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Biologically improved nanofibrous scaffolds for cardiac tissue engineering

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ABSTRACT

Nanofibrous structure developed by electrospinning technology provides attractive extracellular matrix conditions for the anchorage, migration and differentiation of stem cells, including those responsible for regenerative medicine. Recently, biocomposite nanofibers consisting of two or more polymeric blends are electrospun more tidily in order to obtain scaffolds with desired functional and mechanical properties depending on their applications. The study focuses on one such an attempt of using copolymer Poly(L-lactic acid)-co-poly (ε-caprolactone) (PLACL), silk fibroin (SF) and Aloe Vera (AV) for fabricating biocomposite nanofibrous scaffolds for cardiac tissue engineering. SEM micrographs of fabricated electrospun PLACL, PLACL/SF and PLACL/SF/AV nanofibrous scaffolds are porous, beadless, uniform nanofibers with interconnected pores and obtained fibre diameter in the range of 459 ± 22 nm, 202 ± 12 nm and 188 ± 16 nm respectively. PLACL, PLACL/SF and PLACL/SF/AV electrospun mats obtained at room temperature with an elastic modulus of 14.1 ± 0.7, 9.96 ± 2.5 and 7.0 ± 0.9 MPa respectively. PLACL/SF/AV nanofibers have more desirable properties to act as flexible cell supporting scaffolds compared to PLACL for the repair of myocardial infarction (MI). The PLACL/SF and PLACL/SF/AV nanofibers had a contact angle of 51 ± 12° compared to that of 133 ± 15° of PLACL alone. Cardiac cell proliferation was increased by 21% in PLACL/SF/AV nanofibers compared to PLACL by day 6 and further increased to 42% by day 9. Confocal analysis for cardiac expression proteins myosin and connexin 43 was observed better by day 9 compared to all other nanofibrous scaffolds. The results proved that the fabricated PLACL/SF/AV nanofibrous scaffolds have good potentiality for the regeneration of infarcted myocardium in cardiac tissue engineering.

1. Introduction

Globally, cardiovascular diseases and the associated risks leading to death have been continuously noted as the single leading cause of deaths. The Global Health Observatory of the World Health Organization highlights about the Proportional Mortality, an indicator of the percentage of total deaths of all ages. This specifies that cardiovascular diseases lead to about 33% of the total deaths. Cardiovascular disease (CVD) has hiked up to about 17.3 million in the year 2008. This is an increase compared to that of 17.1 million in 2004 [1]. Of these, an estimated 7.3 million were due to Coronary heart disease [2] and remains a significant problem for the global medical community. Acute myocardial infarction (MI) is the principle cause of Coronary heart failure [3]. MI is caused when the supply of oxygen and nutrients to the cardiac muscle is impaired, usually due to occluded coronary arteries. As a result, massive cell death occurs in the affected heart region [4]. The damage incurred to the heart wall is beyond recall as the myocardial tissue has limited regeneration capacity [5,6]. Although the body compensates for LV remodelling initially, mismatch of mechanical and electrical properties of scar with native myocardium ultimately affects the functioning of the heart leading to chronic heart failure, whereby the heart cannot pump adequate blood for all metabolic activities of the body [7]. About 30% patients are unable to survive the acute shock of MI [8]. The survivors are required to endure pharmacological therapy in the form of catecholamines beta-blockers aldosterone or acetylcholine esterase (ACE) inhibitors to pacify piqued immunological activities [9]. However, drugs alone cannot control disease progression competently [10]. As a result, the patients depend upon two lifesaving options: heart transplantation or use of left ventricular assist devices (LVADs). Limitations such as availability of donor organ for the transplantation and cost of LVADs encourage engineering of cardiac aiding constructs that represent a prospective source of advanced therapy in combating Congestive Heart Failure (CHF). Many intriguing modes of regenerating injured myocardium have emerged over time with pioneering research in a variety of technologies including, cell therapy using various cell types, injection of biomaterials, bioengineered patches, 3D construct implantation and even bioreactor treated implants [11,12]. However, in the current scenario models of

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bioengineered cardiac implant that can completely replicate anatomy, physiology and biological stability of a healthy heart are in quest.

Traditionally, cardiomyocytes have been considered terminally differentiated with the response to injury. Recent evidence raises the possibility that a natural system of myocyte repair exists; however, less than 50% of cardiomyocytes are exchanged during a normal life span and the system appears to be inadequate to treat magnitude of an ischemic or heart failure insults [12]. Nonetheless, the capacity of the adult human heart to generate myocytes suggests that it is rational to work towards the development of therapeutic strategies aimed at stimulating regenerative process. Cellular therapeutic approaches involve delivery of an adequate cell dose to the area of interest in the injured region. It is hoped that a favourable microenvironment would aid homing of cells and eventually improve the functioning of the myocardium. Currently available routes of cell administration include an intravenous [13], intracoronary [14], transmyocardial (by direct epicardial injection) [15], catheter-based intra-myocardial system that includes MyoCath [16], Myostar [17] and retrograde coronary sinus delivery [18]. Although these works have reached the Phase 1 and 2 clinical trials [16], their disadvantageous properties such as the cell retention after their delivery retains [18] the quest in search for the best approach for myocardial tissue engineering.

Besides life threatening arrhythmia, damage of muscle tissue in the left ventricle can cause dysfunction and remodelling in terms of progressive dilation imparting structural changes which culminate in the formation of non-contractile fibrotic scar tissue [19,20]. Scar formation is an essential aspect of rapid wound healing, especially in the injured myocardium, which is under constant wall stress. Without rapid wound healing, the ischaemic region would be subject to rupture, which is generally incompatible with life. Scar formation therefore offers protection from immediate danger by providing a rapid mechanical barrier [21]. However scar tissue is largely acellular and lacks the normal biochemical properties of the host cells. This leads to electrical uncoupling, mechanical dysfunction and loss of structural integrity, ultimately resulting in a dilated cardiomyopathy [22,23]. Hence establishing ways to either restrain scar development or to reverse the development will assist in improving the function of the organ. Optimally, myocardial scaffolds should persist long enough to guide the integration of applied cells with native tissue without interference with eventual physiological coupling in the myocardium. Techniques such as varying macromer concentrations and cross-linking of groups have shown to manipulate degradation rates while maintaining mechanical integrity [24]. Procuring control over mechanical properties such as porosity and stiffness of biomaterials, while maintaining their bioactivity is critical. Porosity is shown to influence cell trafficking of cardiomyocytes, which may have cell dimensions more than 100 μm. Large interconnected pores allow integrity via colonization of cells, but excessively large pores may impair vascularization [25]. On the other hand, smaller pores may cause failure of implant due to poor diffusion of oxygen and nutrients [26]. The stiffness of native heart tissue ranges from 10 to 20 kPa at early diastole while it increases to 50 kPa at the end of diastole, which may shoot up to more than 200 kPa in infarcted hearts [27]. Hence, the challenge is to design a biomaterial that provides integration and support to ventricular walls which can synchronize to the nonlinear elastic behaviour of the heart. Such efficacious combination of biomaterials promotes spatial vascularization along with the oxygen and nutrition supply to the tissues.

The electrospinning technology is an impending approach to fabricate fibres with its diameter in the range of nanometers to microns producing scaffolds [28–30]. In an attempt to develop a combination of synthetic and natural polymer to mimic extracellular matrix, this study utilizes the PCL copolymer PLACL and the protein silk fibroin in combination with polysaccharide Aloe Vera. A large variety of polymers have been analysed for its functional compatibility in cardiac tissue engineering [31]. PLACL is a biodegradable copolymer and demonstrates effective mechanical properties. Since it is a synthetic material, it lacks the natural integrin binding domains for proper cellular interaction [32]. The integration of silk fibroin and Aloe Vera in the composition of the scaffold material has been proposed to improve the wound healing aspects and to act as an anti-inflammatory response and effective tissue regeneration [33]. More prominently, Aloe gel assists to increase the collagen content during wound healing process which changes the collagen composition and further increases the degree of cross-linking. This facilitates in accelerated wound contraction and increased the breaking strength of resulting scar tissues [34]. Designing of such bioactive biomaterials that can respond to its environment seems to be a promising approach towards myocardial angiogenesis and regeneration of the myocardium using exogenous cells. In this study, we fabricated poly (L-lactic acid)-co-poly (ε-caprolactone) (PLACL), silk fibroin (SF) and Aloe Vera (AV) blend biocomposite nanofibrous scaffolds and examined their interaction with freshly isolated cardiac cells over a span of 9 days in controlled environment for the regeneration of infarcted myocardium in cardiac tissue engineering.

2. Materials and methods

2.1. Fabrication of nanofibrous scaffolds

Poly (L-lactic acid)-co-poly (ε-caprolactone) (70:30, *Mw* 150 kDa) was prepared by dissolving the polymer in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) to form 10% (w/v) clear solution. PLACL and SF were dissolved in a ratio of 7:3 (w/w) to form 10% (w/v) solution. The PLACL, SF and AV were dissolved in HFP in a ratio of 7:2:1 (w/w) to form 10% (w/v) solution. These solutions were subsequently used for electrospinning to form random nanofibers. For electrospinning, the polymer solutions of PLACL, PLACL/SF and PLACL/SF/AV were separately fed into a 3 mL standard syringe attached to a 27G blunted stainless steel needle using a syringe pump (KDS 100, KD Scientific, Holliston, MA) at a flow rate of 1 mL/h with an applied voltage of 18 kV (Gamma High Voltage Research, USA), respectively. Applying high voltage to the polymer solution was drawn into fibres and collected on 15 mm cover slips spread on collector plate wrapped with aluminium foil kept at a distance of 12–13 cm from the needle tip. These nanofibers were dried overnight under vacuum and used for characterization and cell culture studies.

2.2. Characterization of nanofibers

The surface morphology of electrospun nanofibrous scaffolds was studied under field emission scanning electron microscopy (FEI-QUANTA 200 F, Netherlands) at an accelerating voltage of 10 kV, after sputter coating with gold (JEOL JFC-1200 fine coater, Japan). For measuring the fibre diameter of electrospun nanofibers from the SEM images, $n = 15$ fibres were chosen at random on each of the scaffolds. The average fibre diameter was then calculated along with SD using image analysis software (ImageJ, National Institutes of Health, US).

Tensile properties of electrospun nanofibrous scaffolds were determined with a table-top Tensile Tester (Instron 3345, USA) using load cell of 10 N capacity. Rectangular specimens of dimensions 10 mm × 20 mm were used for testing, at a crosshead speed of 5 mm/min and the data was recorded for every 50 μs. The room conditions were controlled at 25 °C and 34% humidity. Tensile stress strain and elastic modulus were calculated based on the generated tensile stress–strain curve for the nanofibrous scaffolds. The hydrophilic nature of the electrospun nanofibrous scaffolds was measured by sessile drop water contact angle measurement using a VCA Optima Surface Analysis system (AST products, Billerica, MA). The measured contact angle value reflected the hydrophilicity of the scaffolds. Air plasma treatment was conducted to plain PLACL nanofibrous scaffolds by electrodeless radio frequency glow discharge plasma cleaner (Model: PDC-001, Harrick Scientific Corporation, USA). The samples placed on a glass slide were stably put in the chamber of the plasma cleaner. Plasma discharge was applied to samples for 1 min with radiofrequency power set as 30 W under vacuum. FTIR spectroscopic analysis of electrospun nanofibrous