Design of mechanically responsive hydrogels to understand cell response to matrix remodeling

Katherine L. Wiley
Advisor: April M. Kloxin
Committee Members: Wilfred Chen, Millicent O. Sullivan, Kenneth L. van Golen

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The extracellular matrix (ECM) is a complex, ever-changing environment comprised of physically and chemically crosslinked insoluble structures, such as proteoglycans and large insoluble proteins, and soluble chemokines and cytokines. This environment provides key physical and biochemical interactions to the cells that reside within it, and in turn, resident cells remodel the local ECM by degrading and re-depositing matrix components. This matrix remodeling has been associated with several diseases, including the recurrence of breast cancer at metastatic sites, where breast cancer cells are hypothesized to 'awaken' or re-activate after long periods of dormancy upon changes to local ECM properties due to matrix remodeling (e.g., upon injury or with aging). Synthetic hydrogels have been identified as critical tools for three-dimensional (3D) cell culture due to their ability to mimic key characteristics of the ECM (e.g., matrix stiffness and composition) in a controlled fashion appropriate for testing specific hypotheses. The overall goal of this dissertation was to develop and study new synthetic, 3D hydrogel cell culture tools to aid in the understanding of breast cancer cell response to matrix remodeling, particularly matrix softening and stiffening that occur upon injury to the lung a known metastatic site for breast cancer recurrence. Specifically, the aims of this dissertation were to 1) establish a new chemical tool for hydrogel formation and mechanical property control for 3D culture of sensitive cell types, 2) investigate photopolymerization rate as a mechanism for controlling hydrogel stiffness, and 3) probe breast cancer cell response to mechanically static and dynamic synthetic matrices.

First, the nucleophilic addition of thiols to activated alkynes was adapted for 3D cell culture applications, with the goal of creating benign encapsulation conditions for cell types sensitive to free radical polymerization conditions. Hydrogel stiffness, as measured by Young's modulus, and hydrolytic degradation was tuned by changing polymer architecture. Peptides were introduced to the hydrogel structure to encourage cell-matrix interactions, including pendant peptide that mimicked the integrin binding site of an ECM protein, and a thiol functional linker peptide that enables cell-driven local degradation of the hydrogels. Sensitive breast cancer cells (MCF-7) were successfully encapsulated with high cell viability, in contrast to their low survival in hydrogels formed by radically-initiated thiol-ene crosslinking. Bulk hydrogel degradation by ester hydrolysis and local hydrogel degradation by cell-secreted enzymes resulted in rapid formation of growing breast cancer cell clusters within the hydrogels. Overall, this work resulted in a new, adaptable tool for 3D cell culture of sensitive cell types.

Second, a rate-based approach for controlling mechanical properties of hydrogels both initially and temporally with light was investigated. The rate of formation of synthetic hydrogels formed with photoinitiated thiol-ene 'click' chemistry was controlled using irradiation conditions, including light wavelength, intensity, and exposure time, resulting in a range of mechanical properties relevant to mimicking different soft tissues. The differences in mechanical properties

were attributable to hydrogel network defects, including dangling end groups and inelastic loops. The nature of these defects was observed over time by correlation of *in situ* measurements of end group conversion and measurements of hydrogel mechanical properties. Dangling end defects available after hydrogel formation via visible-light initiation were then reacted by a secondary photopolymerization with long wavelength UV light, resulting in hydrogels of increased stiffness (Young's modulus). This work demonstrated that robust control of photoinitiated hydrogel mechanical properties can be achieved simply by altering irradiation conditions.

Finally, bulk enzymatic degradation and secondary photopolymerization mechanical property modulation techniques were integrated to create synthetic, photoinitiated thiol-ene hydrogel cell culture environments with pseudo-reversible mechanical property control. Bulk enzymatic hydrogel degradation was achieved by the incorporation of a peptide linker sequence degradable by an applied thrombin enzyme. Building on previous knowledge of secondary thiol-ene photopolymerization, this method was adapted to incorporate cell degradability. These independent property modulation methods were integrated for either subsequent stiffening and softening or subsequent softening and stiffening to mimic matrix remodeling events that occur upon injury (e.g., matrix degradation followed by deposition and crosslinking). This work established a synthetically accessible, cell compatible method for pseudo-reversible mechanical property modulation and demonstrated its potential to probe the role of changing mechanical properties on key aspects of disease progression.