The Development of Synthetic Polypeptide-Based Hydrogel Systems for Biomaterials

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Statement of Purpose: Cell development is highly dependent on the chemical and physical properties of the material surrounding the cell, the extracellular matrix (ECM). Cell phenotype, migration, and proliferation are heavily influenced by the ECM. In order to determine how the properties of the ECM affect cell development, we must study cells in the highly controlled environment provided by scaffolding materials such as hydrogels. Although hydrogels may be composed of a variety of materials, those composed of synthetic polypeptides have definite advantages. Our group first developed poly(propargyl-L-glutamate) (PPLG), a stable polypeptide with α-helical secondary structure. This synthetic polypeptide system allows for the grafting of a large variety of small molecules, polymers, and biologically relevant molecules to the polymer backbone using the click reaction popularized by Sharpless – copper catalyzed azide alkyne cycloaddition (CuAAC). Grafting efficiency is remarkably high – upwards of 98%. Additionally, using L and D versions of our propargyl monomer, we are able to create a polypeptide (PPDLG) with a random coil secondary structure. The hydrogels produced from PPLG and PPDLG have the same chemical composition, but different physical properties - independently tunable stiffness and permeability. Currently, there are no substrates that allow for the independence of these variables in 3D encapsulation of cells.[1] Furthermore, we are expanding our synthetic polypeptide system by introducing an orthogonal click chemistry, specifically the reaction between a thiol and poly(allyl-L-glutamate) (PALG). In this study, we will describe our current progress toward developing novel synthetic polypeptide hydrogel systems for use in tissue engineering.

Methods: All materials were purchased from Sigma Aldrich or Arcos Organics and used as received. Polypeptides were synthesized using the ring-opening polymerization of *N*-carboxyanhydride (NCA) monomers. The synthesis of PPLG is shown below in Figure 1.[2] PALG is synthesized similarly using allyl alcohol instead of propargyl alcohol.

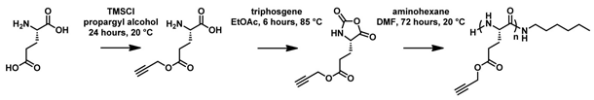

Figure 1. The synthesis of PPLG using the ring-opening polymerization of an NCA.

Grafting of PPLG is accomplished via the CuAAC, while grafting for PALG is accomplished via a thiol reaction with the terminal alkene. PPLG was modified using 4-(maleinimido)phenyl isocyanate (PMPI) and then chemically crosslinked using star polyethylene glycol thiols, forming hydrogels. Nuclear magnetic resonance (NMR), fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), circular dichroism (CD), and gel permeation chromatography (GPC) were used to characterize the chemical and physical characteristics of the synthetic polypeptide systems. After hydrogel formation, atomic force microscopy (AFM) and fluorescence after photo-bleaching (FRAP) were used to investigate the stiffness and permeability of the gels, respectively. These methods allow us to have a thorough understanding of the chemical and physical properties of our polypeptide gel systems.

Results: We have successfully synthesized PPLG and PALG random copolymers of varying degrees of polymerization. Grafting using the thiol alkene system is less robust than CuAAC click chemistry. These results have been confirmed by NMR and GPC.

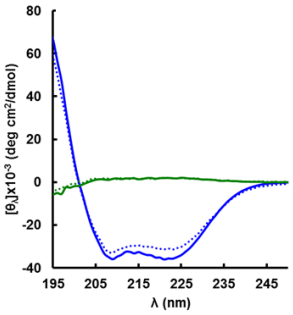

Figure 2. CD spectra for PPLG (blue) and PPDLG (green) based grafted polypeptides. The shape of the CD spectrum indicates the α-helical secondary structure of PPLG and a random coil for PPDLG.

Using CD (Figure 2) and FTIR we confirmed the random coil and α-helical structure of PPDLG and PPLG, respectively. Our AFM and FRAP studies indicate that, although the permeability of the PPLG and PPDLG based hydrogels is the same for polypeptides with the same degree of polymerization and crosslinking, the mechanical stiffness is significantly different. The experimentally-determined elastic modulus for the PPLG system does not agree with that determined by the rubber elastic theory, while that for the PPDLG system does. This indicates that the stiffness of the helical structure of PPLG accounts for the difference in stiffness between the two polypeptide hydrogel systems.

Conclusions: We have successfully synthesized random copolymer polypeptide systems with orthogonal click functionality. Additionally, we have created hydrogel systems composed of synthetic polypeptides that have independently tunable stiffness and permeability. These systems have the potential for numerous applications in tissue engineering - allowing for the study of ligand presentation and the effect of stiffness and permeability.

References:

1. Oelker AM. Soft Matter. 2012; 8:10887-10895.
2. Engler AC. Angew. Chem., Int. Ed. 2009;121: 9498-9502