TOWARD BETTER RECAPITULATION OF NATIVE TISSUES AND TISSUE ENVIRONMENTS

Carly M. Battistoni

Tissue engineering utilizes polymers, cells, and other bioactive factors to promote regeneration within damaged tissue. The main works in this thesis employ naturally derived polymers for use in tissue engineering and explore ways to recapitulate native environments *in vitro*.

Collagen (col) is the most prevalent protein in the body. Col type I, II, and III are all fibril-forming collagens that provide structure to tissues. All three types have been shown to polymerize *in vitro* to form hydrogels, and these hydrogels are commonly studied for use in tissue engineering applications. Other applications include *in vitro* tissue models for drug diffusion models and for drug delivery. Blending collagen types is of particular interest as col I is easier to source and is therefore cheaper than other collagen types. However, to confer biological signals to tissues where col II or III are more abundant (e.g., cartilage or cardiac tissue, respectively), col II or III can be added to col I hydrogels. Additionally, to study drug diffusion through tissues where col II or III are present, adding additional types of col to hydrogel models better recapitulates the native environment and can better capture drug diffusion effects. In this work, col I/II hydrogels polymerize slower, form fibril bundles, result in softer hydrogels, and impede transport of larger macromolecules compared to col I alone. On the other hand, col I/III polymerizes at a similar rate to col I, creates heterogenous fibril structures, are oftentimes stiffer than col I, and impede transport of larger macromolecules. Additionally, this work explored the effect of polymerization temperature on blended gel polymerization and properties. At higher polymerization temperatures, all gels polymerized faster and resulted in more nucleation sites. For col I and col I/III, higher temperatures led to lower mechanical properties. Conversely, col I and II seemed to impede polymerization which led to lower mechanical properties at lower polymerization temperatures.

The second primary chapter of this thesis provides depth to the pro-inflammatory, osteoarthritic model used in the previous chapter. Different pro-inflammatory environments are studied using cytokines found in OA. MSC pellets (used to confirm chondrogenic potential of MSCs) were used to evaluate these inflammatory environments since MSCs are commonly used in tissue engineering. Six treatments were studied: negative control (without chondrogenic growth

factor, TGF-β3), positive control (with chondrogenic growth factor, TGF-β3), and four cytokine treatments all with TGF-β3. IL-1β at 10 ng/mL was utilized as a comparison to literature. TNF-α at 20 ng/mL and OSM at 10 ng/mL were studied as the component parts of the main experimental group: OSM+TNF-α. All cytokine treatment groups limited cartilage production, but OSM decreased production to a statistically lesser extent than other cytokine groups. This observation was similarly observed via immunostaining of cartilage matrix and gene expression of aggrecan. OSM+TNF-α statistically lowered aggrecan gene expression. In terms of degradation, OSM dramatically increased the expression of degradative enzyme matrix metalloproteinase-13 (MMP-13) compared to all other groups. Evaluation of inflammatory markers (IL-6 and IL-8) revealed no signal for OSM-treated pellets. TNF-α yielded some signal after 1 week in culture, but no signal after two weeks. IL-1β and OSM+TNF-α both resulted in sustained IL-6 and IL-8 expression, however, IL-1β exhibited large variance. Thus, each cytokine contributes to unique pathways that are present in OA. As the combination of OSM and TNF-α appeared to lower cartilage gene expression and resulted in sustained and reproducible IL-6 and IL-8 production, it may serve as a better model of OA than a single cytokine such as IL-1β.

The last work evaluates col I/II hydrogels for a specific application: cartilage tissue engineering for osteoarthritic applications. Col II is the primary protein found in cartilage. Other components include: glycosaminoglycans, such as hyaluronic acid (HA) and chondroitin sulfate, chondrocytes (cartilage cells), and other small signaling molecules. Building on prior work in the group, high molecular weight hyaluronic acid (HA) was added to col I/II hydrogels and cartilage differentiation of mesenchymal stem cells (MSCs) was assessed under ideal laboratory conditions and under pro-inflammatory, osteoarthritic conditions (i.e., cytokine-supplemented media of oncostatin M (OSM) at 10 ng/mL and tumor necrosis factor-α (TNF-α) at 20 ng/mL). The addition of HA did not dramatically impact cartilage differentiation of MSCs, however, HA did mitigate the effect of inflammation via downregulation of degradative enzyme production. HA had little impact on inflammatory cytokine production of IL-6 or IL-8, both of which are upregulated during osteoarthritis. However, a linear model suggests that HA and IL-8 are strongly correlated. Thus, this system should be explored further with different concentration of HA or presentations of HA (e.g., chemically modified).