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Poly(L-lactic acid) and polyurethane nanofibers fabricated by solution blow spinning as potential substrates for cardiac cell culture

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ABSTRACT

This paper presents a comparison and evaluation of cardiac cell proliferation on poly(L-lactic acid) (PLLA) and polyurethane (PU) nanofibrous mats fabricated by solution blow spinning (SBS). Three different cardiac cell lines: rat cardiomyoblasts (H9C2 line), human (HCM) and rat cardiomyocytes (RCM) were used for experiments. Cell morphology, orientation and proliferation were investigated on non-modified and protein-modified (fibronectin, collagen, gelatin, laminin, poly-L-lysine) surfaces of both types of nanofibers. Obtained results of cell culture on nanofibers surfaces were compared to the results of cell culture on polystyrene (PS) surfaces modified in the same way. The results indicated that in most cases polymeric nanofibers (PLLA and PU) are better substrates for cardiac cell culture than PS surfaces. All types of investigated cells, cultured on nanofibers (PLLA and PU), had more elongated shape than cells cultured on PS surfaces. Moreover, cells were arranged in parallel to each other, according to fibers orientation. Additionally, it was shown that the protein modifications of investigated surfaces influenced on cell proliferation. Therefore, we suggest that the cardiac cell culture on nanofibrous mats fabricated by SBS could be more advanced experimental *in vitro* model for studies on the effect of various cardiac drugs than traditional culture on PS surface.

1. Introduction

Cardiovascular diseases are the most common cause of death over the world [1]. This problem contributed to the intensification of research on the origin of these diseases and physiology of the heart. Therefore, there is a need for creation experimental *in vitro* models, which will mimic *in vivo* environment and could be used for evaluation of the effect of various cardiac drugs.

In recent years, there is a growing interest in the use of nanofibers for cell culture. Nanofibrous materials have many advantages such as: high porosity, high surface to volume ratio and they are structurally similar to extracellular matrix (ECM). Moreover, nanofibers structure influences on cell organization and orientation [2,3]. This is especially important in the culture of any type of muscle cells, including cardiac muscle cells, which in living organisms, are arranged in parallel to each other forming muscle fibers. In commonly used cardiac cell cultures, carried out in traditional polystyrene culture vessels, cells are randomly oriented. This situation does not correspond to the *in vivo* conditions. The orientation of cultivated cells determines cardiac cell signaling. Random cell organization disrupts cell communication whereby the cells lose their phenotype and functions [4]. Therefore, traditional cardiac cell culture on PS surface, cannot be good experimental *in vitro* model for studies on the effect of various cardiac drugs. The use of polymeric nanofibrous materials could lead to the creation of a cardiac cell culture model with conditions comparable to *in vivo* environment.

Many researches have investigated the effect of nanofibers structure, material composition or modifications of nanofibers surfaces on cell attachment, morphology, orientation and proliferation. Jing et al. [5] reported the use of aligned chitosan-modified poly(propylene carbonate) (PPC) nanofibers for mouse 3T3 fibroblasts culture. They investigated the influence of plasma treatment and chitosan adsorption on cell adhesion, morphology and proliferation. Smooth muscle cell culture on aligned poly(L-lactid-*co*-ε-caprolactone) [P(LLA-CL)] copolymer nanofibrous scaffold was described by Xu et al. [6]. Effect of fibers orientation of random and aligned poly(lactide-*co*-glycolide) (PLGA) and poly(ε-caprolactone) (PCL) nanofibers on cell growth and elastin expression of human vascular smooth muscle cells (HVSMC) was investigated by Zhong et al. [7]. Morphology, orientation and proliferation of human aortic smooth muscle cells were also determined on random and aligned polyurethane and hybrid polyurethane/collagen nanofibers by Jia et al. [8]. The influence of fibers orientation (random or aligned) on cell adhesion, proliferation, and functional gene expression of human smooth muscle cells cultured on poly(ε-caprolactone) (PCL) and PCL–gelatin nanofibers was also described by Kuppan et al. [9].

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The blood outgrowth endothelial cell culture (BOEC) on random and aligned poly(L-lactic acid) (PLLA) nanofibers coated by type-I collagen was reported by Feng et al. [10]. They investigated cell attachment, proliferation, viability and morphology of BOECs on random and aligned nanofibers. Study on another type of muscle cells - skeletal muscle cells was presented in works [11,12]. Ricotti et al. [11] reported the proliferation and differentiation of C2C12 cells on isotropic and aniosotropic poly(hydroxybutyrate) (PHB) nanofibers. Work of Cooper et al. [12] presented polyblend chitosan-polycaprolactone (PCL) nanofibers for skeletal muscle tissue reconstruction. They investigated the effect of the fiber alignment of randomly and unidirectional oriented nanofibers on cell organization and differentiation. Several works report cardiac muscle cell culture on nanofibers. Ricotti et al. [11] described the proliferation and differentiation of rat cardiomyoblasts (H9C2 cells) on isotropic and aniosotropic poly(hydroxybutyrate) (PHB) nanofibers. Zong et al. examined the structural effect of poly(lactide)- and poly(glycolide)-based (PLGA) nanofibers on cell attachment, structure and function of primary rat cardiomyocytes (CMs) [13]. In other work [14], rabbit cardiomyocytes were cultured on random and aligned PCL/gelatin nanofibers to assess the biocompatibility of these scaffolds. Orlova et al. presented proliferation of primary rat cardiomyoblasts on random and aligned polymethylglutarimide (PMGI) nanofibrous materials [15]. Authors of the next work [16] proposed poly(ε-caprolactone) (PCL) nanoscaffolds coated with thymosin β4 for efficient differentiation of murine-derived cardiomyocytes into functioning cardiac tissue. Three-dimensional cardiac co-culture model using chitosan nanofibers coated with fibronectin was presented by Hussain et al. [17]. Ventricular cardiomyocytes from neonatal rats were studied in various culture conditions: mono- and two co-cultures (with 3T3-J2 murine fibroblast and primary rat heart microvessel endothelial cells) for their viability and function. Kitsara et al. presented collagen nanofibers for three-dimensional H9C2 cell culture, which could also be used for *in vivo* testing [18].

In all of the works listed above, nanofibrous materials were fabricated by electrospinning (ES), which is well known and widely used method for nanofibers preparation. However, low efficiency of this technique does not allow to use it for large-scale production of nanofibers [19]. We propose a rarely used method - solution blow spinning (SBS), which enables to fabricate nanofibers with diameter range similar to ES. SBS offers several advantages over ES such as lower cost and higher rate of fiber production, which allows to scale-up the nanofibers manufacturing process to the commercial level [19,20]. Solution blow spinning (SBS) was developed in 2009 by Medeiros et al. [21] as a technique for production of nano- and microfibers. The SBS spinning system consists of concentric nozzles through which a polymer solution and a pressurized gas (air, nitrogen, argon, *etc.*) are simultaneously ejected [21–23]. Polymer solution dosed to the inner nozzle is stretched by compressed gas supplied through the outer nozzle. The high pressure gas is the driving force to stretch the polymer solution into thin strands. When the solvent evaporates from the solution the received dry fibers are deposited onto a collector. Due to the fact that solution blow spinning is based on the application of high-pressure gas, thermo-degradable materials could be also used to generate nanofibers. It is a big limitation of electrospinning, where the high voltage is applied. This feature of ES makes also that SBS is easier and safer to use than ES [24]. Furthermore, solution blow spun nanofibers characterize by larger pore size and higher porosity than electrospun nanofiber mats. This properties are regarded as advantages for tissue engineering [24,25]. Moreover, nanofibers fabricated by SBS show lower elastic modulus than electrospun nanofibers. In case of cardiac cell culture it is an advantage, because the elastic modulus of the culture substrate should be similar to the native myocardium (4–500 kPa) [26,27]. The detailed comparison of these two methods for nanofibers fabrication was previously reported [19,24,25,28]. Some researchers have investigated cell morphology, adhesion, proliferation and differentiation on nanofibers fabricated by solution blow spinning. In most, cases there are reports about the potential use of these materials for bone and vascular tissue engineering. Adal-hay et al. investigated morphology and proliferation of MC3T3-E1 osteoblast cell line on nanohydroxyapatite/poly(lactic acid) (nHA/PLA) hybrid composite nanofibers [29,30] and on poly(vinyl acetate)/hydroxyapatite (PVAc/HA) composite nanofiber mat deposited onto the alkali-treated titanium-substrate [31]. Proliferation and osteogenic differentiation of primary human bone marrow stromal cells (hBMSC) were examined by Tutak et al. [24] on airbrushed nanofiber scaffolds fabricated from four different polymers: poly(D,L - lactic acid) (PDLLA), poly(ε-caprolactone) (PCL), polystyrene (PS) and poly(desaminotyrosyl-tyrosine ethyl ester carbonate) (pDTEc). Similar research, with the same cells, was performed by Hoffman et al. [32] on nanofiber mats fabricated from three biocompatible polymers: poly(D,L-lactic acid) (PDLLA), poly(ε-caprolactone) (PCL) and poly(methyl methacrylate) (PMMA) composited with zirconium-modified amorphous calcium phosphate (Zr-ACP). Study on the use of poly(lactic acid) (PLA) nanofibers for bovine aortic endothelial cell culture (BAEC) was presented in work [33]. Viability of human coronary atrial endothelial cells (HCAEC) and murine fibroblasts (L929) was investigated by Behrens et al. [34] on poly(lactic-*co*-glycolic acid) (PLGA) nanofibers. They also directly deposited PLGA nanofiber mats in multiple surgical models in *in vivo* environment. In other work [35] application of poly(lactide-*co*-ε-caprolactone) (PLCL) and poly(L-lactide) (PLLA) nanofibers for human saphenous vein endothelial cell (HSVEC) culture was described. Bolbasov et al. [25] presented comparison of cell adhesion on vinylidene fluoride-tetrafluoroethylene copolymer (VDF-TeFE)-based nonwoven materials fabricated by electrospinning and solution blow spinning. They observed higher adhesion of multipotent mesenchymal stromal cells (MMSC) and endothelial hybrid EA-hy 926 cell line to nanofibers produced by SBS. There are also some reports about antimicrobial activity of airbrushed nanofibers. Bonan et al. [22] presented poly(lactic acid) and polyvinylpyrrolidone nanofibers loaded with Copaiba oil (containing β-caryophyllene - a known antimicrobial agent), which demonstrated antibacterial activity against *Staphylococcus aureus*. Yuan et al. [36] reported antimicrobial activity of liquid-infused poly(styrene-*b*-isobutylene-*b*-styrene) (SIBS) nanofibers against *Pseudomonas aeruginosa*. Additionally, this material suppressed effectively blood cell adhesion, reduced hemolysis, and inhibit blood coagulation *in vitro*. To the best of our knowledge there are any reports presented the use of nanofiber mats fabricated by SBS for cardiac cell culture.

This work presents a possibility of using nanofibrous mats as potential substrates for cardiac cell culture. The polymeric nanofibrous materials were fabricated of poly-(L-lactic acid) (PLLA) and polyurethane (PU) by solution blow spinning (SBS). PLLA and PU are common used biodegradable materials for nanofibers fabrication. They were investigated as biomaterials for many biomedical applications. PLLA nanofibers were widely used in such biomedical areas as: drug delivery systems [37], bone [38], vascular [33] and heart tissue engineering [13]. PU nanofibers were also used for drug delivery systems [39], wound dressing [40] and for vascular tissue engineering [41].

For our experiments three cardiac cell lines differing in age and origin: rat cardiomyoblasts (H9C2 line), human cardiomyocytes and rat cardiomyocytes were used. Cell cultures were performed for 7 days on non-modified and protein-modified (fibronectin, collagen, gelatin, laminin and poly-L-lysine) nanofibers surface. Before and after surfaces modification, hydrophilic properties (contact angle measurements) and porosity of investigated nanofibrous materials were characterized. Cell morphology and organization were evaluated by microscopic observations after calcein-AM staining. For the evaluation of cell proliferation commercially available cell proliferation assay (alamarBlue) was performed. Obtained results of cardiac cell culture on the investigated surfaces of nanofibers were compared to the results of cardiac cell culture on polystyrene (PS) surfaces modified in the same way. According to the best of our knowledge, this is the first report of the use of nanofibrous mats fabricated by the SBS method for cardiac cell culture.