Laser Alignment of Adult Myocytes on Agarose Gel

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Statement of Purpose: In response to damage, stress and cell death cardiac muscle undergoes remodeling in which cardiomyocytes de-differentiate and re-differentiate. An understanding of the mechanisms involved in this process may lead to therapies to promote and enhance the repair of damaged cardiac tissue. In vitro studies of this process have helped to further this understanding by setting up controlled environments with greater monitoring ability. In vivo, cardiac myocytes have an aligned arrangement. To mimic in vivo physiology, we have implemented our laser cell micropatterning system (Pirlo RK. Biotechnol J. 2006 Sep;1(9):1007-13) to create an aligned row of adult myocytes on agarose gel, to study re-differentiation mechanisms and the relation to geometric configuration, alignment, and proximity.

Methods: The cells used were harvested from Adult feline cardiomyocytes. Standard enzymatic digestion using collagenase type II (155 U/mL) was employed to obtain reproducible yields of calcium-tolerant left ventricular cardiomyocytes. Buffer used (mM): 120 NaCl, 5.8 KCl, 1.5 MgSO4, 1.2 NaH2PO4, 4.3 NaHCO3, 2.5 CaCl2, 10 dextrose and 10 HEPES.

The substrate was coated with agarose gel. Cells were suspended in MEM (Minimum Essential Media, FisherScientific) at a density of 100,000/mL and loaded into a 50µL syringe coupled to a hollow optical fiber using MicroTight® fittings. Cells were injected using a MicroPump II® from World Precision Instruments.

Exploiting the phenomenon of laser guidance, whereby a weakly focused laser exerts a radial force trapping the cell horizontally, and an axial force which pushes the cell down in the direction of the beams propagation (Figure 1.), we have developed a laser micropatterning system.

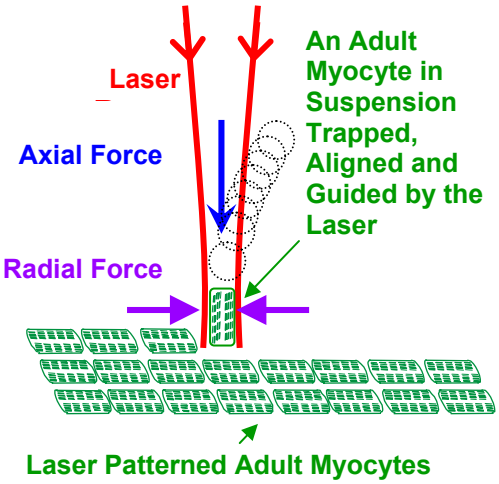

Figure 1. Conceptual illustration of laser micropatterning of aligned adult myocytes.

A Ti:Sapphire laser from Spectra-Physics tuned to produce an 800nm Gaussian beam was weekly focused using a 20X L-Plan objective. Single cells injected through the fiber were captured at the focus of the beam, manipulated to a vertical orientation and moved to the appropriate position over the cell pattern. The cell was then guided toward the substrate, deposited at the end of the previous cell, and then laid across the substrate in the direction of cell alignment.

Results/Discussion: With the laser patterning system we were able to create rows of aligned cardiomyocytes on an agarose coated glass substrate. Using a weekly focused beam it was possible to capture single cells injected into the cell deposition chamber, orient them vertically, move them to the desired place on the substrate, and then lay them horizontally in an aligned manner as shown in Figure 2. Previous attempts using magnetic or mechanical techniques to influence the alignment of cells have not have not achieved the highly adjacent end to end alignment achieved here.

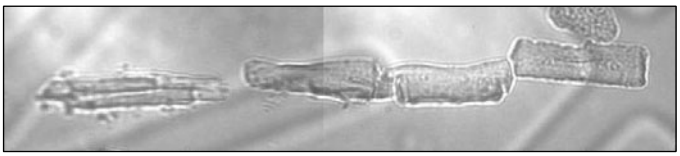

Figure 2. One row of laser aligned adult cardiomyocytes. Photo was taken immediately after cell deposition and spliced to extend the field of view for the length of the pattern.

Conclusions: The results have shown that the laser cell micropatterning system developed in our lab can effectively align adult myocytes onto a 3D agarose substrate for the systematic study of in vitro cardiac myocyte re-differentiation.