INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: **Silk Based Tissue Engineered Strategies to Repair Damaged Intervertebral Disc**

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SHORT ABSTRACT

Low back pain (LBP) is an important clinical and socio-economic condition affecting the population indiscriminately across the globe. Intervertebral disc (IVD) degeneration is the major cause of LBP. The conventional therapies are only effective in symptomatic relief of pain without restoring the biomechanical function of native discs and are also associated with high expenditure of the treatment. The emergence of tissue engineering technology provides a promising alternative strategy for the treatment of IVD degeneration by replacing the damaged discs with tissue engineered discs that may resemble the native disc structure and functions. In this thesis, we tried to develop biomimetic IVD constructs that included annulus fibrosus (AF) anatomical equivalents and a suitable hydrogel system as a nucleus pulposus (NP) substitute using silk fibroin as biomaterial.

To mimic the internal intricacy of AF anatomy, a silk protein-based multilayered, disc-like angle-ply construct was fabricated, consisting of concentric layers of lamellar sheets. The developed constructs supported primary AF cells and human mesenchymal stem cells (hMSCs) viability, proliferation and deposition of AF specific ECM components with time. Furthermore, a seamless, full thickness disc-like angle-ply construct was fabricated using SF protein. This improved design restricted the possible delamination of multiple lamellar sheets upon implantation. To mimic the gradual transition of mechanical gradient from inner to outer region of native AF tissue, SF proteins from two different sources (namely *Bombyx mori*, BM SF as mulberry, and *Antheraea assamensis*, AA SF and *Philosamia ricini*, PR SF as non-mulberry) were blended that provided differential mechanical and cell binding properties. Furthermore, for precise replication and high throughput reproducibility, 3D printing technology was adopted wherein silk-carrageenan based biomaterial ink was deposited in layer-by-layer pattern mimicking the native AF anatomy. These AF cells/adipose derived stem cells (ADSCs) compatible 3D printed constructs possessed requisite compressive modulus showing their potential in load bearing applications.

Formulation of biologically active hydrogels with desirable characteristics is one of the prerequisites for successful application in nucleus pulposus (NP) tissue engineering to address disc degeneration. For this, two naturally derived silk fibroin proteins (*Bombyx mori*, BM SF and *Antheraea assamensis*, AA SF) were blended together to allow self-assembly and transformation into hydrogels in absence of any cross-linker or external stimuli. The self-gelling properties were thoroughly investigated under several physiological conditions *e.g.,* pH, temperature, and ionic strength. Tunable gelation time (~ 8-40 min) and mechanical properties were achieved depending on the silk fibroin

combinations. The NP cells compatible hydrogel system showed good recovery of mechanical properties when tested in an *ex vivo* model, thus validating its *in situ* applicability in a minimally invasive manner towards potential disc regeneration therapy. This hydrogel system was further functionalized with decellularized human Wharton's jelly extracellular matrix (dWJECM) as an ampule of bioactive cues. The developed NP cells compatible bioactive hydrogel showed the rheological and mechanical properties similar to the native NP tissue, hence used as minimally invasive injectable hydrogel as NP substitute.
Overall, the AF tissue mimicking constructs and NP tissue equivalents supported cell proliferation, differentiation and ECM deposition resulting in IVD tissue features based on ECM deposition and morphology, indicating their potential for future studies related to IVD replacement therapy.