Synchronized Mechanical and Electrical Stimulation of Primary Heart Cells with a Stretchable Microelectrode Array

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ABSTRACT

The culture of contractile cells such as cardiomyocytes is typically performed in environments that do not resemble the in vivo conditions in the heart. Adult murine cardiomyocytes are accustomed to both electrical pacing and mechanical stretch. We present initial culture experiments of contractile cells in an integrated electromechanical system with a stretchable microelectrode array. We have demonstrated synchronized electrical and mechanical stimulation of cardiomyocytes and the capability to provide asynchronous electrical and mechanical stimulation and are probing the effects on cell phenotype, function, and survival.

KEY WORDS: primary cells, adult cardiomyocytes, stretchable electrode array, synchronized stimulation

INTRODUCTION

The in vivo environment of cardiac tissues is highly dynamic, with cells experiencing both regular cyclic strains and electrical pacing. Primary cardiac tissue is particularly difficult to culture because within 24-72 hours the myofibrils break down and lose their structure, thereby losing their ability to function as contractile tissues[1]. To create a more physiologically accurate cell culture environment, we hypothesize that cell culture of cardiac cells may be enhanced by the incorporation of synchronized stretch [2-4] and electrical stimulation in the culturing protocol (Fig 1). We have previously reported [5] the creation of a stretchable electrode array for cell culture. Here, we present an integrated electromechanical stimulation system for use with a stretchable electrode array. This system allows for synchronous and asynchronous mechanical stimulation and electrical pacing of cultured cardiomyocytes. In addition, because thousands of cells can be cultured per device, this stretching system is better matched to conventional cell culture techniques than the majority of existing MEMS force applicators and sensors [6]. Ultimately, larger arrays of planar, stretchable electrodes will allow a culture to be paced with a traveling potential waveform vs. the conventional pair of pacing electrodes used in myocyte culture (e.g. Ionoptix Myopacer). A finer granularity of electrodes will also allow readout of depolarization potentials in regions of a beating cell culture and timing from pacemaker cells in the culture. We seek to improve cellular function of primary cardiomyocytes for long term culture using such electromechanical stimulation systems.

EXPERIMENTAL SETUP

Stretch system

The integrated stimulation system unites both our stretching and pacing systems. Our stretching system consists of a serial-controlled linear actuator (Zaber T-LA60) mounted to micromanipulator (ST-Japan) via a linear stage (Newport 443 Series). Our pacing system is a modified commercial cell culture pacing device (IonOptix Myopacer). A computer interface allows communication with a microcontroller (dsPIC33) which then commands both our stretching system and electrical pacing system (Fig 2). Stretch waveforms and pacing parameters are set by the microcontroller for the stimulation systems and provide synchronous or asynchronous continuous stretching and pacing of the stretchable electrode array.

Figure 1. We hypothesize that coupled mechanical and electrical stimulation of adult cardiomyocyte cell culture will enhance cell phenotype maintenance.

Figure 2. Control of mechanical stretch and electrical pacing is achieved by a microcontroller interfaced with a serial linear actuator-based (Zaber) micromanipulator (ST-Japan) and a pacing system (IonOptix).
Cell adhesion and device preparation

We treated the stretchable electrode arrays and simple all-PDMS control films with an O2 plasma to create a hydrophilic surface then permanently bonded PDMS retaining rings to them. We coated the devices with Natural Mouse Laminin (Invitrogen 10µg/mL) for one hour and aspirated off the excess solution. Cardiac myocytes were extracted from the hearts of adult mice (FVB strain). We deposited the cells on the devices and allowed them to attach to the laminin for approximately 2 hours. We aspirated the excess buffer and added a HEPES-buffered salt solution (1mM CaCl2, 137 mM NaCl, 5.4 mM KCl, 15 mM dextrose, 1.3 mM MgSO4, 1.2 mM NaH2PO4, 20 mM HEPES (Invitrogen)), to the culture wells.

Substrate stretching and pacing

For our initial experiments cell substrates were mechanically stretched and electrically paced at 1Hz at various offsets. The electrical pacing stimulation was a 10V biphasic 10ms high/10ms low waveform (Fig 2); while mechanical stretch was applied uniaxially with a triangular waveform from 0% to 10% strain.

RESULTS AND DISCUSSION

We have fabricated and demonstrated a system for applying synchronized electrical and mechanical stimulation, which utilizes a stretchable microelectrode array cell culture platform. Because cardiac cells are extremely sensitive to stimulation parameters and since this system more accurately mimics the in vivo cell environment, we can now work to determine those electromechanical stimulation parameters that lead to enhanced cell phenotype, function, and survival.

CONCLUSIONS

We have demonstrated a combined stimulation system for applying cyclic mechanical strain and electrical stimulation to cell culture on a stretchable electrode array. This system opens the door to a more controlled investigation of the relationship between cardiomyocyte electromechanical stimulation and growth. Cardiac cells are more sensitive to their environment than other cells such as smooth muscle cells. Future research will focus on 1) improving viability of primary adult cells through tuned stimulation parameters, 2) characterizing development and maintenance of neonatal cells and stem cells under stimulation, 3) enhancing device biocompatibility during dynamic stimulation, and 4) miniaturizing the stretchable electrode arrays to allow electrical readout of depolarization potentials from regions of the cell culture.

REFERENCES