Journal of Molecular and Cellular Cardiology 76 (2014) 227–234

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

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

Arrhythmogenic role of the border between two areas of cardiac cell alignment

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ARTICLE INFO

Article history:
Received 31 March 2014
Received in revised form 29 August 2014
Accepted 2 September 2014
Available online 16 September 2014

Keywords:
Excitation
Cardiomyocytes
Tissue culture
Optical mapping
FitzHugh–Nagumo model

ABSTRACT

The goal of this study is to develop experimental and computational models of the excitation transition between areas of cardiac tissue with different anatomical anisotropy. Alignment of seeded neonatal rat cardiomyocytes was achieved with the aid of guiding polymer (PMGI) nanofibers, and two areas with orthogonal alignment were placed into a contact. It was found that the excitation wave crossing border between the areas with different alignment direction experiences substantial perturbation, up to the complete conduction block. In addition to the experimental study, this effect was analyzed computationally using generic FitzHugh–Nagumo reaction–diffusion model. It was shown that the non-monotonous changes of the excitation wave velocity on this boundary may be explained by the source/sink mismatch. *Thus, the border may play pro-arrhythmogenic role.*

1. Introduction

Bioengineered cardiac implants are believed to play very important role in a contemporary regenerative medicine of heart [1,2]. In general, such an implant constitutes a cultured tissue patch based on a specific scaffold which provides structural and functional environment for the cardiac cells grown on it. Usually, scaffolds represent polymer matrices made of the non-toxic, biocompatible and, desirably, biodegradable materials. The polymer matrices may be as simple as sponge-like slabs, containing cells [3,4], as well as highly ordered assemblies of the cell sheets [5]. Nowadays it is clearly understood that the architecture of the tissue patch greatly influences its functionality [6]. In designing scaffolds for the prospective implants, a lot of attention was paid to mechanical and structural matching of the cultured tissue patches to the genuine cardiac tissue [7,8]. It was also shown that this mechanical matching increases efficiency of pluripotent cells differentiation into cardiomyocytes [9]. In this study, we attract attention to the important aspect of the implanted cardiac tissue: matching of AP conduction of the host tissue and the implant. The mechanical contraction of the heart tissue follows AP propagation and is orchestrated by it. The heterogeneities in the heart tissue may perturb propagation of AP and serve as the sources of re-entry formation—the most dangerous heart arrhythmia [10–12]. If the conduction properties of the host tissue and the implant are mismatching, the border zone may create problems for the excitation propagation. This mismatching may be based on the different physiological properties of the cells, constituting the tissue, such as activity of ion channels, coupling between cells (gap-junction protein expression), and architectural differences between the tissue parts. In addition, it is known that the intact heart area with an abrupt change of the fiber orientation may be arrhythmogenic [13,14] and development of the model representing such system would be of general interest in studies of the reentry formation.

We developed an experimental model for the study of the excitation propagation at the border zone between two areas with differently aligned cardiac cells. It was shown earlier, that propagation speed greatly depends on the cell alignment, being faster along cells than in the perpendicular direction [15–18]. The model is based on the polymer nanofibers, providing guiding support for the cardiomyocytes seeded on them and controlling their elongation in the direction of fibers [18]. The matrix, representing two areas of nanofibers with the different directions of alignment (in extreme case, placed orthogonally), creates a template for the grown cardiomyocyte tissue with two parts where the cells are aligned in different directions and exhibit different anisotropy of the excitation propagation. The propagating front of excitation was observed by tracking fluorescent probes, i.e. optical mapping [19]. It was found that the border between two alignment zones serves as a perturbing feature for the excitation propagation, the more perturbing as the higher degree of misalignment was set. In general, the perturbation of the propagating wave front was either delay, or acceleration at the border, depending on the direction of the propagation. The progressing delay may lead to the conduction block and the re-entry (rotating wave) origination.

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http://dx.doi.org/10.1016/j.yjmcc.2014.09.003
0022-2828/

2. Materials and methods

2.1. Preparation of solid substrate with designed surface topography

2.1.1. Materials

An electrospinning ready solution of polymethylglutarimide (PMGI, viscosity 339 cst at 25 C) having a concentration of 13% being dissolved in a mixture of cyclopentanone and tetrahydrofurfuryl alcohols (9:1) was purchased from MICROCHEM Corp. (MA, USA). The ionic surfactant sodium dodecyl sulfate (SDS, obtained from Wako Chemicals Inc., Japan) dissolved in ethanol was admixed into the polymer solution to a final concentration of 1.44 g/L, to prevent beading and obtain smooth fibers during electrospinning.

Polydimethylsiloxane (PDMS) was prepared by the thorough mixing of two liquid components (purchased from Dow Corning Toray Corp., Japan) at a ratio of 1:10 in a flat Petri dish. After being kept at 80 °C for 1 h, the polymerized PDMS layer was separated from the dish, cut into 20 × 20 mm squares and cleaned with ethanol before use. The PDMS squares were used as temporary holders with PMGI nanofibers for preparation of glass cover slips micro-imprinted with nanofibrous pattern.

All other chemicals were purchased from Wako Chemicals Inc., Japan.

2.1.2. Electrospinning of PMGI nanofibers

The prepared solution of PMGI polymer with SDS was electrospun using a high voltage power supply (Matsusada Precision Inc., Japan) with an 8 kV potential between the solution and the grounded collector. The solution was loaded into a 1 ml syringe and delivered through a 27 gauge blunt-tip needle at a flow rate of 0.8 ml/h using a programmable syringe pump (Fusion 100, Chemyx Inc., Japan). Fibers were deposited onto a grounded stationary collector at a distance of 10 cm from the syringe tip. The collector was round in shape (5 cm in diameter), made of 0.01 mm thick aluminum foil. The alignment of the nanofibers was ensured by the presence of a rectangular hole in the middle of the collector. The size of the hole was 20 mm in width and 25 mm in height. In compliance with the desired positioning density of the nanofibers as 50 fibers/mm spraying time was set to 1 min.

2.1.3. Preparation of solid substrate with nanofibrous surface topography

Our primary task was to create a nanofibrous pattern on the surface of the solid substrate (glass cover slips) in order to prepare anisotropic cardiac tissue cultures with abrupt change in cardiac fiber orientation by 90°. For this purpose collected PMGI nanofibers were transferred onto the above-mentioned PDMS square holders in two steps. At first collected nanofibers from the hole were transferred to PDMS square, then half of these fibers were orthogonally cut and removed from the PDMS. After that procedure was repeated prepared samples were stored for 24 h to remove the solvent residues.

For our study it was necessary to prepare substrates with the sharp changing of fiber direction (90°). For the first step we prepared 10 mm long fibers and transferred them onto PDMS, and then we gently cut a half of the fibers in the orthogonal direction. For the second step we prepared 20 mm long fibers and transferred them onto the cleaned side of PDMS orthogonally to fibers which were transferred before (see Fig. 1a). For cell seeding and cultivation, the electrospun PMGI nanofibers were transferred from the PDMS onto 22 mm glass cover slips by micro-contact printing to fasten the fibers to a smooth glassy surface. After all, the surface with fibers was coated with fibronectin (0.16 mg/ml) for better cell adherence.

For the studies of unidirectional block we have cut the samples and divided into narrow stripes (3–4 mm width) for better wave collimation. Cuts were made in perpendicular to the border direction using a sharp razor. Thus, we obtained several narrow specimens with short uniform borders instead of a large one with a long non-uniform border.

We have also added lidocaine to suppress spontaneous activity and to raise the threshold in order to make an effect more pronounced [20,21]. Lidocaine was diluted in Tyrode solution to 180–200 μM concentration.

2.2. Cells

Cardiac cell isolation, seeding and cultivation performed according to Worthington protocol (http://www.worthington-biochem.com/NCIS/default.html). Cardiac cells were isolated from the ventricles of 1–3 day old neonatal Wistar rats (SLC Inc., Japan).

All procedures were performed according to the institutional requirements for the care and use of laboratory animals. All studies conformed to the Guide for the Care and Use of Laboratory Animals, published by the United States National Institutes of Health (Publication No. 85-23, revised 1996) and approved by MIPT Life Science Center Provisional Animal Care and Research Procedures Committee, Protocol #A2-2012-09-02.

2.3. α-Actin staining

α-Actin staining of cells attached to nanofibers was performed as described at [18].

2.4. Optical mapping

Optical mapping was done in 5–6 day-old cell culture samples that were grown on glass cover slips covered with PMGI nanofibers. To monitor activity and visualize the excitation wave propagation, the cells were loaded with the Ca^{2+} sensitive indicator Fluo-4-AM (Invitrogen,

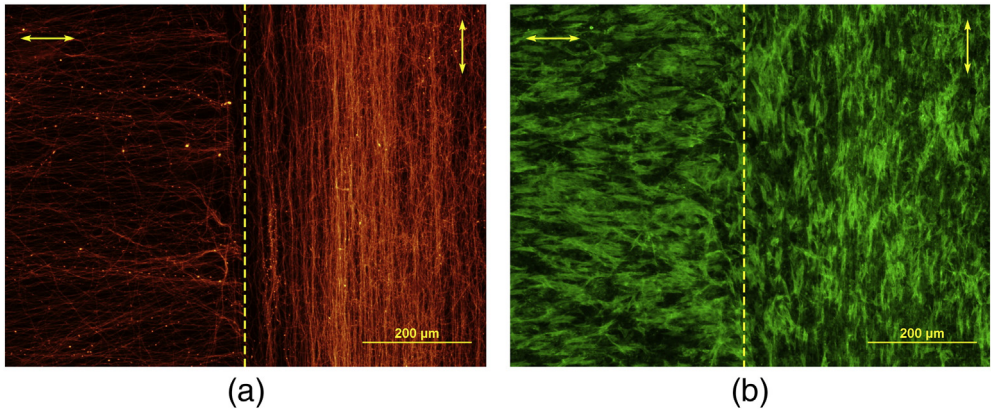

Fig. 1. Cardiac tissue grown in two adjacent areas of nanofibers with orthogonal orientation. (a) Nanofibers stained with Rhodamine. (b) Actin staining of the cells grown on nanofibers.