Bioinspired Microenvironments for Modulating Human Immune Cell Responses

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The immune system protects the body from foreign pathogens and maintains tissue homeostasis through coordinated innate and adaptive immune responses. The innate immune system provides rapid, non-specific defense through cell types including macrophages, dendritic cells, neutrophils, and natural killer cells, while the adaptive immune system generates antigen-specific responses through T and B cells. Although these responses are essential for maintaining health, dysregulated immune activity contributes to chronic inflammation, fibrosis, autoimmune disease, and cancer. Developing strategies to direct immune cell behavior therefore requires a deeper understanding of how immune cells respond to the complex microenvironments present in healthy and diseased tissues.

While animal models have been paramount in preclinical modeling, *in vitro* engineering model systems are needed to answer specific mechanistic questions about how the local microenvironments affect human responses. Cells exist not in two-dimensions, but in a complex 3D extracellular matrix (ECM)—a network of proteins and other bioactive molecules that give our tissue defined structure and instruct cellular functions. Hydrogels, made through engineered synthetic building blocks, can be used to create *in vitro* ECMs that have tunable complexity, mechanics, and bioactivity. In this thesis, I engineer hydrogel-based platforms to create synthetic ECMs that enable investigation of immune cell–microenvironment interactions and establish biomaterial design principles for directing both innate and adaptive immune responses.

I first focused on macrophages, critical tissue-resident immune cells that coordinate host defense, inflammation, and tissue repair, to investigate how the physical and biochemical properties of the ECM regulate their behavior. I develop bioprinting workflows for developing more consistent, high-throughput hydrogel platforms—engineered with a synthetic backbone of a poly(ethylene) glycol (PEG) and bioactive ECM peptides. Workflows included differentiation from monocytes to macrophages and downstream analysis of immunophenotype through gene expression, protein secretion, and surface marker expression. Using primary and immortalized murine macrophages, I identified differences in macrophage responses between 2D and 3D culture. I subsequently translated these workflows to human THP-1 macrophages and demonstrated that ECM stiffness influences baseline gene expression and macrophage responses to Pseudomonas aeruginosa, a clinically relevant bacterial lung pathogen. I then increased the

physiological complexity of these models by incorporating primary blood-derived macrophages with fibroblasts and epithelial cells to develop a multicellular *in vitro* lung model. Systematic evaluation of ECM stiffness and multicellular interactions demonstrated that both matrix properties and tissue complexity influence macrophage phenotype and responses to bacterial challenge, highlighting the importance of incorporating relevant 3D microenvironmental cues when modeling human immune responses.

Building on these studies of immune–ECM interactions, I developed and applied biomaterials for driving immune pro-regenerative and anti-inflammatory functions. I engineered synthetic collagen-inspired ECMs with increased complexity, using multifunctional collagen-mimetic peptides (CMPs) with hierarchical fibrillar structures. Using PEG molecular crowding, I generated CMP-rich molecular condensates—creating synthetic ECMs with microstructure and heterogeneity. These synthetic ECMs reveled that collagen integrin-binding cues, mainly GFOGER, drive pro-regenerative macrophage phenotypes through $\beta 1$ integrin engagement.

Finally, I extended my knowledge of synthetic biomaterials for generating immunotherapy platforms for increasing the effectiveness of regulatory T-cell (Treg) therapy. I evaluated how PEG-based activating hydrogels together with anti-inflammatory macrophages could be used to convert T-cells to Tregs and maintain Treg stability. I showed that tunable, soft hydrogel scaffolds performed better at generating Tregs compared to the industry standard nanomatrix platform, demonstrating the potential of hydrogel-based approaches for engineering immune cell therapies for treating autoimmune disease.

In conclusion, my thesis established synthetic ECMs are versatile, tunable platforms for understanding and directing immune cell behavior. This thesis advances our understanding of macrophage biology by revealing how macrophages respond to mechanical, biochemical, and multicellular cues within engineered 3D microenvironments.