Title:

A Piezoelectric Straining Device and Cellular Mechanics during Acute Oscillatory Loading of a Tissue-Engineered Micro-Construct

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Introduction:

Although we do not fully understand the role that the extracellular environment has on cell response, we do know that it has profound effects on regulating cellular phenotype. For this reason, the use of three-dimensional tissue engineered constructs in biomedical research will likely increase in the coming years. In previous work, we developed a passive 'microtissue' model of airway smooth muscle (ASM).[1] In this model 100 to 200 cells in a collagen matrix self-assemble around a pair of cantilevers into a 3D arrangement consistent with intact tissue. Apart from its physiological relevance, the microtissue model also allows for high-throughput experiments, direct measurement of contractile force, and *in situ* live-cell staining for protein expression, making it an extremely compelling system to probe cellular mechanics.

In contrast to this previous work, we now present a novel active microtissue model with piezoelectric cantilevers that are both actuators and force sensors. This model permits the study of acute and chronic dynamic mechanical strain, a known stimulus for cell response, and contractile function. After characterizing the actuator/sensor performance and assessing cell survival within the device, we have investigated the hypothesis that a large acute strain will lead to decreased tissue stiffness and is able to reverse cellular contractile force through fluidization of the cytoskeleton.

Materials and Methods:

A mathematical model of actuator dynamics with tissue loading was used to optimize device design. Standard microfabrication techniques were used to manufacture arrays of miniature piezoelectric bimorph actuators/sensors out of lead zirconate titanate (PZT). The actuators were coated with parylene C to act as a barrier for electrical insulation and biocompatibility. Tissues consisting of ASM cells and 3T3 fibroblasts at a ratio of 80:20 in a collagen matrix were fabricated within the device to assess tissue formation. An *in stitu* dapi/propidium iodide stain was used to assess cell survival at the end of three days. At this time, the tissues received acute, oscillatory strains. A video analysis program, designed in Labview, was developed to track the peak deflection of the actuators. Using the mathematical model for the actuator dynamics with tissue loading, and by comparing peak deflections with and without the presence of the tissue, the stiffness of the tissues were determined at strains ranging from +/- 0.5% to 5% when at baseline and after 20 minute incubations with 80mM potassium chloride (KCl), 0.1mM forskolin, and 10μM cytochalasin D. A linear regression analysis was used to examine the relationship between strain and stiffness with an analysis of covariance to examine for differences between the pharmacological conditions. For all statistical comparisons, $p<0.05$ was taken to be significant.

Results:

An array of bimorph actuators with dimensions of 2mmx400umx70um (lxwxt) was fabricated and gave an average peak deflection of 12.39± 0.49 um when driven with a ±20 volt sinusoidal wave at breathing frequency (0.3 Hz). In a similar manner to the passive 'microtissue' model, we confirmed that live tissues formed around the tops of the actuators with an even distribution of cells and negligible cell mortality. We found that tissue stiffness significantly decreased as the amplitude of mechanical strain increased (n=4). When <1% strain was applied, addition of the contractile agonist KCl (41.9 ± 3.4 uN/um) did marginally increase tissue stiffness compared to the baseline (33.90 ± 3.0 uN/um) while forskolin (28.9 ± 9.1 uN/um) and cytochalasin D (27.7 ± 9.6 uN/um) led to a decreased stiffness.

Discussion:

We have developed a novel platform for the study of cellular biomechanics in tissue-engineered constructs in a micro-array format. The system has the ability to apply continuous oscillatory strain. It also enables the ability to measure changes in stiffness and visualize the structural organization within the tissues in response to the presence of dynamic mechanical strain.

We found that acute mechanical strain decreased the tissue stiffness. This finding is consistent with previously published results on individual ASM cells and *ex-vivo* airway tissue strips, and has been attributed to the fluidization of the cytoskeleton.[2] Fluidization in response to strain may explain the similar responses under the different pharmaceutical conditions: at high strains the contractile apparatus is fluidized, and therefore, cannot generate force or contribute to stiffness. These findings directly reflect the ability of a deep inspiration to reverse ASM contraction and expand the airways in healthy subjects.

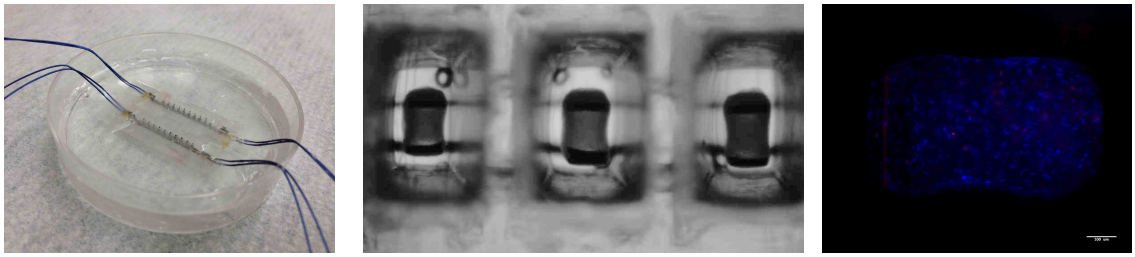

Figure 1: A photograph of the device (left). The bimorph actuators/sensors exist in a micro-array format within PDMS wells. A tissue forms across a pair of actuators and is stretched by the bending of the bimorphs (middle). A representative image of a fluorescently stained microtissue (right). There was negligible cell death (red) compared to the number of cells within the tissue (blue).

References:

1. West AR, Zaman N, Cole DJ, Walker MJ, Legant WR, Boudou T, Chen CS, Favreau JT, Gaudette GR, Cowley EA, Maksym GN. Development and Characterisation of a 3D Microtissue Culture Model of Airway Smooth Muscle. *Am J Physiol Lung Cell Molecular Physiol.* (2013); 304(1):L4-16.
2. Maksym, G.N., et al., *Beneficial and harmful effects of oscillatory mechanical strain on airway smooth muscle.* Can J Physiol Pharmacol, 2005. 83(10): p. 913-22.