AFM Investigation of Fibroblast-Fibroblast Interactions on Collagen Substrates

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Statement of Purpose: Cardiac fibroblasts are known to regulate the ECM component during fibroblast-myocyte-ECM interaction and thus modify the mechanical property of the heart muscle. Fibroblasts have been shown to form junctions with themselves as well as with myocytes in cultures [1] but the nature of these interactions is poorly understood. Recently, the atomic force microscope (AFM) has emerged as a useful tool for the study of cell mechanics [2-4] and binding processes [5-6] at the micro-/ nano-scale. In a few recent studies [5-6], cells themselves were individually bound to AFM tips for the measurement of adhesion to modified surfaces. The goal of this study was to determine the strength of interaction and the time required for cell-cell junctions to form between two cardiac fibroblasts on collagen gels.

Methods: Thin (~100µm) collagen gels were made on glass cover slips. 100µl of a collagen solution (Invitrogen) was spread onto 1cm^2 glass cover slips. In half of the cover slips, the collagen fibers were aligned using mechanical scraping. Fibroblasts were isolated from neonatal rat hearts. The cells were seeded at low density (<40,000 cells/cm2) on bare glass cover slips and at a higher density (~80,000 cells/cm^2) for 36 hours. AFM contact mode probes (Veeco, spring constant ~ 0.6N/m) were incubated in 0.5mg/ml biotinylated bovine serum albumin for 24hrs in 37°C.The probes were put in 0.5 mg/ml streptavidin for 10min at room temperature, then incubated with biotinylated concanavilin A for 24hrs[5] and rinsed with phosphate buffered saline (PBS). In a Dimension 3100 AFM (Veeco), the modified probe was positioned over a cell on the low density glass surface using the AFM's optical microscope. The tip was brought into the cell and held under 3.5nN constant force for 30s. After successful cell-probe binding, the cell was no longer visible in the optical microscope. To further confirm proper cell-probe binding, force-displacement curve were taken with the same tip on bare glass cover slips before and after cell attachment. The collagen samples were placed in the AFM with the cell-attached probe. The probe was positioned over a cell using the optical microscope and then the two cells (the probe cell and the surface cell) were brought together. The cells were held together for a varied delay (0, 1, and 5s) at 3.5nN of force, which translates to ~3µm of displacement passed the point of cell-cell contact, and then pulled apart.

Results / Discussion: The curves before cell attachment show the usual small surface forces and no indentation (Fig. 1). The curves after cell-probe attachment are characteristic of cell nanoindentation [3] and show the typical non-elastic indentation hysteresis behavior on retraction previously reported to be found upon cell indentation [3]. This further confirms good cell-probe attachment as well as gives a good characterization of the mechanical properties of the single cell on the probe. The approach curve was fit to the Hertz model for a pyramid indenter and the modulus value (~0.3kPa) agrees well with the literature [2-3]. When two cells were brought together, the approach and initial part of the retract curves (Fig. 2) were very similar to single cell indentation (Fig. 1) indicating that the cell on the tip and the cell on the surface have similar mechanical stiffness and nonelastic properties. However, when the cells were allowed time to interact at the maximum force (Fig. 2) the adhesion force on retract increased and peaks indicative of unbinding were observed. The magnitude of this adhesion force and the number of unbinding peaks increased with delay time (Fig. 2). There was no statistical difference in the force of interaction between cells on the aligned collagen gels as opposed to the unaligned gels. Collagen alignment at the nanoscale has been known to affect myocyte properties but it seemed to have little effect on the fibroblast-fibroblast adherent junction. This may be possibly because the mechanical scraping method produced only moderately aligned films as determined from AFM imaging. Also, fibroblasts have been shown to exert significant forces on their surrounding matrix. After 36 hours of culture, this might be enough to completely disrupt the alignment of the collagen gel around the cell.

Conclusions: Fibroblast-fibroblast adhesive interactions begin to form very quickly (within a couple of seconds) after physical contact is made. Given this time scale, these interactions are probably due to proteins on the cell surface that are already present at the start of the experiment. Longer time course studies are underway using a closed fluid cell to observe changes in interactions as junction proteins are synthesized. Unlike previous studies on myocyte interactions, there were no observable differences between cardiac fibroblast interactions on aligned vs. unaligned collagen. However, further studies on well defined patterned substrates need to be undertaken to determine whether collagen alignment does not affect fibroblast interactions.

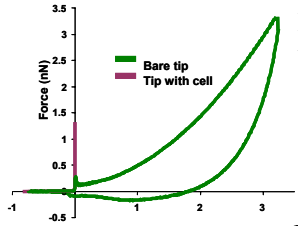

Fig. 1 Force curves of the tip before (purple curve) and after (green curve) a cell is attached on bare glass in PBS.

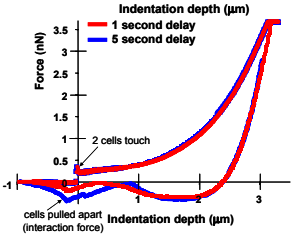

Fig. 2 Force of two fibroblasts cells being pushed together, held at constant (3.5nN) load for 1 second (red curve) or 5 seconds (blue curve), and then pulled apart.

References: Rook+ AJP-Cell Physiology, 32:C959-977, 1992; Lieber+ AJP-Heart Circ., 287(2), 2004; Mahaffy+, J. Biophys. 86(3), 2004; Costa+, J. Biomech. Eng., 121: 462-471, 1999; Li+, Biophys. J. 84(2), 2003; Zhang+, AJP- Heart Circ., 28 6 :359-367, 2004