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β-PVDF based electrospun nanofibers – A promising material for developing cardiac patches

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ABSTRACT

Necrosis in heart muscles can permanently hinder the natural healthy rhythm of heart pumping mechanism. The damaged muscular tissues are replaced by scar tissues and burdens the healthy muscles resulting in further attenuated functioning of heart. Since, human heart muscles cannot regenerate naturally or it has been thought so, pharmacological procedures such as using a heart assist device are followed to restore the lost function of heart. Stem cell engineering and cardiac patches offers promising prospects with their cutting edge research reports. Cardiac patches offers a viable solution as they can also function as an implant to assist in offering the mechanical support the damaged muscles were capable of. Designing cardiac patches to suit multiple functions is not only challenging but also perilous due to the target organ with which it will be interfaced. Sensor based, electrically active, miniaturized circuitry etc., poses a huge threat to the individual in whom the device/patch is implanted. In this paper, we propose a hypothesis on choosing β-PVDF based nanocomposites as the inimitable material for designing implantable cardiac patches. β-PVDF based nanocomposite materials is expected to exhibit piezoelectric effect and contribute to the adherence, proliferation and maturation of stem cells. Physico-chemical characterizations followed by in vitro cell line studies were performed in ought to confirm the same. The results revealed that the β-PVDF based nanocomposite material was mechanically stable and supportive in cardiomyocyte adherence and differentiation when compared to standard non piezoelectric scaffolds (control). Hence, an implantable β-PVDF based novel electrospun nanocomposite scaffold is hypothesized to be the hour of need in conjugation with stem cell engineering for repairing damaged heart muscles.

Introduction and background

A severe myocardial infraction cause permanent damage to muscles of heart resulting in a weak or reduced functioning of the pumping organ. Severity of muscle damage is measured by the size of infarct. In most case, a myocardial infraction that last between 6 and 30 s can permanently damage 20% of heart muscle. The damaged heart muscle becomes a scar tissue and contributes to the malfunctioning of heart by affecting the ejection fraction. The malfunctioning of heart could lead to heart failure and death [1]. The alteration of ejection fraction is due to the breakage in the collective coordination of the heart muscles, caused by the damaged heart muscle or scar tissue. The scar tissue unlike the healthy heart muscle, cannot conduct and transfer the electrical signal that architects the pumping mechanism of heart. Since, adult cardiomyocytes cannot proliferate on their own or when given an external stimuli, other ways to repair the heart is sought [2–4]. Recently, in ought to rectify the malfunctioning of the heart muscles, stem cell therapy have been given a nod by the experts in cardiology. In this procedure, a healthy stem cell from a potent donor or from the patient is isolated and cultured in lab for 2–3 weeks. The multiplied cells were infused at the site of the scar tissue of the recipient by catheterization. The infused cells were allowed to proliferate and be part of the heart musculature and help in recovering the lost rhythm of the heart [5–7]. Though, it has been reported to be an effective way in recovering or improving the ejection fraction and infarct size reduction [7], it is a very slow process. Though the method has been proved to be successful, the immunological rejection by the recipient still poses a threat to the entire process. Experts claim that, even when they are rejected

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completely by the host immune system, the infused cells perform by creating a favorable environment for the development of new cardiac cells and blood vessels by secreting growth factors, prior to getting rejected. Another method of recovering the lost function of heart muscle is to surgically remove the scar tissue and replace it with a scaffold that assist in the formation of the new cardiomyocytes. The scaffold is termed as cardiac patch and is generally a biocompatible polymer or a composite material or a bio prosthetic membrane with an engineered surface or morphology [8]. These implants are designed to match the mechanical properties, porosity and match the extracellular matrix of the cardiac cells, to offer a favorable environment for the adherence, differentiation and proliferation of the host cells or the implanted stem cells and also to measure electrical signals from cells [9,10]. The same are also set as the prerequisite for the material to be considered for implanting. Sharon Fleischer et al, designed and validated the effectiveness of electrospun albumin scaffold with microgroove and microchannels with side cage structure, in aligning cardiac tissues and endothelial cell growth [9]. Sharon Fleischer et al, also reported that electrospun fibers scaffolds with spring like coiled fiber structures favor cardiac tissue engineering [11] Other morphologies such as honey comb structure have also been reported to favor cardiac tissue engineering [12]. More recently, carbon based and PVDF based electrically charged, conductive and piezoelectric scaffolds are found to be more supportive in vitro studies [13–17]. Conductive, piezoelectric, charged biomaterials have been used in controlled as well as programmed drug release, regulate proteins, neurotrophic factors etc. Electrically charged as well as conductive scaffolds have also been reported to favor the adherence, proliferation maturation of a wide variety of cell lines. A wide range of electrically active scaffolds have been reported for their positive influence on the growth, development, differentiation of fibroblasts, neonatal cells, stem cells, etc. but very less insights are shed on their mechanical behavior and long term durability with respect to size. In this research article, by combining the stem cell engineering and the scaffold technology, use of a novel nanocomposite material is hypothesized to be a solution for repairing heart muscles. A β-PVDF based nanocomposite is hypothesized to favor the adherence, proliferation and maturation of cardiomyocytes. The same is also been experimentally verified by in vitro cell line studies using H9C2 cells and by hemolysis test. From the experimentals, it is confirmed that β-PVDF based materials positively influences cardiomyocyte adherence and development.

Hypothesis

It is hypothesized that replacing the scar tissue and implanting a β-PVDF based nanocomposite scaffold/cardiac patch, can serve as template for the stem cells to adhere, mature and proliferate. The same is hypothesized to be a viable solution to regenerate damaged heart muscles. The β-PVDF based nanocomposite scaffold proposed is a novel electrospun β-PVDF-PMMA/HAp/TiO_2 (PPHT) composite nanofibers for developing cardiac implant applications. The proposed hypothesis and the novel material is expected to be a viable solution for developing implantable cardiac scaffolds. The mechanically durable electrospun PPHT composite nanofibers are expected to match the extracellular matrix of cardiac cells and favor the adherence, proliferation and maturation of newly seeded cells. The electrospun PPHT scaffolds are also expected to generate a surface charge density when they are subjected to the pumping action of the heart muscles. The surface charge density will favor vascularization and thus support the development of newly seeded cells. The proposed hypothesis is presented as an illustration in Fig. 1.

Evaluation of the hypothesis

Myocardial infraction leaves behind a scar tissue which results in lack of coordination between the muscles of heart. Remodeling process of scar tissue takes place immediately after infraction which will result in formation of thick, nonfunctional and nonconductive muscle which will pose a huge threat to the neighboring muscles. The cardiac remodeling process changes the morphology of the cardiac muscle as well the biomolecular composition resulting in loss of conductive and elastic properties. The remodeling process could affect the neighboring cells by stopping or restricting the flow of nutrients to the active cells. So, removing the scar tissue and using stem cell treatments are recommended by the medical professionals. Proliferation of existing cardiomyocytes or the development and maturation of new stem cells into cardiomyocytes are regulated by a complex system of signaling pathways and by several cardiac-specific transcription factors and regulators [18–21]. Hence, the success of stem cell therapy by and large is still debated similar to the hypothesis and theories on proliferation of existing cardiomyocyte in adult humans. Electrically active scaffolds have been reported to yield promising results with respect to bone repair applications and nerve repair applications [22–24]. Li Ming et al and Lins LC et al, reported the use of PVDF based materials as a template to support and match the new nerve cells and extra cellular matrix, respectively [25,26]. Similar to cardiac muscles, bone is also a complicated organ with a complex vasculature system. The electrically active scaffold like a nanofiber mesh has been hypothesized to support the bone repair mechanism by modulating Ca^{+2} and controlling the feedback mechanism of the cytokines. Myosin and actin are regulated by Ca^{+2} for the polarization and depolarization process of individual heart cells. Hence, piezoelectric or conductive scaffolds for cardiac tissue engineering can accelerate the development of new cells. Of all electrically active scaffolds PVDF-TrFE with fiber morphology have been most researched and reported for their successive role in tissue engineering of cardiac stem cells, nerve stem cells, soft endothelial cells for blood vessels, etc.[27–29]. PVDF-TrFE scaffolds were reported to support development of mouse embryonic stem cell-derived cardiomyocytes which had mature phenotype, platelet endothelial cell adhesion molecule, endothelial nitric oxide synthase, arterial specific marker and Notch-1, similar to the cardiomyocytes. With respect to the above considerations, it is expected that the hypothesized implantable PPHT nanofiber scaffold can effectively assist in replacing the damaged cardiac muscles.

Theory and rationalization

It is theoreticized that, PPHT nanofibers with hydrophilic nature, porosity, conductivity and hemocompatibility assist in adherence of the stem cells as well as their differentiation into cardiomyocytes. Primarily, the PPHT nanofibers are surgically interfaced with the cardiac muscles after the scar tissue is removed, by suturing. Healthy Stem cells cultured in lab are seeded on PPHT nanofiber. The seeded stem cells are expected to adhere to the mesoporous morphology of the scaffold, differentiate and mature into cardioyocytes to become part of the cardiac muscle. The PPHT nanofibers due to their biocompatibility and hemocompatibility nature shall not interfere with the immune system and vice versa. The PPHT nanofibers will resist deformations as well as fracture due to their high elasticity and tensile strength. The β phase of the PVDF-PMMA polymer matrix acts as the power house reservoir and gets charged up when it deforms and relaxes with the movement of the heart muscle. The PPHT nanofiber scaffold will get electrically charged and creates a cloud of charges around the scaffold. These cloud of charges attract the fundamental supplies for the newly seeded cells. The mesoporus nonwoven morphology will also favor the vascularization which is crucial for the supply of nutrition for the cells. The PPHT nanofiber scaffold shall continue to act as the skeletal frame work of the newly formed cells and contribute to the electrical conduction and rigorous pumping actions. In due course of time, the PPHT nanofiber scaffold is expected to be part of the heart muscle system as a permanent bioprosthetic. The development of electrospun ultrafine β-PVDF-PMMA/HAp/TiO_2 (PPHT) composite nanofibers and the

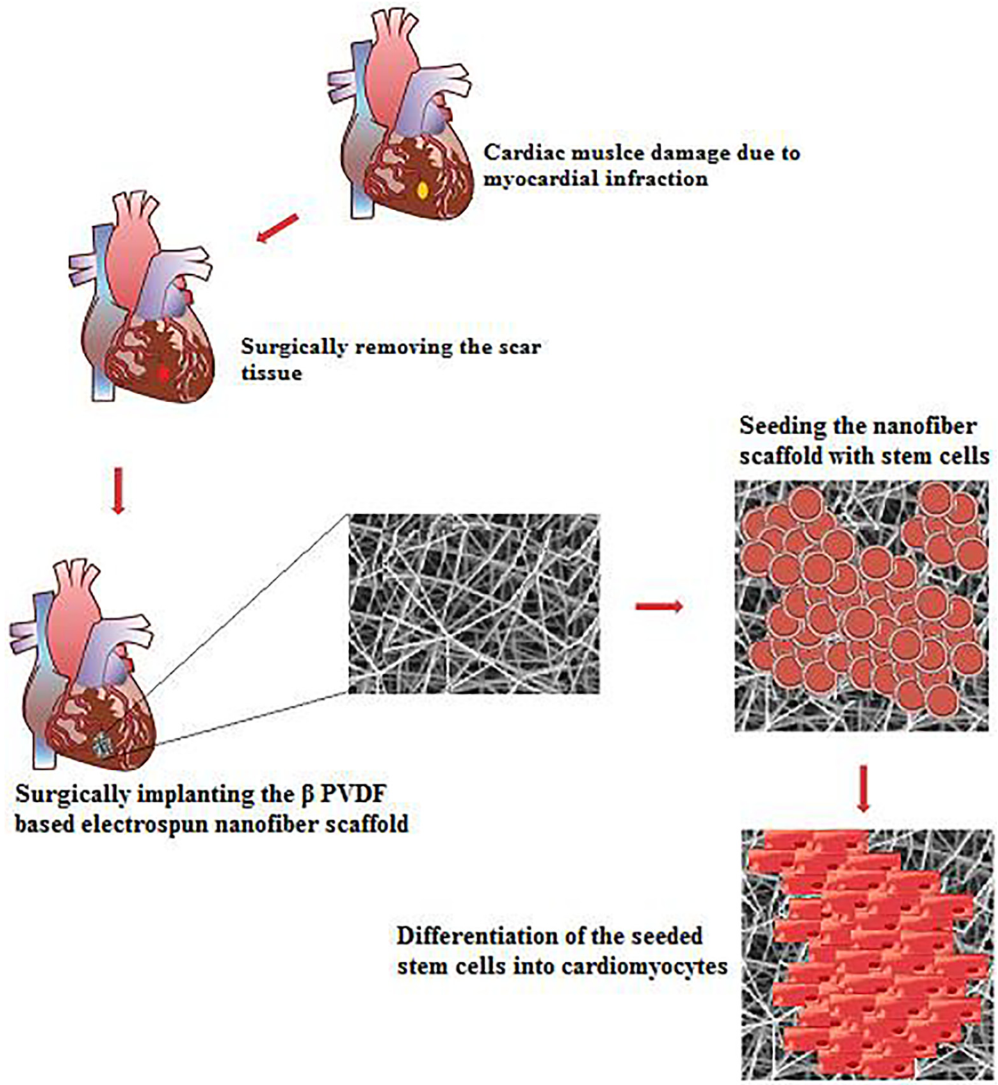

Fig. 1. Illustration of using the prepared β-PVDF based nanofiber scaffold along with stem cell therapy.

characterizations performed to confirm and investigate their phase, structure, morphology, elemental composition, and wettability by XRD, FTIR, SEM, EDS and Goniometer are presented in Supplementary 1 (S1). The in vitro cell line studies using H9C2 cells and hemolysis tests performed to validate the proposed hypothesis on the cardiomyocyte compatibility and hemocompatibility are also presented in S1.

Experimental results and discussion

The XRD and FTIR characterization confirmed the β-phase of the PVDF based polymer matrix and the crystallinity of HAp and TiO_2 nanofillers since, β-phase exhibits piezoelectric property. From wettability tests, it was found that the PPHT nanofibers were hydrophilic and hemophilic with contact angles of 39° and 53°, respectively. In vitro cardiomyocyte compatibility tests performed using H9C2 cells showed that the PPHT nanofibers assisted in adherence and differentiation of the H9C2 cells. The tensile strength of PPHT nanofibers were found to be around 78–88 MPa with a minimum of 80% elongation when subjected to maximum stress and before fracture. The in vitro hemocompatibility tests revealed that the PPHT nanofibers did not induce hemolysis to the red blood cells at the end of 6 h exposure time, insisting the fact that the PPHT scaffold can be interfaced with direct blood contact organ such as heart. The obtained results stresses the functionality of the prepared PPHT nanofibers as a scaffolds for cardiomyocyte repair applications.

Conclusion

The proposed hypothesis of using a β phase PVDF or PVDF based material as implantable scaffolds along with stem cell therapy can be a viable solution for the replacement of the scar tissues of heart due to infraction, is discussed. The prepared novel PPHT nanofiber scaffold material due to the β phase, hydrophilicity and hemophilicity can effectively offer a substrate for the seeded stem cells to adhere, develop, mature and proliferate into healthy cardiac cells. The PPHT nanofiber scaffold can also provide sufficient vascularization for the growth of the new cells. The exemplary tensile strength and elongation when subjected to maximum stress makes it suitable for the cardiac patch applications as it can withstand the vigorous pumping action of heart muscles. The theory and the experimental results both confirm the hypothesis of β phase PVDF based material for developing scaffolds or cardiac patch applications.

Conflict of interest

I, Prof. N. Satyanarayana, Department of Physics, Pondicherry University, hereby declare that the research hypothesis presented in the manuscript titled, β-PVDF based electrospunnanofibers – A promising material for developing cardiac patches, co-authored by Ratnakar Arumugam and Endu Sekhar Sreenadhu is an original research work of my team. I assure you that there is no conflict of interest with respect to the same.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2018.10.005.

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