Electron-Beam Patterned Surfaces to Control *S. aureus* and Osteoblastic Cell Adhesion

Yi Wang[a], Guruprakash Subbiahdoss[b], Jan Swartjes[b], Henny C. van der Mei[b], Henk J. Busscher[b], Matthew Libera[a]
[a] Department of Chemical Engineering and Material Science, Stevens Institute of Technology, Hoboken, New Jersey 07030
[b] Department of Biomedical Engineering, University Medical Center Groningen, University of Groningen, The Netherlands

Introduction and Purpose: A broad range of biomaterials synthesis and surface modification techniques have been introduced to provide good tissue integration properties. Typically, however, surfaces optimized to enable good tissue integration are also adhesive to bacteria, and the infection of implants has thus become a leading failure mechanism [1]. Based upon differences in their characteristic size and adhesion mechanisms, there is a window within which to design surfaces with nano and micro scale modulations in cell adhesiveness whose interactions with tissue cells and bacteria are substantially different. Here we study interactions of an osteoblast cell line and staphylococcal bacteria with surfaces whose cell adhesiveness is modulated by cell-repulsive gel particles patterned on an otherwise cell-adhesive surface. The patterns are formed by electron-beam processing of PEG (poly(ethylene glycol)) hydrogels, a material whose antifouling properties are well established. We explore inter-gel spacings of 0.5 µm to 3 µm and assess how these surfaces differentially interact with desirable tissue cells and undesirable bacteria. We use flow-cell based *in vitro* experimental models to study both the individual (monoculture) and simultaneous (co-culture) adherence and proliferation of both bacteria and eukaryotic cells. A "race for the surface" *in vitro* co-culture experimental model was developed recently [2] by studying staphylococcal bacteria and osteoblastic cells during a simulated surgical process in a flow chamber.

Methods: A low-energy electron beam (2 keV) was used to create surface-patterned hydrogels of PEG on glass. By controlling the specific electron exposure conditions, the resulting crosslinked gel diameter was about 400 nm. The patterns were designed to be 3x6 arrays with 6 different inter-gel spacings (0.5 µm, 1 µm, 1.5 µm, 2 µm, 2.5 µm, 3 µm). Each pattern was copied three times on the same substrate. Details of the substrate preparation and electron-beam patterning process have been described [3]. Osteoblastic sarcoma cells (U2OS) and S*taphylococcus aureus* (*S. aureus*) were cultured and harvested following a procedure established using a modified culture medium composed of 98% DMEM + FBS (tissue growth medium) and 2% TSB (bacterial medium) [2]. The *S. aureus* adhesion experiment with a flow shear rate of 11 s^{-1} was used to define the proper density of bacteria in the medium before adding the U2OS solution (~10^5 cm^{-2}). The flow chamber was flushed using PBS after initial adhesion to remove the bacterial suspension. The U2OS cell solution was then introduced and sustained for 1.5 hrs to allow adhesion and spreading. Modified medium was then continuously flowed in at a low shear rate of 0.14 s^{-1} for 48 hrs.

Results: Images of *S. aureus* on gels patches were captured at 3 min intervals to determine the number of *S. aureus* as function of time. After 30 min, the density of *S. aureus* reached 10^5 cm^{-2} on the unmodified bare glass surface used as a control in these experiments. Importantly, fig. 1 shows that the ability of the gel-modified surfaces to repel *S. aureus* is a strong function of the inter-gel spacing. Relative to unmodified glass, patterns with 1 µm and 1.5 µm inter-gel spacing enable 77% and 60% fewer bacteria to adhere, respectively, after 3 hrs of *S. aureus* exposure. Fig. 2 shows SEM images of U2OS cells cultured on PEG gels with a 1.5 µm inter-gel spacing for 40 hrs. 0.5 µm inter-gel spacings are not only repulsive to *S. aureus* but also to U2OS cells, because 400 nm dia gels spaced at 0.5 µm form an almost continuous cell-repulsive surface. Increasing the inter-gel spacing exposes more cell-adhesive surface between the PEG gels. U2OS cells start to adhere on patches with gels spaced at 1 µm and spread well on patches with gels spaced at 1.5 µm despite the fact that such surfaces contain a significant portion of cell-repulsive character.

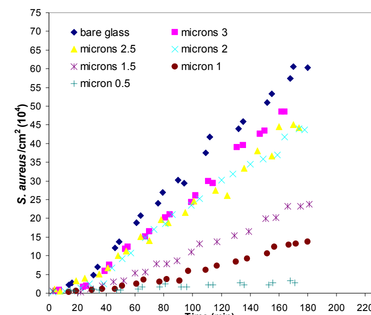

Figure 1. Initial adhesion of *S. aureus* monoculture in PBS solution.

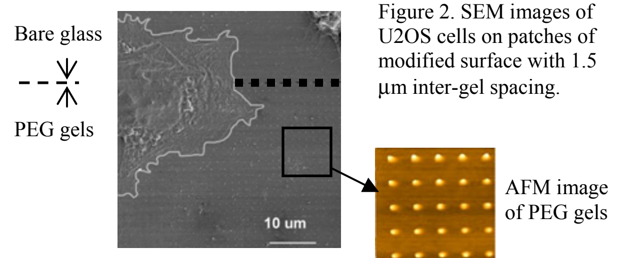

Figure 2. SEM images of U2OS cells on patches of modified surface with 1.5 µm inter-gel spacing.

Conclusions: By modulating cell adhesiveness using submicon cell-repulsive features on an otherwise cell-adhesive surface, we have begun to identify a regime where *S. aureus* adhesion can be significantly reduced while osteoblast adhesion and spreading is preserved. Such structures may lead to new implant surfaces that can preserve heathly tissue integration while reducing the susceptibility of such surfaces to infection.

References:

1. D. Campoccia, Biomaterials 2006; 27; 2331-9
2. G.Subbiahdoss, Acta Biomaterialia 2009; 5; 1399-1404
3. P. Krsko, Langmuir 2003; 19; 5618-5625