Biodegradable Hydrogels based on PEGylated Proteins for Tissue Engineering Applications

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Introduction: Biological proteins covalently modified with synthetic polymers have been used extensively to prolong the half-life and enhance the therapeutic potential of important biological macromolecules. Perhaps the most notable example of such modification schemes is based on the use of poly(ethylene glycol) (PEG) for improving the performance of therapeutic agents such as interferon. PEG modification of the proteins (PEGylation) is associated with several beneficial characteristics including decreased enzymatic degradation rates, reduced immunogenicity, and improved physicochemical properties. Although there are commercially available PEGylated therapeutic proteins, few have applied this technological paradigm to structural proteins for biomaterials discovery. Our group employs the PEGylation approach for designing biodegradable hydrogels for tissue regeneration and drug delivery. The PEGylated protein hydrogels convey biofunctional signals to cells through the protein backbone, including adhesion and protease degradation sites, while the synthetic PEG precursor is used for controlling the physicochemical properties of the matrix. Using PEGylated fibrinogen, collagen, and albumin, we create hydrogels having protein-specific biofunctionality and tunable structural properties. This class of biodegradable hydrogels has proven to be highly compatible with a variety of cell and tissue types both *in vitro* and *in vivo*, for tissue regeneration and tissue engineering.

Materials and Methods: Sulfhydryl chemistries are used to covalently bind multiple PEG-diacrylate (PEG-DA) macromers to thiol-containing protein molecules by Michael-type addition reaction to form a PEGylated protein precursor. For fibrinogen and albumin PEGylation, high excess of PEG-DA was reacted with denatured protein under reducing conditions. Thiolation of collagen was done by a succinimidylacetyl-thioacetate (SATA) reaction scheme. The resulting acetylated SH groups on the lysine residues were deprotected before the collagen was PEGylated by Michael-type addition. The 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 6-kDa PEG-DA during PEGylation, or by including 1%, 3%, and 5% additional PEG-DA prior to photopolymerization. Biocompatibility was tested using dispersed 3-D cultures of different cell types, including smooth muscle cells, cardiomyocytes, fibroblasts, and embryonic stem cells. Cellular outgrowth experiments were performed on vascular smooth muscle tissue, neuronal tissue, and cartilage tissue explants.

Results: PEGylated collagen and fibrinogen hydrogels containing adhesion and degradation sites in the protein backbone supported cell sprouting, cell migration, and proliferation within the hydrogel network with each cell type we tested. Additional PEG-DA increases the proteolytic resistance of the protein backbone and restricts the extent of cell extension and migration of dispersed cell cultures in the PEGylated collagen and fibrinogen hydrogels. PEG-albumin hydrogels exhibit poor cell spreading and survival by virtue of the fact that albumin lacks degradation or adhesion sites. Cellular invasion experiments using vascular tissue reveal the regenerative potential of the PEGylated fibrinogen based on the widespread smooth muscle outgrowth observed into these hydrogels, compared to PEGylated collagen and albumin hydrogels. Neuronal outgrowth experiments using dorsal root ganglion embedded in PEG-fibrinogen hydrogels containing nerve growth factor (NGF) show widespread 3-D invasion of schwann cells and neurons from the chick DRG into the surrounding hydrogel (Fig 1).

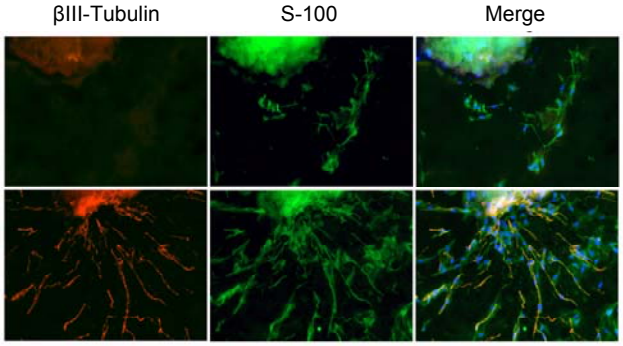

Figure 1: Dorsal root ganglion 3-D outgrowth into PEGylated fibrinogen hydrogels with (bottom) and without (top) NGF; shown: schwann cells (S-100) and neurons (βIII-tubulin).

Discussion and Conclusions: The photopolymerizable PEGylated protein hydrogels are an alternative to biological or synthetic biomaterials in use today. The physicochemical characteristics of the hydrogels, including hydration and degradation properties, can be controlled by the composition of PEG and protein backbone. The interchangeable protein backbone retains much of its inherent biofunctionality, thus providing specific biocompatibility using a number of different cell and tissue types. The availability of the endogenous proteins, the simplicity of manufacturing, and the compatibility with living tissues, make these hydrogels a choice alternative biomaterial for tissue engineering.

Reference: 1. Lutolf M.P. and Hubbell J.A., *Nat Biotechnol*, 23(1) 2005; 2. Almany L. and Seliktar D., *Biomaterials*, 26(15) 2005; 3. Dikovsky D. *et al.*, *Biomaterials*, In press.