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# Electrospun fine-textured scaffolds for heart tissue constructs

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**Abstract**

The structural and functional effects of fine-textured matrices with sub-micron features on the growth of cardiac myocytes were examined. Electrospinning was used to fabricate biodegradable non-woven poly(lactide)- and poly(glycolide)-based (PLGA) scaffolds for cardiac tissue engineering applications. Post-processing was applied to achieve macro-scale fiber orientation (anisotropy). In vitro studies confirmed a dose–response effect of the poly(glycolide) concentration on the degradation rate and the pH value changes. Different formulations were examined to assess scaffold effects on cell attachment, structure and function. Primary cardiomyocytes (CMs) were cultured on the electrospun scaffolds to form tissue-like constructs. Scanning electron microscopy (SEM) revealed that the fine fiber architecture of the non-woven matrix allowed the cardiomyocytes to make extensive use of provided external cues for isotropic or anisotropic growth, and to some extent to crawl inside and pull on fibers. Structural analysis by confocal microscopy indicated that cardiomyocytes had a preference for relatively hydrophobic surfaces. CMs on electrospun poly(L-lactide) (PLLA) scaffolds developed mature contractile machinery (sarcomeres). Functionality (excitability) of the engineered constructs was confirmed by optical imaging of electrical activity using voltage-sensitive dyes. We conclude that engineered cardiac tissue structure and function can be modulated by the chemistry and geometry of the provided nano- and micro-textured surfaces. Electrospinning is a versatile manufacturing technique for design of biomaterials with potentially reorganizable architecture for cell and tissue growth.


*Keywords:* Electrospinning; Poly(glycolide) and poly(lactide)-based (PLGA) scaffold; Cardiomyocytes; Cardiac tissue engineering

## 1. Introduction

The area of tissue engineering has been driven by biomimicry-inspired design of materials to recreate the natural three-dimensional environment for better cell and tissue growth [1]. An important aspect of these efforts is to mimic the fibrillar structure of the extracellular matrix (ECM), which provides essential guidance for cell organization, survival and function. Only recently, success has been reached in developing biomaterials with sub-micron fibers and morphological similarity to the ECM, thus possibly providing an innate setting for cell assembly and growth. Electrospinning has emerged as a simple yet versatile method in manufacturing such biomaterials out of synthetic [2–6] or natural [7,8] polymers. Recently, electrospun non-woven nanofiber membranes have been demonstrated in multiple biomedical applications, including the production of scaffolds for tissue engineering, wound healing, drug delivery, and medical implants [3,9–14]. The

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attraction of electrospun biomaterials in the context of tissue engineering is manifested in several ways. First, very high surface area-to-volume ratio and high porosity can be achieved for better cell incorporation and perfusion. Second, the process allows for control of structure at the nano-, micro- and macro-scales for flexible tissue design. It has been suggested that nanofibrous ECM-mimicking features may improve cellular response and biocompatibility because of the morphological similarities to the three-dimensional ECM protein fiber network [15]. Third, the electrospinning process is versatile, since it offers the ability to incorporate multiple polymers and bioactive ingredients [4,16]; it can also be used to enhance mechanical properties of the obtained nanofibrous materials compared to solid-walled equivalents [17].

A wide variety of biodegradable and biocompatible polymers have been investigated to fabricate nanofibrous membranes including synthetic polymers, such as poly(lactide), poly(glycolide) and their copolymers (PLGA), poly(ε-caprolactone), poly(ethylene-*co*-vinyl alcohol), and natural polymers, such as collagen, protein, and fibrinogen [2–15]. Among them, PLGA and PCL materials hold a great potential for success since they are FDA approved, low-cost materials for use in humans, and they are easy to process to achieve controllable microstructure and morphology. In addition, the degradation rate and mechanical properties of PLGA can be easily tailored for different targeted applications.

A particular macro-feature in cell organization found often in vivo—directional growth or anisotropy—can be effectively addressed using the process of electrospinning. An example was demonstrated recently by employing a spinning disk in combination with electrospinning to fabricate oriented non-woven scaffolds [10]. For myocardial tissue, anisotropy carries functional importance and has been the focus of extensive research to orient the ECM or the cell growth in a 2D setting [18–20], and electrospun oriented fibers can effectively help in solving this problem.

In this study, sub-micron nanostructured PLGA membranes with different compositions were fabricated through the electrospinning process. Compositions varied slightly in hydrophobicity as well as in degradation rate. The electrospun matrices were used as scaffolds for the generation of cardiac tissue constructs. The surface and degradation properties of these membranes were examined and correlated with cellular response. Cell behavior and cell–scaffold interactions were observed using scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). Electrical activity of cardiac myocytes (CMs) was evaluated with a custom-designed optical system, as described elsewhere [21,22]. Cell behaviors on the scaffolds with different chemical compositions were compared to determine the optimal composition for the design and future applications of these nano- and micro-textured scaffolds for cardiac tissue regeneration.

## 2. Materials and methods

### 2.1. Materials

Poly(glycolide-*co*-lactide) (PLA10GA90, LA/GA 10:90) random copolymer sample was obtained from Ethicon, Inc. (Somerville, NJ). This copolymer had an intrinsic viscosity of 1.56 ml/g in 0.1 g/ml hexafluoroisopropanol (HFIP), equivalent to a weight-average molecular weight ($M_w$) of about $7.5 \times 10^4$ g/mol and a polydispersity index ($M_w/M_n$) of about 3.1. Amorphous poly(lactide-*co*-glycolide) (PLA75GA25, LA/GA: 75:25) sample with an inherent viscosity of 0.55–0.75 dl/g was purchased from Birmingham Polymers (Birmingham, AL). Semi-crystalline poly(L-lactic acid) (PLLA) sample was an experimental polymer made by DuPont (Wilmington, DE), having a weight-average molecular weight of about $1.0 \times 10^5$ g/mole, a polydispersity index of about 2.0 and D-stereo configuration molar percentage of 5. D,L-lactide monomers were purchased from Sigma (St. Louis, MO). They were re-crystallized from ethyl acetate, and dried under vacuum before use. Methoxy poly(ethylene glycol) with a molecular weight of $5 \times 10^3$ g/mol was also purchased from Sigma. Poly(ethylene glycol)–poly(D,L-lactide) diblock copolymer (PEG–PLA, 5k–5k) was synthesized from D,L-lactide and methoxy poly(ethylene glycol) by ring-opening polymerization using a procedure described elsewhere [3]. The molecular weight of PEG–PLA diblock copolymer was measured by gel permeation chromatography (GPC) in THF solvent. 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) was used as the solvent to prepare 10 wt% polymers in solution for electrospinning.

### 2.2. Electrospinning

The detailed description of the electrospinning apparatus used in this study was published previously [5]. In short, the polymer solution was delivered by a programmable pump to the exit hole of the electrode (spinneret), which was connected with a positive high-voltage supply to generate a high electric field between the spinneret and the grounded collecting plate. The electric field strength applied in this study was 2 kV/cm, having a distance of 15 cm between the spinneret and the ground. The polymer solution feed rate was 100 μl/min. The recovered samples were placed in a vacuum oven at room temperature over 1 week to fully remove the solvent.

### 2.3. Post-processing to produce oriented electrospun scaffolds

Electrospun PLGA membranes were uniaxially stretched to achieve anisotropic fiber architecture for cardiac cell culturing. A modified Instron 4410 tensile stretching apparatus with a custom-built heating chamber was used for this purpose; the detailed description of the instrument was illustrated elsewhere [23]. The modification of the Instron apparatus allowed the sample to be stretched symmetrically along the uniaxial direction. Each membrane was first drawn to the desired extension ratio of 200% at a constant rate of 4 mm/min at 60 °C, and then cooled down to room temperature under the tension.

With the increase in the stretching extension ratio, the porosity of the membrane would often decrease. If this is a concern, the sequential biaxial stretching process with asymmetric draw ratios can be effectively used to control both orientation and porosity of the electrospun scaffold. However, in the present study, only uniaxially drawn scaffolds were used.

### 2.4. Contact angle measurements

Static contact angles of distilled water on the surface of the electrospun membranes were measured by a Cam 200 optical contact angle meter (KSV Instruments, Monroe, CT), equipped with a CCD camera (KGV-5000). This measurement was used to evaluate the hydrophilicity of the electrospun membranes with different chemical compositions. The experimental procedure was as follows: The electrospun membranes were attached to a silicone wafer. About 5 μl of distilled water was pipetted onto the membrane. Temporal images of the droplet were taken after 5 min. The contact angles were calculated by computer analysis of the acquired images. The chosen experimental conditions were 25 °C and 65% humidity.

### 2.5. In vitro degradation study

Electrospun membranes were cut into a rectangular shape with dimensions of $50 \times 10 \times 0.2\,mm^3$ for in vitro degradation studies. The cut electrospun specimens were placed in closed bottles containing 50 ml phosphate buffer solution (PBS, pH 7.30) and incubated in vitro at a temperature of $37.0 \pm 0.1$ °C for different periods of time. Three specimens were recovered at the end of each degradation period, dabbed dry with a tissue, and then dried in a vacuum oven at room temperature for 1 week. The weight of the degraded sample ($g_d$) was measured and mass loss percentage (MLP) of the sample was calculated ($MLP = (g_d - g_0)/g_0 \times 100$), based on the initial mass of each sample ($g_0$) before incubation. The pH value change at the end of each incubation period was also monitored with a pH meter (Orion, Model 290A).

### 2.6. Cardiomyocyte primary cell culture

Electrospun membranes were cut into a rectangular shape with dimensions of $12 \times 7 \times 0.2\,mm^3$ for ex vivo cell culturing studies. Primary CMs were harvested from the ventricular portion of the hearts of 2–4-day old Sprague-Dawley rats. The myocytes were plated on the designed scaffolds at a density of $400\,k/cm^2$. The matrices with CMs were kept in medium M199 supplemented with 2% (10% for the initial 3 days) fetal bovine serum (FBS) at 37 °C, 5% $CO_2$ and 95% humidity with change of culture medium every other day. The detailed cell culture procedure has been described elsewhere [21,24].

### 2.7. Microstructural assessment

Cell–scaffold samples for scanning electron microscope images were first fixed in 3.7% formaldehyde (Electron Microscopy Sciences, Fort Washington, PA) in phosphate-buffered saline (PBS) for 30 min, replaced with fresh fixative every 15 min and rinsed with PBS three times afterward. The samples were then dehydrated with increasing concentrations of acetone in water. Dehydrated specimens were placed in a series of spur resin and acetone and dried in a dry oven set at 65 °C overnight. After drying, the specimens were mounted on aluminum stubs, sputter-coated with gold, and reviewed in a scanning electron microscope (SEM, JEOL JSM5300).

For CLSM, cell-scaffold samples were fixed in 3.7% formaldehyde in PBS. F-actin and nuclei of the cells were co-stained with Phalloidin-Alexa 488 (Molecular Probes) and TOTO-3 (Molecular Probes), respectively. Samples were then mounted on a glass slide with VectaShield (Vector Laboratories, Burlingame, CA) and imaged with a CLSM composed of a Nikon E800 microscope integrated to a BioRad Radiance 2000 confocal laser scanning system. Samples were excited simultaneously at 488 nm for actin and 637 nm for TOTO-3. Emission at 535 nm was mapped to the green channel and 680 nm to the blue channel in a 24-bit RGB format containing $1024 \times 1024$ $pixels^2$ representing $195 \times 195\,\mu m^2$ area.

### 2.8. Functional cell assessment

Electrical activity in cultured CMs after 7-day plating was measured optically using voltage-sensitive dyes [21]. These are fluorescent molecules, which respond with sub-microsecond change in fluorescence to alterations in the voltage across the cell membrane. Combined with fast and sensitive detectors, these dyes provide the

means to optically measure electrical signals (action potentials) in excitable cells [25]. The scaffolds with cells were transferred to a custom-made experimental chamber, and the cells were superfused with normal Tyrode's solution (135 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 0.33 $NaH_2PO_4$, 5 HEPES, 5 glucose in mM), adjusted to pH 7.4 with NaOH. The cells were incubated with about 50 μM of voltage-sensitive dye (di-8-ANEPPS, Molecular Probes) for 5 min at room temperature. Electrical stimulation was provided through embedded platinum electrodes on the sides of the chamber. Stimulation threshold was 2–5 V/m and the frequency was varied. The optical mapping technique to track electrical activity in cultured cells via fluorescence has been reported elsewhere [20,24,25].

## 3. Results and discussion

### 3.1. Scaffold composition and nanofibrous structure

As indicated in Table 1, three nanofibrous PLGA membranes with different compositions were fabricated via electrospinning as scaffolds for in vitro degradation and ex vivo CM culturing studies. The compositions of three kinds of membranes were poly(L-lactide) (sample ID: PLLA); 75 wt% poly(glycolide-*co*-lactide) blending with 25 wt% poly(L-lactide) (sample ID: PLA10-GA90 + PLLA); and 85 wt% poly(lactide-*co*-glycolide) blending with 15 wt% poly(ethylene glycol)-b-poly(D, L-lactide) (sample ID: PLGA + PEG–PLA), respectively. The electrospinning conditions were controlled so that all membranes with different compositions had a similar morphology as shown in Fig. 1(A–C). All membranes demonstrated a large distribution of fiber diameters with the average around 1 μm (Table 1). The fibers were randomly oriented and formed many interconnected pores with a large pore size distribution. The surface hydrophilicity of the membranes decreased in the following order PEG–PLA + PLA25GA75 (hydrophilic) > PLA10GA90 + PLLA > PLLA (hydrophobic) as measured by the contact angle measurement (Table 1).

Typical SEM images of oriented electrospun scaffolds with different chemical compositions by uniaxial stretching (stretching ratio of 200%) are shown in

Table 1
Scaffold compositions and some physiochemical properties

| Sample | Composition | Average fiber diameter (μm) | Porosity[a] (%) | Contact angle |
|---|---|---|---|---|
| PLLA | Poly(L-lactide) | 1 | 71 | 107 |
| PLA10GA90 + PLLA | 75 wt% Poly(glycolide-*co*-lactide) + 25 wt% poly(L-lactide) | 1 | 78 | 85 |
| PLA75GA25 + PEG–PLA | 85 wt% P(lactide-*co*-glycolide) + 15 wt% poly(ethylene glycol)–poly(D,L-lactide) | 0.9 | 75 | 60 |

[a]Porosity $= (1 - \rho/\rho_0) \times 100$, $\rho$: density of electrospun membrane; $\rho_0$: initial raw pallet density.

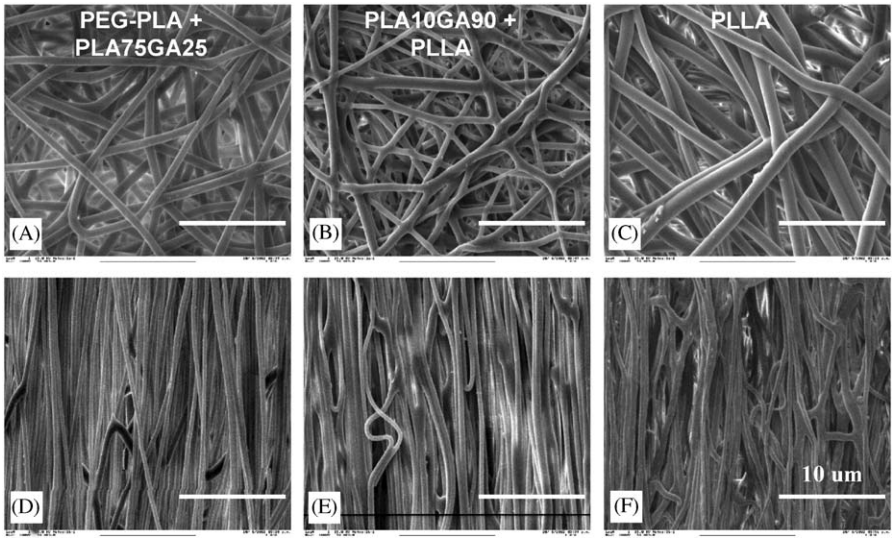


Fig. 1. SEM images of as-prepared electrospun membranes: (A) PLA75GA25 + PEG–PLA; (B) PLA10GA90 + PLLA; (C) PLLA. Images of the corresponding oriented electrospun membranes are shown in (D), (E) and (F), respectively.

Fig. 1(D–F). It is seen that the overall orientation of the non-woven scaffold increases significantly, the degree of orientation is similar to the level reported in the study employing a spinning disk in combination with electrospinning [10], but the porosity of the membranes decreased by about 20% (using the equation outlined in Table 1). Dependence of scaffold parameters on the process settings, as well as mechanical properties of electrospun compositions similar to the ones used here, have been discussed in detail in recent studies by our group [5,6]. In this study, the uniaxially oriented membranes were directly used with the further adjustment of porosity (e.g. by sequential biaxial stretching of asymmetric draw ratios).

### 3.2. In vitro degradation

The chemical compositions of the three scaffolds used were carefully chosen to minimize mechanical shrinkage during the in vitro degradation. The degradation rates of the three as-spun (non-oriented) samples were compared and results are shown in Fig. 2A. The PLA10GA90 + PLLA electrospun membrane exhibited the fastest weight loss rate during in vitro degradation due to the incorporation of the faster degrading PGA component. More than 20 wt% of initial weight was lost within 7 days. The corresponding pH value of the buffer solution decreased from 7.3 to 7.1 (Fig. 2B) with the

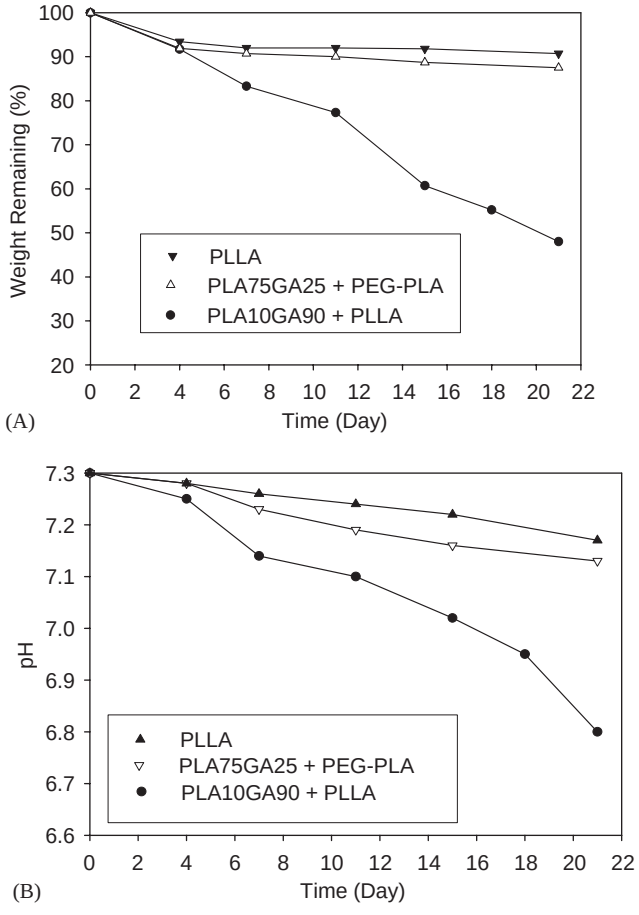


Fig. 2. In vitro degradation rates and pH value changes for PLGA-based electrospun membranes with three different compositions over 3 weeks: (A) degradation rates; (B) pH values in buffer solution during degradation.

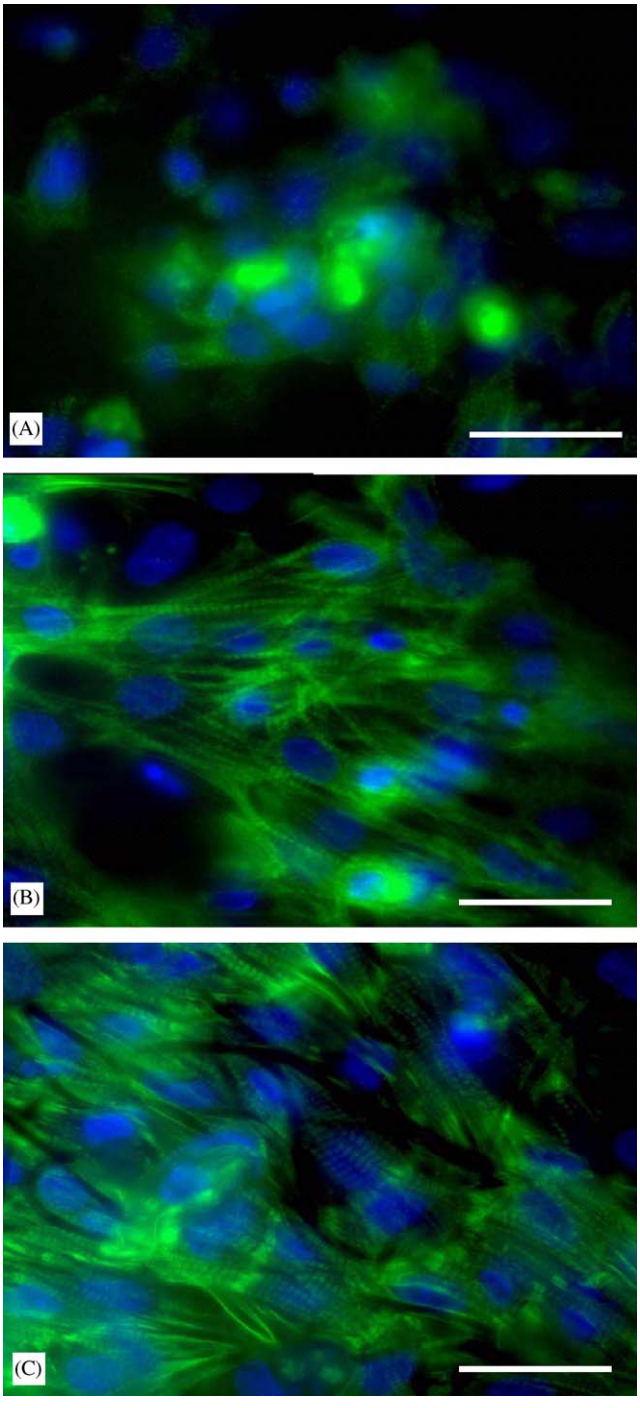


Fig. 3. CLSM images of cardiac myocytes on as-spun (non-oriented) membranes with different compositions: (A) PLGA + PEG–PLA, (B) PLA10GA90:PLLA (75:25), and (C) PLLA. Actin cytoskeleton is shown in green and cell nuclei labeled with TOTO-3 in blue. Scale bar is 20 μm.

release of degraded acidic byproducts from the scaffolds. Both PLGA + PEG–PLA and PLLA electrospun membranes degraded much slower with less changes in the pH values compared with the PLA10GA90 + PLLA sample.

### 3.3. Ex vivo cell culturing and response to scaffolding

CMs demonstrated different ability to attach and function on the electrospun PLGA membranes with different compositions. Fig. 3 shows confocal microscopy images of cell–scaffold (non-oriented) samples 7 days after plating. CMs were found to be very sensitive to the scaffold compositions and preferred the relatively hydrophobic surface. The hydrophilic surface of PLGA + PEG–PLA membrane affected the cell behavior negatively, producing clumps of structurally compromised cells. The faster degradation rate of the scaffold (PLA10GA90 + PLLA) also negatively affected the cardiomyocytes and caused them to lose spatial organization and cluster together. Among the three tested compositions, the most hydrophobic PLLA electrospun scaffolds provided the best support for CM attachment and structural development. It was observed that the cell nuclei (stained blue) were surrounded with mature cytoskeleton (stained green) on both PLLA and PLLA + PLA10GA90 electrospun scaffolds. The cells on PLLA membranes exhibited well-defined periodic units in the contractile machinery (sarcomeres) and intercalated disks (cell-to-cell contacts) typical for well-connected adult tissue (brighter green regions). The relative cell density increased progressively as follows: PLLA > PLA10GA90 + PLLA > PLA75-GA25 + PEG–PLA.

Fig. 4 shows CMs 7 days after culturing on as spun (non-oriented) PLLA membranes within different depths from the top surface of membranes. The membrane surface was totally covered with CMs (Fig. 4A). CMs also seem to have migrated to some degree into the non-woven fiber matrix (Fig. 4B–D), showing cytoskleleton-related fluorescence at deeper layers than a typical monolayer on a flat surface.

The SEM images of CMs on non-oriented PLLA electrospun membranes revealed that the CMs organized themselves according to the scaffold provided guidance, in some cases developing filopodia-like extensions along the fibers (Fig. 5A). Some cells were seen under thin fibers, possibly getting there by crawling and

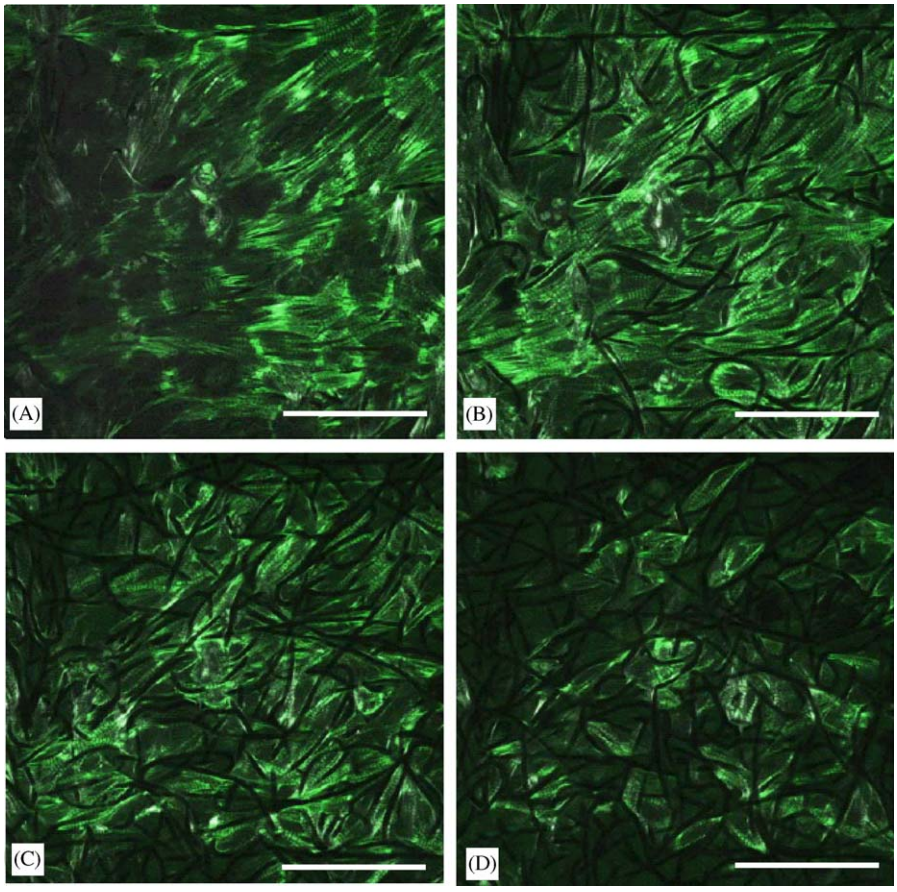


Fig. 4. CLSM images of cardiac myocytes on as-spun (non-oriented) PLLA membranes at different depths from the upper surface: (A) 2 μm from upper surface; (B) 4 μm; (C) 6 μm; and (D) 8 μm. Scale bar is 50 μm.

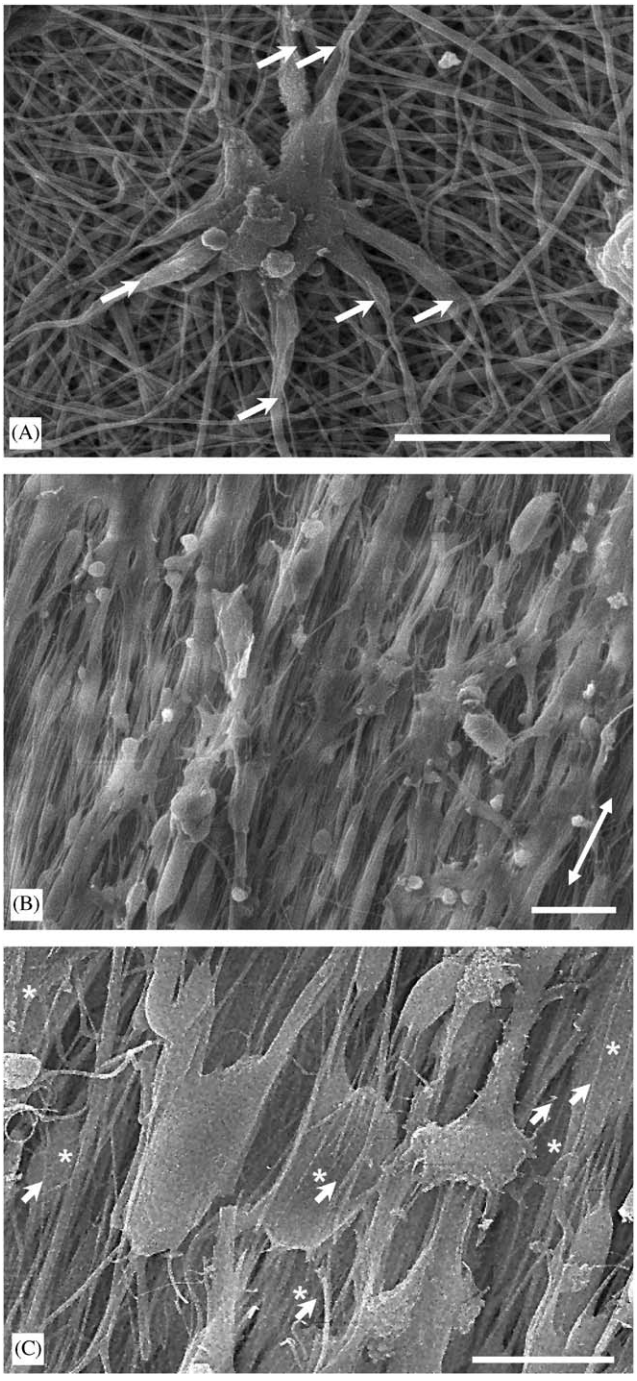


Fig. 5. SEM images of cardiac myocytes cultured on randomly oriented (A) and uniaxially stretched aligned PLLA electrospun membranes at two different magnifications (B) and (C). Arrows in (A) point to fiber-guided filopodia-like cell extensions; the arrow in (B) indicates the matrix fiber direction; stars in (C) indicate cells with fine fibers running on top of them; arrowheads indicate some of the scaffold fibers covering these cells. Scale bar is 40 μm.

spreading inside the scaffold. The SEM image of CMs on oriented PLGA membranes on day 7, shown in Figs. 5B and C, illustrates this interaction of the cells with the provided matrix.

To confirm matrix guidance on cell morphology and growth, we cultured CM on all three matrices of different composition (PLGA + PEG–PLA, PLA10-GA90:PLLA 75:25, and PLLA) with oriented fibers produced by uniaxial stretching (Fig. 6). Again, we verified that the most hydrophobic PLLA scaffold

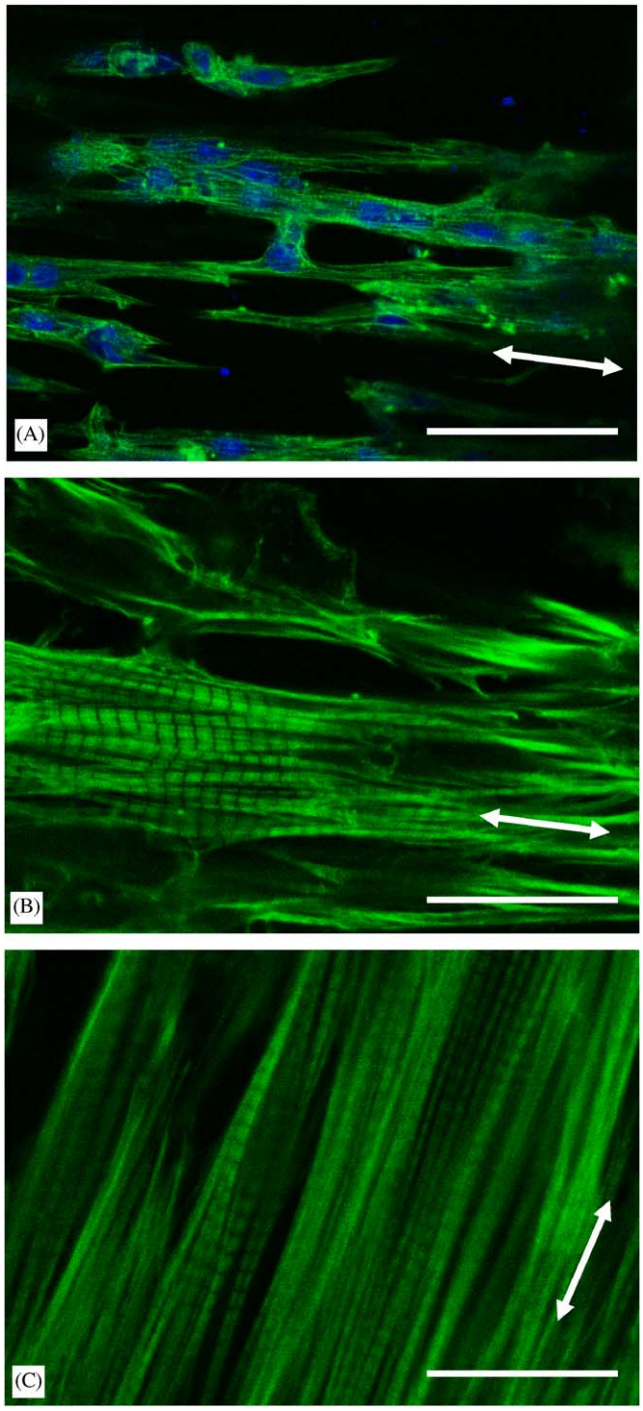


Fig. 6. CLSM images of cardiac myocytes on predefined oriented fibers with different compositions: (A) PLGA + PEG–PLA, (B) PLA10GA90:PLLA (75:25), and (C) PLLA. Scale bar is 20 μm. Arrows indicate matrix fiber direction.

provided the best support for CM attachment and structural development (Fig. 6C). The observed order of CM cell density on the oriented electrospun membranes was also PLLA > PLA10GA90 + PLLA > PLA75-GA25 + PEG–PLA. In Fig. 6C, it was found that cells aligned along the PLLA fiber direction with elongated nuclei, where post-processed fiber alignment provided guidance for cells to grow along the stretching direction.

Functional studies of CM on the scaffolds using optical mapping of electrical activity demonstrated that cells on PLLA performed better than cells on PLA10-GA90 + PLLA, and significantly outperformed cells on PLGA + PEG–PLA (Fig. 7). For example, cultured CMs on PLLA membranes followed external pacing rates up to 6 Hz with overall shorter action potential durations (APD) at all frequencies, while CMs on PLA10GA90 + PLLA were able to follow up to 4 Hz. Comparing the APD for cells grown on these two types of scaffolds and stimulated at 2 Hz, we find a significant difference (Fig. 7B). CMs cultured on PLGA + PEG–PLA membranes could only follow up to 1 Hz pacing and had prolonged APDs of 0.5 s. Abnormally long APDs and inability to maintain high pacing rates can be arrhythmogenic and thus is undesirable.

As seen from Table 1 and Figs. 1 and 2, the three examined electrospun matrices were similar in fiber architecture and porosity, but differed in their degradation profiles (loss of mass and rate of pH change in the medium) and their surface hydrophobicity. Based on the differential cellular response to these three matrices (Figs. 3–7), we believe that the scaffold hydrophobicity was a major factor sensed by the cardiomyocytes.

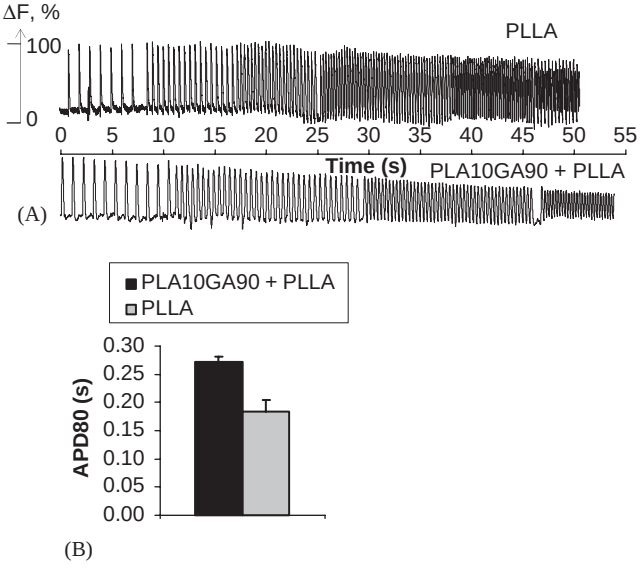


Fig. 7. Electrical response of cardiac myocytes on electrospun scaffolds. Action potentials were measured using a voltage-sensitive dye di-8-ANEPPS and a microscale optical recording system: (A) example electrical traces from PLA10GA90 + PLLA (bottom) and PLLA (top) are shown. Time (*x*-axis) is in seconds, transmembrane voltage (*y*-axis) is presented as normalized fluorescence response. PLA10GA90 + PLLA followed pacing from 1 to 4 Hz, had longer action potentials. PLLA followed pacing from 1 to 6 Hz, with shorter action potentials. (B) Comparison of the action potential duration at 80% recovery (APD80) at 2 Hz pacing.

## 4. Conclusions

Electrospun PLGA-based scaffolds were examined to investigate the effects of their chemical composition, degradation rate and surface properties on the attachment and growth of cardiac myocytes (CMs) to form well-connected tissue. Scanning electron microscopy revealed that CMs interacted with the provided nano- and microfibrous network trying to organize their growth in order to follow the scaffold-prescribed direction. We applied simple post-processing steps (mechanical stretch of the electrospun membranes) to achieve oriented scaffold texture, enforcing anisotropic cell growth. Confocal microscopy of fluorescently labeled intracellular structures demonstrated that the CMs were very sensitive to the composition of the electrospun PLGA-based scaffolds, with a preference to relatively hydrophobic surfaces. The CM cell density was lower on hydrophilic and faster degrading electrospun scaffolds. Poly(L-lactide) scaffolds promoted better cell adhesion and mature cytoskeleton structure with well-defined periodic units in the contractile machinery (sarcomeres). Reestablished cell-to-cell contacts and intercalated disks, typical for mature adult cardiac tissue, could be clearly identified. Functional studies of CMs on the scaffolds further confirmed the superior response on PLLA scaffolds compared to PLA10-GA90 + PLLA and PLGA + PEG–PLA. In summary, nano- and microstructured electrospun non-woven scaffolds provide both flexibility and guidance for CM growth and can be successfully applied to obtain structurally and functionally competent cardiac tissue constructs.

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