GENETIC AND EPIGENETIC NANOCOMPOSITE SCAFFOLDS FOR CARDIAC REPAIR AND REGENERATION

SUMMARY OF DOCTORAL THESIS

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Myocardial infarction is the predominant cause of death worldwide. The human adult heart lacks the efficiency to regenerate on its own. Repair of these damaged myocardia is the only cardiac therapy available at present as current therapies are restricted to heart transplantation. Despite, many advances in regenerative strategies, mortality remains high. At this juncture, direct reprogramming raises the possibility of changing the somatic cells to direct patient-specific cell bypassing stemness. This approach can eliminate the limitation of stem cells such as genetic aberration and altered expression of defined /chemical/genetic factor for reprogramming which causes permanent side effects like cancer. Thus, this approach helps in patient-specific precision medicine after myocardial infarction, but the ability to efficiently generate a more considerable number of functional cardiomyocytes remains a challenge. Besides, extensively research on direct conversion of cells to a different lineage. The drawback, such as the use of viral vectors and low transfection efficiency necessitates an alternative strategy for reprogramming cells. The advent of direct reprogramming or transdifferentiation has come to a stage where it needs to be fine-tuned with a combinational approach for efficient reprogramming. Previous reports, in the field of direct lineage conversion, has led to the identification of different cocktails for reprogramming and have successfully demonstrated the expediency of this strategy. However, now, the field of transdifferentiation entrails a systematic approach to reprogram cells for a high yield of the fully

functional cardiomyocyte. In this thesis, we aim to fabricate electrospun scaffold with engineered genetic and epigenetic regulators to overcome the current limitation of direct reprogramming such as lack of an ideal delivery system and reprogramming efficiency to convert more functional cells. Thus, scaffold mediated cell-free therapy helps to mimic myocardial microenvironment that helps to modulate the cell fate of hearts most abundant cell type, adult cardiac fibroblast into fully functional cardiomyocyte bypassing pluripotency. To achieve this goal, we have explored the potential of 3D electrospun Poly (L-lactic acid) (PLLA) fiber with different topography, such as porous and smooth. Electrospun scaffolds were used as it structurally mimic the naive extracellular cellular matrix, provides in-situ scaffold mediated sustained and controlled genetic and epigenetic factors delivery and also serves an ideal biomimetic composite scaffold for dictating the cell fate for non-stem cell therapy by mimicking the myocardial environment. The thesis entitled "**Genetic and Epigenetic Nanocomposite Scaffolds for Cardiac Repair and Regeneration**" comprises of nine chapters which describe how we explored different strategies to engineer biomimetic scaffolds with genetic and epigenetic regulators. Following sections highlight the summary of each chapter of the thesis.

Chapter 1, entitled "**Cardiac Direct Reprogramming with Engineered miRNA Scaffold",** deals with the introduction, review of literature, aim and objective of the thesis. It covers the current state of art and methods to develop efficient cell-free scaffold for direct reprogramming with miRNA and epigenetic factors. Furthermore, chapter 1 describes the crucial role of miRNA and critical factors needed for efficient scaffold mediated in-situ miRNA delivery systems in direct reprogramming. It also provides with a new insight to understand the role of biophysical cue for

reprogramming. In conclusion, this chapter illustrates the important parameters for fabrication of nanocomposite scaffold with nanoparticles for direct cardiac reprogramming.

Chapter 2, entitled **"Bio-Instrumentation",** outlines the basic operating principles of sophisticated instruments used in this study for the characterization of nanomaterials for direct reprogramming. The instrument such as (i) Electrospinner used for fabrication of nanocomposite scaffold (ii) FTIR, XPS, TGA, SEM and TEM for characterization of physicochemical, thermal, morphological, topographic properties of scaffolds are dealt. Furthermore, other molecular techniques used for the evaluation of change in cell fate are also discussed in detail.

Chapter 3, entitled **"Effect of Electrospinning Parameters on fiber morphology of Electrospun Porous and Smooth Random Poly (L-lactide) Fiber",** comprehends the important fabrication parameters used for fabrication electrospun porous and smooth scaffolds with different solvent systems. The distinguish the effects of polymer solution parameters such as viscosity, an ambient condition such as humidity, temperature and also fabrication parameters such as voltage, tip to collector distance, flow rate, for successful fabrication of electrospun scaffolds as the parameters can influence the morphology of fabricated fibers. Herein different weight percentage of polymers, flow rate and solution parameters were varied to attain a defect-free fiber. For instance, in the case of porous fiber, the dual solvent system was used to fabricate highly porous PLLA scaffolds, whereas, smooth fibers were fabricated with a single solvent system to achieve a smooth morphology.

Subsequently, these fabricated PLLA scaffolds were further used for its respective applications.

Chapter 4, entitled **"Surface Functionalization and Surface Characterization of Electrospun Scaffolds for Cardiac Tissue Engineering and Regeneration",** deals with the surface functionalization of protein using simple drop coating method and characterization of fabricated PLLA electrospun fiber. The fibers were surface functionalized by ECM mimetic proteins such as collagen, gelatin, fibronectin, and poly L-lysine to enhance its property. The functionalized scaffolds were further characterized using XPS, FTIR and TGA to confirm the successful protein functionalization. Apart from physiochemical characterization, the degradation property of the scaffolds was evaluated to understand its property for its potential application in cardiac tissue engineering and direct reprogramming.

Chapter 5, entitled **"ECM Mimetic Electrospun Porous Poly (L-Lactic acid) Poly (L-lactic acid) Scaffolds as a Potential Substrate for Cardiac Tissue Engineering",** demonstrates the potential property of PLLA scaffolds in cell attachment, proliferation and migration. The results were conducive that these scaffolds could be manipulated for its possible application in cardiac tissue engineering. In this chapter, the human cardiac fibroblast was grown on surface functionalized scaffolds with different ECM proteins. The results showed PLLA scaffolds provided excellent cell anchorage irrespective of the surface functionalization. Further, evaluation of cell behaviour on PLLA scaffolds analytes using Ki67 ELISA and protein expression profiles revealed that cardiac fibroblast cells grown on different fiber substratum expressed crucial proteins which had a

major role in cardiac fibroblast growth and differentiation, which illustrates its exciting avenues for co-culture systems to mimic the myocardial microenvironment, heart on-chip applications.

Chapter 6, entitled **"Poly (lactic-co-glycolic acid)/Polyethylenimine Nanocarriers for Direct Genetic Reprogramming of miRNA Targeting Cardiac Fibroblasts",** accentuates the role of non-viral Poly (lactic-co-glycolic acid) (PLGA)-Polyethyleneimine (PEI) nanocarriers with different cocktails of muscle-specific miRNA such as miR-1 and miR-133a to target cardiac fibroblast. PLGA Nanospheres were compared with most widely used PEI-polyplexes and lipoplexes to identify efficient non-viral gene delivery system for targeting cardiac fibroblast for direct genetic reprogramming. We have exploited PLGA nanocarriers to overcome the current limitation of gene delivery system. Here, PLGA nanospheres were synthesized by encapsulating PEI-miRNA complexes. The PLGA nanosphere was synthesized using PEI-miRNA complexes instead of lipoplexes as these complexes possess drawbacks such as lipid stability in the serum despite its high transfection efficiency by facilitating the lipoplexes to non-bilayer. The polyplexes stability was evaluated to ensure successful condensation of the gene with polymer interaction by various characterization techniques. Further, to enhance the biocompatibility of the polymer for direct reprogramming. The polyplexes were encapsulated with PLGA nanosphere. Upon encapsulation, the average diameter of the formulation ranged within a few hundred nanometers which ensures its application in intramyocardial administration for direct reprogramming. Further, the phenotype of reprogrammed cells was confirmed by screening for early cardiac markers such as cTnT and alpha-actinin by immunocytochemistry. Our findings

suggest that PLGA-PEI-miRNA nanocarriers improved the intracellular internalization of cargo, exhibited pH-dependent release of the genetic material and efficiently reprogrammed cardiac fibroblast into cardiomyocyte like cells with minimal dosage. Thus, nanovector mediated gene delivery serves as an ideal system for direct cardiac reprogramming.

Chapter 7, entitled **Polyethylenimine-miRNA Loaded Nanocomposite Poly (L-lactic acid) Scaffolds-Mediated Delivery of Dual Muscle Specific miRNA for Cardiac Direct Reprogramming",** engrosses the role of scaffold mediated delivery of miRNA for direct reprogramming of heart. Based on work carried out in the previous chapters, we found that miRNA is a promising candidate for reprogramming of cardiac fibroblast *invitro*. Based on the previous results of chapter 6, In this chapter, we explored microenvironmental signals such as substrate topographic cue and genetic reprogramming cocktail of dual microRNA for direct genetic reprogramming. In this study, dual muscle-specific miRNA was complexed with PEI by the method of self-assembly to protect the miRNAs from extracellular degradation during entry to the cell. Further, these polyplexes were immobilized to electrospun smooth scaffolds and porous nanofibrous scaffolds with high porosity, for scaffold with mediated in situ delivery of dual miRNA as this strategy can overcome the uncontrolled off-target effects by providing two-stage controlled release of miRNA from the scaffolds. Results were suggestive that sustained-release enables to achieve a high transfection efficiency as high levels of miRNA is maintained for efficient reprogramming.

Further manipulating PLLA scaffolds with miRNA enhanced the transfection efficiency to 2-fold in comparison to our previous findings. A holistic approach of

modulating biophysical cue, genetic reprogramming cocktail helps in modulating the cell fate of somatic cell to cardiomyocyte like cells. To summaries, *in-situ* scaffold mediated microRNA delivery targeting cardiac fibroblast serves as a new alternative strategy to investigate the role of microRNAs in dictating cell fate. It also serves as an exciting avenue for gene therapy. The scaffold mediated delivery of miRNA acts as a novel cell-free therapeutic strategy for direct cardiac reprogramming.

Chapter 8, entitled **"Bioactive Genetic and Epigenetic Nanocomposite Poly (L-lactic acid) Porous Scaffolds for Cardiac Direct Reprogramming",** illustrates the fabrication of Epigenetic and genetic nanocomposite scaffolds for direct cardiac reprogramming and tissue engineering. An electrospun scaffold is an ideal biomaterial for developing substitutes for the regeneration of partially or fully damaged organ or tissues. However, in some case, reprogramming becomes tough due to the uncertain role of epigenetic and genetic factors. It also limits the transfection efficiency. To overcome these drawbacks, direct conversion of the somatic cells provides an exciting propitious opportunity to reprogram functional cardiomyocytes. To directly reprogram, targeting most abundant cell type is an ideal strategy. A systemic approach to reprogram fully functional heart by manipulation of biophysical cues, genetic and epigenetic cues can assist the direct conversion of cell fate of somatic cell such as cardiac fibroblast. Herein, in this chapter, PLGA nanospheres were used to encapsulated with muscle-specific miRNA for genetic reprogramming and DNA methylation inhibitor for epigenetic reprogramming. We hypothesis that controlled expression of chromatin regulators has a crucial role in direct cardiac reprogramming. Thus, the nanocarriers with an epigenetic regulator for DNA targeting and miRNA for genetic manipulation was immobilized on PLLA

scaffold for two-stage release. The scaffolds provide a microenvironment for somatic cells to efficiently reprogram. Therefore, this strategy serves as an ideal alternative method for efficient direct reprogramming

Chapter 9, entitled **"Conclusion"**, concludes the key findings, study limitations and future perspective of the research work outlined in this thesis.