Stimuli-Sensitive Hydrogels for Reactive Oxygen Species-Mediated Controlled Degradation and Release

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Statement of Purpose: Development of stimuli-sensitive biomaterials that respond to changes in the tissue microenvironment is a potentially promising approach for solving challenging problems in regenerative medicine. Among other stimuli, overproduction of reactive oxygen species (ROS) has been implicated in major diseases and host responses to biomaterial implants, including inflammation and angiogenesis. A controlled change in biomaterial properties (e.g., drug release and degradation) in response to ROS production will be advantageous to program "right timing" of regulatory effects. Therefore, the goal of the current project is to develop stimuli-sensitive hydrogels that enable control of degradation kinetics and subsequent release of functional molecules in a ROS level-dependent manner. Proline oligomers have been previously shown to be sensitive to chemical degradation by oxidation. Therefore, we have formulated biamino PEG-P_n-PEG crosslinkers to make hydrogels using a library of combinatorial polymers with a general formula, x%PCL-*co*-y%carboxylatedPCL-*co*-z%PEG, where x, y, and z represent molar % of the corresponding units. The purpose of this design is to provide a wide range of physicochemical and mechanical properties of hydrogels. Controlled degradation of the crosslinkers and the hydrogels were then assessed through a variety of methods including HPLC, GPC and SEM. The material properties of these hydrogels are well-suited for applications involving the modulation of inflammatory and angiogenic activity, as well as stem cell differentiation.

Methods: The proline oligomers Ac-KP_5K, Ac-KP_7K, and Ac-$KP_{10}K$ were synthesized by standard Fmoc- chemistry on a Rink amide resin to fashion two free amines for the coupling of Fmoc-PEG_{11}-COOH. To evaluate the oxidative degradation of the peptides and bi-PEGylated peptides, these materials were reacted for several days at 24°C or 37°C in the presence of 50μM Cu^{2+} and 100mM H_2O_2 in PBS. Products were then analyzed by HPLC-MS and GPC. We then formed chemically-crosslinked hydrogels of x%PCL-*co*-y%carboxylatedPCL-*co*-z%PEG with equimolar amounts of the PEG-P_n-PEG linkers and assessed the oxidation-dependent material properties of these materials by mechanical testing, SEM, and mass.

Results: HPLC analysis showed that the P_5 and P_{10} oligomers are indeed degraded following four days of treatment. The biPEGylated P_n crosslinkers retained their oxidative degradability as shown by GPC following 2 days of treatment (Figure 1). x%PCL-*co*-y%carboxylatedPCL-*co*-z%PEG were then formed and chemically crosslinked with the biPEGylated-P_n linkers. To generate an oxidative environment mimicking those in physiological inflammatory scenarios, hydrogels were incubated with 3-morpholinosydnonimine (SIN-1), which generates superoxide, over several weeks. Preliminary results from mass and mechanical analysis confirm the gradual oxidative degradation of the hydrogels.

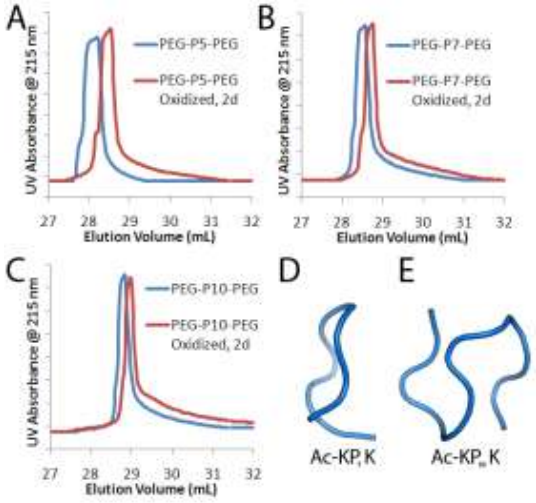

Figure 1. Analysis of oxidative degradation of biPEGylated proline oligomers by GPC (A-C). PEP-FOLD-generated models of the P_7 and P_{10} peptides support the later elution times exhibited by PEG-P_7-PEG and PEG-P_{10}-PEG versus PEG-P_5-PEG (D-E).

Conclusions: Proline oligomers and their biPEGylated counterparts exhibit oxidative degradability. The resulting biPEGylated-P_n linkers have since been used to chemically crosslink hydrogels. Preliminary evidence shows degradation kinetics of hydrogels changes in response to ROS level and type. This study will provide a novel toolbox that enables ROS-mediated controlled degradation and release of biomaterials for therapeutic applications.

References:

1) J Biol Chem. 1989; 264: 3341-3346.

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