HL-1 Cardiomyocyte Mechanistic Responses to Micro-Patterned Culture Environment

Kuangyi Zhao, Carina J. Lee, William J. Agnew, Sioun Lee, and William C. Tang, *Senior Member, IEEE*
University of California, Irvine
E-mail: wctang@uci.edu

***Abstract*—The work presented in this paper is aimed at manipulating HL-1 tumor-cell derived cardiomyocyte's orientation for their contractile force measurement. The cells' ability to rhythmically contract autonomously once reaching confluence *in vitro* offers the opportunity to observe how the contractility is influenced by external stimuli. However, different from cardiomyocyte in human hearts, these cells are oriented randomly *in vitro*. One of the key components of this research is to align the cells on polymer substrates predictably and repeatedly to allow for systematic quantification of their contractility. Micron-sized grooves and ridges are fabricated photolithographically on SU-8 and replicated using soft lithography onto biocompatible polymer surfaces. We will report on the quantitative efficiency of HL-1 cardiomyocyte alignment with various micro-patterned surfaces.**

BACKGROUND

In 2012, there were 17.5 million death resulting from cardiovascular diseases (CVDs), accounting for 31% of global mortality rate [1]. The goal to reduce CVD mortality rate by 20% in year 2020 [2] calls for new drugs, resulting in a great demand for improved drug discovery with a faster and more reliable platform for early-stage drug testing. To study cardiotoxicity, the idea of using cardiomyocyte biomechanical response to drugs as an *in vitro* surrogate was developed. To replicate the *in vivo* anisotropic morphology on a micromechanical platform, however, requires guided cell orientation since cells are distributed randomly *in vitro*. Studies have been done to test the contractile force of aligned neonatal cardiac cells on pegs and grooves on PDMS micro-patterns [3]. Our group is using HL-1 cell line [4] and manipulating its alignment using micron-sized ridges and grooves with different spacing and width. Recent microscopic results have shown some cell alignment behavior on the lines, but further studies need to be done.

CURRENT RESULTS

HL-1 cardiomyocyte was incubated in cell culture flasks pre-coated with gelatin/fibronectin (FN). Claycomb Medium was used for the HL-1 culture supplemented with 10% Fetal Bovine Serum, 100 U/ml: 100 µg/ml Penicillin/Streptomycin, 0.1mM Norepinephrine, and 2mM L-Glutamine. The HL-1 cells reached a confluent state four days after they were passaged 1:2, and synchronized autonomous cell beating was observed from the third day post-passage (Fig. 1).

Micron-sized ridges and grooves on SU-8 mold were fabricated using standard photolithographic technique. Based on the measured size of HL-1 cardiomyocyte at around 15µm in diameter [5], photomasks were designed to have dark lines of 5µm, 10µm, 15µm, 20µm, 30µm and 50µm in width, resulting in grooves with these widths on the SU-8 mold, which replicated on PDMS as ridges. Conversely, the transparent areas on the mask resulted in different ridge separations of the PDMS replicates with the same widths range. Five PDMS substrates were used in the current experiments, with ridges fixed at 5µm width separated by 5µm, 10µm, 15µm, 20µm and 30µm grooves (Fig. 2). The PDMS replicates were cut into 1cm^2 squares after demolding, with the straight-line structures within an area of 0.5cm X 0.5cm. In this experiment, the PDMS substrates were autoclaved for one hour for sterilization before being seeded. After sterilization, PDMS substrates were placed into the 24-well plates. The stamps and wells were coated with FN for at least one hour before cells were seeded on them.

Cell orientation would be analyzed with ImageJ and Matlab®. The method to measure random orientation of cells was shown in Fig. 3 and the orientation profiles generated by Matlab® was shown in Fig. 4. After cells reached confluent state, the flask was passaged into two half. Half of the cells were seeded into another flasks for incubation, and the other half was seeded into 24-wells plates onto the five patterned substrates and one flat PDMS stamp as control (each pattern has two stamps), with 1ml supplemented Claycomb Medium in each well and was changed daily. Under our microscopic observation, the HL-1 cardiomyocytes were clustered on PDMS stamps on the third day of incubation (Fig. 5). The single cells and clusters showed some evidence of cell alignment, but nuclei and actin staining needs to be done to show evidence of alignment on single cells. For future work, the PDMS surfaces need to be treated with oxygen plasma to increase their wettability for better cell migration.

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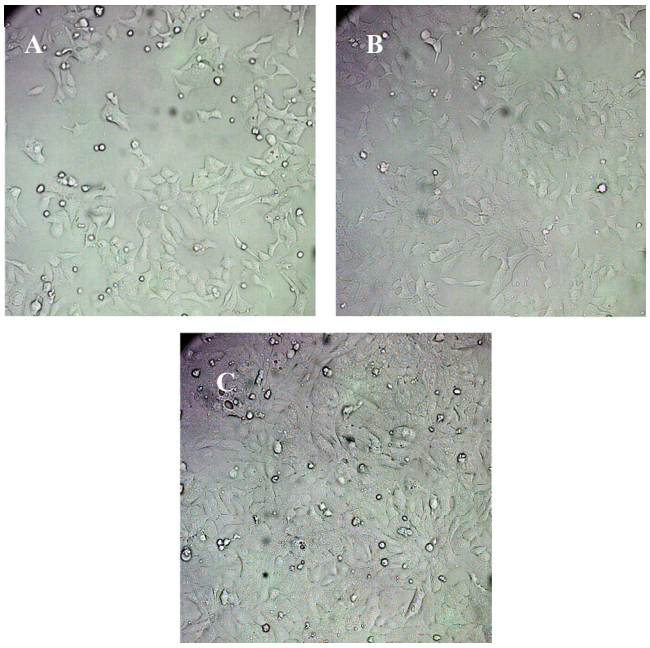

Fig. 1. Time-lapsed image of HL-1 cell growth after seeding into FN-coated flasks at (A) 24 hours, (B) 48 hours, and (C) 72 hours. Strong synchronized autonomous cell beating was observed after 72 hours cell growth. Magnification was 20X.

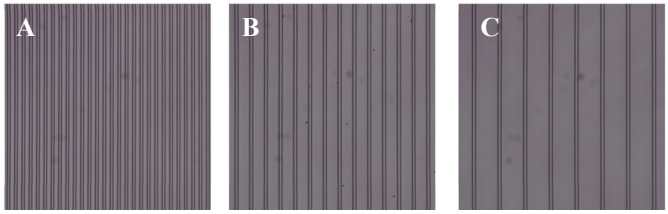

Fig. 2. Resulting PDMS patterns with 5µm-wide ridges spaced at (A) 5µm, (B) 15µm, (C) 30µm. (those spaced at 10µm and 20µm were not shown). Magnification was 40X.

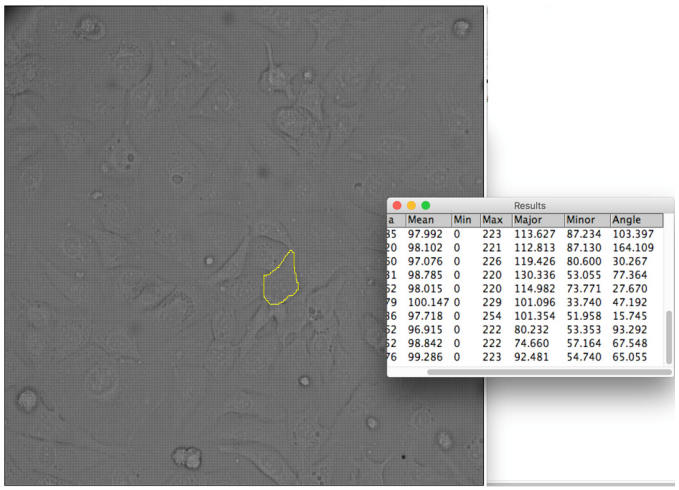

Fig. 3 ImageJ analysis of cells orientation by manually circling the cell shape and measuring the angle from the horizontal direction using the built-in ellipse-fit of random shape.

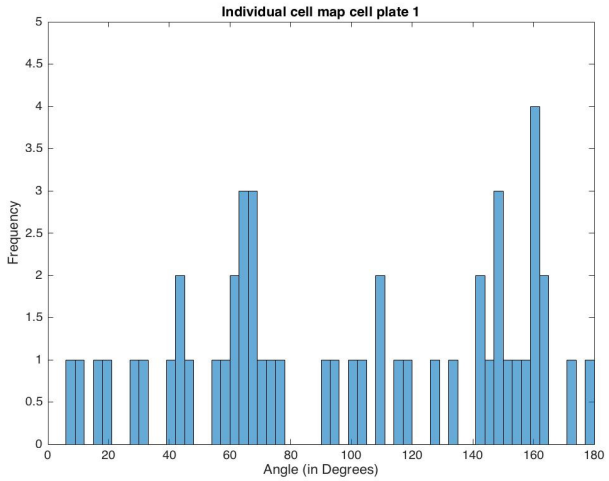

Fig. 4. Orientation bar chart generated by Matlab® using the angle column in Fig. 3. Each bar represents the cell count within 3 degrees. The total measured cell number of this figure was 50, indicating roughly random orientation.

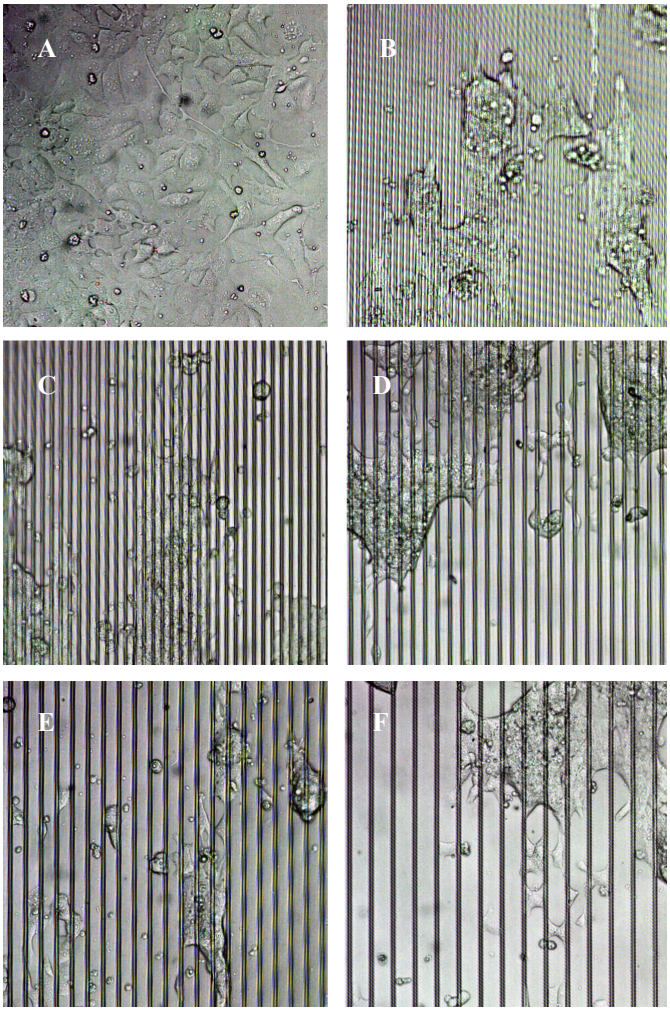

Fig. 5 Cell elongation on various PDMS substrates three days after being seeded. (A) was the blank control of PDMS stamp. PDMS patterns were 5µm ridges separated by (B) 5µm, (C) 10µm, (D) 15µm, (E) 20µm, and (F) 30µm. Clusters was obviously affected in (B) and (E), while the others remained vague.