414c Comb-like Peptide-Protein Polymer Conjugate Hydrogels

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A class of high-molecular weight peptide-protein polymer conjugates were created to act as substrates for enzymatic crosslinking into a hydrogel for tissue engineering applications. First, high-molecular weight protein polymers were created by bacterial expression in *E. coli*. The synthesis of these protein polymers by genetic engineering allowed for the creation of large proteins with a specifically tailored amino acid sequence and controlled molecular length (monodispersity). A highly repetitive DNA template encoding the amino acid sequence $(GKAGTGSA)_n$, with n = 30, 60, and 120 was created by "controlled cloning"[1]. The DNA was ligated into the expression vector pET-19 and expressed as a fusion protein with a 10X Histidine tag, which allowed for purification by nickel affinity chromatography. The target protein was isolated by cyanogen bromide cleavage and cation exchange chromatography. Protein purity and identity were verified by both MALDI mass spectrometry and amino acid analysis. The resulting target protein contains an evenly spaced lysine residue that allows for chemical grafting of synthetic peptides. Transglutaminase (TG) substrate peptides were grafted onto the reactive lysine residues, producing a comb-like peptide-protein polymer conjugate. The addition of TG human recombinant Factor XIII (hrFXIII) enzymatically crosslinks the conjugates into a hydrogel with viscoelastic properties. This enzymatically crosslinked hydrogel has a uniform and controlled pore architecture due to the protein polymer monodispersity. Additionally, the peptide-protein polymer conjugate hydrogel contains a reactive crosslinking site, which facilitates customizable grafting of bioactive peptides that can be chosen for the cell-signaling requirements of particular tissue applications.

1) J. Won, A. E. Barron, *Macromolecules* **2002**, *35*, 8281.