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3D screening device for the evaluation of cell response to different electrospun microtopographies

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ABSTRACT

Micro- and nano-topographies of scaffold surfaces play a pivotal role in tissue engineering applications, influencing cell behavior such as adhesion, orientation, alignment, morphology and proliferation. In this study, a novel microfabrication method based on the combination of soft-lithography and electrospinning for the production of micro-patterned electrospun scaffolds was proposed. Subsequently, a 3D screening device for electrospun meshes with different micro-topographies was designed, fabricated and biologically validated. Results indicated that the use of defined patterns could induce specific morphological variations in human mesenchymal stem cell cytoskeletal organization, which could be related to differential activity of signaling pathways.

Statement of Significance

We introduce a novel and time saving method to fabricate 3D micropatterns with controlled micro-architectures on electrospun meshes using a custom made collector and a PDMS mold with the desired topography. A possible application of this fabrication technique is represented by a 3D screening system for patterned electrospun meshes that allows the screening of different scaffold/electrospun parameters on cell activity. In addition, what we have developed in this study could be modularly applied to existing platforms. Considering the different patterned geometries, the cell morphological data indicated a change in the cytoskeletal organization with a close correspondence to the patterns, as shown by phenoplot and boxplot analysis, and might hint at the differential activity of cell signaling. The 3D screening system proposed in this study could be used to evaluate topographies favoring cell alignment, proliferation and functional performance, and has the potential to be upscaled for high-throughput.

1. Introduction

Surface topography influences the physico-chemical interactions at the interface between biomaterials and cells by affecting cell attachment [1–3], viability [1,4], and morphology, providing cell guidance and inducing differentiation [1,5–7]. One of the main purposes of regenerative medicine recent strategies is the fabrication of scaffolds with tailored micro/nano surface topographies to stimulate biological interactions similar to those of the extracellular matrix (ECM) [7]. In order to replicate natural regenerative processes, a scaffold may mimic the composition and the structure of biological tissues to promote an adequate phenotypical response. Furthermore, scaffolds topography proved to directly influence cell adhesion, differentiation, genetic expression, migration, morphology, orientation and proliferation providing contact guidance cues and influencing the cytoskeletal arrangement [8,9].

Several studies reported that structures with defined patterns promote and favour cell orientation, maturation and regulation of different cellular biological mechanisms, while randomly patterned surfaces promote non-oriented cell growth and less

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structured cellular responses [1,10,11]. Truckenmüller et al. demonstrated that the use of systems with regular and aligned channels can promote contact guidance [12], while Chew et al. assured cell elongation and alignment using solid matrices with aligned patterns [13]. Dalby et al. studied the impact of surface micro- and nano-topographies on cell behavior demonstrating that their dimensions play a pivotal role influencing cell adhesion, growth and differentiation [14,15]. A similar result was obtained by Elias et al. with the use of carbon nanofibers with diameters ranging from 60 nm to 200 nm [16]. These phenomena are probably due to differences on local surface free energy [10] or to preferential cell orientation depending on the presence of a minimal length of 2 μm required for cell attachment [17].

The architecture of scaffolds used in regenerative medicine can be characterized by a macro-, micro-, and nano-structure. The macro-structure is related to the shape and dimensions of the entire scaffold, while the micro- and nano-structures refer to the scaffold surface features, which are known to act both at a cellular and sub-cellular level [7]. The macro-structure depends on the dimensions of the damaged tissue and on the implantation site, while the micro- and nano-structures are related to the structural properties of the tissue itself. When specifically looking at surface topographies of scaffolds, both micro- and nano-topographies are able to influence specific cellular responses, resulting from influencing cell morphology and adhesion [7].

Topologically, the ECM is characterized by pores, ridges and fibers of different dimensions [18]. Among the different scaffold microfabrication techniques used today to attempt mimicking such a degree of complexity, electrospinning (ESP) is a suitable alternative to fabricate random or aligned fibrous meshes to mimic the native ECM environment. Considering this possibility, researchers focused their interest on the controlled spatial arrangement of fibers to fabricate patterned structures able to mimic the micro-structure of the tissues [19]. Combining electrospinning and plasma polymerization techniques, Guex et al. developed a plasma-coated, parallel-oriented electrospun PCL scaffold that provides structurally and chemically adhesion sites for cellular attachment, thus allowing epicardial implantation and cell delivery without signs of chronic inflammations [20]. Baker et al. fabricated a dual-component (PCL-PEO) aligned nanofibrous scaffold via a dual-spinneret electrospinning setup and showed that MSCs were highly sensitive to their 3D microenvironment [21]. In particular, MSCs seeded on aligned nanofibers expressed an increase of type I collagen and a aggrecan's down-regulation and showed a highly polarized cell body with pronounced actin stress-fibers demonstrating that an aligned nanofibrous microenvironment is suitable for the production of organized fibrocartilaginous matrix [21].

Recent studies demonstrated the possibility to fabricate electrospun scaffolds with microtopographies using a custom-made collector plate [22] or combining additive manufacturing (AM) approaches with ESP [19,23]. Vaquette et al. investigated the use of patterned collectors to increase the pore size of electrospun scaffolds to improve cell infiltration [22]. In particular, the pattern was reproduced on the electrospun network and SEM analysis showed an increase of the pore size and pore size distribution. In addition, mechanical analysis revealed the possibility to tailor the mechanical properties according to the pattern. Rogers et al. proposed a reproducible method to design and fabricate electrospun scaffolds with defined microtopographies using a projection-microstereolithography method to generate a patterned resin formed in a layer-by layer process [19].

However, the design of these topographies is still based on arbitrary inputs. This makes the process of identifying the optimal topography to elicit a desired cellular function still bound to a trial and error approach. The use of screening technologies could be therefore instrumental to discover optimal topographies to influence cell behavior through contact guidance. Several researchers worked on the development of 2D and 3D screening systems for different surface topographies. Lovmand et al. used a combinatorial screening approach for the fabrication of a BioSurface Structure Array two-dimensional platform for the systematic screening of cellular responses to a large variety of nano- and microstructured surfaces [24]. A tantalum array was produced on boron-doped p-type Si-wafers and a standard lithography process was used for transferring the designed arrays of patterns to the substrate. The combinations of size, gap and height of structures which enhance mineralization as well as the expression of osteogenic markers of a preosteoblastic murine cell line were identified. The same approach was used by Kolind et al. to analyse human fibroblast proliferation and mechanical response on micro-structured surfaces composed by pillars [25]. They demonstrated that altering the inter-pillar gap size of the structures caused a significant change in fibroblast proliferation and stress induced modifications in the cytoskeleton and focal adhesion morphology. We have previously developed an array of surface topographies designed using randomized algorithms to screen cell-surface topographic interactions [11]. With this technology, we demonstrated that surface topography enhanced the osteogenic differentiation of hMSCs [26,1], supported proliferation and cell–cell adhesion of induced pluripotent stem cells [26], and precisely controlled cell shape [1].

Despite these studies demonstrated the importance of surface topographies, no studies attempted to develop a method to systematically screen ESP scaffolds with different microtopographies. In this study, a novel, flexible, scalable, fast and reproducible microfabrication method based on the combination of soft-lithography and an ESP technique was used to produce micropatterned electrospun scaffolds. A screening device for electrospun meshes with different microtopographies that has the potential to be upscaled for high-throughput analysis was designed, fabricated and biologically validated. The system was used to screen for topographies favoring cell alignment, proliferation and functional performance. The main advantages of this approach are related to its flexibility, scalability and fast reproducibility. The system allows to design topographies based on specific inputs, to fabricate 3D micropatterned scaffolds with a large variety of personalized and controlled geometries and dimensions, which can be as well upscaled for series production.

2. Materials and methods

2.1. Microfabrication technique

The microfabrication technique used for the production of a 3D screening device is based on the combination of Soft-lithography and ESP. It consisted on the fabrication of a PDMS mold with a defined microtopography that was subsequently used as a target for the ESP jet.

2.2. Design and fabrication of the 3D screening device

The design and fabrication of the 3D screening device was divided into three phases. Initially, a PDMS mold was fabricated from a silicon wafer with different topography geometries and sizes (Fig. 1c-d and table 1). Subsequently, the mold was positioned on top of a custom made electrode, and a polylactic co-glycolic acid (PLGA) structure was electrospun. Then, a 3D structure composed of 25 squared wells was fabricated using stereolithography in order to isolate each topography. Finally, the ESP mesh was peeled off from the PDMS mold, glued (3140 RTV Coating non-corrosive silicone rubber, Dow Corning) on a Petri dish, and the 3D structure was glued on the top of it (Fig. 1a-b).