LOW COST MICRO-PLATFORM FOR CULTURING AND STIMULATION OF CARDIOMYOCYTE TISSUE

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ABSTRACT

This paper reports a low cost rapid prototyping platform to culture cardiomyocyte tissue with embedded biocompatible stimulation electrodes based on micro-structured conductive PDMS. The polymeric platform was fabricated using conventional soft lithography based on a non-photolithographic method to obtain molds, combined with the inclusion of micro-structured polymeric electrodes in the replicas. Device fabrication, electrical characterizations of the conductive polymer, as well as mechanical response of cultured tissue under spontaneous and stimulated activity are presented. Through this method, synchronization and coupling bio-electromechanical activity of different cell aggregates has been observed.

INTRODUCTION

Understanding the bio-electromechanical behavior of cardiac tissue and its activity pattern under different experimental conditions has potential for clinical and biotechnological applications. Relevance of cardiac tissue engineering is to create *in vitro* functional bioreactor that can be used as a biological model for studies of cardiac tissue development, electrophysiological properties and/or disease processes, and eventually as implants to repair injured myocardium. Successfully engineered 2D and 3D tissue should therefore exhibit the important properties of native cardiac tissue, like phenotype dependence on protein substrate, contractile and electrophysiological functionality among others [1].

Several approaches for making micro-engineered platforms for cell culturing and manipulation have been reported [2] where devices were built under traditional fabrication processes that require sophisticated technological resources to pattern and etch microstructures either on glass substrates, or similar processes to obtain molds for soft lithography. Additionally, cell culture platforms that incorporate electrical stimulation usually requires high cost biocompatible metal deposition and patterning (e.g. Au, ITO,etc) [3]. The purpose of this work is to provide a platform for non-invasive electrical stimulation of a well localized cultured heart tissue and to analyze its bio-electromechanical behavior without the need of an advanced micro-fabrication facility.

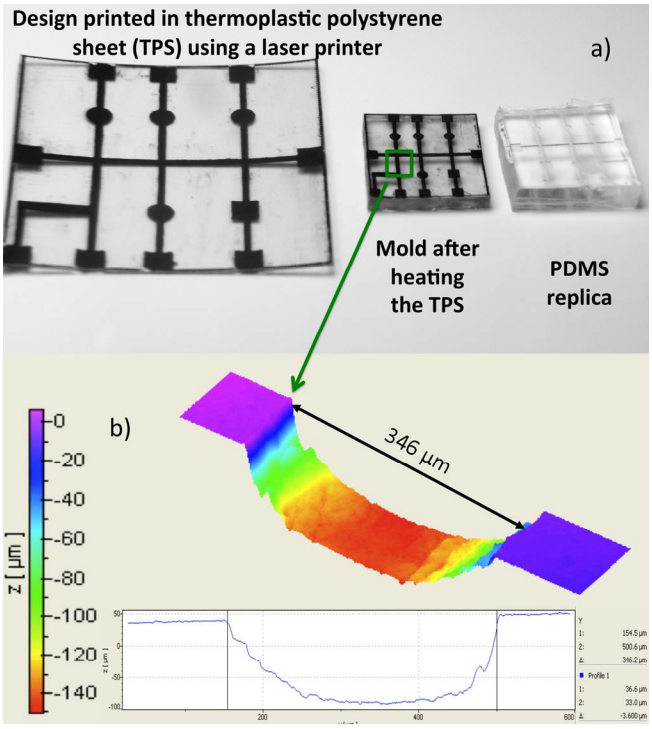

Figure 1:(a) Photography of the fabrication process showing: printed design on TPS sheet, mold obtained after heating the TPS and its PDMS replica; and in (b) the results of optical profilemeter of the trenches cross-section taken by MSA-400 Polytec optical topography system are presented.

There exist previous efforts on developing an inexpensive molding method for soft lithography [4] based on a pre-stressed thermoplastic polystyrene (TPS) molds, commercially known as Shrinky Dinks. The pattern is transferred directly on to TPS sheets through a laser printer and its size shrinks when it is baked in a conventional oven. This procedure requires neither optical lithographic patterning (e.g. in SU8 resin) nor Deep Reactive Ion Etching (DRIE) Silicon etching to form bulky trenches. Furthermore, the inclusion of conductive particles into polydimethilsyloxane PDMS matrices to produce polymeric micro-structured electrodes has been reported [5]. To achieve our goal, both techniques were combined to obtain a method that is amenable to any lab since it requires no dedicated photolithography equipment.

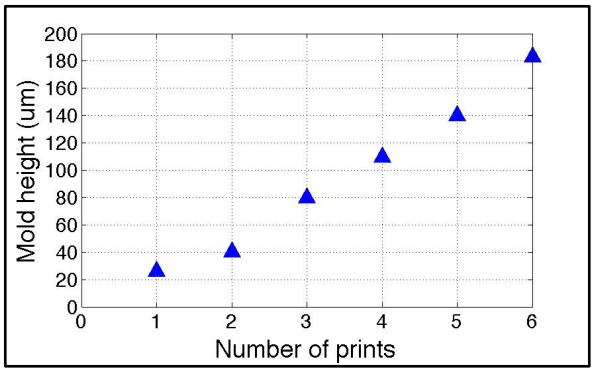

Figure 2: Mold height measurements obtained by feeding through the TPS sheet in the laser printer several times.

FABRICATION OF CELL CULTURE PLATFORM

The pattern was design in order to obtain different sizes of squared and rounded wells to trap and concentrate cells in a confined area, obtaining three dimensional cardiomyocyte arrays. The master pattern was produced using CAD software and printed directly onto the polystyrene sheet using a standard Hewlett-Packard LaserJet 1320 printer, with 1200 dpi resolution, which leaves a layer of 5-7µm height. The printer resolution mainly affects the height of the imprint and as described in [4], these sheets can be fed through the printer several times for additional channel height.

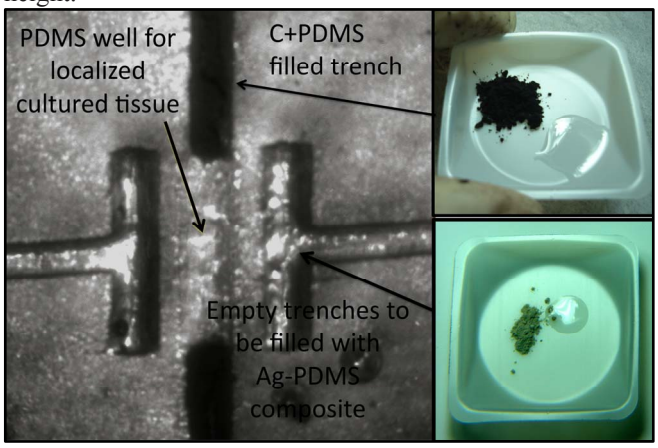

Figure 3: Mixtures of C+PDMS and Ag-PDMS were prepared to be filled into previously defined trenches. Notice how the design defines an area where the tissue culture will be located.

To shrink the design, the printed TPS sheet was placed on a toaster oven at 160° Celsius during 3-5 min, reducing the size by 60-65% along the plane and increasing the ink height by 450-500%. It has been found that the shrinkage/expansion ratios depend on the original feature size and its proximity with other structures. From the same design, repeatable features over 100µm and 30-160µm in depth have been achieved by this method. Once the mold is cleaned, PDMS (10:1 Sylgard 184 kit) is poured over the master and cured. Finally, the PDMS is peeled off containing a negative copy of the master pattern. The whole process from design conception to working device can be completed within minutes. Figure 1a) shows the design printed on the TPS before and after shrinking, and the PDMS replica. In Figure 1b), the profile characterization of the replica is presented. Notice that with the TPS molding method, rounded channels are easily achieved, which is desirable for replica peeling, microfluidic purposes and (more important in our case) for obtaining smooth wells where cell aggregates can be formed. Profile characterization of the mold heights increased by printing several layers of ink, with this particular printer, is presented in Figure 2. These measurements were carried out by optical metrology using the Polytec MSA-400 topography system based on white light interferometry.

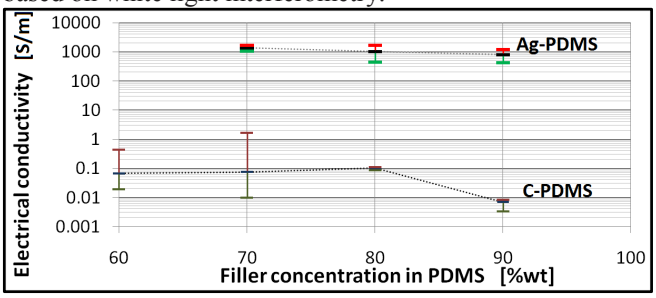

Figure 4: Electrical conductivity of C-PDMS and Ag-PDMS composites as a function of filler concentration and operating voltage. Average values and maximal dispersions are presented over a 0-30V range.

BIOCOMPATIBLE ELECTRODES BASED ON CONDUCTIVE PDMS

Electrodes were constructed (see Figure 3) by filling trenches with PDMS-based conducting composites, which were synthesized by uniform mixing of conductive 2-3µm silver (Ag) and 2-12µm carbon (C) powder (327085 and 484164 Sigma-Aldrich respectively) with PDMS, thus changing its resistivity properties. Different Ag-PDMS and C-PDMS concentrations were electrically characterized in ambient conditions with a BK 889B LCR-meter and the characteristic trend is presented in Figure 4.

Resistivity of these mixtures was measured at different operating voltages over time and discrepancies were noticed. This seems to be caused by the material expansion when the applied voltage is increased (in a mixture filled on a glass channel, this data deviation was not presented). Although electrical conductivity levels at different filling concentrations are consistent with the literature for the two composites [6-7], characterization conducted over a 30V range clearly indicates a dependence on the full voltage range and results were less stable for C-PDMS composites. This phenomenon has already been observed and reported for carbon-based PDMS composites, but it was only significant at lower voltages [8]. Moreover, it was noticed that current passing through the structures under constant high voltages decrease with time before eventual

stabilization. This effect is probably due to filler powder percolation inside the PDMS matrix.

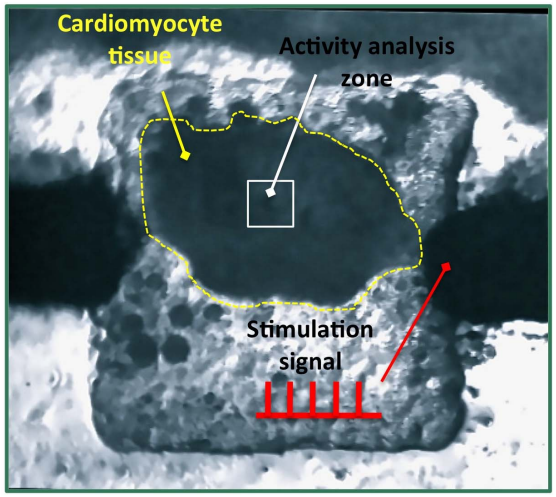

Figure 5: Optical micrograph of the cardiac cell aggregate formed in between the electrodes terminals. Survival of the cells can be visually corroborated most of the time given the spontaneous activity of these cells. Otherwise electrical stimulation is used.

Ag-PDMS electrodes present a stabilization phase around ten times shorter than C-PDMS, a much lower voltage-dependency on electrical conductivity, and required less powder as the optimized concentration corresponds to 70% in weight, so those were the ones used in further. Higher concentrations led to a more rigid paste unable to be filled into the micro-patterned trenches.

CELL CULTURE AND STIMULATION

To carry out the cell culture, cardiomyocytes isolated from chick embryos with seven-days in ovo were used. Cells were dissociated through an enzymatic process and cultured on the top of the polymeric micro-platform inside a sterilized and temperature controlled incubator. Modified Eagle's Medium (MEM) supplemented with fetal bovine serum and 1% of streptomycin was used to maintain the cells. After 36-48 hours a 3D cell aggregate was successfully formed inside wells and between the electrodes, and it was straightforward to stimulate them non-invasively (Figure 5).

Once the cellular structure is obtained, it is possible to apply different stimulation protocols. By now, we have been using trains of electric pulses at different frequencies because we are interested in synchronization and coupling processes.

RESULTS AND DISCUSSION

We report here two significant experimental results. Video of spontaneous contractions of the tissue as activity evidence was recorded through phase contrast microscopy using a Pulnix monochromatic progressive scan CCD camera and an inverted microscope. Image acquisition is made by means of NI-PCI 1409 board and LabView v8.0 interface. Sample results of image processing (utilizing ImageJ software) are plotted in Figure 6. Notice how the irregular basal spontaneous activity of the cell aggregate becomes regular and periodic after being driven by electrical stimulation of 1.5 Hz, although the cells do not follow the pulse frequency.

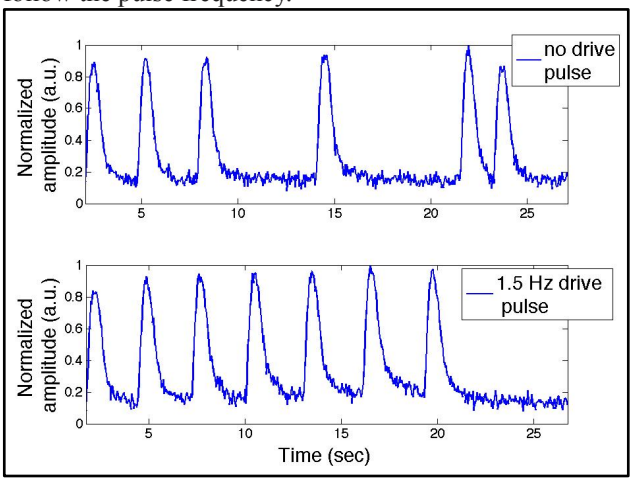

Figure 6: Comparison of a recorded bio-mechanical activity of (top) contractile spontaneous (bottom) stimulated by 1.5 Hz electrical pulse. Observe how irregular spontaneous activity becomes regular during the electrical stimulation delivered by the embedded electrodes.

Results from a different aggregate are shown in Figure7. Observe that the first 10 seconds correspond to spontaneous activity (with no stimuli), being an unconditioned response with an alternating pattern. Once 2Hz pulse train stimulation is applied, there is a transient behavior that lasts 18 seconds until a more regular, synchronized and slower beating occurs. This result is very interesting given its similarity to conditions observed in more complex cardiac models. One such example is an alternate tachycardia, a known condition in clinical environment, associated with the risk of a dangerous arrhythmia and has been demonstrated that possible "healing" of this condition can be done with electrical stimulation.

Comparable results to that in Figures 6 and 7 have been obtained in our laboratory using tiny Pt wired electrodes placed around the cardiomyocytes by means of a micromanipulator, which is very tricky to maintain in place without damaging the cells. Furthermore, cultures were developed directly on petri dishes where tissue is generated in a random way along the dish. By contrast, the procedure presented here has various important advantages, including having wells in the micro-scale size to facilitate the confinement of cells that will be aggregated, helping the tissue to remain inside the well. Another advantage is to have a robust and safe method to study electrical perturbations and resynchronization protocols in a well

controlled, non-invasive and inexpensive environment. This is useful not only for cell cultures but for tissue slices or monolayers of cardiac tissue that we have tried as well.

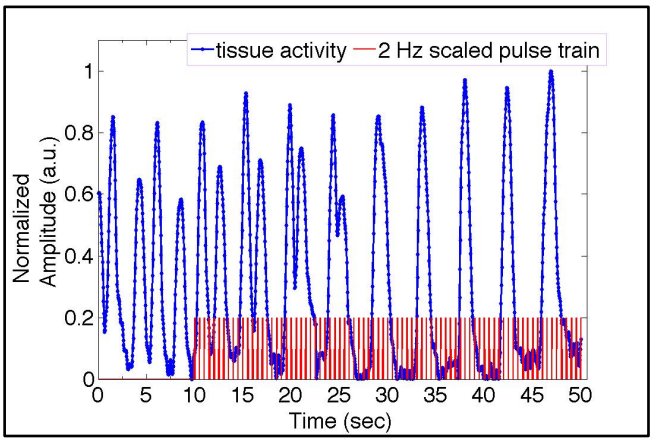

Figure 7: Spontaneous alternate contractile activity from embryonic cardiac cells. The lower trace marks the start and duration of electrical stimulation with the embedded electrodes. Notice the cells aggregate initially exhibits alternant activity with cycles of two variable amplitudes and period. After a short transient, the behavior regularizes into a slower rhythm than the pulse train since the cells cannot follow the applied stimulation frequency.

We are interested in exploring others possible applications for these methods. Action potentials (Vm) and intracellular calcium fluctuations in cardiac myocytes can be measured optically using voltage- and/ or calcium-sensitive dyes, which respond by instantaneous change in fluorescence. The experimental chamber needed to maintain or grow the cells may have different arrays of embedded electrodes, wells or induced geometries, and should allow the cells to be super-fused with normal saline solution or with added drugs solutions of pharmacological interest. Different protein substrate can be used to study adherence, effect on metabolism, phenotype expression or contractility.

CONCLUSIONS

A low cost platform for cell culturing and stimulation of cardiomyocyte cells aggregate was designed, fabricated and characterized. The design includes wells of different size and shapes to help the cells to aggregate in confined areas. Thus, 3D tissue was easily bio-engineered in localized zones. The device fabrication was achieved by soft lithography, combining a non-photolithographic method to create TPS molds and replicas with conductive biocompatible electrodes. As mentioned earlier, the complete platform can be prototyped within minutes. This procedure allow us to successfully cultured cell aggregates, as well as electrically and non-invasively stimulated cardiomyocyte tissue in the biocompatible device, without the need of sophisticated facilities.

Given the ubiquity of cellular electrical phenomena, such a procedure that allows inclusion of biocompatible electrodes will be useful in a variety of applications involving electrical field stimulation of cells. Cell culture and tissue engineering methods benefit from the use of versatile inexpensive and micro-patterned materials that can be modified easily.

Micro-scale engineering approaches represent a powerful tool for controlling the cellular microenvironment. Biocompatible micro-devices can be developed by accessible MEMS-based methods presented in this report, and expanded capabilities as sensors and control device technologies can be incorporated in this low cost biocompatible platform.

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