Biomaterials 32 (2011) 4267–4274

Contents lists available at ScienceDirect

Biomaterials

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

Patterned cardiomyocytes on microelectrode arrays as a functional, high information content drug screening platform

Anupama Natarajan [a,b], Maria Stancescu [a], Vipra Dhir [a,c], Christopher Armstrong [a], Frank Sommerhage [a], James J. Hickman [a,b,c,*], Peter Molnar [a,b,d,*]

[a] University of Central Florida, NanoScience Technology Center, 12424 Research Parkway, Suite 400, Orlando, FL 32826, United States
[b] Burnett College of Biomedical Sciences, University of Central Florida, Orlando, FL, United States
[c] Department of Mechanical, Materials and Aerospace Engineering, University of Central Florida, Orlando, FL, United States
[d] Department of Zoology, University of West Hungary, Szombathely, H-9700, Hungary

ARTICLE INFO

Article history:
Received 8 November 2010
Accepted 14 December 2010
Available online 31 March 2011

Keywords:
Biosensor
Cardiac tissue engineering
Cardiomyocyte
Electrode
In vitro test
Micropatterning

ABSTRACT

Cardiac side effects are one of the major causes of drug candidate failures in preclinical drug development or in clinical trials and are responsible for the retraction of several already marketed therapeutics. Thus, the development of a relatively high-throughput, high information content tool to screen drugs and toxins would be important in the field of cardiac research and drug development. In this study, recordings from commercial multielectrode arrays were combined with surface patterning of cardiac myocyte monolayers to enhance the information content of the method; specifically, to enable the measurement of conduction velocity, refractory period after action potentials and to create a functional re-entry model. Two drugs, 1-Heptanol, a gap junction blocker, and Sparfloxacin, a fluoroquinone antibiotic, were tested in this system. 1-Heptanol administration resulted in a marked reduction in conduction velocity, whereas Sparfloxacin caused rapid, irregular and unsynchronized activity, indicating fibrillation. As shown in these experiments, patterning of cardiac myocyte monolayers solved several inherent problems of multielectrode recordings, increased the temporal resolution of conduction velocity measurements, and made the synchronization of external stimulation with action potential propagation possible for refractory period measurements. This method could be further developed as a cardiac side effect screening platform after combination with human cardiomyocytes.

1. Introduction

The development of a high-throughput, high information content device to study and understand cardiac electrophysiology would be important for the fields of cardiac physiology, tissue engineering and drug research. More than 850,000 people are hospitalized for arrhythmias each year and ventricular fibrillation (VF) is a leading cause of cardiac death [1]. Despite the intensive research in this area, the mechanism of VF is still poorly understood [2–5].

Arrhythmia is a known side effect of commercial drugs. One of the mechanisms by which drugs can cause a potentially fatal form of ventricular tachy arrhythmia, called Torsades de pointes (Tdp), is through the prolongation of the QT interval (in an ECG the length of the ventricular action potential). It has been reported that approximately 2–3% of all prescribed drugs can cause long QT syndrome [6,7]. A broad range of cardiovascular drugs and antibiotics also have the potential risk of causing drug induced Tdp [8,9]. At the same time, prolongation of the QT interval does not necessarily lead to Tdp; lengthening of the QT interval could even be anti-arrhythmogenic, as it is considered a mechanism of action of the class III anti-arrhythmics [8,9]. Thus, a relatively high-throughput method to identify cardiac side effects and differentiate between arrhythmic and anti-arrhythmic effects at an early stage of drug development would have a significant impact on the field.

Gap junctions play an important role in the propagation of excitation in cardiac tissue. Changes in gap junction function affect major cardiac parameters, such as conduction velocity (CV). It has been observed in several cardiovascular diseases that the expression of connexins (protein molecules that form gap junction channels) is decreased or their distribution is changed, leading to a malfunction in gap junction coupling [10]. Understanding the pharmacological modulation of cardiac gap junction channels would further aid the drug development enterprise.

* Corresponding authors. University of Central Florida, NanoScience Technology Center, 12424 Research Parkway, Suite 400, Orlando, FL 32826, United States. Tel.: +1 407 823 1925; fax: +1 407 882 2819.
E-mail address: jhickman@mail.ucf.edu (P. Molnar).

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2010.12.022

Introduction of an in vitro method for cardiac side effect testing, which has high predictive value, would have a significant impact on drug development as it could also reduce the cost, time and the number of drugs failing in clinical trials [11]. In vitro testing would also reduce the need for animal testing and could be used to study drug effects with a functional assay, but at the cellular level. Other in vitro methods, such as whole heart experiments (Langendorff heart model) or the Purkinje fiber preparation, are difficult and time consuming [11]. Traditional methods used to study QT interval prolongation at the cellular level include patch-clamp experiments. However, these experiments are time intensive, require a skilled operator and cannot be used to study action potential (AP) propagation or parameters such as CV and re-entry. Moreover, evidence suggests that prolongation of QT intervals is not the best predictor of Torsades de pointes. The measurement of the length, or the variability in the length, of the refractory period after a cardiac action potential may have more relevance for predicting arrhythmic behavior [9].

Cardiac myocytes cultured on microelectrode arrays (MEA) have several benefits compared to either traditional patch-clamp electrophysiology or isolated organ methods. The use of MEA's in the investigation of cardiac side effects would provide information in a relatively high-throughput and low cost manner compared to standard patch-clamp electrophysiology. However, at this time, it is still a low information content method and this has limited its use. Cardiac myocytes on MEAs have been used in a number of studies to investigate the effect of toxins, such as pesticides [12] and cardioactive drugs [13] on cardiac field potentials. A commercial system has also been introduced to measure QT intervals in a relatively high-throughput fashion [14], but, to date, it has only limited applications. However, cardiac myocytes can now be maintained over longer periods of time [15], thus chronic experiments, such as the monitoring of network remodeling for specific diseases, is now feasible. In addition, serum-free formulations for cardiac culture have also been introduced, which would increase the reproducibility of such a system [16].

All of the above mentioned studies utilized unorganized monolayers of cardiomyocytes on the MEAs. Development of a patterned cardiac myocyte layer that is aligned with the electrodes of a MEA could solve several problems associated with the random spread of excitation in a cardiac monolayer, which makes evaluation of the obtained data, such as CV, difficult. It would also enable the development of specific open-loop or closed-loop stimulation protocols to measure critical parameters, such as the length of the refractory period after the action potential. It could also be used to create a high-throughput, low cost functional re-entry model.

There are several lines of evidence indicating that not only contact interaction with the surface but the shape of the attachment area determines the physiology of cardiac myocytes [17]. Pattern geometries determine the extent of the alignment of the long axis of cardiac myocytes, and this alignment determines CV [18] and other physiological and pharmacological properties of cardiac tissues [19,20].

Several different methods have been developed for cell patterning. One category of this technique is based on direct placement of cells or extracellular matrix molecules on desired locations and includes patterning through microfluidic channels [21–23], microcontact printing [24,25] and inkjet printing [26]. Cardiac myocytes have previously been patterned on glass using photoresist [27] as well as other techniques [15,17,19,20,25]. Another method utilized photolithography following surface modification with self-assembled monolayers (SAMs) for neurons [28–30] as well as myocytes [15,31]. The benefit of this method is the compatibility of the technique with cheap automated silicon manufacturing steps and the ability of the cells to self-assemble after random plating.

SAMs are one molecule thick monolayers attached to a surface composed of organic molecules, which have been extensively used for surface patterning [31–33]. Surface modification with SAMs is also compatible with advanced photolithography methods [30,34]. Studies have also shown that cells survive on these surfaces for extended periods of time [35,36], do not migrate off the patterned areas [34] and exhibit the typical morphology and physiology of the specific cell type [16,37].

The goal of this study was the development of patterned, rat, cardiomyocyte cultures on MEAs in a serum-free medium for the study of cardiac physiology and pharmacology utilizing a high-throughput technique, but with high information content. An adsorbed fibronectin layer was used as the foreground because it supported cardiac myocyte attachment and growth and a 2-[Methoxy(Polyethyleneoxy) Propyl]TrimethoxySilane (SiPEG) SAM was used as the cell repellent background because of its excellent protein adsorption resistant properties [38]. The measurement of CV with the patterned cardiac myocyte monolayers and the feasibility to apply different stimulation protocols to the MEA/cardiac system was demonstrated. The action of 1-Heptanol and Sparfloxacin was also assessed.

2. Materials and methods

2.1. Experimental

2.1.1. Surface modification of microelectrode arrays with PEG silanes

MEA's containing 60, 10 μm diameter electrodes (Multichannel-Systems, Germany) were cleaned by soaking the arrays in a detergent solution for 2 h followed by sonication for 10 min. The arrays were then oxygen plasma cleaned for 20 min. Surface modification was completed by incubation of the MEAs in a 3 mM PEG silane, 2-[Methoxypoly(ethyleneoxy)propyl]trimethoxysilane (SiPEG) (MW = 460–590, Gelest), solution in toluene, with 37% concentrated HCL added to achieve a final value of 0.08% (0.8 ml HCL/L), for 45 min at room temperature. The arrays were then rinsed once in toluene, twice in ethanol, twice in water and sonicated in water for 2 min to remove the non-covalently linked material [39]. The arrays were air dried with nitrogen and stored in a dessicator overnight.

2.1.2. Laser ablation and patterning of the microelectrode arrays

The MEAs were patterned using a deep UV (193 nm) excimer laser (Lambda Physik) at a pulse power of 230 mW and a frequency of 10 Hz for 45 s through a quartz photomask (Bandwidth foundry, Eveleigh, Australia). The arrays were sterilized using 70% isopropanol and then incubated with 5 μg/ml of fibronectin in a Phosphate buffered solution (Invitrogen) for 20 min at room temperature. The solution was removed and the surface was first rinsed with PBS, followed by the plating medium, and then dried before the cells were plated.

2.1.3. Neonatal rat cardiomyocyte culture

The neonatal rat cardiomyocyte culture was prepared using the cardiac isolation kit from Worthington [40]. All animal work was approved by the UCF IACUC and followed NIH guidelines. Briefly, two day-old rat pups were euthanized in a pre-charged CO_2 chamber. Hearts were dissected and minced in ice cold Hanks balanced salt solution (HBSS). Cardiac myocytes were dissociated by incubation of the hearts in trypsin (100 μg/ml in HBSS) for 16 h at 2–8 °C. The hearts in the trypsin solution were briefly warmed with a trypsin inhibitor before adding collegenase (300 units/ml in L-15 medium) for 45 min in a water bath at 37 °C followed by mechanical trituration. The cell solution was filtered to remove any remaining tissue and centrifuged at 50 g for 5 min at 22 °C. The cells were resuspended in high glucose Dulbecco's modified eagle medium (DMEM, Gibco/Invitrogen) supplemented with 10% Fetal Bovine Serum (Gibco/Invitrogen) and 1% penicillin streptomycin (Gibco/Invitrogen), preplated in Petri dishes and incubated at 37 °C and in 5% CO_2 for 45 min. This was necessary to eliminate the fibroblasts. The supernatant from the Petri dishes was centrifuged at 50 g for 5 min at 22 °C. The cells were then resuspended in the plating medium. The serum-free plating medium consisted of: 100 ml Ultraculture medium (Bio Whittaker Cambrex) supplemented with 10 ml B27, 1 ml L-glutamine (Gibco/Invitrogen), 1 ml Penicillin Streptomycin, 0.375 g dextrose (Fisher Scientific) in 800 μl water, 1 ml non-essential amino acids and 1 ml of Hepes buffer (Gibco/Invitrogen) [41]. Additional growth factors were also added to improve cell survival in the serum-free conditions. They included 0.1 μg/ml of L-thyroxine, 10 ng/ml of Epidermal growth factor (Sigma–aldrich) and 0.5 μg/ml of Hydrocortisone (BD biosciences). Cells were plated at a density of 1000 cells/mm^2 on the MEAs. The medium was changed 24 h after plating. Subsequent changing of the medium was performed every third day.

2.1.4. Immunostaining

Patterned Cardiomyocytes were immunostained for F-Actin with Rhodamine Phalloidin (Invitrogen, R415), using a protocol provided by the company. Briefly, the cells were washed with PBS and fixed using 3.7% Formaldehyde. The coverslips were extracted with 0.1% Triton X. The staining solution (with 1% Bovine Serum Albumin