Electrospinning is one of the versatile techniques used to fabricate scaffolds having structural resemblance with native myocardial matrix. The extracellular matrix of heart comprises collagen as the major component with small amounts of elastin, fibronectin and laminin. The size scale of collagen fibrils ranges from nano to micro scale. Both natural and synthetic polymers were electrospun into fibers of nano to micro scale dimensions for wide range of tissue engineering applications. Synthetic polymer scaffolds may be produced using defined processes and have highly tunable mechanical and chemical properties. However hydrophobic properties of synthetic polymers leave them with insufficient cell-recognition signals for cell attachment and proliferation. In contrast, naturally derived polymers have the potential advantages of cell recognition motifs. Fibrin is natural wound healing matrix having angiogenic potential and comprehensively used for tissue engineering applications. It provides a natural environment for cell attachment, migration and proliferation. However electrospinning of fibrin (ogen) require harsh organic solvents and synthetic cross linking agents which may compromise the bioactivity of the scaffolds which make them inappropriate for tissue engineering applications. So the first objective of this thesis was to develop a method for electrospinning fibrin nanofibers without using any harsh organic solvents. In the first part of the thesis, the process, characterization, and suitability of the fibrin nanofibrous scaffolds for tissue engineering applications.
is demonstrated. The potential of the scaffold for supporting the differentiation of Mesenchymal stem cells (MSCs) into cardiomyocytes is also evaluated. Our results demonstrate that the average diameter of the electrospun fibrin nanofibers ranges from 50–500 nm, which resembles the natural ECM in dimension. Cell attachment and proliferation studies revealed that the scaffold supports the attachment, spreading, and proliferation of human umbilical cord blood-derived MSCs. The scaffolds also supported the differentiation of cardiomyocytes from MSCs when provided with 5-azacytidine containing culture medium under suitable culture conditions.

Thereafter, we demonstrated the fabrication and characterization of a multiscale fibrin based composite scaffold with polycaprolactone (PCL) by sequential electrospinning of PCL microfibers and fibrin nanofibers. Results showed that this multiscale scaffold has great potential for tissue engineering applications due to the combined benefits of fibrin nanofibers such as cell attachment and proliferation and that of PCL microfibers such as open structure, larger pore size and adequate mechanical strength. Since PCL has a slow degradation rate, in the next step, PCL is replaced with Poly (lactic-co-glycolic acid) (PLGA) which is a fast degrading polymer, to modulate the degradation and mechanical properties of the scaffold to make it suitable for myocardial tissue engineering applications. Morphological, chemical, and mechanical characterization of the scaffolds was done by scanning electron microscopy, fibrin-specific phosphotungstic acid hematoxylin staining, and mechanical testing. The fiber diameters of fibrin nanofibers range from 50 to 300 nm and that of poly (lactide-co-glycolide) microfibers range from 2 to 4 µm, which mimics the structural hierarchy of native myocardial tissue. Experimental results indicate that this scaffold enhances the differentiation of mesenchymal stem cells into cardiomyocytes when provided with cardiogenic differentiation inducers.

Another important challenge in cardiac tissue engineering is the formation of a thick viable tissue. Single layer of electrospun membrane have only ~ 100 µM thickness, so 6-8 layers of the cell seeded membranes were stacked together to form a thicker patch having ~ 1 mm thickness with viable cells. Cardiac cells, rely on
electrical signals to coordinate their contraction, that’s a big challenge in engineering a functional tissue construct. Good coupling is needed between the cells to allow the propagation of action potential for which we used a bioreactor to mimic the dynamics of natural heart muscle. The cells showed enhanced expression of connexin-43 a gap junction protein involved in cell-cell coupling. Gene expression studies showed enhanced expression of cardiac specific genes GATA4, NkX-2.5, MEF2C, MYH, Troponin and ATF upon stimulation. Also, the cells electromechanically integrated to generate a beating myocardial tissue.

Now to translate this research into clinical practice, pre-clinical testing should be done in suitable animal models. Since rat coronary ligation model is the commonly used animal model to test cardiovascular implants, in vitro differentiation studies were conducted using rat bone marrow MSCs, which were seeded on to the scaffold, differentiated in to cardiomyocytes and made in to thick viable beating patch in a bioreactor. Collectively, the findings in the thesis work shows the promising potential of this myocardial patch with contractile cardiomyocytes differentiated from MSCs as an alternative myocardial tissue replacement for congenital and reconstructive heart surgery.