GENETIC AND EPIGENETIC NANOCOMPOSITE SCAFFOLDS FOR CARDIAC REPAIR AND REGENERATION

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報告・学位記番号	甲第474号（甲（バ）第十二号）			
学位記授与の日付	2020年9月25日			
学位記授与の要件	本学学位規程第3条第1項該当			
学位論文題目	GENETIC AND EPIGENETIC NANOCOMPOSITE SCAFFOLDS FOR CARDIAC REPAIR AND REGENERATION（和訳：ジェネティクスおよびエピジェネティクスなナノ粒子を含む機能性細胞足場の作製とそれらを用いた心臓修復および再生）			
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学位論文審査結果報告書〔甲〕

【論文審査】

Direct reprogramming of damage myocardium holds a great potential in treating patients with end stage heart failure. Despite the lack of critical insight into how to regenerate adult human heart myocardium following injury. Repair/regeneration of damage heart through the different cell types present with the heart myocardium is a promising approach. Generally, reprogramming approaches aims to modify the phenotype of one cell type to another either by indirect stem cell reprogramming or direct reprogramming bypassing pluripotency via delivery of various reprogramming factors. However, despite the superior quality and growing interest towards stem cell, the risk to benefit ratio is high thus necessitates an alternative strategy that meet all criteria necessary for safe and efficacious reprogramming. Till date, no consensus has been reached about the ideal reprogramming strategy with optimal delivery method for genetic reprogramming. On the other hand, extensive research in the past has made it possible to identify an ideal reprogramming cocktail for high yield of reprogrammed cells. Now that, a comprehensive understanding of reprogramming cocktail based on many reports in the past has attracted many scientists to explore noncoding RNA based cocktails for efficient direct reprograming. In the present work, electrospun scaffold with epigenetic and genetic factors were fabricated for attaining controlled delivery of these factors and also to overcome, the off-target effect and safety concerns of present delivery systems. Thus, well designed biomaterial-based scaffolds with engineered miRNA not only co-exist with the tissue by providing intramyocardial cellular environment but also can provide precision control of the release of miRNA that may be crucial for cardiac direct reprogramming.

Chapter 1 deals with the introduction, review of literature, aim and objective of the thesis. In conclusion, this chapter illustrates the important parameters for fabrication of nanocomposite scaffold with nanoparticles for direct cardiac reprogramming. Chapter 2 outlines the basic operating principles of sophisticated instruments used in this study for the characterization of nanomaterials for direct reprogramming. Chapter 3 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 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 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 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 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. The 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 engrosses the role of scaffold mediated delivery of miRNA for direct reprogramming of heart. Based on work carried out in the previous chapters, it was found that miRNA is a promising candidate for reprogramming of cardiac fibroblast invitro. 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 illustrates the fabrication of Epigenetic and genetic nanocomposite scaffolds for direct cardiac reprogramming and tissue engineering. 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 concludes the key findings, study limitations and future perspective of the research work outlined in this thesis.

【審査結果】

The thesis entitled “GENETIC AND EPIGENETIC NANOCOMPOSITE SCAFFOLDS FOR CARDIAC REPAIR AND REGENERATIO focuses on repair/regeneration of damage heart by using well designed biomaterial-based scaffolds.

Three first-authoring papers and one co-authoring paper have been published by international journals such as Polymers (MDPI),Applied Nano Materials (The American Society of Chemistry), Current Pharmaceutical Design, and International Journal of Pharmaceutics.

Judging by the results shown in the thesis and the number of international papers published so far, the level of the present research results is definitely high by international standards.

Direct cardiac reprogramming has been an emerging interest for many regenerative medicine scientists for cardiac regeneration after Ischemic heart disease. Priya has demonstrated in her research that with the advancement in nanotechnology, genetic reprogramming of cardiac fibroblast to cardiomyocyte like cells in vitro can be achieved with minimal dose of reprogramming cocktail using nanoparticle-based delivery systems. Based on these findings, in this work, she has fabricated electro spun nanocomposite scaffolds as cell free therapy to successfully reprogram cardiac fibroblast to cardiomyocyte like cells using genetic and epigenetic factors.

In conclusion, the thesis is considered as a high quality, high standard one by international standards.