Chapter 4

SUMMARY AND FUTURE PERSPECTIVES

4.1 SUMMARY

The research work carried out in this thesis was focused on developing a functional cardiac patch *in vitro*, using a multiscale scaffold composed of natural and synthetic polymers for myocardial regenerative purposes. For this purpose, the candidate materials selected were fibrin and PLGA, which are expected to provide enhanced biological response and mechanical integrity respectively.

The work started by devising a new electrospinning method for the fabrication of fibrin nanofibers. The nanofibers of fibrin generated by this method showed fiber diameters in the range of 50-500nm which mimic the dimensions of native ECM. The fibrinogen rich cryoprecipitate used in this study contained considerable amounts of the natural cross-linking agent plasma transglutaminase (Factor XIII), which eliminated the use of external cross-linking agents that can be deleterious to the bioactivity of the scaffold. This novel electrospinning method has a striking advantage over other conventional coating techniques wherein fibrin is coated over various scaffold surfaces. This advantage lies in the uniform distribution of fibrin with predictable fiber morphology and dimension that can be obtained by electrospinning, which promotes cell attachment and spreading more efficiently and rapidly. Cell-interaction studies with cord blood derived hMSCs revealed the application potential of this scaffold in various avenues of tissue engineering. The hMSCs seeded on the scaffolds were differentiated into cardiomyocytes, when exposed to cardiomyogenic inducers and provided with specific growth medium for cardiomyocyte growth. However, the scaffold was found to have poor mechanical integrity to be used for engineering tissues such as myocardium.
which needs better mechanical stability to withstand the pressure developed during heartbeat.

In the second part of the thesis, emphasis was laid on developing a multiscale composite scaffold with better mechanical integrity and porosity. The technique was optimized using PCL as a candidate polymer, which is comparatively cheaper than other biodegradable polymers and widely used for tissue engineering applications. A sequential electrospinning of fibrin nanofibers and PCL microfibers was employed to create the multiscale scaffold. The developed multiscale scaffold showed improved mechanical properties than natural protein fibers alone, with better cell attachment and spreading in comparison to the synthetic polymer PCL in fibrous form. The multiscale nature of the scaffold resulted in larger pore size which favored cellular infiltration throughout the scaffold. The in vitro inflammatory response evoked by this scaffold was minimal.

Having established the feasibility of fabricating a multiscale scaffold with uniformly distributed fibrin nanofibers for tissue engineering applications, we next sought to select another biodegradable polymer which has a mechanical and degradation profile matching with the requirements of cardiac tissue engineering scaffolds.

Third part of the study was thus focused on developing a multiscale composite scaffold of PLGA microfibers and fibrin nanofibers. PLGA 75:25 was found to be a better polymer for myocardial tissue engineering applications, as its degradation time (5-8 months) matches with the myocardial regeneration time frame. The composite scaffold showed better cell attachment, viability and proliferation compared to its synthetic counterpart. Although the fibrin component was very less in the scaffold compared to the synthetic polymer component, its bioactivity was found to be adequate in promoting initial attachment and proliferation of hMSCs and their subsequent differentiation into cardiomyocytes. However, the culture of the cell seeded scaffold under static conditions did not yield any functional contractile myocytes.

Subsequently, a design strategy was developed to create a functional myocardial patch using a bioreactor, which generated a biomimetic microenvironment by providing electromechanical stimulation. Electromechanical stimulation resulted in an efficient cell-
cell coupling and the differentiated cardiomyocytes organized into a beating tissue construct \textit{in vitro}. Individual cell seeded PLGA-fibrin membranes were then made into a functional 3D patch by layer-by-layer assembly of individual electrospun sheets glued with fibrin.

Finally, as a preliminary attempt to test the \textit{in vivo} integration efficacy of the developed 3D cardiac patch with host myocardium in rat MI model, the same design principles were used to generate a functional myocardial patch from rat bone marrow MSCs.

This thesis work is a proof of the concept that, the stem cells can be directed to differentiate in to functional tissues if provided with a tissue mimetic microenvironment. Preliminary \textit{in vitro} results indicate that there is an increased possibility that the patch would perform better in \textit{in vivo} conditions. However, the present study has the following limitations.

4.2 LIMITATIONS OF THE THESIS

There are numerous potential applications for this \textit{in vitro} generated cardiac patch such as regenerative medicine, cell therapy, and toxicity studies. Hence it is essential that the tissue engineered construct should recapitulate the native cardiac physiology in order to be useful for the above clinical applications. The clinical application of the engineered patch with stem cell derived cardiomyocytes will ultimately be determined by the functional properties of the patch. In this thesis, the functional nature of the constructs was determined by the expression of gap junction protein connexin-43, which is critical for the electrical coupling between adjacent myocytes. Also, the contractile behaviour was assessed by recording video clips and quantifying the beating rate. For further proving the validity of this patch for clinical applications, more functional assays like electrophysiological properties and contractile force measurements needs to be done. Tools like microelectrode or patch clamp are required to determine the electrophysiological parameters. Direct measurement of contractile force requires the use of force transducers. In addition to that, \textit{in vivo} studies in suitable animal models need to
be done to completely validate the clinical applicability of the in vitro generated myocardial patch.

### 4.3 FUTURE PERSPECTIVES

The technique developed for generating fibrin based multiscale composite fibrous scaffolds can be adopted for engineering other tissues as well. The biological response of fibrin nanofibers can be coupled with any other synthetic or natural polymers befitting tissue specific applications.

In this PhD thesis, we could develop a myocardial patch in vitro, with cardiomyocytes differentiated from cord blood hMSCs and rat bone marrow MSCs. As a part of the future work it would be useful to explore other cell sources like iPSCs, adipose derived stem cells to create functional tissue equivalents.

Improved cell seeding and culture techniques are essential to make a cardiac tissue construct with clinically relevant dimensions and cell density.

Since prevascularization is a critical requirement of myocardial tissue engineering scaffold, a co-culture system with other cell populations of heart tissue can be developed to improve the functionality of a bioengineered tissue construct.

Finally, long term and short term in vivo implantation studies are required to evaluate the performance of the developed patch in suitable animal MI models. Previous studies have shown that functional integration with the host myocardium can fail if the implanted construct does not communicate electrically to the host myocardium.

This is only the beginning of a journey towards a successful cardiac implant which can be used as an alternative tissue replacement in reconstructive cardiac surgery. We will have to address numerous biological issues to translate this research work from bench to bedside.