Biomimetic Micropatterns Based on the Embryonic Heart for 2D Cardiac Tissue Engineering

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Statement of Purpose: The goal of cardiac tissue engineering is to create muscle with structural and functional properties similar to those of the native myocardium. One of the key characteristics of myocardium is uniaxial orientation of the cardiomyocytes (CMs) comprising it. The extracellular matrix (ECM) plays an important role in the formation of the aligned tissue. It has been previously shown[1] that fibronectin (FN), one of the key ECM proteins during development and wound healing, can be microcontact printed onto a 2D-substrate in a line pattern to create a confluent, aligned monolayer of CMs. We sought to improve this technique by modifying the ECM protein micropattern on the substrate to produce 2D myocardium with improved cell alignment. Further, we wanted to know how cell-cell interactions between CMs and cardiac fibroblasts influenced alignment.

Methods: Microcontact printing[2] was used to deposit FN onto poly(dimethylsiloxane)-coated glass coverslips into one of three patterns (Fig. 1A): 3 µm wide lines with 3 µm wide spacing (3x3), a biomimetic pattern, and the universally used 20 µm wide alternating lines of high and low density fibronectin that served as a control (20x20). The biomimetic pattern was derived from 3D-images of FN in chick embryonic hearts. Primary chick CMs were seeded onto the substrates. After 3 days of incubation samples were stained for nuclei, actin, and α-actinin, and imaged using confocal microscopy. Images were analyzed for actin alignment using MATLAB.

Results: We were able to successfully grow confluent cardiac monolayers using all three patterns (Fig. 1B). It was found that the orientation order parameter (OOP, ranging from 0 for an isotropic tissue to 1 for a perfectly aligned one) for CMs in a monolayer was the highest for the 3x3 pattern (0.94), the lowest for the biomimetic pattern (0.88) and intermediate for the 20x20 pattern (0.91) (Fig. 1D). However, the OOP values were close to each other for all patterns.

Next, in order to study individual cell-cell interactions, CMs were seeded at low density (Fig. 1C) and the actin alignment of isolated CMs was analyzed. It was found that, whereas cells on the line patterns have similar OOPs to those of the confluent monolayers, isolated cells on the biomimetic pattern have significantly lower OOP compared to the high cell density case (Fig. 1D).

To further investigate this observed phenomenon, CMs were seeded onto the biomimetic pattern at 4 different densities and for each cell density OOP was calculated. It was found that OOP increased with the area fraction occupied by cells (Fig. 1E). This suggests that cell-cell interactions play a significant role in the uniaxial alignment of 2D-monolayer of cardiac muscle cells on the biomimetic pattern, but not on the 3x3 and 20x20 patterns.

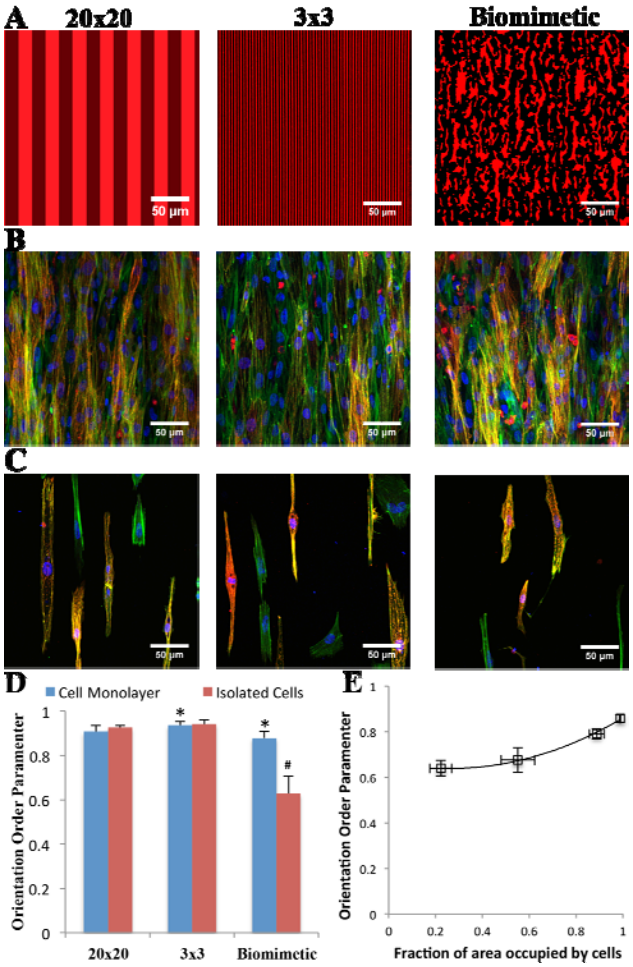

Figure 1. (A) FN patterns used. (B) Immunostained cardiac monolayers on FN patterns (blue – nuclei, green – actin, red – α-actinin). (C) Immunostained isolated cells on FN patterns. (D) OOP values for cells in a monolayer (* – $p<0.05$ compared to the 20x20 pattern) and isolated cells (# – $p<0.05$ compared to the cell monolayer for the same pattern) on different patterns. (E) OOP of CMs on the biomimetic pattern for different cell densities. Error bars represent standard deviations.

Conclusions: The currently used technique of engineering cardiac monolayers was improved in terms of cell alignment by modifying the ECM pattern. We also showed that for the FN pattern that mimics the structure of FN in embryonic myocardium, tissue alignment significantly depends on the cell density. It suggests that cell-cell interactions can play an important role in tissue formation as the cues provided by ECM proteins.

References:

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