Fibrous Hyaluronic Acid Scaffolds with Engineered Degradation through MMP Sensitivity

Ryan J. Wade[1], Jason A. Burdick[1,2]
[1]Department of Materials Science and Engineering, [2]Department of Bioengineering: University of Pennsylvania

Statement of Purpose: Electrospun nanofibers have shown promise as tissue engineering scaffolds to replicate the size, mechanical structure, and anisotropy found in native extracellular matrix (ECM) [1]; however, there has been little work towards engineering nanofibrous scaffolds that degrade through matrix metalloproteinases (MMPs), proteases involved in the cell-mediated degradation of native ECM. In particular, recent studies with MMP-sensitive hydrogels have demonstrated the importance of MMP susceptibility in temporally modulating cell-material interactions [2]. Thus, incorporating MMP sensitivity into a synthetic macromer capable of nanofibrous formation would allow for tissue engineering scaffolds that mimic ECM structural features and biologically relevant degradation mechanisms in response to MMPs. Towards this goal, we introduce a new hyaluronic acid (HA) (a linear polysaccharide found throughout the ECM that is known to influence cell behavior) macromer that is both photopolymerizable and MMP sensitive to create nanofibrous scaffolds.

Methods: HA-tetrabutylammonium (HA-TBA) [3] was dissolved in DMSO (Fisher) and reacted with N-(2-Aminoethyl)maleimide trifluoroacetate salt (Sigma) and BOP coupling agent (Sigma) under N_2 to yield maleimide HA (MaHA). MMP susceptible peptides (GCNS-*GGRM-SMPV*-SNGG-Methacrylate) were next synthesized using a PS3 automated solid phase peptide synthesizer (Protein Technologies, Inc) and standard FMOC chemistry. MaHA was then reacted with the peptide (maleimide and thiol from cysteine group) in DI H_2O to yield the final product MePHA (*Me*thacrylated *P*eptide HA) (Fig.1A).

50 µl solutions of 2 wt% MePHA (15% of HA repeats modified with methacrylated peptide) were exposed to 15 mW/cm^2 UV light (365 nm) for 15 minutes to induce gelation, then equilibrated for 48 hours in 500 µl of TTC buffer (0.05% Triton X-100, 50mM Tris-HCl, 1mM $CaCl_2$). Gels were then degraded in the presence of 18 U/ml Type II Collagenase (Worthington Biochemical) before mass loss analysis via uronic acid assay, as previously described [3]. Nanofibers were electrospun using a 1 wt% solution of MePHA (40% of HA repeats modified) with 4 wt% PEO (900kDA) in DI H_2O through an 18 gauge needle set to 1.2ml/hr flow rate, 22kV voltage, and 18 cm collection distance. SEM images were taken using a FEI Quanta 600 ESEM. Fluorescent images were taken using an Olympus BX51 microscope.

Results: Rheology data indicated that gelation occurred within 5 minutes when 2-3 wt% MePHA solutions were exposed to UV light (365 nm) with maximum storage moduli ranging from 1-6kPa, depending on the degree of HA functionalization, intensity of UV light, and wt% of MePHA. 2 wt% gels of MMP sensitive MePHA fully degraded in the presence of Type II Collagenase after 4 days; however, MMP insensitive gels (scrambled amino acid sequence), remained stable after 20 days under identical conditions (Fig. 1B).

To demonstrate electrospinning capability, a 1 wt% solution of MePHA with 4% PEO as a carrier polymer was electrospun, collected onto an aluminum sheet, and imaged under SEM (Fig. 1C). The fibers crosslinked with exposure to UV light and swelled in an aqueous environment. To visualize hydrated fibers, fluorescent peptides were synthesized (sequence: GCKK-FITC) and conjugated to the macromer prior to electrospinning (Fig. 1D). Swollen fibers were 2.0 ± 0.5 µm in diameter and regular in shape. Fiber orientation is altered through the rate of spinning of a collecting rotating mandrel.

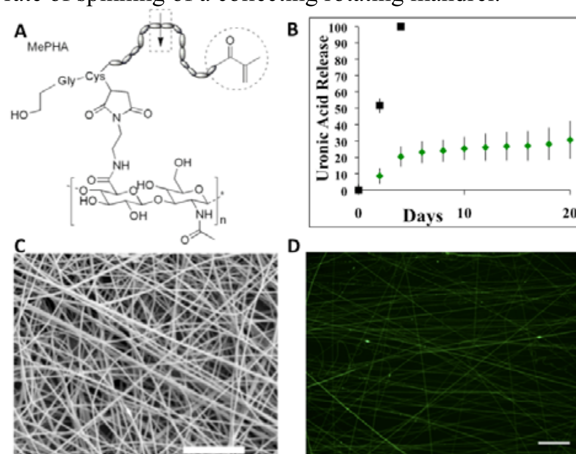

Figure 1: (A) MePHA: the down arrow indicates the site of MMP cleavage (dotted rectangle) while the methacrylate group is capable of photopolymerization (dotted circle). (B) Uronic acid release of MMP sensitive (■) or insensitive (◆) gels in Type II Collagenase. (C) SEM and (D) fluorescent image of a MePHA nanofibrous scaffold (Scale Bar: (C) 10 µm, (D) 100 µm).

Conclusions: While the importance of fibrous topography in directing cell-material interaction is appreciated, there has been little work in fibrillar scaffolds towards engineering MMP sensitivity, a biologically relevant mode of degradation. Collagen, a natural component of ECM that is degraded in the presence of MMPs has been electrospun (type I, II, and III) into fiber form; however, electrospun fibers require non native cross-linking (typically gluteraldehyde vapor), and the conditions required to isolate and reprocess collagen into fibrous form inhibits its native conformation [4]. Here, we developed an MMP sensitive, photopolymerizable network that can further be tuned to alter mechanics, cellular adhesion sites (RGD), and fiber alignment. Future work will investigate cell-mediated degradation with applications in cellular morphogenesis as a function of scaffold biochemical and biophysical properties.

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