Modulus and Proteolytic Resistance of PEGylated Fibrinogen Hydrogel Biomaterials Affects 3-D Smooth Muscle Cell-Mediated Spreading and Remodeling

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Statement of Purpose: Our research is focused on the design of engineered biomaterials that can harness natural cellular and molecular healing pathways to enhance functional tissue regeneration. Two important considerations for tissue regeneration are induction and remodeling. Although the healing process that leads to functional regeneration relies on numerous biological events, it can often be catalyzed and sustained by a single inductive biological factor. Ideally, one can engineer a synthetic biomaterial to possess inductive healing properties using protein immobilization techniques and also to be susceptible to cell-mediated remodeling. Toward this goal, we developed a novel biomimetic material that can harness the inductive properties of the natural blood clot protein fibrinogen. Using synthetic polymer conjugation chemistry, we modify the fibrinogen molecule with poly(ethylene glycol) (PEG) to create a biosynthetic precursor with tunable physicochemical properties based on the molecular relationship between the two constituents. A hydrogel matrix is formed from the biocompatible liquid precursor by non-toxic free-radical polymerization using light activation (photopolymerization). The susceptibility of this hydrogel biomaterial to protease degradation and consequent cell-mediated remodeling is controlled by the amount and size of the PEG constituent in the polymer network. The protein-based biomaterial also conveys inductive signals to cells through bioactive sites on the fibrinogen backbone.

Methods: Sulfhydryl chemistries are used to covalently bind multiple PEG-diacrylate (PEG-DA) macromers to thiol-containing fibrinogen molecules by Michael-type addition reaction to form a PEGylated protein precursor. High excess of PEG-DA was reacted with denatured fibrinogen for 3 hours under reducing conditions to allow for complete fibrinogen PEGylation. The purified PEGylated protein macromolecules were assembled into a hydrogel network using photopolymerization of the unreacted acrylate end groups on the protein-bound PEG. The molecular relationship between protein and PEG was changed by using 10-kDa and 20-kDa PEG-DA during PEGylation, or by including 1% (w/v) additional 4-arm star-PEG-acrylate prior to photopolymerization of the PEGylated fibrinogen precursor. The mechanical properties of the hydrogels were determined using a strain-rate controlled shear rheometer with dynamic time-sweep tests. The reported shear modulus was taken as the real part of the complex shear modulus G* = G' + iG" at the conclusion of the time-sweep test. Biocompatibility and cell-mediated remodeling was tested using encapsulated smooth muscle cells (SMCs) which were dispersed in the liquid precursor and three-dimensionally entrapped in the hydrogel during photopolymerization.

Results: A set of PEGylated fibrinogen hydrogels were designed and characterized by shear rheometery. Figure 1 summarizes the shear modulus of these materials as a function of precursor protein concentration using different PEG chain lengths (10 kDa and 20 kDa) and additional 4-arm star-PEG-acrylate (+). The cell-mediated remodeling of the hydrogel, as indicated by SMC spreading, was documented by phase-contrast micrographs (Fig 1, bottom). SMC spreading within the dense hydrogel network was observed in all variations of the hydrogels shown in the Fig 1 graph. The shear modulus of the material had a significant effect on the extent of SMC spreading. The additional star-PEG-acrylate, which was shown to increase the proteolytic resistance of the protein backbone, also restricted the extent of SMC extension within the hydrogels. Higher concentrations of precursor had a similar effect on SMC extension in the hydrogels.

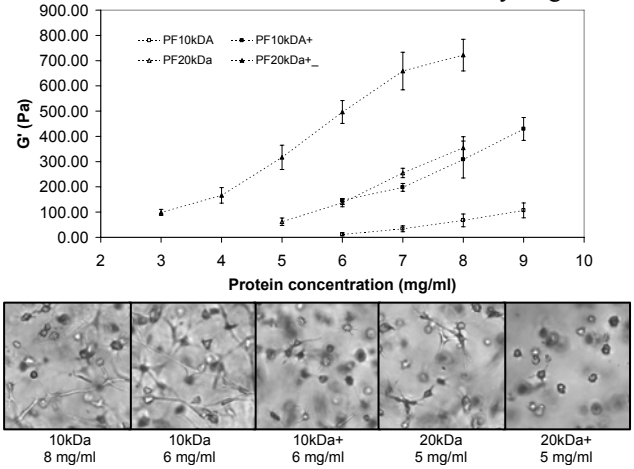

Figure 1: Shear modulus and three-dimensional SMC spreading within PEGylated fibrinogen hydrogels.

Discussion and Conclusions: The photopolymerizable PEGylated fibrinogen hydrogels are an alternative to biological or synthetic biomaterials in use today. The physicochemical characteristics of the hydrogels, including stiffness, and biodegradation properties can be controlled by the compositions of the PEG and the fibrinogen backbone. We have demonstrated that controlling the stiffness and biodegradation of the biosynthetic hydrogel alters the way cells are able to remodeling the material in vitro. Further experiments are required to identify which of these parameters dominate the cell-mediated remodeling process.

Reference: Shapira, K., Seliktar, D., *Acta Biomaterialia* In Press; Almany L., Seliktar D., *Biomaterials*, 26(15) 2005; Dikovsky D. *et al.*, *Biomaterials*, 27(8):1496-506, 2006.