Controlling *S. epidermidis* Colonization by Surface Micropatterning

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Purpose: Infection as a failure mode of orthopedic implants is being increasingly recognized. There is often no alternative to resolve infection except to remove the implant altogether. The revision surgeries have significant consequences on both patient health and cost. Importantly, implant surfaces have not been engineered to control their interactions with bacteria. They have instead been optimized based on their mechanical and osseointegration properties. Our interest is to create surfaces that preserve osseointegration but which preferentially repel bacteria or hinder their proliferation and, hence, reduce the risk of infection. We have been exploring how to control staphylococcal interactions with surfaces modified by sub-micron cell-repulsive features patterned at micro length scales on otherwise cell-adhesive surfaces. We have recently shown that the adhesion of staphylococcal bacteria can be substantially reduced while preserving significant cell-adhesive character, and that lateral confinement affects the phenotypic behavior of growing *S. epidermidis* colonies [1]. Here we describe experiments to study how laterally confined *S. epi* colonies interact with each other with implications on how confinement may reduce infection.

Methods: We constructed patterns of discrete PEG hydrogels on Si wafers (5 mm x 7 mm) by irradiating solvent-cast thin films of monoamine-terminated poly(ethylene glycol) [PEG; M_w=5 kDa] using 10 keV focused electron beams in a field-emission LEO 982 SEM [2, 3]. These irradiated regions become both crosslinked and bound to the substrate. After e-beam exposure, unirradiated PEG was removed by water washes. We created various patterns following methods similar to those of e-beam lithography. The various surfaces were exposed for 5 min to ~5 µl of *S. epi* inoculum (10^8 cfu/ml), flooded with Tryptic Soy Broth (TSB), and then cultured at 37 ^oC for periods ranging from 5 min to 24 hrs. After multiple PBS rinses, the samples were fixed for 15 min in 4% paraformaldehyde, rinsed in DI water, air dried, and examined either in the SEM (2 kV) or in a Nikon E1000 optical microscope.

Results: Fig. 1 describes the area fraction of unpatterned Si covered by *S. epi* as a function of culture time. Digital images were collected via light microscopy (1 kx) from samples removed at different time points. The images were binarized and the area fraction covered by bacteria was calculated. A confluent layer forms after ~10 hours. The inset images show that the inoculum contains few multi-cell clusters and that the early stages of growth are primarily in 2D. Importantly, the images show that a retiform (networked) structure develops as the colonies try to connect with each other. Figure 2 shows SEM images of *S. epi* on patterned surfaces after 5 hours of culture. *S.epi* was confined in the circles (20 µm dia; light contrast fig. 2 center) of Si surrounded by cell-repulsive e-beam patterned PEG gel (black fig. 2 center). The independent variable here was the distance between adjacent cell-adhesive circles. The circles were separated by PEG gels 2 µm, 5 µm, or 8 µm wide. The growing bacterial colonies can bridge 2 and 5 µm distances but not the 8 µm or higher (not shown). Ongoing work addresses other spatial patterns, bacterial culture times, and the effects of flow on laterally confined *S. epi* colonization.

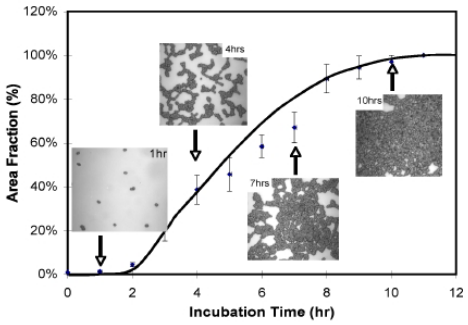

Fig. 1 – *S. epi* surface coverage on silicon.

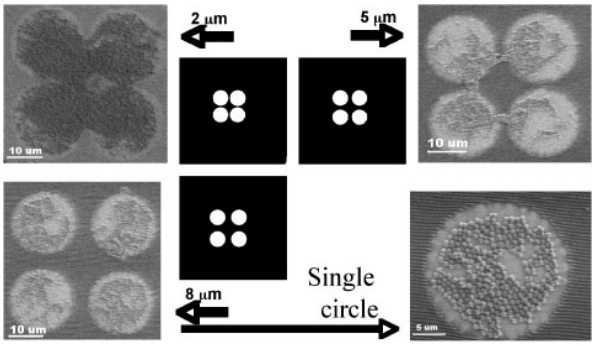

Fig. 2 - Bridging of *S. epi* colonies across cell-repulsive patterned hydrogels

Conclusions: Surface micro and nano patterning by PEG hydrogels can modulate the adhesion of S. epi on an otherwise adhesive surface. Our results suggest that lateral confinement can hinder biofilm development even if adhesion has occurred. The ability of adjacent colonies to network across cell-repulsive surface suggests that mechanisms exist – e.g. quorum-sensing - which can influence the cooperative colony development.

References: [1] P. Krsko, J. Kaplan, M. Libera, Acta Biomaterialia, in press (2008). [2] P. Krsko et al. in: Nanofabrication: Technologies, Devices, and Applications II, SPIE, Bellingham, 2005, p. 600201. [3] P. Krsko, S. Sukhishvili, M. Mansfield, R. Clancy, M. Libera, Langmuir 19 (2003) 5618-5625.

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