Mechanisms underlying bacterial interactions with breast implant textures

Sang Won Lee[1,2], Erick L. Johnson[3], J. Alex Chediak[2], Hainsworth Shin[2], Yi Wang[2], K. Scott Phillips[2]*, and Dacheng Ren[1,4,5]*

[1]Department of Biomedical and Chemical Engineering, Syracuse University, Syracuse, NY 13244, [2]United States Food and Drug Administration, Office of Medical Products and Tobacco, Center for Devices and Radiological Health, Office of Science and Engineering Laboratories, Division of Biology, Chemistry, and Materials Science, Silver Spring, MD 20993, [3]Mechanical and Industrial Engineering, Montana State University, Bozeman, MT 59717, [4]Department of Civil and Environmental Engineering, Syracuse University, Syracuse, NY 13244, [5]Department of Biology, Syracuse University, Syracuse, NY 13244, United States

Statement of Purpose: In 2011, the U.S. FDA first identified an association between textured breast implants and breast implant-associated anaplastic large cell lymphoma (BIA-ALCL)[1]. With additional evidence in the following years, it has become clear that the risk of BIA-ALCL is higher for textured implants, resulting in recall of some products in 2019. While the cause of BIA-ALCL is not well understood at this time, it has been hypothesized that bacterial biofilms on the implants may contribute to the pathogenesis of BIA-ALCL. In particular, it is believed that greater surface areas on textured implant surfaces lead to greater microbial bioburden, increasing the probability that chronic antigen stimulation will cross a hypothetical threshold to promote onset of ALCL. However, from existing evidence, the specific link between breast implant textures and bacterial interaction is still not clear. Thus, it is important to understand how such textures affect microbial adhesion and biofilm formation.

In this study, we developed a method to screen a library of polydimethylsiloxane (PDMS) textures composed of varying sizes of recessed patterns and distances between patterns. This high-throughput microplate assay allows us to quantify bacterial adhesion to different patterns in the library and better understand how surface topography affects bacterial adhesion. Confocal microscopy and computational simulation were used to validate the method and to identify the patterns associated with enhanced bacterial adhesion.

Methods: To obtain surfaces with topographic patterns of interest, a Si wafer with complementary patterns was fabricated at Cornell NanoScale Science & Facility. The Si pattern was then used as a master to fabricate PDMS, a mixture of 10:1 weight ratio of Sylgard 184 base and curing agent. *Escherichia coli* RP437/pRSH103 was cultured in tryptic soy broth (TSB) or lysogeny broth (LB) supplemented with 30 μg/mL of tetracycline and used in all tests of bacterial adhesion. A plate washer and a plate reader were used to conduct high-throughput assay measurements for bacterial attachment, and confocal and inverted fluorescence microscopy were used to visualize bacterial biomass in 3D.

Summary of the results: To systematically characterize how relevant surface topographies affect bacterial adhesion, 10 μm-deep recessive square-bottom patterns were fabricated with side lengths (2, 5, 10, 50, 100, 200, and 300 μm) and inter-feature (between recessive patterns) distances (2, 5, 10, 50, and 100 μm). The high-throughput adhesion assay was developed using the optimized conditions of the plate washer and the plate reader. In addition, the uniformity of the optimized assay protocol was validated using StarCCM+ computational software.

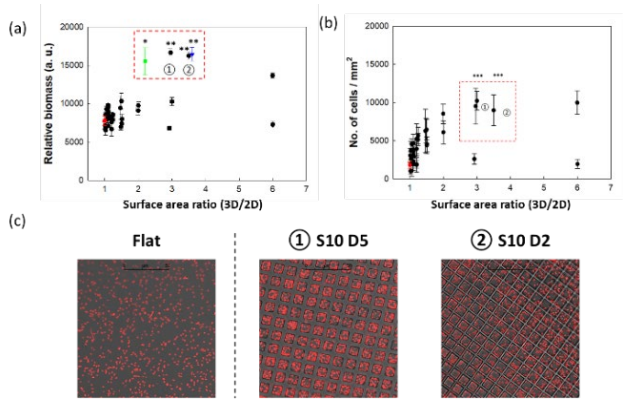

Figure 1: (a) Relative biomass of *E. coli* RP437/pRSH103 on up-right patterned PDMS surfaces after 4 h attachment under static condition (Red circle: flat control. Green square: commercial textured breast implant A. Blue triangle: commercial textured breast implant B. ①: S10D5. ②: S10D2) * $p<0.05$ and ** $p<0.01$. (b) The number of attached *E. coli* RP437/pRSH103 cells on upside down patterned PDMS surfaces after 4 h attachment under static condition. (Red circle: flat control. ①: S10D5. ②: S10D2) *** $p<0.001$. Representative fluorescent confocal microscopic images of up-right (c) flat, S10D5, and S10D2 are shown. S: a dimension of feature side (μm), D: a dimension of the distance between features (μm). Scale bar = 10 μm.

Bacterial adhesion (4 h) for red fluorescent *E. coli* RP437/pRSH103 on the library of surface topography patterns and coupons of two commercial implants (green and blue points) were tested. The three outlier patterns from the PDMS library, S5 D2, S10 D2, and S10 D5, plus two commercial implants showed significantly higher bacterial attachment than the flat control. In addition, we provided evidence that on the outlier patterns, cells prefer to attach on the convex edges inside the patterns.

Conclusions: A high-throughput microplate assay was developed and characterized to screen bacterial adhesion on different PDMS patterns. The results showed that *E. coli* adhesion did not follow a monotonic, linear relationship with surface roughness and surface area ratio but was rather affected by the specific topography (size and spacing) of these recessive patterns.

References: 1. Questions and Answers about Breast Implant-Associated Anaplastic Large Cell Lymphoma (BIA-ALCL). U.S. Food and Drug Administration, 2019.