Online Proceedings

Poster Session 2

Commons East, Easel 71

1:00 PM to 2:30 PM

Controlling 4D Stem Cell Differentiation in Hydrogels using Site-Specifically Modified Growth Factors

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NASA Space Grant Scholar, Washington Research Foundation Fellow

Mentor: Cole DeForest, Chemical Engineering & Bioengineering

Mentor: Jared Shadish, Chemical Engineering

Mentor: Christopher Arakawa, Pathology/ Bioengineering

The ability to recapitulate the dynamic presentation of signals in a stem cell's microenvironment remains a major hurdle in tissue engineering. Controlling cell growth and differentiation in 4 dimensions (i.e., time and 3D space) would allow for heterogeneous synthetic tissues to be produced that match the complexity of their native counterparts. Combining strategies in both light-programmable hydrogels and recombinant protein engineering, we control the cellular microenvironment using proteins site-specifically modified with a bioorthogonal handle and able to covalently bind to a photocaged reactive group in the hydrogel. Previously we've demonstrated the ability to photopattern gels with fluorescent proteins that excite at different wavelengths, and have shown that multiple proteins can be patterned independently within the same material with 4D control. These techniques have many potential applications, including improving joint replacement. Current joint replacement therapies often involve use of metals, plastics, and ceramics, and commonly require future revision surgeries. We propose that through our techniques, we can generate a patterned bone/cartilage interface to improve joint replacement, creating a longer-term option for joint replacement. Towards this, we have generated two photopatternable recombinant growth factors, BMP-2 (bone morphogenic protein 2) and TGF-β (transforming growth factor β), known to direct human mesenchymal stem cell (hMSC) osteogenesis and chondrogenesis. I will encapsulate hMSCs in a hydrogel and direct cell differentiation and growth in 4D using a combination of photopatterned BMP-2 and TGF-β proteins. This research will have significant impact in tissue engineering, as it will enable recreation of complex physiological structures, grown outside the body with the patient's own cells, that can be used for personalized medicine. Our approach is unique in that it allows for unprecedented control over microscale tissue structures, ultimately matching the complexity of native tissue.

Poster Session 4

MGH 241, Easel 145

4:00 PM to 6:00 PM

Optimization of Sortase-Tag Expressed Protein Ligation (STEPL) for Yield and Robustness

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Mentor: Cole DeForest, Chemical Engineering & Bioengineering

Mentor: Jared Shadish, Chemical Engineering

Proteins are naturally-occurring biopolymers that are useful in a wide range of therapeutic and imaging applications. Though natural proteins are comprised of just 20 monomeric amino acids, they can be post-translationally decorated to introduce expanded chemical functionality. Though several techniques have been developed, the majority do not provide site-specific modification nor stoichiometric conjugation necessary to preserve protein bioactivity. Additionally, proteins functionalized through these methods have proven difficult to purify after modification, thereby limiting potential clinical and industrial applications. Sortase-Tag Expressed Protein Ligation (STEPL) is a protein functionalization/purification method that solves many of these problems. This method allows functional probes to be site-specifically conjugated onto a protein, while simultaneously separating modified and unmodified proteins. Though this technique has been successfully demonstrated in the DeForest lab, a remaining challenge is to increase yield during protein expression. In our group, we exploit several microbiology techniques to optimize this process. Bacterial strain identity, growth conditions, reaction temperature, and protein solubility have all been shown to influence protein expression. Using Green Fluorescent Protein (GFP), we found that the STEPL yield increased when conducted for 4 hours at 37C versus overnight at 18C. This was determined through mass spectrometry, which showed hydrolyzed proteins after prolonged reaction. For some difficult to express proteins, including growth factors, the STEPL protocol resulted in a negligible protein yield. Through experimenting with a dialysis refolding protocol, functional growth

factor Bone Morphogenic Protein-2 was produced at dramatically increased yields. Furthermore, experimenting with E. coli growth and STEPL reaction conditions has resulted in increased yield for many different functionalized proteins. Although progress has been made in producing functionalized protein with the STEPL method, yield remains below that obtained through other methods. Further experimentation to improve the robustness and yield of the STEPL protocol will increase the availability of functionalized proteins in biotherapeutics.