Chapter 4
SUMMARY AND FUTURE PERSPECTIVES

For regeneration of complex organs such as liver, besides good biocompatibility and biodegradability, the scaffolds ideally should possess the 3D spatial architecture of the liver as well to promote gene expression related to cell growth and liver-specific functions. Thus, 3D scaffolds embedded with protein nanoconstructs have drawn increasing interest in the biomedical arena owing to their additional ability to deliver drugs at optimal doses, often resulting in increased therapeutic efficacy of the drug, weakened side effects, and improved patient compliance. Initial part of this research work explains the combinatorial strategy adopted for the synthesis and characterization of biomimetic scaffolds having Agarose- nano Fibrin (AnF) milieu. Research interests were also given to synthesize hepatocyte growth factor (HGF) loaded nano fibrin constructs and to study its efficacy to induce hepatocyte proliferation within this bioengineered matrix. Since a major obstacle in engineering liver tissues for clinical use is the limited availability of human cells, later part of this research work demonstrated the prospect of in vitro differentiation of the readily available human mesenchymal stem cells (hMSCs) from umbilical cord blood to hepatocytes. The AnF cryogels that mimic hepatic microenvironment developed through this study could be a promising 3D platform for inducing matrix assisted differentiation of multipotent stem cells to hepatocytes under static in vitro cell culture conditions. The use of multipotent stem cells like hMSCs for in vitro differentiation to adult human cells can help to overcome the hurdles of cell shortage, which is a major research constrain. Under circumstances in which a small, but functional liver tissue system could be engineered to provide the equivalent biological function proportional to a few percent of a normal, well-functioning liver, such an approach would be feasible. Such hepatic tissues can be engineered rapidly to produce therapeutic effects, allowing this approach to become an effective modality in the treatment of acute liver failure. The later part of this research work envisages the potential of developing a perfusion cassette that can hold 3D hydrogel matrix bed of Agarose- nano Fibrin (AnF) milieu. Use of hepatocyte growth factor (HGF) loaded nano Fibrin (nF) moieties was experimented within such hydrogel matrices for nurturing human stem cell derived hepatocytes for prolonged durations. High density cell laden Alginate micro beads(AMBs) are
synthesized and used to encapsulate the hepatocyte biomass within such hydrogel matrix bed. Physico-Chemical and *in vitro* biological evaluations of these matrix beds further proved the practicability of using it as a Live Cell based Bioartificial Liver Support System (LC-BALSS). In order to study the prolonged response of embedded hepatocytes to the *in situ* liver fluid dynamics, the liver assisted system thus designed was positioned in a perfusion bioreactor system by giving a controlled fluid flow (5 ml/min) of cell culture media and body fluids (blood plasma and serum) that enabled a detail investigation on metabolic and detoxification activities of embedded hepatocytes. Hepatocytes are progressively proliferated by spontaneously aggregating within the hydrogel matrices of LC-BALSS that helped in retaining their metabolic and detoxification activities for extended durations. The enhancement in expression of OATP and Connexin-32 protein during prolonged perfusion of cell culture media proved the functional activity of cellular surface proteins of the embedded hepatocytes within the hydrogel matrix. The elevated level of ALT activity, net albumin synthesis and EROD activity was further observed during this experiment that further proved the efficacy of hepatocyte microsomal enzymes. Detoxification activity of the system exposed to the perfusion of human blood plasma was further proved through the quantification of ammonia to urea conversion and by quantifying the conversion of Alkyloxyresorufin and Propafenone to their respective fluorescent by products. Biosynthetic activity of the resident hepatocytes was demonstrated further by quantifying the net synthesis albumin a fibrinogen while perfusion of blood plasma and pooled serum through the matrix. The fundamental success attained through the development of LC-BALSS can further be translated from ‘bench to bedside’ directly as described strategy in Figure 4.1 or through modifications using similar combinatorial cell-supportive biomatrices in an extracorporeal bioreactor setup to further enhance the metabolic and detoxification hepatic functionalities through successful liver organoid growth.
Figure 4.1. Representation of the clinical translational perspective of LC-BALSS

Clinical translation perspective

Considering that 5 litres of patients whole blood is needed to be used for hepatic dialysis (i.e., about 3 L of plasma separated out), using a single perfusion unit of LC-BALSS with a perfusion rate of 5 ml/min, the dialysis can be completed within 10 hours. The feasibility of connecting five perfusion units of LC-BALSS is also discussed. If that is the case, the dialysis can be completed within 2 hours.