Development of Nanoscale Hydrogel Coatings for High Speed Cell Sorting

Jacob Lilly[1], Gabriela Romero[1a], Weijie Xu[1b], Hainsworth Y. Shin[2], Vivek Balasubramaniam[3], Brad J. Berron[1]

1 – Department of Chemical and Materials Engineering, University of Kentucky, Lexington, KY

2 – Department of Biomedical Engineering, University of Kentucky, Lexington, KY

3 – Department of Pediatrics, University of Wisconsin, Madison, WI

a – Currently at Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA

b – Currently at DSM Biomedical, Exton PA

Statement of Purpose: Antigen-Specific Lysis is a new method for high-purity, high-speed sorting of cells. The process consists of depositing a protective coating on only antigen-expressing cells, lysing all unprotected cells by chemical attack, and the removal of the protective coatings. In this work, we examine the relationship between the structure of the hydrogel coating and the protection afforded against a damaging chemical species. This work primarily focuses on the transport of macromolecular species through ~100 nm thick PEG diacrylate coatings. These thin coatings bridge the thickness gap between existing PEG diacrylate coatings and layer-by-layer materials. The significance in these materials also extends to immunoprotection, where these coatings are 1000X thinner than previously described PEG diacrylate coatings used for the immuno-protection of pancreatic islets (Cruise GM. Biomaterials. 1998;14:1287-1294).

Methods: Cell coating: Human Jurkat cells were sequentially incubated in biotinylated mouse anti-human CD45 and streptavidin-eosin conjugates. The cells were dispersed in 300 μL of 420 mM PEG diacrylate, 21 mM triethanolamine, and 35 mM vinyl pyrrolidinone (aq.). The solution was purged with N_2 for 5 minutes. Then, a photopolymerization reaction was initiated with an LED lamp (THORLabs, 530 nm light, 30 mW/cm^2) for 10 minutes. After polymerization, the cells were rinsed with PBS. Nucleus Coating: The nuclei of human dermal fibroblast cell samples were permeablized in 0.01% Triton X-100 in PBS for 7 minutes and coated with PEG diacrylate, as described previously (Avens HJ. J Histochem Cytochem. 2011; 59:76-87). Diffusion Kinetcs: Human dermal fibroblast cell samples were washed twice with transport buffer (Mohr, D. Embo Journal. 2009;28:2541-2553). Diffusion kinetics were analyzed with a Leica AOBS TCS SP5 confocal microscope (Leica Microsystems, Germany) equipped with a HyD photon-counting hybrid detector. The focus was adjusted to inside a coated nucleus. An automated timed scan was initiated, with an image taken every 1.5 seconds. After 3 scans, 100 μL of FTIC dextran in transport buffer was pipetted directly onto the scanning region and the automated scanning program was allowed to run for at least 160 seconds.

Results: The nucleus of a fibroblast was coated with a PEG diacrylate hydrogel coating, and the fluorescence inside the nucleus was measure with respect to time after introduction of fluorescent dextran to the permeablized cell. Large molecular weight (10 and 20 kDa) dextrans were completely excluded from coatings made from PEG diacrylates of monomer lengths 575 and 3500 kDa. On this basis, coatings were estimated to have a mesh size <3.7 nm. Low molecular weight materials (fluorexcein and DAPI) crossed the hydrogels unimpeded. 4kDa dextrans slowly permeated across the coating. The transit of 4kDa dextrans supported a mesh size to be >1.3 nm in these two coating materials. This molecular weight cutoff behavior supports these coatings behave similarly to bulk scale PEG diacrylate hydrogels, and the magnitude of this cutoff agrees well with similar bulk PEG diacrylate materials, despite being only ~100 nm thick. Further, the diffusion constant of the 4 kDa dextran were estimated for coatings of both molecular weight precursors. The greater diffusivity of the larger precursor, again support these coatings behave analogously to their bulk counterparts. Finally, these coatings have been used to specifically protect cells from lysis, where cell populations in blood have been purified to >99% purity.

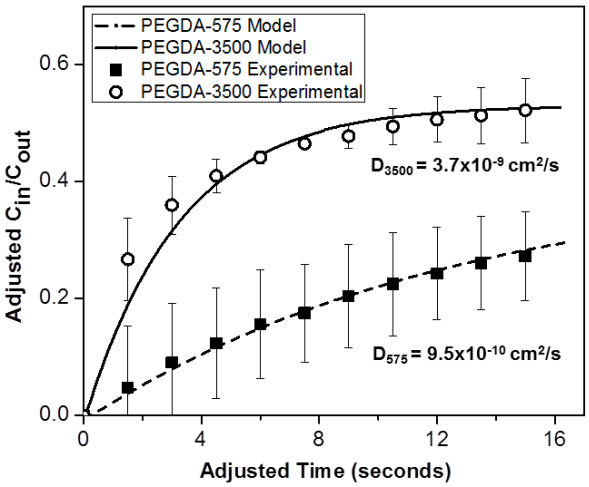

Figure 1. Diffusivity modeling of 4 kDa dextrans across ~100 nm coatings on cellular substrates.

Conclusions: In this work we determined the mechanism of action for protective nanoscale hydrogel coatings is largely size-exclusion based. Time-dependent, photon-counting confocal microscopy provides a polymer mesh size estimate of 1.3 - 3.7 nm for coatings formed from PEG diacrylate macromers of 575 and 3500 kDa in length. As such, we expect these antigen-specific coatings to protect cells against all harmful materials greater than 4 nm across (e.g., complement proteins). Fluorescent analysis indicates films are only 100-200 nm in thickness, which is consistent with previously reported data on glass substrates. Diffusion coefficients of 4kDa FD permeation through films are in good agreement with reported trends and magnitudes in bulk crosslink hydrogel materials. Additionally, we expect this photon-counting CSLM diffusion measurement technique to serve as a convenient assay of coating integrity and diffusive performance on other encapsulated cellular materials.