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Designing Dynamic Protein Scaffolds for Tissue Engineering

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Abstract – A key attribute missing from many current biomaterials is the ability to independently tune multiple biomaterial properties without simultaneously affecting other material parameters. Because cells are well known to respond to changes in the initial elastic modulus, degradation rate, and cell adhesivity of a biomaterial, it is critical to develop synthetic design strategies that allow decoupled tailoring of each individual parameter in order to systematically optimize cell-scaffold interactions. We present the development of a family of biomimetic scaffolds composed of chemically crosslinked, elastin-like proteins designed to support tissue regeneration through a combination of cell adhesion and cell-induced degradation and remodeling.

The engineered synthetic proteins used in this application are composed of alternating structural and bioactive sequences. The structural sequences are similar to repeated sequences from elastin, a fibrous protein found in connective tissue that is known to provide both elasticity and resilience. The bioactive sites consist of peptide sequences that have been previously shown to mimic actions performed by proteins in the extracellular matrix environment. Two types of bioactive sites are included: cell binding domains and protease cleavage sites. Cell binding domains, such as the RGD domain of fibronectin, can offer the scaffold a capacity to adhere and communicate with cells via their cell surface integrin receptors. The inclusion of protease cleavage sites can allow the encapsulated cells, which secrete serine proteases, to dynamically remodel through the scaffold as they alter their morphology and migrate through the matrix. Currently we have demonstrated that the proteolytic degradation rate of these protein-based biomaterials can be tuned independently from the initial elastic modulus and the cell adhesivity. Furthermore, we have demonstrated that these biomaterials are compatible with *in vitro* cultures of human embryonic stem cell-derived cardiomyocytes, skeletal myoblasts, endothelial cells, and neuronal cultures. Variations in RGD domain density are positively correlated with neuronal adhesion and neurite outgrowth.

These engineered proteins are produced using recombinant techniques and chemically crosslinked into highly swollen hydrogels with controllable mechanical properties. Through a modest 3% change in the chemical identity of twelve otherwise identical engineered proteins, we can modify the protease substrate specificity resulting in tunable changes in protease degradation half-life over two orders of magnitude. Under high concentrations of constant protease exposure, the designed scaffolds exhibit systematic variation of scaffold lifetime, from being fully degraded within a single day to showing no noticeable degradation within several weeks.

Recent advancements in biology and medicine have placed significant emphasis on the development of fabrication strategies that generate three-dimensional biochemical, mechanical, and spatial patterns within soft, biocompatible hydrogels. Patterned hydrogels often are designed to mimic natural tissue structures to enable fundamental studies of cellular response to chemical or physical cues. Current technologies for patterning hydrogels have focused on creating features using layer-by-layer stacking (fused with heat, adhesives, or light), molding, 3D printing, electrochemical deposition, and photolithography. These technologies have proven to be successful in producing intricate patterns within hydrogels but only address applications that require static features present before material implantation or experimentation.

In this work we propose a new, dynamic, three-dimensional hydrogel patterning method, involving layered spatial deposition of our chemically crosslinked, biodegradable protein polymers. By arranging proteins with widely differing and controllably tuned degradation rates within a single, composite hydrogel material, patterns can be triggered to emerge over time in response to biologically relevant enzymes. By designing the polymeric degradation fragments to be smaller than the hydrogel pore size, we have evolved internal void structures inside the hydrogel with both spatial and temporal resolution. The material released during pattern formation can be additionally modified to serve as drug delivery vehicles. Using this strategy, we have developed a family of protein-based, polymeric hydrogels with highly tunable biodegradation rates, used these polymers to evolve 3D patterns within composite materials at pre-determined rates, and demonstrated spatial and temporal delivery of multiple fluorochromic molecules with distinct delivery profiles from a single composite structure.