Mechanically- & Biologically-Tunable Polyethylene Glycol Hydrogels for Quantitative Neutrophil Migration Assays

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Statement of Purpose: Extracellular matrix (ECM; proteins, proteoglycans, growth factors) is highly varied and specialized throughout the body. ECM physical (e.g. pore architecture, Young's modulus) and biological (e.g. cell binding site type/density) properties govern local cell behavior. ECM properties are dynamic; pathological ECM remodeling is associated with destructive inflammation in chronic inflammatory disorders. The mechanisms behind this remodeling are difficult to elucidate *in vivo,* and *in vitro* models can fail to accurately replicate human ECM. Here, we present two *in vitro* porated polyethylene glycol (PEG) gels models of human ECM for inflammatory assays. First, we use urea salt leached PEG gels to study the role of ECM mechanics, architecture and cell binding sites on neutrophil (PMN) chemotaxis along a interleukin (IL)-8 gradient in extravascular space. Second, we created thin, basement membrane-like porated PEG membranes by dissovling embedded polystyrene beads to asses the role of the vascular BM in PMN extravastion.

Methods: Porated PEG networks for PMN chemotaxis were created using 4, 10 and 20kDa PEG and a urea salt leaching protocol. PEG (Polysciences) was modified to form PEG-diacrylate (PEG-DA). RGD (Arg-Gly-Asp) and human plasma fibronectin (hpFN) were conjugated to acryloyl-PEG-N-hydroxysuccinimide (3500Da, JenKem) for biologically functionalized gels. PEG-DA (± protein), urea, and acetophenone/n-vinylpyrrolidone cross linker were solubilized in phosphate buffered saline. The solution was dried to allow urea crystal nucleation and grow. Gels were then cross-linked with UV light and rinsed to remove the urea. Load-deformation analysis was used to determine the gels' Young's moduli. Pore size and density were determined by scanning electron microscopy (SEM). Fresh human PMNs were isolated from donated blood via plasma separation and a Ficoll gradient and used in chemotaxis assays (IL-8 gradient); results from gels were compared to polycarbonate transwells (TWs; Corning, Inc.) and human amniotic membrane (AM). To create BM-like PEG gels, PEG-DA was solubilized with 3-μm diameter polystyrene beads (Polysciences.) and Irgacure 2959 (1 or 3%). Cross-linked gels were rinsed in tetrahydrofuran and methanol to selectively dissolve polystyrene and leave PEG. SEM and mechanical testing were conducted as stated above to determine the gels' physical properties and to confirm the polystyrene removal. Optical coherence tomography (OCT; Thor Labs) was used to determine BM-like gel thickness.

Results: Urea salt leaching formed complex, 3D, inter-connected pore networks in PEG similar to ECM (Fig.1A-C). PEG molecular weight (MW), drying time, and PEG and urea concentration dictated pore size and density. AM was found to have small pores (0.47 ± 0.03μm) and a high pore density ($7.08\times10^5 \pm 3.84\times10^4$pores/mm^2). PEG pore diameter ranged from 0.46±0.04μm to 10.92±0.83μm and pore density ranged from $4.67x10^3$±553 pores/mm^2 to 2.84×10^5±4.13×10^4 pores/mm^2 across all conditions tested. Porating PEG gels lowered their Young's moduli to ~3kPa (Fig.1E); this is comparable to AM, in contrast to TWs (E: ~2GPa). Gels were functionalized with RGD and hpFN; small peptides did not influence the gels' physical properties, but whole protein decreased pore diameter and increased Young's modulus. Chemotaxis assays with human PMNs were conducted with and without IL-8 (Fig.1G); IL-8 significantly increased PMN movement confirming active chemotaxis in this system. Replicating chemotaxis assays across PEG gels of varying physical and biological properties confirmed that PMN chemotaxis is sensitive to ECM physical and biological remodeling.

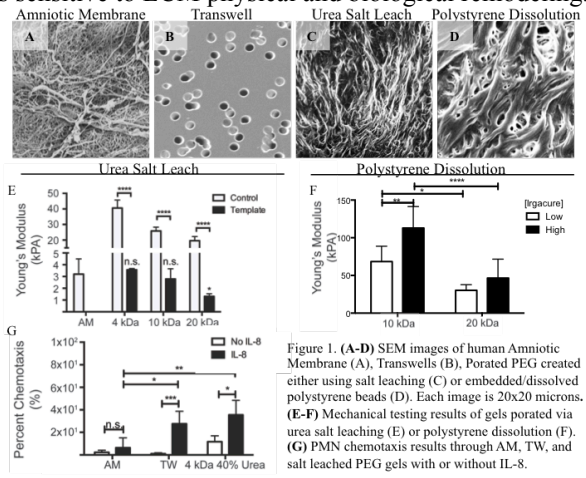

Figure 1. **(A-D)** SEM images of human Amniotic Membrane (A), Transwells (B), Porated PEG created either using salt leaching (C) or embedded/dissolved polystyrene beads (D). Each image is 20x20 microns. **(E-F)** Mechanical testing results of gels porated via urea salt leaching (E) or polystyrene dissolution (F). **(G)** PMN chemotaxis results through AM, TW, and salt leached PEG gels with or without IL-8.

Thin, BM-like porated gels had higher Young's moduli than salt leached gels (Fig.1F), but remain more similar to soft tissue than TWs. PEG MW and crosslinking dictated gel mechanics. Gel thickness, as determined by OCT, ranged from 0.08 to 0.018 mm (TWs: 0.01mm thick).

Conclusions: Porated PEG gels can be used for modular, biomimetic, immunoassays to replicate the complexity and diversity of human ECM *in vitro*. Urea salt leached PEG gels serve as mechanistic models to explore chemotaxis in 3D ECM, such as interstitial tissue. Using these gels, we demonstrate that within a physiological range of soft tissues (<5 kPa), less stiff (lower Young's moduli), more locally elastic gels (higher PEG MW) are more supportive of PMN migration. Similarly, small pores and high pore densities further promote PMN chemotaxis in response to IL-8. We confirmed prior results showing that a dearth or an excess of cell binding sites reduces chemotaxis. We next presented thinner, porated gels that replicate specialized ECM, such as vascular BM. These gels offer mechanical and biological advantages to current models, i.e. TWs, while maintaining their thin structure. Overall, PEG gels improve *in vitro* ECM models and provide more relevant results of immune cell migration in mechanically and biologically complex settings.

References: Zawko, SA. Acta Biomater. 2010; 6: 2415–2421. Liu, KK. J. Phys. D Appl. Phys. 2001; 34: L91-L94. Parkhurst, M. Biophys. J. 1992; 61, 306–315. Lauridsen, H. Technology. 2014; 02, 133-143. Lauridsen H. FASEB J. 2014; 28, 1166-80.