“Exploring Potential of Silk 3D Matrices for Bioartificial Liver and Regenerative Applications”

ABSTRACT

Advanced liver cirrhotic patients are limited with liver transplants as the only therapeutic option; indeed, the practical applications of liver transplantation possess several drawbacks. The criteria for engineering 3D hepatic constructs for liver regenerative medicine are (i) cell-cell and cell-matrix interactions assisting long-term cell viability, (ii) heterotypic culture of liver cells maintaining cell functionality, (iii) long-term liver-specific synthetic, metabolic, and detoxification functions, and (iv) facilitating the regeneration of damaged tissue. A suitable 3D matrix with appropriate biocompatibility, hemocompatibility, topography, and physicochemical attributes facilitates hepatocyte aggregation, polarity, differentiation, and proliferation. Herein, mulberry *Bombyx mori* (BM) silk fibroin, non-mulberry *Antheraea assamensis* (AA) silk fibroin, and decellularized liver extracellular matrix (ECM) are explored in the domain of liver tissue engineering owing to their biochemical composition, mechanical stiffness, biocompatibility, and biodegradability. The presence of intrinsic RGD (arginine-glycine-aspartic acid) motifs and the high mechanical strength of AA silk fibroin has made it a potential biomaterial as it enhances cellular attachment and cell-matrix interactions. The prominent role of LECM hydrogel in liver tissue engineering has been emerging, owing to the presence of growth factors, cytokines, and cell-secreted exosomes.

In the first objective, a 3D silk biomatrix niche was developed and investigated for its potential in maintaining long-term functional hepatocyte spheroids. The porous and hemocompatible blend 3D silk scaffold (BA) fabricated by amalgamating mechanically resilient RGD containing AA silk fibroin and BM silk fibroin assisted the self-aggregation of functional hepatocyte clusters and maintained albumin secretion, ureagenesis, and cytochrome P450 activity over 3 weeks, as evaluated using HepG2 and primary rat hepatocytes. In the second objective, a physiologically relevant *in vitro* liver zonation model recapitulating healthy liver's mechanical strength, periportal functions, and pericentral functions was developed. The blend silk scaffold was functionalized with decellularized LECM solution to resemble tissue-specific biomimetic cues and dynamic interplay between cells and matrix to accomplish hepatocyte polarity, growth, liver functions, and long-term stability. In the third objective, a bioprinted *in vitro* liver model recapitulating the spatiotemporal arrangement and cellular composition of the native liver was developed with a sinusoidal lumen-like network, alternate cords of parenchymal and non-parenchymal cells, and investigated for its potential for drug screening applications. The developed clinically relevant human vascularized liver model maintained hepatic functions over 2 weeks, showed increased metabolic competence, and predicted hepatotoxicity. In the fourth objective, the host response and macrophage phenotype activations state towards mulberry and non-mulberry silk scaffolds were investigated and compared to liver ECM, small intestinal submucosa ECM (SIS), and polypropylene surgical mesh (PP mesh) in a partial thickness abdominal wall defect model and *in vitro* primary murine bone marrow-derived macrophages. The findings showed that *Antheraea assamensis* silk implants supported constructive remodeling with fewer multinucleate giant cells, high CD206 expression, and increased M2-like: M1-like macrophage response with potential therapeutic applications. In the fifth objective, the therapeutic role of liver ECM and AA in attenuating liver fibrosis was examined in the acetaminophen-

induced liver fibrosis mouse model. The intraperitoneal administration of liver ECM and AA hydrogel attenuated liver injury through reduced necrosis, inflammation, and NAPQI accumulation, and promoted early liver regeneration by activating cyclin D1 and Ki67.

Thus, the current thesis deliberates on designing an ideal 3D matrix and employs advanced fabrication techniques and perfusion culture systems to generate liver models. Various crucial facets were addressed in this thesis for the development of a scaffold platform that could provide physiochemical attributes, microarchitectural features of the native liver, hepatocyte metabolic zonation, minimal inflammation, and promote hepatocyte regeneration. Thus, the positive findings from this work hold promise for the viable 3D matrices with biomimetic cues from LECM and AA silk fibroin supporting the long-term hepatocyte polarity and functionality for its applications in regenerative medicine for clinical translation.