INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title:
Silk Based Three Dimensional Bioprinting for Meniscus Tissue Engineering

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SHORT ABSTRACT

Meniscus is a fibrocartilaginous avascular tissue present in the knee joint between the femoral condyles and tibial plateau. The heterogenous cellular composition of fibrogenic, chondrogenic and fibrochondrogenic phenotypes and extracellular composition of collagen, glycosaminoglycans and adhesion glycoproteins dictates the physiological function of this loadbearing tissue. Meniscus is prone to tears and injuries due to various trauma, accidents, and physiological conditions. These tears are conventionally treated with resection, suturing and implants. Most of them have their respective drawbacks related to patient-specificity for size and shape, material properties, cellular make-up and mechanical resilience being the major factors. This thesis identifies the requisite design considerations for treatment of full and partial thickness meniscus tears of both larger and smaller dimensions. 3D printing, injectable hydrogel and ultrasound-based strategies have been explored to potentially treat meniscus defects. Silk fibroin from mulberry (*B. mori*) and non-mulberry (*A. assama*) sources have been blended with various other bioactive polymers to formulate shear-thinning and thermo-reversible hydrogel inks for 3D printing and injection.

In the first objective, a silk fibroin/gelatin based bioink was formulated to 3D print physiologically relevant meniscus constructs to recapitulate the tri-layered macro-architecture of the native meniscus. The seeded fibrochondrocytes were found to be proliferating and maintaining their phenotype on these constructs. In the second objective, we formulated a photo-polymerizing silk-fibroin methacrylate(silkMA)/gelatin methacrylate (gelMA)/A. assama ink for encapsulation of growth factor loaded microspheres. These microspheres were loaded with fibrogenic and chondrogenic differentiation factors and used for fabricating constructs that aided in mimicking the zonal biochemical and cellular makeup of the red, red-white and white zones of the meniscus. In the third objective, we further enhanced the biomaterial-ink composition with polyethylene glycol di-methacrylate (PEGDMA) and autologous growth factor rich plasma (GFRP) to formulate silkMA/gelMA/PEGDMA/GFRP ink. The constructs 3D printed using this ink composition enabled sustained release of growth factors, slowed degradability and significantly enhanced mechanical resilience. The printed constructs also aided in fibrochondrogenic differentiation of neonatal human stem cells *in vitro*. This photo-polymerizing ink was further improved with polyethylene glycol di-acrylate (PEGDA) to formulate silkMA/gelMA/PEGDA/GFRP hydrogel in the fourth objective for minimally invasive injectable treatment approach for smaller meniscus tears. This hydrogel composition aided in neonatal stem cell proliferation, migration and fibrochondrogenic differentiation *in vitro*. *In vivo* evaluation yielded healing of full-thickness tears of the meniscus using this hydrogel composition. In the fifth objective, the injectable hydrogel composition was further used for 3D printing of constructs that were seeded with neonatal human stem cells and stimulated with low intensity pulsed ultrasound (LIPUS). This augmented the differentiation towards fibrochondrogenic phenotype and extracellular matrix deposition.

Thus, the biomaterial inks, injectable hydrogel composition and the LIPUS stimulation approach evaluated and demonstrated in this thesis could be envisaged for the development of holistic patient-specific clinically applicable meniscus tear remediation strategies in the future.