Measuring Oxygen Concentration Under *Staphylococcus aureus* Biofilms in Response to Chemical Gradients in a Microfludic Device

Adit Dhummakupt[1], Philip C. Samson[2], Dmitry Markov[2], John P. Wikswo[2], Leslie M. Shor[3]

We have developed a procedure for creating patterned *Staphylococcus aureus* biofilms inside a microfluidic device capable of producing chemical gradients and detecting oxygen concentrations. Using this device, we were able to observe and quantify the reaction of a biofilm population that comes in contact with a harmful chemical, such as formaldehyde or phenol. Its defensive reaction is to decrease the permeability of the biofilm, thus shielding the core bacteria from the diffusion of chemicals and consequently lowering the oxygen concentration.

***Keywords* — Staphylococcus aureus, biofilms, oxygen concentration, microfluidic,**

I. Background

A microbial biofilm is a community of several species of bacteria attached to a surface through an extracellular matrix [1]. This extracellular matrix is composed of polymeric proteins, polysaccharides and DNA, and allows the bacterial colony to attach itself to both biotic and abiotic surfaces [2]. These structures are highly dynamic and include several layers of bacteria, each with different phenotypes [3], pores and networks for the flow of nutrients and waste, and the ability to quickly respond to external conditions [4]. Compared to the planktonic form of bacteria, this complex structure is highly resistant to antibiotics, toxins and even macrophages [5].

There are several advantages to studying these populations with a microfluidic device. They allow for the precise control of bacterial growth and the introduction of chemicals of interest [6]. These devices can also be easily coupled with sensitive detectors and sensors for quantitative data [7]. We have devised a method to form biofilms of *S. aureus* inside a microfluidic device that is capable of creating up to 4 chemical gradients. This device also features an oxygen-sensing film that fluoresces in the absence of oxygen.

Acknowledgements: This project was funded by NSF grant # 0649883 and the Searle-SyBBURE program at Vanderbilt University.

[1]Searle-Systems Biology and Bioengineering Undergraduate Research Experience, Vanderbilt University

[2]Vanderbilt Institute for Integrative Biosystems Research and Education, Vanderbilt University

[3]Department of Chemical, Materials and Biomolecular Engineering and Center for Environmental Science and Engineering, University of Connecticut

II. Experimental Data

A. Methods

The biofilms were formed using a micro-contact printing process that uses a PDMS stamp to transfer *S. aureus*. Bacterial dots were stamped onto a glass slide coated with a platinum porphyrin compound which acts as our oxygen-sensor. After incubation, a microfluidic gradient generator was placed on top of the biofilms and loaded with various chemicals. These chemicals diffused into the central chamber where the biofilms are housed.

B. Results

By measuring the fluorescence of the oxygen-sensing film over time, were discovered that *S. aureus* responded to a front of bactericide by substantially decreasing the permeability of the biofilm, consequentially decreasing the oxygen levels inside. The controls showed no response.

III. Conclusion

We present a device that can provide information on a biofilm's dynamic response to harmful chemicals. When subjected to a harmful chemical, the bacteria will respond by decreasing its permeability in an effort to reduce diffusion of the chemical to its core.

References

[1] O'Toole, G., Kaplan, H. B. & Kolter, R. (2000) Biofilm formation as microbial development. *Annual Review of Microbiology* **54**, 49-79.

[2] Davey, M. E. & O'Toole, G. A. (2000) Microbial biofilms: from ecology to molecular genetics. *Microbiology and Molecular Biology Reviews* **64**, 847-+.

[3] Ramos, C., Licht, T. R., Sternberg, C., Krogfelt, K. A. & Molin, S. (2001) in *Microbial Growth in Biofilms, Pt B* Vol. 337 *Methods in Enzymology* 21-42.

[4] Sutherland, I. W. (2001) The biofilm matrix - an immobilized but dynamic microbial environment. *Trends in Microbiology* **9**, 222-227.

[5] Costerton, J. W., Stewart, P. S. & Greenberg, E. P. (1999) Bacterial biofilms: A common cause of persistent infections. *Science* **284**, 1318-1322.

[6] Eun, Y. J. & Weibel, D. B. (2009) Fabrication of Microbial Biofilm Arrays by Geometric Control of Cell Adhesion. *Langmuir* **25**, 4643-4654, doi:10.1021/la803985a .

[7] Richter, L. *et al.* (2007) Development of a microfluidic biochip for online monitoring of fungal biofilm dynamics. *Lab on a Chip* **7**, 1723-1731, doi:10.1039/b708236c.