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Bioartificial liver constructs

The liver with high regenerative capability performs a complex array of synthetic, metabolic, immunologic and detoxification functions to regulate the physiological homeostasis of the human body. Viral infections, drug induced liver injury, hepatocellular carcinoma, autoimmune disorders, alcoholic and non-alcoholic fatty liver diseases results in a non-regenerative end-stage liver disease called cirrhosis. Each year, over 10% of the world population is affected by cirrhosis and is also estimated to be 12th leading cause of mortality by 2020. Liver transplantation, a primary successful therapy cannot meet the increasing demand inspite of the surgical advancements. Unfortunately, 50 million deaths occurs worldwide due to organ shortage, high cost and immune rejection. As a result, alternate liver support technologies like bioartificial liver (BAL) devices analogus to kidney dialysis units, cell and tissue-engineering based therapies acts as a clinical bridge by performing some functions and providing sufficient time for injured liver to recover. An *in vitro* functional hepatic tissue simulating micro-environmental niche of native liver is in great need to achieve maximum liver functions.

Fig. 1. Dr. Biman B Mandal (team lead/corresponding author) and Ms. Janani G (first author).

A three dimensional (3D) scaffold considering the material properties is essential for culturing hepatocytes to bring improvements in the culture period and cellular metabolism of developed hepatic construct. Current research focuses on fabricating an economical, biocompatible matrix that promotes hepatocyte functionality through scaffold based biomimetic approach to exploit in BAL devices. Silk, a fibrous protein and a natural biomaterial possess valuable bioactive properties like biocompatibility, hemocompatibility, mechanical strength, low immunogenicity and ability to acquire various formats. Explicitly, silk sutures have been used in healthcare for many years and recently explored for various tissue engineering applications. Silk fibroins isolated from mulberry (*Bombyx mori*, BM) and non-mulberry (*Antheraea assama*, AA) silkworm own unique amino acid sequences with difference in hydrophobicity and crystallinity. Physicochemical attributes of a matrix influence the behavior of cultured hepatocytes in terms of attachment, morphology and functionality. Considering this, the present study emphasizes on (i) developing a suitable bioactive matrix by blending mechanically resilient, cell adhesion motif (RGD) rich AA with BM to fabricate blend (BA) porous scaffold and (ii) validating their potential in supporting hepatocyte adhesion, growth and functionality using human HepG2 (Hepatocarcinoma) cells and primary neonatal rat liver cells. Silk scaffolds presented interconnected pores in the range of 70–130 µm diameter that recapitulate *in vivo* liver microarchitecture to maintain hepatic physiology. The mechanically stable scaffolds displayed porosity around 88-92%, thus assisting bidirectional mass transfer of nutrients to diffuse in and metabolites to diffuse out of the cell-seeded construct.

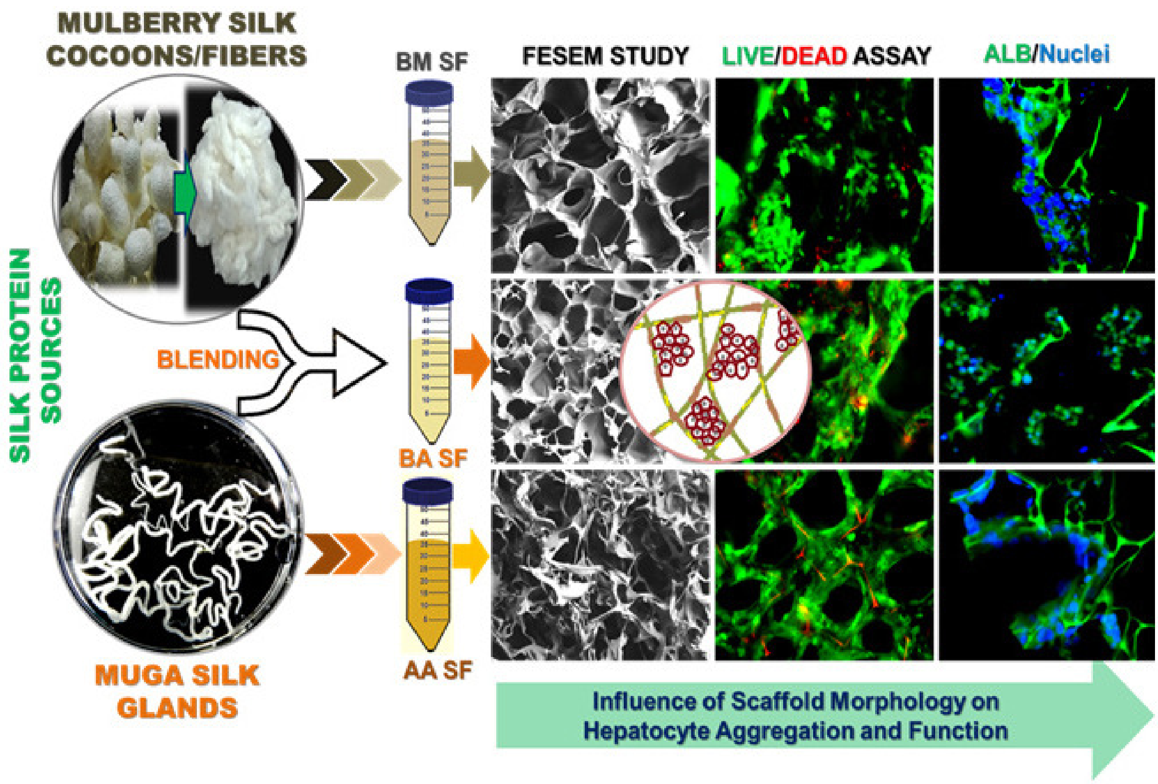

Fig. 2. Schematic representation of the fabrication of bioactive blend silk scaffolds for the development of functional liver constructs.

Surface topography and physicochemical attributes of scaffolds influenced the cell attachment pattern and the growth profile of cultured hepatocytes in the matrix. Culturing primary rat liver cells signifies the physiologically relevant system for BAL by accomplishing the major liver functions. Live cell imaging and histology of cell cultured scaffolds evidenced the formation of stable and optimal sized hepatocyte clusters (< 100 µm) in the blend silk scaffolds. Improved nutrient and oxygen diffusion to the hepatocyte clusters supported high cell density highlighting both cell-cell and cell-matrix interactions. Hepatocyte clusters in blend silk scaffolds were metabolically active and retained liver-specific functions including albumin synthesis, urea synthesis and cytochrome P450 enzymatic activity over 3 weeks as compared to hepatocytes cultured in BM and AA scaffolds. Further, a functionally engineered *in vitro* liver construct for BAL systems demands hemocompatibility and immunocompatibility to minimize thrombogenesis and host immune response respectively. Subcutaneous implantation of silk scaffolds revealed graft remodeling with macrophage infiltration, cellular ingrowth and vascularization confirming the immunocompatibility of silk matrices. Engineered bioactive 3D hepatocyte construct with long-term functional attributes would find its future prospects in biohybrid devices and liver tissue engineering.

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Publication

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