Engineered Block Copolymer Surface Scaffolds for High Avidity, Lectin-Based Microbe Capture

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Statement of Purpose: Using lectin proteins as capture molecules in cellular screening assays allows for isolation of target cells based on extracellular glycan content. This method offers the advantage of highly specific, non-destructive, reversible catch and release of cells. Despite these advantages, capture lectins are often limited by their inherently weak association with oligosaccharides (K_D ~ 10-100 μM). We aim to address this limitation by developing surfaces containing patterns of micro-structured, dually reactive block copolymer films that can be functionally modified with high concentrations of lectins, creating high-avidity surface scaffolds for improved cell capture. In this report, we discuss our current efforts to optimize scaffold structure and shape to fully promote cell capture efficiency.

Methods: The block copolymer poly(glycidyl methacrylate)-block-4,4-dimethyl-2-vinylazlactone (PGMA-b-PVDMA) was polymerized in solution to block lengths of 56 and 175 monomers, respectively, using RAFT polymerization according to previous protocols.[1] Patterned films of PGMA-b-PVDMA were formed over silicon and quartz surfaces by patterning photoresist using standard photo-lithography, followed by spin coating a 1 wt % solution of the copolymer (1500 rpm, 15 s), and annealing (110 °C, 18 hrs) to allow for reaction of the PGMA block with the oxide-bearing surface. Photoresist was then removed with wet lift-off. Wheat germ agglutinin (WGA) lectin was coupled to the polymer films through reaction with the azlactone groups present in the PVDMA blocks to create the lectin-functionalized scaffolds. *Pseudomonas fluorescences* (GM30) and *E. coli* (HB101) microbes expressing extracellular N-acetyl-D-glucosamine (specific to WGA) were then incubated over the polymer scaffolds.

Results: Circular scaffolds of the PGMA-*b*-PVDMA copolymer enhanced the lectin-based capture of microbes by up to 43% relative to non-polymeric control scaffolds.[2] To further investigate the effect of scaffold shape with microbe capture, the polymer scaffolds were patterned into lines, grids or circles of varied dimensions with feature sizes down to 2 μm. AFM and SEM images of these scaffolds (Fig. 1 and 2A) revealed structures with

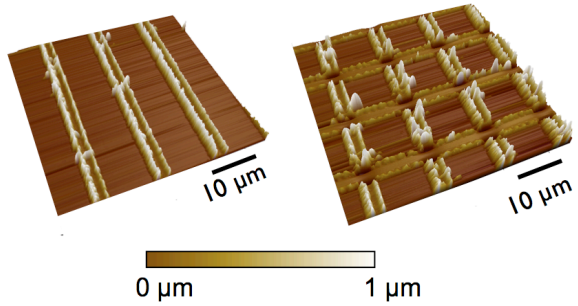

Fig 1. AFM images of polymeric line (left) and grid (right) scaffolds.

pronounced surface topology and high surface roughness towards the scaffold edges. Upon functionalization of the scaffolds with WGA lectins and contact with *P.fluorescence* microbes, preferential adhesion and aggregation on the scaffold edges was noted (Fig 2B). This suggests that the combination of high surface roughness, micron-scale topology and lectin functionality is critical for promoting efficient microbe capture.

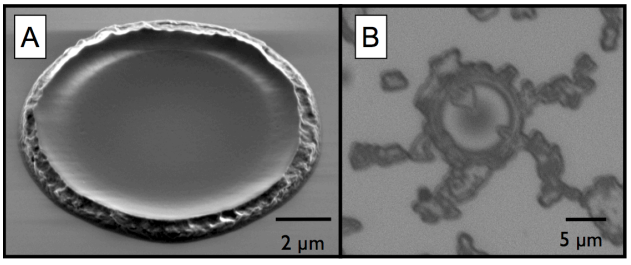

Fig 2. A) SEM image of a 10 μm diameter circular polymer scaffold. **B)** 100X brighfield image of microbial aggregation around the edges of a lectin-functionalized polymer scaffold.

Conclusions: The PGMA-*b*-PVDMA copolymers can be patterned over solid substrates to create high-avidity surface scaffolds for microbe capture based on extracellular glycan composition. The continued refinement of this approach will identify optimal scaffold shapes, dimensions and surface roughness to maximize microbe contact area and promote improved capture. This material can then be integrated into static or flow-based screening assays aimed at isolating specific microbes from complex media or from larger microbial communities.

References: ([1]Lokitz BS. et. al., Macromolecules 2012; 45, 6438-6449; [2]Hansen RR. et. al., Biomacromolecules 2013;14:3742-3748)