INDIAN INSTITUTE OF TECHNOLOGY, GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Development of Silk Based Matrices for Cartilage & Osteochondral Tissue Engineering

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SHORT ABSTRACT

The osteochondral tissue of the knee joint is a multi-tissue organ composed of articular cartilage, subchondral bone, and the synovial membrane, with effective functioning dependent on sustained joint homeostasis. The articular cartilage is incapable of self-healing and affects entire osteochondral tissue when damaged. In the absence of intervention, it ultimately leads to Osteoarthritis (OA) and limits the functioning of the entire joint. The extant non-availability of a cure for OA mandates the use of external regenerative strategies to repair the damaged osteochondral tissue.

The current thesis explored silk fibroin as a biomaterial-based regenerative strategy, sourced from mulberry (*Bombyx mori*) and non-mulberry/wild (*Antheraea assamensis, Antheraea mylitta,* and *Philosamia ricini*) silk types. The subsequent fabrication of matrices for cartilage and osteochondral tissue engineering was achieved through conventional or manual fabrication methods such as freeze-drying. The developed agarose and silk fibroin blended hydrogel nurtured the positive facets of the agarose gold standard and offset the negative aspects. It resulted in hydrogels that were biodegradable and immunocompatible, with the ability to support extracellular matrix (ECM) synthesis. The subsequent requirement of mechanical compliance, crucial in load-bearing articular joints, was achieved through fiber-reinforced composite scaffolds. The fiber-reinforced scaffolds supported improved growth of chondrocytes with an increased compressive modulus and stiffness (nearly 8-fold), in comparison to the fiber-free control groups. Since the entire osteochondral unit is implicated in OA, a biphasic silk scaffold was developed that mimicked the native osteochondral joint. It contained a spongy fiber-free phase for cartilage, a fiber-reinforced phase for bone revival, and a connecting interface, fabricated using a facile reproducible process. The developed hierarchically structured biphasic silk scaffold displayed phase-specific porous structures, with suitable mechanical

strength, and positive characteristics of in vitro ECM deposition, and in vivo regeneration (in rabbit) of osteochondral tissue.

Efforts were subsequently directed towards advanced additive manufacturing techniques such as 3D bioprinting. The self-gelling ability of silk fibroin blends *(B. mori* and *P. ricini*) was used along with gelatin as a bulking agent to develop the bioink, to encapsulate chondrocytes for cartilage bioprinting. The bioprinted constructs demonstrated *in vitro* cartilage-specific ECM formation and *in vivo* biocompatibility. The developed bioink showed good print fidelity for bioprinting cartilage grids along with state-of-art anatomical structures like the human ear. Disease model development was central to the final objective of understanding the molecular mechanisms of OA initiation and progression. An inflamed in vitro model was generated by 3D bioprinting an osteochondral unit using primed stem cells encapsulated silk-based bioink and cytokine-induced pathological conditions. The model was validated using anti-inflammatory drugs viz. Rhein and Celecoxib. It successfully mimicked the inflamed OA unit observed in the early stages of OA, and displayed the mitigative effects of the applied drugs, consequently, catering to the demand for a robust, high-throughput platform for screening novel anti-inflammatory drugs towards OA therapeutics.

Therefore, the current thesis explored and developed novel silk biomaterial-based matrices and bioinks for cartilage and osteochondral repair and regeneration; and validated their pre-clinical functionality both *in vitro* and *in vivo*.