UNIVERSITY OF Southampton

Title:	Infective endocarditis: a microfluidic approach to disease prevention and the development of bioinspired prosthetic replacement valves
From:	Mr Alex Daly with Dr Tim Millar
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Alex Daly is now a third year Medical student at the University of Southampton and was chosen as the applicant for the BMS summer studentship following a process open to all year two Medical students in the Faculty He was chosen from the 13 applicants on the basis of his personal statement and his interest in vascular research. He spent the summer months from July to August in my lab where he used his training in anatomy to dissect the aortic valve from pig hearts. Infective endocarditis is a disease that is difficult to treat, can disseminate rapidly around the body and can lead to permanent damage to the valve leaflets of the heart. Prevention of infection, dissolution of the biofilm and replacement valves are all potential goals of the current research.

Alex collected pig hearts from the local butcher and removed the valve leaflets and processed them in order to isolate cells for further culturing. He then compared different techniques of endothelial removal from the valve leaflet and also compared two different growth media.

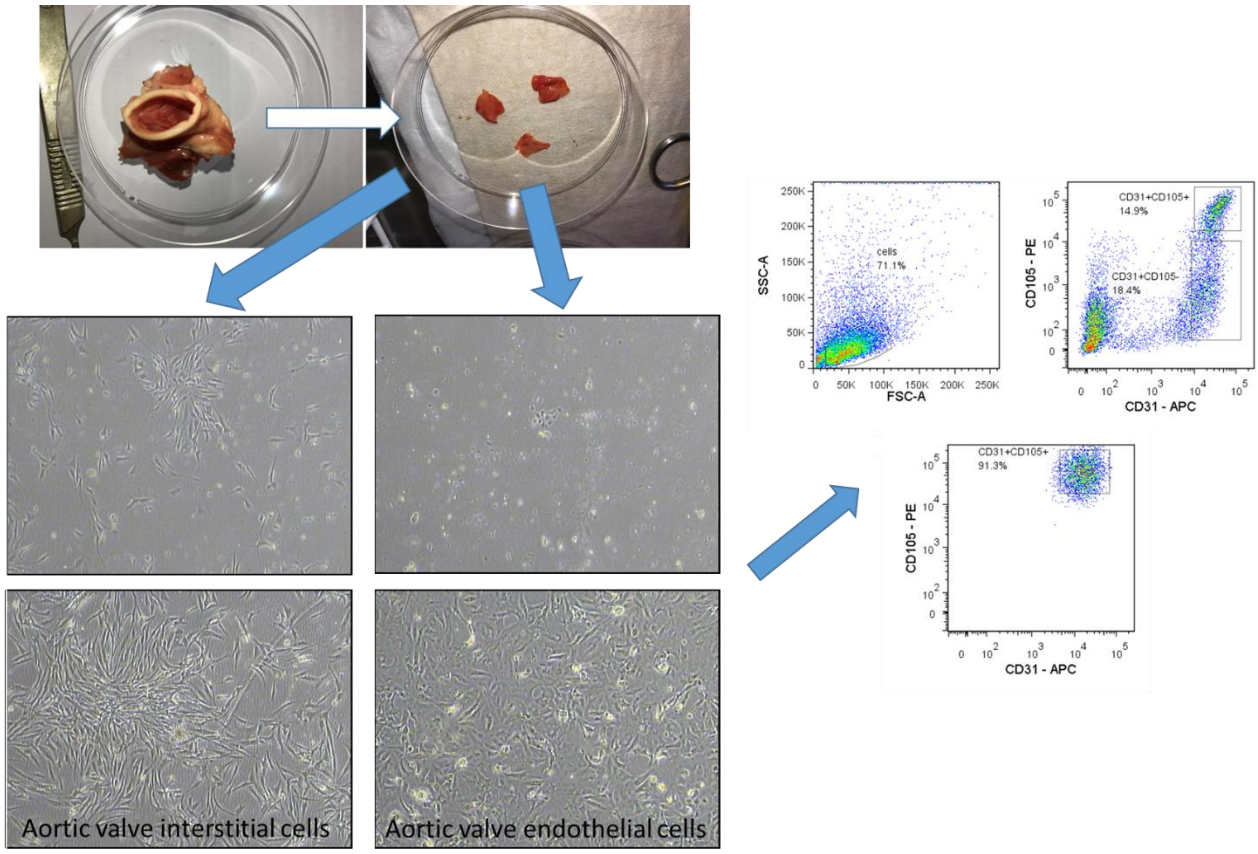


Figure 1. Dissection, culture and phenotyping of porcine valve aortic cells. The aortic valve was dissected from the pig heart and the individual valve leaflets of the tricuspid valve were removed. Incubated in collagenase, scraped and then cultured before phenotyping using endothelial marker CD31 and CD105 coexpression.

The aortic valves were dissected from the heart and their support before being incubated in collagenase solution. Alex then compared the removal of cells using a cell scraper or a cotton wool bud. The valves were gently scraped and the resulting cells plated onto collagen coated tissue culture plates. Initial experiments showed that cells could be removed aseptically and cultured without subsequent infection. The morphology of the cells isolated with the two techniques differed. Using the scraper, cells resembled a non-endothelial morphology whereas the cotton bud method generated the expected cobble stone morphology. Cells initially grew slowly in a 10% fetal calf serum based media. This was later replaced with a 20% human serum based media and cells proliferated much more quickly to reach confluence and retained their morphology. Using flow cytometry and comparing to HUVEC, the cotton bud method produced a mixed population that included endothelial cells whereas the cell scraper method looked to produce interstitial cells alone.

Alongside his endothelial culture work, Alex was using bacteria in microfluidic flow systems to see how they attach to matrix proteins under shear stress. This was to be followed by measuring the interaction of bacteria with porcine aortic valve endothelial cells when sufficient numbers and purity had been isolated. We had previously observed the interaction of *S. pyogenes* with matrix proteins and the endothelium under flow so we looked to repeat this with a lab strain of *E. coli* to allow us the future potential of genetic modification of the bacteria to increase or decrease adhesive capability. Compared to the *S. pyogenes* previously used, the *E. coli* strain showed fewer adherent bacteria to a variety of matrix proteins and to HUVEC under static and non-turbulent shear stress.

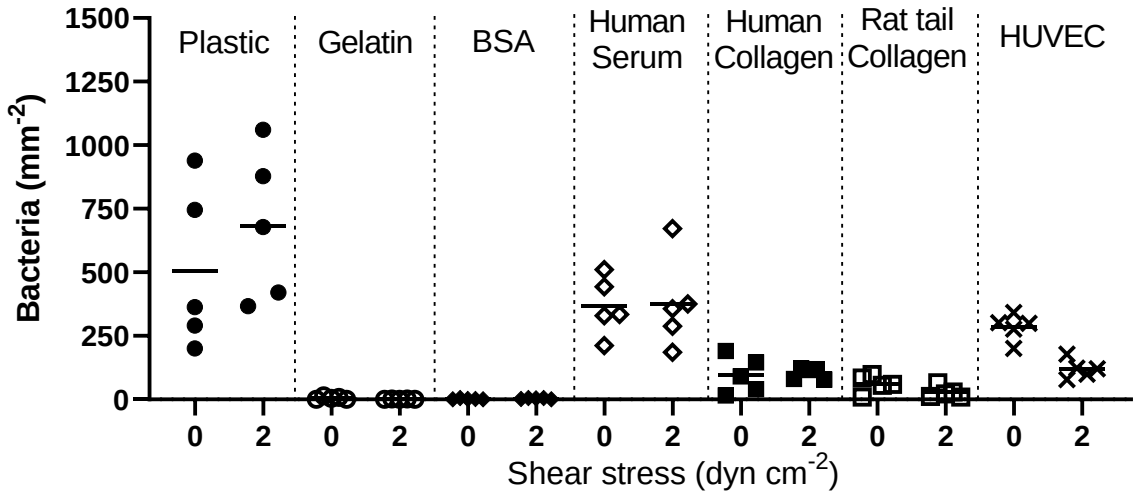


Figure 2. Bacterial adhesion to matrix proteins and endothelial cells under shear stress

Alex also established two assays of bacterial interaction: “autoaggregation” where bacteria form biofilms in solution and “Substrate biofilms” where bacteria attach to substrates. These processes are under the influence of signalling molecules and Alex looked at how these might be disrupted. We used uncoated 96 well plates initially with the intention of adding matrix proteins to see if substrate biofilm formation is affected.

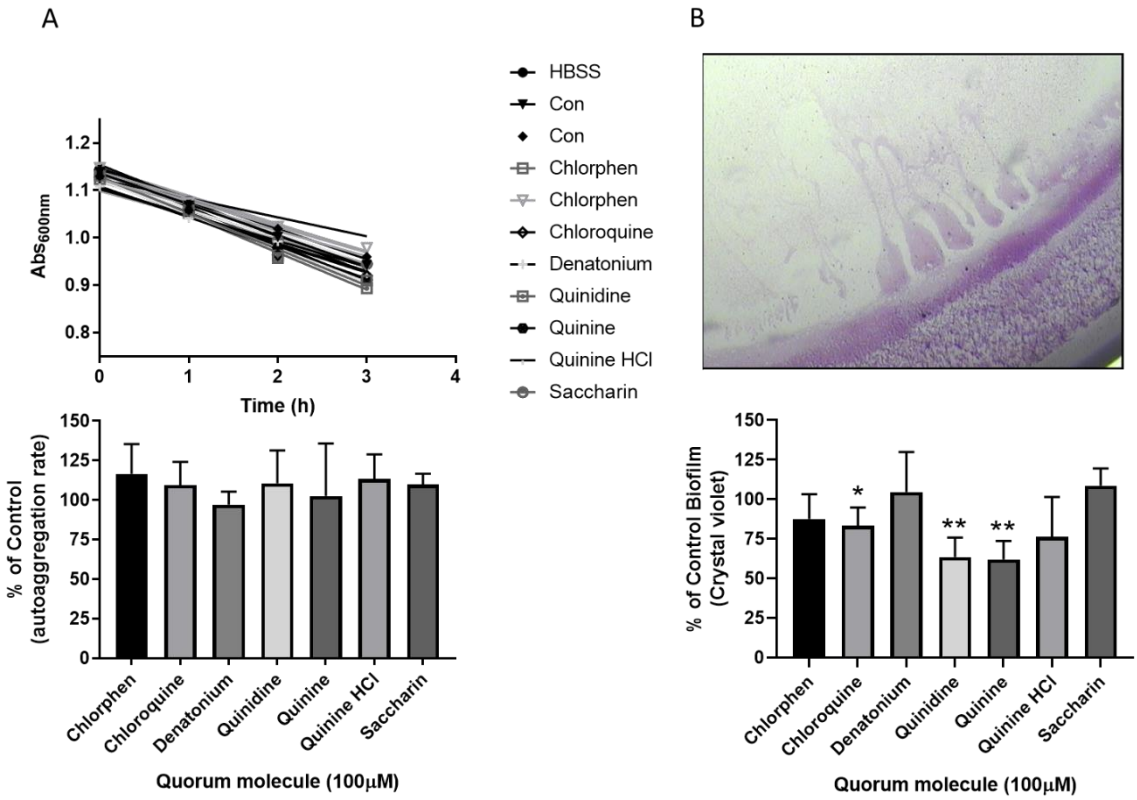
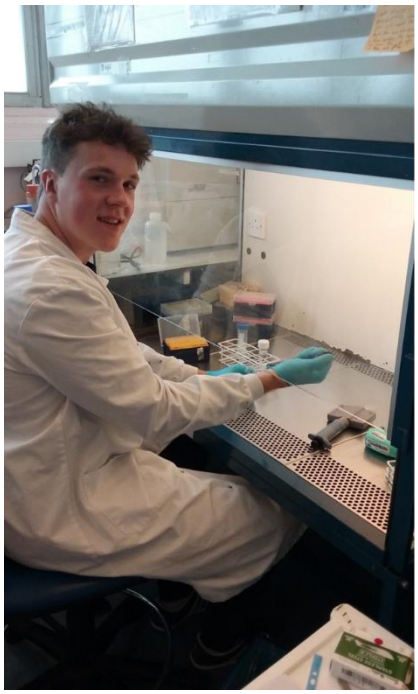


Figure 3. Bacteria – bacteria interaction and bacteria - substrate interaction in assays of biofilm formation

The bacteria were allowed to aggregate at 25C in Hanks buffer and the rate of clearing of the culture gives an indication of how aggregated the cells are. The quorum molecules did not cause any significant change to the rate of aggregation. However, the cultures were allowed to stand and a surface dependent biofilm form for 24h. The wells were then washed, fixed and stained for biofilms using crystal violet staining compared to non bacterial controls and bacteria in the absence of quorum molecules. Images were taken and then the crystal violet stain was solubilised and the absorbance measured as an indication of the amount of surface biofilm formed under each condition. Three of the quorum molecules, chloroquine, quinine and its stereoisomer quinidine reduced absorbance suggesting an effect on the *E. coli* biofilm.

Future direction

The data generated during this eight week project has shown that using a gentle isolation technique and the standard culture medium used in the lab, aortic endothelial cells can be isolated, cultured in the absence of infection and characterised. Alex was also able to generate data to compare against previous results from the lab with a different bacterial species to show how similar or different the phenomenon we wish to study is. By developing a new assay in the lab, this has expanded the techniques we can use to interrogate the host pathogen interaction. Hopefully, these preliminary data will help in generating grant income to pursue this research further.



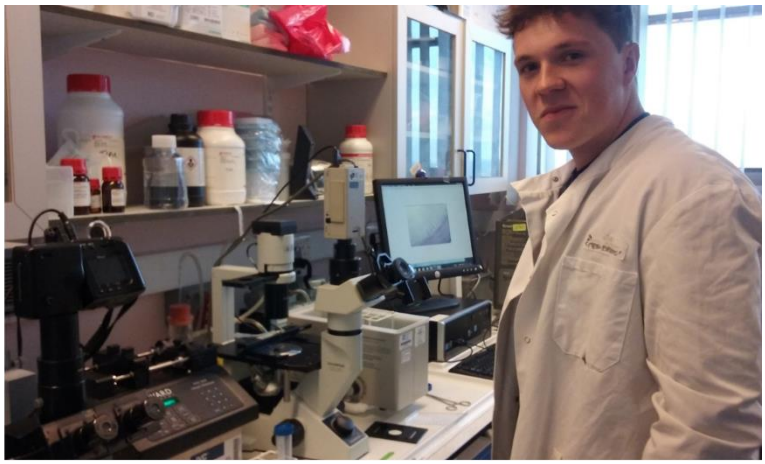


Figure 4. Alex Daly in the lab

Acknowledgements

Thanks must go to the BMS for providing Alex with this opportunity to carry out basic research in the lab that will hopefully provide him with a future interest in all things vascular. Also I would personally like to thank Jemma Paterson my PhD student who helped out with this project and made sure Alex was able to keep on track. Finally, my own personal thanks as we managed to make some progress in this project that is now being written in to an MRC Systems and Populations grant application with my colleagues in infection and engineering at the University of Southampton. Alex was excellent to work with and keen to develop his skills in the lab often making suggestions especially around dissections. He also organised organ collections and helped out in the lab when required. I would recommend that the BMS continue to support this initiative for as long as possible as it adds value to the individuals involved and lab where the works takes place as well as spreading the word of the good work that the society does.

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