The Electrophysiological Properties of Cardiac Fibroblast in a Surface Patterned Cell Culture Model

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Statement of Purpose: In the infarct area of a heart, cardiac fibroblast is known to deposit extra collagen, which promotes fibrosis. This fibrous tissue acts as an insulator in the damaged area thereby influencing local electrical signal conduction. This is fibroblast's "passive role" in myocardium signal conduction. However, studies suggest that fibroblast may have a more "active role" in myocardium conduction. In culture it has been shown that when placed in physical contact with viable myocytes, fibroblasts can form functional gap junctions and conduct electrical signal. This coupling event is suggested to play a role in re-entrant arrhythmias that occurs at the infarct tissue - healthy tissue border zone. There is still little known about fibroblast's involvement in the electrical communication within the diseased heart. Our interest is in designing an in vitro model using cell micropatterning to study and characterize the electrophysiological properties of fibroblasts cocultured with myocytes. In this study we evaluate the hypothesis that fibroblast will conduct electrical signal between myocytes regulated by the number of fibroblast involved.

Methods:
We created a model to test fibroblasts' ability to conduct electrical signals between groups of myocytes. With photolithography we created two islands of myocytes separated by a set distance (50-600 μm). Then using the laser cell micropatterning system, developed in our lab, we positioned fibroblast between the myocyte islands to form a fibroblast bridge.

Cell culture:
Primary neonatal-day 3 rat cardiac myocytes and fibroblasts were harvested, purified and kept in Dulbecco's Modified Essential Medium supplemented with antibiotics and serum.

Surface patterning:
Polydimethyl siloxane (PDMS) stencils, created by photolithography, are used to provide the growth pattern for the myocyte islands. We created PDMS stencil patterns which consisted of 200 μm wide and 4 mm long islands separated by varying distance (50 μm to 600 μm) (fig1). This enabled us to form fibroblast bridges of various lengths (50 μm to 600 μm).

Laser technique:
A weakly focused laser beam generates functional optical forces with two components on the cell entering into the focal region: a radial component directing towards the beam axis; and an axial component in the direction of beam propagation. Consequently, the cell is drawn into the beam center and propelled along the axis. Using this technique fibroblasts were deposited into the myocyte cultures to form cell bridges.

Seeding of cells:
PDMS stencils aligned to treated glass coverslips (22mm x 22mm) were seeded (1,400 cell/mm^2) with myocytes. The culture was incubated for a 24 hour period to allowed cell adhesion. After which the unattached cells were washed away using Hank's Balance Salt Solution. The PDMS stencil was removed leaving islands of myocytes. Using laser cell micropatterning fibroblast cells were deposited between the myocyte islands to form cell bridges.

Results/Discussion: Preliminary data have shown that myocyte island contraction could be synchronized within a couple of minutes after being connected by fibroblast strands. After 24 hour incubation period the fibroblast showed normal morphology and the myocytes island synchronization via the fibroblast bridge was still observed. Studies are currently ongoing to examine this coupling event over longer distances. In addition, patch clamp and immunocytochemistry techniques are being employed to further study the electrical properties of the fibroblast-myocyte cultures.

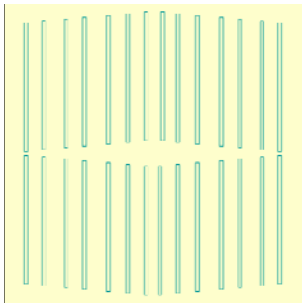

Fig 1

Conclusions: This laser cell micropatterning has the following advantages: 1) temporal characteristics of conduction through the myocyte fibroblast circuit can be obtained 2) specific patterning with high spatial resolution can easily be achieved by precisely moving the target substrate during cell deposition. Therefore, laser micropattern is an effective approach to study the electrical coupling between myocytes and fibroblasts.

References:
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