Acta Biomaterialia 68 (2018) 214–222

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Short communication

High resolution 3D microscopy study of cardiomyocytes on polymer scaffold nanofibers reveals formation of unusual sheathed structure

Victor Balashov [a], Anton Efimov [b], Olga Agapova [b], Alexander Pogorelov [c], Igor Agapov [b], Konstantin Agladze [a,*]

[a] Moscow Institute of Physics and Technology, Institutskiy Pereulok 9, Dolgoprudny, Moscow Region 141700, Russia
[b] V.I. Shumakov National Medical Research Center of Transplantology and Artificial Organs, Shchukinskaya 1, Moscow 123182, Russia
[c] Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Institutskaya 3, Pushchino, Moscow Region 142290, Russia

ARTICLE INFO

Article history:
Received 15 August 2017
Received in revised form 4 December 2017
Accepted 22 December 2017
Available online 27 December 2017

Keywords:
Biomaterials
Tissue engineering
3-D reconstruction
Transmission electron microscopy
Atomic force microscopy
Nanotomography

ABSTRACT

Building functional and robust scaffolds for engineered biological tissue requires a nanoscale mechanistic understanding of how cells use the scaffold for their growth and development. A vast majority of the scaffolds used for cardiac tissue engineering are based on polymer materials, the matrices of nanofibers. Attempts to load the polymer fibers of the scaffold with additional sophisticated features, such as electrical conductivity and controlled release of the growth factors or other biologically active molecules, as well as trying to match the mechanical features of the scaffold to those of the extracellular matrix, cannot be efficient without a detailed knowledge of how the cells are attached and strategically positioned with respect to the scaffold nanofibers at micro and nanolevel. Studying single cell – single fiber interactions with the aid of confocal laser scanning microscopy (CLSM), scanning probe nanotomography (SPNT), and transmission electron microscopy (TEM), we found that cardiac cells actively interact with substrate nanofibers, but in different ways. While cardiomyocytes often create a remarkable "sheath" structure, enveloping fiber and, thus, substantially increasing contact zone, fibroblasts interact with nanofibers in the locations of focal adhesion clusters mainly without wrapping the fiber.

Statements of Significance

We found that cardiomyocytes grown on electrospun polymer nanofibers often create a striking "sheath" structure, enveloping fiber with the formation of a very narrow (~22 nm) membrane gap leading from the fiber to the extracellular space. This wrapping makes the entire fiber surface available for cell attachment. This finding gives a new prospective view on how scaffold nanofibers may interact with growing cells. It may play a significant role in effective design of novel nanofiber scaffolds for tissue engineering concerning mechanical and electrical properties of scaffolds as well as controlled drug release from "smart" biomaterials.

1. Introduction

Polymer nanofibers play a unique role in the development of regenerative medicine [1–5]. Scaffolds based on such fibers are used successfully for the construction of implants designated for the replacement of soft and elastic tissues [6,7]. Polymer matrices for tissue culture are often produced using the electrospinning process [8–10]. They may have various architectures [11] and physical features, such as elasticity [12,13] and electrical conductivity [14,15]. In the "smart" scaffolds, polymers also can be loaded with biologically active molecules which are released either spontaneously or in response to external stimuli [16,17].

The interaction of the cell with the scaffold adds substantially to the degree of freedom of the cell-fiber system, making it even more multidimensional. Consequently, such properties as the mechanical elasticity and robustness of the entire cell-substrate system will depend on the cell-fiber connection as much as on the polymer features. Also, the electrical conductivity of the scaffold would only provide an electrical link between the cells if it is electrically isolated from the cell-surrounding tissue's ionic solution. The release of the biologically active molecules from the scaffold would only

* Corresponding author.
E-mail address: agladze.ki@mipt.ru (K. Agladze).

https://doi.org/10.1016/j.actbio.2017.12.031
1742-7061/

work if their concentration reaches physiologically relevant values which would depend on the tissue architecture. Furthermore, nanoscale features of cell-scaffold interface may significantly influence and regulate cell growth, migration, shape and functionality, and it is especially important if we aim for proper cardiac cell organization in the engineered tissue [18]. Previous studies have suggested that fibroblasts [19] and cardiomyocytes [20] may encompass nanofibers, but no detailed high-resolution microscopy study was performed to reveal nanoscale features of cell-fiber interactions. In order to build a detailed and mechanistic model of the cell-fiber nanointerface, we studied it with the aid of three types of microscopy: confocal laser scanning microscopy (CLSM) [21], scanning probe nanotomography (SPNT) [22] and transmission electron microscopy (TEM) [23].

The necessity of combining these three methods is based on their individual strengths and weaknesses. CLSM gives the most intact image of the living cell, appropriate for three-dimensional (3D) reconstruction, although its resolution is restricted by the light's wavelength, which is about 500 nm. TEM provides the highest possible resolution, typically resolving objects of a few nanometers size; however, it works best for the samples which are the more distant from the living state, and it is hardly appropriate for the 3D reconstruction. The recently designed SPNT, which combines scanning probe microscopy (SPM) and ultramicrotomy in an integrated "slice-and-view" manner, achieves resolution approaching that of TEM and is specially designed to restore the 3D structure of the biological sample, although also in the far from living state. SPNT is based on the direct measurement of the sample surface with the SPM tip immediately after sample sectioning with the ultramicrotome diamond knife. Successive SPM measurements of the object's surface after removing a layer of material by ultrathin sectioning enable 3D tomographic reconstruction of the nanoscale sample structure by software integration of the layer-by-layer SPM images obtained [24–27].

The combination of the above methods allowed us to discover some unusual features of the interface between the polymer fiber and the cell, which were quite different from the well-known focal adhesion of the cell attaching to the plane substrate [28,29]. It appears that cardiomyocytes tend to create a sheathing shape and envelop the polymer fiber. The fiber is not "swallowed" by the cells, but rather is pushed deeply inside, remaining separated by the cell membrane from the inner cell space, as we will show below.

2. Materials and methods

2.1. Electrospinning of suspended PLA/FN nanofibers

Poly(l-lactic acid) (PLA, $M_w \sim 700\,000$, Polysciences Inc., USA, 21512) was dissolved in the hexafluoroisopropanol (HFP, Sigma-Aldrich Co., USA, 105228) at a concentration of 25 mg/ml. In order to enable confocal fluorescent microscopy studies the PLA solution was supplemented with Rhodamine 6G fluorescent dye at a final concentration of 0.01 μg/ml. Polydimethylsiloxane (PDMS, Dow Chemical, USA) blocks with dimensions of 10 × 20 × 3 mm were used as a substrate for electrospun fibers. Each block had a middle triangle groove of 2 mm width and 1 – 2 mm depth along the longer side. PLA solution was electrospun using Nanon-01 electrospinning setup (Mecc Co., Japan). The solution was loaded into the 3 ml syringe and ejected through the 22 gauge blunt tip needle at a flow rate of 0.5–1 ml/h towards the drum collector of 20 cm diameter rotating at 500 rpm speed. The voltage applied between the syringe tip and the grounded collector was in the range from 4 to 7 kV and the distance from needle tip to the collector was 10 cm. PDMS substrate blocks were attached to the drum collector with the grooves perpendicular to the movement direction, therefore the nanofibers were deposited across the groove (see Fig. 1). The electospinning procedure was carried out for 5 min. The average distance between individual electrospun nanofibers was ~3–6 μm. The specimens were coated with a solution of human plasma fibronectin (10 μg/ml in PBS, Imtech, Russia) by 12 h incubation at 37 °C to produce a cell adhesive matrix.

2.2. Cardiac cell isolation, seeding, culturing

Cardiac cells were isolated from the ventricles of 1–3 day old neonatal Wistar rats, according to the Worthington protocol (http://www.worthingtonbiochem.com/NCIS/default.html). Suspension of isolated cells in Dulbecco-modified Eagle plating medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin streptomycin and 1% kanamycin was preplated for 1 h in a 75 cm^2 cell culture flask (Corning Inc., USA) in order to reduce the number of fibroblasts. Unattached cells were collected and then seeded on the sterile specimens with elecrospun fibers at 10^5 cells/cm^2 cell density.

After 24 h incubation under humidified 5% CO_2 atmospheric conditions at 37 °C, unsettled cells were washed away and the culture medium was replaced with DMEM supplemented with 5% FBS.

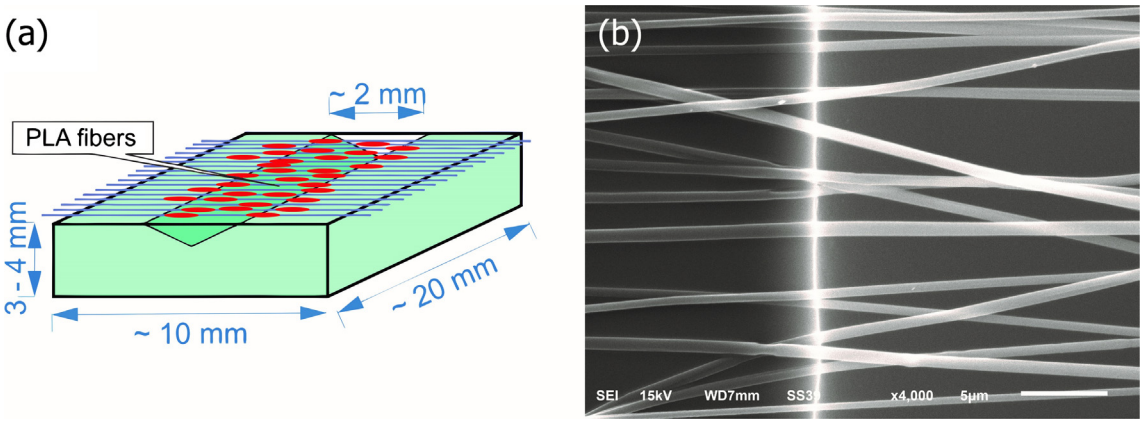

Fig. 1. (a) Scheme of preparation of substrates with suspended PLA/FN nanofibers. (b) SEM image of electrospun PLA/FN nanofibers.