, Havelin, LI & Furnes, O 2007, 'Future clinical and economic impact of revision total hip and knee arthroplasty', *JBJS*, vol. 89, pp. 144-51.
3. Mandell, JB, Orr, S, Koch, J, Nourie, B, Ma, D, Bonar, DD, Shah, N & Urish, KL 2019, 'Large variations in clinical antibiotic activity against Staphylococcus aureus biofilms of periprosthetic joint infection isolates', *Journal of Orthopaedic Research*, vol. 0, no. 0.
4. Römling, U & Balsalobre, C 2012, 'Biofilm infections, their resilience to therapy and innovative treatment strategies', *Journal of Internal Medicine*, vol. 272, no. 6, pp. 541-61.
5. Magana, M, Sereti, C, Ioannidis, A, Mitchell, CA, Ball, AR, Magiorkinis, E, Chatzipanagiotou, S, Hamblin, MR, Hadjifrangiskou, M & Tegos, GP 2018, 'Options and Limitations in Clinical Investigation of Bacterial Biofilms', *Clinical microbiology reviews*, vol. 31, no. 3, pp. e00084-16.
6. Macia, M, Rojo-Molinero, E & Oliver, A 2014, 'Antimicrobial susceptibility testing in biofilm-growing bacteria', *Clinical Microbiology and Infection*, vol. 20, no. 10, pp. 981-90.