Fibrous Biomimetic Hydrogels for Tissue Engineering

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Introduction: Microstructured fibrous materials have been shown to enhance cell proliferation, migration, and differentiation in many studies (1). To this end we are developing an extracellular matrix (ECM) like material, such that the ECM can be thought of as a hydrated network of proteins, a fibrous hydrogel system. Our lab has investigated the use of poly(ethylene glycol) (PEG) based photopolymerizable hydrogels with tunable mechanical properties in which ECM mimetic components can readily be incorporated (2). In a continued effort to develop biomimetic materials for the advancement of tissue engineering and the investigation of events in cell biology, we have developed microstructured fibrous photopolymerizable PEG-based hydrogels via electrospinning. Variations in cell response (NIH 3T3) between a traditional hydrogel system and the electrospun fibrous hydrogel membranes were investigated.

Methods: NIH 3T3 fibroblasts were cultured in high glucose DMEM with 10% FBS and 1% GPS at 37 °C until 80% confluent, and were used between passage 6 and 10. The cells were seeded on all samples at 5000 cells/ cm2. The 3.4 kDa and 10 kDa poly(ethylene glycol) diacrylate (PEGDA) synthesis and the acryloyl-PEG-RGDS (PEG-RGDS) conjugation were described previously (3). Two sets of PEGDA-based hydrogels were investigated, one set was PEGDA, denoted as the control (no bioactivity), and the second set was PEGDA plus an adhesive ligand, monoacrylate-PEG-RGDS (Table 1). The hydrogels were crosslinked under long wavelength UV light using photoinitiator, 300 mg/mL acetophenone-NVP (2,2 dimethoxy-2-phenolacetophenone in 1-vinyl-2-pryyolidone).

Table 1. Hydrogel Formulations

Sample	wt % 10kDa PEGDA	µmol PEG-RGDS	Acetophenone NVP solution	Exposure Time
Control	10		10 µL/mL	30 sec
Bioactive	10	2.5	10 µL/mL	30 sec

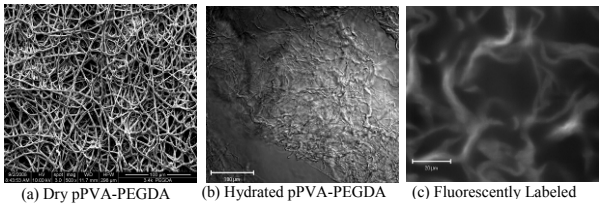
To electrospin fibrous hydrogels a high molecular weight, 134 kDa, photoactive poly(vinyl alcohol) (pPVA), from BioCure, was mixed with 3.4 kDa PEGDA to facilitate fiber formation. The pPVA was chosen because its biocompatibility is similar to PEG, and allows for little or no protein adsorption or cell attachment without the incorporation of bioactive moieties. Also, the pPVA can be crosslinked under the same conditions as PEGDA.

Table 2. Electrospinning Solution Conditions

Sample	wt % pPVA	wt % 3.4kDa PEGDA	µmol PEG-RGDS	Acetophenone NVP solution	Exposure Time
Control	5	30		40 µL/mL	60 sec
Bioactive	5	30	2.5	40 µL/mL	60 sec

Two sets of electrospun fibrous hydrogels were investigated, one control (pPVA+PEGDA) and one bioactive (pPVA + PEGDA + PEG-RGDS) (Table 2). The pPVA + PEGDA mixtures were dissolved in 70% ethanol. The electrospun samples were crosslinked using acetophenone-NVP as a photoinitiator under long wavelength UV light. The electrospinning apparatus was composed of a syringe pump and a high voltage power supply. The pPVA+PEGDA solutions were placed in a syringe with a 21 gauge needle; at an accelerating voltage of 10 kV, a 15 cm gap between the needle and the collection plate, and a 3 mL/ hr flow rate were fibers were readily formed. The fiber morphology was investigated via scanning electron microscopy (SEM) and confocal microscopy.

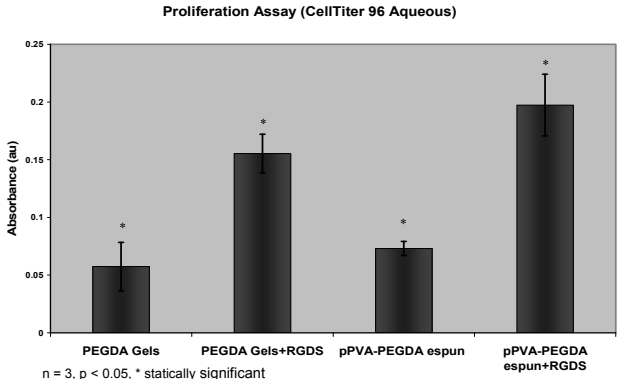
Results: Electrospun pPVA-PEGDA fibrous membranes were readily produced. No differences were seen in the fiber structure before and after crosslinking or with the addition of monoacrylate-PEG-RGDS. The dry fiber (as spun) diameters range between 500 nm and 1.5 µm (Fig. 1a). The hydrated fiber diameters range between 2 µm and 5 µm (Fig. 1b and 1c).



(a) Dry pPVA-PEGDA (b) Hydrated pPVA-PEGDA (c) Fluorescently Labeled

Figure 1. Electrospun pPVA-PEGDA fibers (a) dry (100 µm), (b) hydrated (100 µm), and (c) fluorescently labeled hydrated fiber network (20 µm).

To evaluate differences in cell proliferation the samples were seeded with 3T3s and allowed to attach overnight. The greatest cell proliferation was on the bioactive electrospun membranes (+RGDS), as compared to the other samples (Figure 3). Each group is statistically different for one another based on an ANOVA with Fisher post hoc evaluation.



Conclusions: These results demonstrate the ability to electrospin fibrous hydrogels. Cell studies have shown increased cell proliferation on the bioactive electrospun pPVA-PEGDA. Investigations are underway to evaluate cell morphology and cell migration.

References: (1) Goldstein AS, Biomaterials 2006;27:596-606. (2) West JL, Biomaterials 2008;in press. (3) West JL, Biomaterials 200;23;4325. **Acknowledgment:** NIH Bioengineering Research Partnership Program