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Novel PGS/PCL electrospun fiber mats with patterned topographical features for cardiac patch applications

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ABSTRACT

Nano- and micro-scale topographical features play a critical role in the induction and maintenance of various cellular properties and functions, including morphology, adhesion, gene regulation, and cell-to-cell communication. In addition, recent studies have indicated that the structure and function of heart tissue are also sensitive to mechanical cues at the nano- and micro-scale. Although fabrication methods exist for generating topographical features on polymeric scaffolds for cell culture, current techniques, especially those with nano-scale resolution, are typically complex, prohibitively expensive and not accessible to most biology laboratories. Here, we present a simple and tunable fabrication method for the production of patterned electrospun fibers that simulate the complex anisotropic and multi-scale architecture of cardiac tissue, to promote cardiac cell alignment. This method is based on the combination of electrospinning and soft lithography techniques, in which electrospun fibers, based on a blend of poly(glycerol sebacate) and poly(caprolactone), were collected on a patterned Teflon-coated silicon wafer with imprinted topographical features. Different surface topographies were investigated, such as squares and grooves, with constant or different interspatial distances. In vitro cell culture studies successfully demonstrated the alignment of both C2C12 myoblasts and neonatal rat cardiomyocytes on fabricated electrospun patterned surfaces. C2C12 cells were cultured over a period of 72 h to study the effect of topographical cues on cell morphology. Cells attached within the first 8 h after seeding and after 24 h most of the cells started to align responding to the topographical cues. Similarly, cardiomyocytes responded to the topographical features by aligning themselves and by expressing Connexin 43 along cellular junctions. Summarizing, we have developed a new method with the potential to significantly promote cardiac tissue engineering by fabricating electrospun fibers with defined topographical features to guide and instruct donor and/or host cells.

1. Introduction

Contact guidance involves introducing topographical patterning on a substrate material at the nano- and micro-scale to induce controlled cellular responses like desired orientation and morphology [1]. Oriented cells in turn induce the self-assembly of additional consistently organized layers of cells and extracellular matrix [2]. Such a phenomenon, called contact guidance, has been observed in a variety of cell types, such as epithelial cells [3–5], fibroblasts [5–8], oligodendrocytes [9], and astrocytes [10]. In vitro cell culture studies have demonstrated that the use of tunable biomimetic surface topographies results in a more appropriate phenotypic and physiologic response of individual cells and tissue constructs. While the importance of micro-scale topography is well known, recent findings have also revealed the importance of nano-scale topography [11–14] and of hierarchical surface structures.

The anisotropic architecture of the heart synchronizes the required mechanical contraction and electrical propagation of stimuli [11]. This architecture, both at nano- and micro-scale level, is based on a complex organization of the cardiac extracellular matrix (ECM). When cardiomyocytes are cultured in vitro on traditional porous scaffolds or flat surfaces, they do not receive the appropriate conditions to morphologically, mechanically, or physiologically develop, as in the native tissue [11,15]. However, the introduction of topographical features mimicking the native environment has been shown to promote the generation of a well-developed functional cardiac tissue [16–19].

Previous studies have been carried out to examine the alignment of cardiomyocytes on patterned surfaces, presenting micro-topographical (10–100 μm) features, fabricated using various methods, such as micro-contact printing, abrasion, photolithography, hot embossing,

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electrospinning, and laser ablation [20,21]. Additionally, micro-topographical features have been shown to have greater influence than electrical cues on the alignment of cells [22]. Moreover, it has recently been reported that the heart is sensitive to nano-scale topographical features. In fact, the cardiac ECM is made up of aligned fibrils of the order of 100 nm in diameter [11]. The electrophysiological functionality of the heart is direction-dependent [23]. Finally, nano-scale topographical features play an important role in controlling cell mechanics, protein expression and anisotropic action potential propagation [24].

Currently available nano-fabrication methods, such as *e*-beam lithography and nano-imprint lithography, are relatively cost-intensive requiring complex facilities. However, achieving guided anisotropic growth of cardiomyocytes is critical, as it is important for the final success of engineered cardiac constructs (patches). Therefore, to address this aspect, in this study we present a controllable, fast, and inexpensive fabrication approach to produce cell culture substrates with tunable nano- and micro-scale topographical features. This innovative fabrication method is based on the combination of electrospinning and soft lithography. The polymeric material chosen for scaffold fabrication is a blend of poly(glycerol sebacate) and poly(caprolactone) (PGS/PCL). Morphological and biological tests were carried to verify the success of the approach in obtaining the desired topography and to investigate the effect of the topographical features on the behavior of C2C12 myoblasts and neonatal cardiomyocytes.

2. Materials and methods

2.1. Materials

All materials were purchased from Sigma Aldrich if not stated otherwise.

2.2. Scaffold preparation

2.2.1. PGS preparation

PGS was synthesised as previously described [25], according to the process reported by Wang et al. [26]. Briefly, it involves the pre-polycondensation of an equimolar mixture of glycerol and sebacic acid. The mixture was heated at 120 °C under inert nitrogen atmosphere to form a prepolymer.

2.2.2. Fabrication of electrospun PGS/PCL fibers with tailored topography

Fabrication of PGS/PCL fibers was carried out using the established technique of electrospinning. For the fabrication of PGS/PCL fibers, PGS prepolymer was mixed with PCL (Mn = 70.000–90,000 g/mol) in a weight ratio of 2:1, in a solvent system containing Dimethyl carbonate:Methanol in 7:3 volume ratio. The PGS/PCL blend solutions were placed in a glass syringe (lure-lock type, 10 ml) and connected to a metal syringe needle (20 gauge, inner diameter 0.8 mm) and placed on a pump (Razel R99-E syringe pump, Italy). The final concentration of the polymer in the mixture was 15 wt%. The fibers were electrospun for 4 h using the following parameters: voltage = 15 kV which was supplied directly from a high DC voltage power supply; collector distance = 15 cm and flow rate = 1.6 ml/h for 4 h. The obtained fiber mats had an average fiber thickness of 100 ± 0.1 μm.

To produce topographical features on PGS/PCL fiber matrices, a surface-patterned silicon wafer covered by Teflon was used as the negative mold. Fabrication of the silicon mask was carried out by Fraunhofer Institute for Integrated Systems and Device Technology IISB, Erlangen, Germany. The underlying silicon merely acts as the support of the Teflon top layer, which has been structured through photolithography with different surface patterns. Fig. 1 depicts a schematic view of one of the patterned mold structures and of the electrospinning process. The PGS/PCL electrospun fibers were collected on the patterned Teflon-coated silicon wafer used as target during electrospinning. No further curing was required.

2.3. Characterization of the prepared scaffolds

2.3.1. Surface characterization of PGS/PCL fibers

In order to study the surface morphology of different imprinted PGS/PCL fiber matrices, a LEO 435 VP scanning electron microscope (SEM) (Germany) was used. Samples were prepared by placing them on 8 mm diameter aluminum stubs, which were sputtered with gold-palladium for 1 min. SEM images were captured at an acceleration voltage of 10 kV. The apparent porosity of the fiber mats, fiber diameter, and the dimensions of the topographical features were determined from SEM micrographs using the software ImageJ. Three samples of different experimental preparation were analyzed for each topographical feature as well as the control.

The roughness of the scaffolds was evaluated using a UBM laser profilometer (UBM Meßtechnik GmbH, Ettlingen), which obtained 3D images of the surface topography of samples using a reflection of a 670 nm laser beam and a stage positioning system with maximum measurement frequency of 10 kHz. This measurement allowed to investigate and quantify the roughness and topography of the surface. The roughness was represented as the average root mean square (Ra) value.

2.3.2. In vitro cytocompatibility studies with C2C12

PGS/PCL patterned fibers were cut into circular discs (diameter: 15 mm) for in vitro cell culture studies. All samples were pretreated with 70% v/v EtOH/H_2O for 24 h prior to seeding. The EtOH treatment has a dual effect: in addition to sterilization, it leached out any unreacted monomers or toxic reagents used during scaffold fabrication. The samples were then washed 3 times in phosphate buffered saline (PBS) before cell seeding. C2C12 mouse skeletal myoblasts (DSMZ, Germany) were cultured with RPMI, supplemented with 10% fetal bovine serum (FBS) and 100 U/mg/ml penicillin/streptomycin, at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were trypsinized after 3 days of culture, using a solution containing 0.05% trypsin. C2C12 cells were seeded in wells containing samples at a concentration of 10^5cells/well. The medium was changed every other day throughout the entire experiment and the samples were processed after 8, 24 h and 72 h for further characterization.

2.3.3. In vitro studies with neonatal cardiomyocytes

Primary cardiomyocytes were isolated from 3-days-old Sprague Dawley rats as previously described [27]. The isolated cardiomyocytes were pre-plated for 2 h in 2 mM L-Glutamine, 10% FBS and 100 U/mg/ml Pen/Strep in DMEM/F12 medium. Subsequently, unattached cells, of which >90% were cardiomyocytes, were collected and centrifuged. Cells were then re-suspended in neonatal cardiomyocyte medium (100 U/mg/ml Pen/Strep in DMEM/F12 medium) supplemented with 0.5% horse serum and seeded in a 24-well plate for 5 days on different matrices at a density of 0.5 million cells/well. A coating with gelatin and fibronectin was performed on glass coverslips, which were used as controls, as previously described [23]. Cells were cultured in 5% CO_2, 95% air humidified atmosphere at 37 °C. The cell culture medium was changed every other day.

Prior to the cell culture studies with cardiomyocytes surface, modification of the PGS/PCL fiber mats with fibronectin was conducted. The PGS/PCL fibers were coated with fibronectin in order to enhance cell attachment and to study the effect of the surface topography only, the samples were pre-treated with NaOH for 5 min then washed with distilled water followed by a treatment with hydrochloric acid for 25 s and finally washed three times with distilled water, as previously described [25]. The modified samples were immersed each in 1 ml fibronectin of concentration 25 μg/ml in PBS for 16 h. Prior to cell seeding the fibronectin solution was removed and the samples were washed with PBS.