

Design and Application of Soft Multiscale Biomaterials for Understanding the Role of Extracellular Matrix Properties in Collagenous Tissue Repair

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Injuries and disorders involving the musculoskeletal system are among the greatest causes of pain and disability worldwide. Tissue engineering approaches are used in clinical applications for injuries involving these collagen-rich tissues (e.g., tendon, ligament, bone). Such treatments include traditional autografts and allografts, and more recently, have expanded to include ceramics, natural and synthetic scaffolds, and composite materials as an attractive alternative to natural tissue grafts. Development of new biomaterials scaffolds are of interest to the medical community for application as musculoskeletal injury treatment, including materials systems that enable *in situ* formation and afford control of biophysical and biochemical properties to promote desired healing responses with limited batch-to-batch variation. In particular, materials designed to capture key aspects of native collagenous tissues are of interest for both translational applications to stabilize tissues and promote their healing and for use in fundamental studies to probe key factors in directing cellular responses. Such bioinspired materials provide opportunities for hypothesis-driven testing of materials properties and impact on cell behavior in cell culture applications, which can lead to new insights into wound healing and tissue remodeling, further informing materials designs for tissue engineering applications.

Toward improving upon current biomaterials designs, this work aimed to design and apply fully synthetic hydrogel matrices that more fully capture aspects of native collagenous tissues by incorporating collagen mimetic peptides (CMPs). These matrices were intended to allow for independent control over various matrix properties including mechanics, hierarchical structure, and biochemical function for 3D cell culture applications. The goals of this dissertation were to (1) establish approaches to more quickly and consistently synthesize multifunctional CMPs (mfCMPs), (2) establish tools to characterize the structure of assembled mfCMPs after incorporation into a hydrogel, (3) examine the impacts of mfCMPs on cell responses, specifically cell viability, morphology, motility, and directional migration, and (4) determine the suitability of these materials for use in preclinical tissue engineering applications.

First, mfCMPs were designed to self-assemble into triple helices and elongate into fibrils similar to the fibrillar collagen type I found in loose connective tissues and in the provisional matrix formed in tissues undergoing healing after injury. These assembled structures then were covalently incorporated into a larger hydrogel network to form a fully synthetic hydrogel composite with tunable properties. These materials demonstrated mechanical properties within the range of soft collagenous tissues, and when used for 3D cell culture applications, supported high viability of human mesenchymal stem cells. Further, the presence of relatively high concentrations of mfCMPs (2.5 mM) resulted in significantly more elongated cell morphologies compared to materials with lower concentrations of mfCMP or no mfCMP.

Second, new methods were established for the synthesis and characterization of mfCMPs. Expanded experimental use of mfCMPs demanded the development of more rapid synthesis protocols and consistent purification techniques. Microwave-assisted synthesis of mfCMPs was established, where a methodical approach was taken to identify residues prone to deletions and employ targeted use of dual chemistry couplings to improve purity of the crude product. Purification techniques incorporating heating and ion displacement were utilized to more consistently separate mfCMPs from unwanted side products and prepare the peptides for use in cell culture applications (i.e., remove cytotoxic ions). Further, an approach for the fluorescent labeling of assembled mfCMPs after covalent incorporation into a hydrogel was established, enabling visualization of the final hierarchical structure using confocal microscopy techniques and is of relevance for future studies of structure-property relationships.

Next, a list of *in vitro* and *in situ* success criteria for soft biomaterials intended for tendon repair was established and tested using these multifunctional, cell-instructive hydrogels. Specifically, the hydrogel scaffolds were shown to have mechanical properties within the range of developing tendon. Hydrogel cytocompatibility was tested: both complete gel formation and individual hydrogel components were found to be noncytotoxic to human and rat mesenchymal stem cells. The multifunctional hydrogels were formed in tendon defects and incubated under fluid shear for 2 weeks, where the hydrogels were retained in the defect during that time. Further, hydrogels formed in defects provided mechanical support to the surrounding tissue at the time of formation compared to tissues with empty defects.

Finally, this rational approach to hydrogel design allowed for independent control over fibrillar content, biochemical identity, and hydrogel degradability to explore key properties in promoting cell migration into and within these materials. An approach to study directional cell migration into 3D scaffolds was established and used with these materials. At the time scales explored in this work, cell invasion of the hydrogels was limited; however, studies of cell motility indicated that even at low concentrations (1 mM) mfCMPs appear to promote increased matrix sampling and general cell motility. The materials, tools, and methods established in this dissertation have provided insights into composition-structure-property relationships that will inform designs of new synthetic extracellular matrices both for fundamental studies of cell response and for translation into a range of tissue engineering applications.