GENETIC AND EPIGENETIC NANOCOMPOSITE SCAFFOLDS FOR CARDIAC REPAIR AND REGENERATION

PRIYADHARSHNI MUNIYANDI

Student ID: 4R10170002

Abstract

In recent years, in the field of regenerative medicine, immense interest has been directed towards the direct lineage conversion bypassing pluripotency. The direct conversion of somatic cells to fully mature functional cells is an enduring goal in the field of regenerative medicine. The indirect conversion of cells to a different lineage has been extensively researched ever since the discovery of iPSC cells. The drawback of the iPSC cells such as genetic aberrations 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. Extensive research 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. Now, the field of transdifferentiation entrails a systematic approach to reprogram with a high yield of fully functional cells. This thesis aims to fabricate electrospun scaffold with engineered genetic and epigenetic regulators for cell-free therapy which mimics myocardial microenvironment that helps to modulate the cell fate of somatic cell into fully functional cardiomyocyte bypassing pluripotency. To accomplish this goal, we have explored the potential of 3D electrospun fiber with different topography, for its application in direct cardiac reprogramming. Poly (L-lactic acid)

(PLLA) fibrous nanocomposite scaffolds were fabricated using electrospinning. Its application in regenerative medicines was explored due to its expedient property. Electrospun fibers were used to (i) structurally mimic the naive ECM, (ii) *in-situ* scaffold mediated sustained and controlled genetic and epigenetic factors delivery (iii) ideal bioactive composite scaffold for dictating the cell fate for non-stem cell therapy.

The thesis encompasses nine chapters. Following sections accentuates the summary of each chapter of the thesis entitled "**GENETIC AND EPIGENETIC NANOCOMPOSITE SCAFFOLDS FOR CARDIAC REPAIR AND REGENERATION**".

Chapter 1, entitled **"Cardiac Direct Reprogramming with Engineered miRNA Scaffold,"** reviews the recent progress in cardiac reprogramming as a new technology for cardiac regeneration with a special emphasis on different biomaterial scaffolds for engineering miRNA for its application in direct reprogramming. It also 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 has subsections comprehends the (i) role of miRNA in direct reprogramming (ii) role of the biophysical cue for reprogramming (iii) nanocarriers for nanocomposite scaffold (iv) parameters for fabrication for ideal scaffold for direct reprogramming of cardiac fibroblast (v) critical factors for efficient miRNA delivery systems for cardiac regeneration.

Chapter 2, entitled "**Bio-Instrumentation",** introduces to various sophisticated instrumentation techniques exploited for characterization of nanomaterials and for application of nanomaterials for *invitro* cardiac reprogramming. The chapter rationalize the basic principle of the instruments such as electrospinning used for fabrication of

electrospun fiber, electron microscope such as SEM, TEM for characterization of morphology, spectroscopic techniques for studying the physicochemical properties of the material, the laser microscope for analyzing the cellular internalization of the nanocarriers used for gene delivery and finally various other instruments used for analyzing the molecular signature of the reprogrammed cells.

Chapter 3, entitled **"Effect of Electrospinning Parameters on Porous and Smooth Random Poly (L-lactic acid) Fiber Morphology,"** covers the critical parameters for fabrication of electrospun porous scaffolds with a dual solvent system and smooth scaffolds with single solvent systems. The subsections include 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.

Chapter 4, entitled **"Surface Functionalization and Surface Characterization of Electrospun Scaffolds for Cardiac Tissue Engineering and Regeneration,"** emphasis the surface modification of the electrospun scaffold using physical drop-casting of ECM mimetic proteins. Cell behaviour is dependent on the surface property of the scaffold. However, fiber substrate has certain limitations such as swelling, poor degradation and wettability. To enhance the property of the substrate and to overcome the inherent limitation of the electrospun scaffold, different ECM proteins was used to surface functionalize the fibers fabricated. Furthermore, surface-functionalized scaffold was characterized using XPS, FTIR, TGA to investigate the successful immobilization of protein.

Chapter 5, entitled **"ECM Mimetic Electrospun Porous Poly (L-Lactic acid) Scaffolds as a Potential Substrate for Cardiac Tissue Engineering,"** describes the potential property of PLLA scaffolds in cardiac tissue engineering. In this chapter, electrospun nanoporous scaffolds were fabricated and surface functionalized with different ECM proteins such as collagen, gelatin, fibronectin and poly L-lysine. Further, the unmodified and surface functionalized PLLA scaffolds were subjected to in vitro evaluation of scaffold properties for cardiac tissue engineering, following which adult human cardiac fibroblasts were cultured on these PLLA unmodified, and different protein functionalized scaffolds to investigate its potential role in cell adhesion, migration and proliferation. The results demonstrated that fabricated electrospun porous scaffolds exhibited excellent cell adhesion, proliferation on unmodified and different ECM protein modified substrates. Additionally, these substrates expressed different angiogenic proteins and other specific protein for differentiation regardless of the surface modification, which has more prospective applications in regenerative medicine and co-culture systems to mimic the heart microenvironment for better reprogramming.

Chapter 6, entitled **"Poly (lactic-co-glycolic acid)/ Polyethylenimine Nanocarriers for Direct Genetic Reprogramming of miRNA Targeting Cardiac Fibroblasts,"** outlines the role of PLGA nanocarrier for targeting cardiac fibroblast for direct cardiac reprogramming. Genetic reprogramming using microRNAs (miRNAs) holds promising avenues in cardiac reprogramming. Targeting the most abundant cells type is an ideal choice to reprogram the injured heart directly. To deliver the cargo to intramyocardial administration necessitates a nanoscale particle to enhance the internalization of the particle. Hence, we exploited Poly (lactic-co-glycolic acid) (PLGA)-Polyethyleneimine (PEI) nanocarrier to efficiently target cardiac fibroblast with

dual muscle-specific miRNA such as miR-1 and miR-133a. The miRNA-Polyplexes were encapsulated in biodegradable PLGA nanospheres. Furthermore, the cytocompatibility of the nanocarriers is assessed by *invitro* cytotoxicity, ROS production, GSH assay, live dead assay and actin staining. Besides the cytotoxicity assay, the reprogramming of cardiac fibroblast to cardiomyocyte like cell is determined by the expression of late mature 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. Thus, nanoscale approach serves as an ideal system for gene delivery and promising therapeutic strategy 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,"** epitomizes the use of nanocomposite scaffold for cell-free therapy. The electrospun fibrous scaffold was fabricated with random orientation with porous and smooth architecture for directly reprogramming adult cardiac fibroblast with miRNA as these miRNA holds great promise for restoring heart function. In this chapter, the microenvironmental signals such as substrate topographic cue and genetic reprogramming cocktail of dual miRNA were used to change the cell fate. In this study, electrospun fibrous porous and smooth scaffolds were fabricated to modulate the topography of the substrate for dictating cell fate. Here, scaffold not only serves as a biophysical cue but also serves as an efficient platform for the release of cargo in a two-stage controlled manner from the scaffolds. The sustained release from the scaffolds helps to achieve a high transfection efficiency. The results demonstrated that upon

engineering, the scaffolds with miRNA enhanced the transfection efficiency to 2-fold in comparison to our previous findings. A combined approach of modulating biophysical cue, genetic reprogramming cocktail helps in modulating the cell fate of somatic cell to cardiomyocyte like cells. In conclusion, in-situ scaffold mediated miRNA delivery targeting cardiac fibroblast provides new insight to investigate the role of miRNA in controlling molecular network that regulates cardiac cell fate. It also serves as a new research model, as a novel cell-free therapeutic strategy for repair and regeneration for end-stage heart failure.

Chapter 8, entitled **"Bioactive Genetic and Epigenetic Nanocomposite Poly (L-lactic acid) Porous Scaffolds for Cardiac Direct Reprogramming,"** deals with fabrication and manipulation of epigenetic and genetic nanocomposite scaffolds for direct cardiac reprogramming and tissue engineering. Cardiovascular disease is a predominant cause of death worldwide. Despite advances in reprogramming strategies, the mortality in cardiovascular disease remains high. The drawback of iPSC reprogramming for end-stage cardiac patients has many limitations, so an alternative reprogramming strategy such as direct reprogramming is a promising method, which raises the possibility of converting the cell fate of somatic cells to direct patient-specific cell bypassing stemness. This non-stem method can eliminate the limitation of stem cells and allow precision medicine in regenerative medicine. Although initially, the yield of reprogrammed new cardiomyocyte from cardiac fibroblast was low, various recent advances in reprogramming approach have improved the in vitro efficiency. However, epigenetic barrier renders the reprogramming efficiency. For instance, the chromatic alteration caused that altered accessibility of DNA greatly contribute to epigenetic changes. So as to eliminate the epigenetic barrier, in this study, we intend to target DNA which is one

of the promising strategies to manipulate epigenetic and genetic factors to enhance regeneration. Thus, based on our previous findings, we hypothesis that the use of small molecule which inhibits DNA methylation can increase the transfection efficiency. Here, small molecule and miRNA loaded PLGA nanospheres were immobilized on the PLLA scaffold for two stage-controlled delivery. Collectively, PLLA nanocomposite scaffolds provide a niche for the cells. Subsequently, the cell-material interface will enhance the reprogramming efficiency by providing an *invivo* myocardial environment and thus serves as an ideal choice to directly reprogram cardiac fibroblast to cardiomyocyte.

Chapter 9, entitled "**Conclusion,**" focuses the key findings, study limitations and future perspective of the research work outlined in the chapters above.