EXTRACELLULAR MATRIX (ECM) MICROSTRUCTURE DETERMINES ECM-CELL STRAIN TRANSFER IN 3D TISSUE CONSTRUCTS

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INTRODUCTION

Micro-biomechanical signals between the extracellular matrix (ECM) and cells regulate cellular processes and ultimately define tissue form and function. Although mechanical loads experienced by tissues have been shown to affect fundamental cellular behavior, little is known regarding the mechanisms by which loads applied to tissues ultimately affect cellular function. This mechanotransduction pathway can be broken down into several steps. First, applied loads are transmitted through tissue microstructure from the macro- to the micro-level. At the micro-level, mechanical loads are transferred from the ECM to resident cells. Previous studies by the present investigators have demonstrated that the macro-level stress-strain behavior [1] and the micro-level 3D strain response of an ECM [2,3] are dependent upon ECM microstructure. In the present paper, the mode by which specific properties of ECM microstructure alter ECM-cell strain transfer is defined. Tissue constructs (TCs), consisting of fibroblasts cultured in 3D collagen ECMs with varied collagen fibril density, were mechanically loaded in tension. Strains in individual cells and the surrounding ECM were measured and compared. Such fundamental knowledge regarding cell-ECM biomechanics will assist in the establishment of design criteria to be used in the next generation of tissue engineered devices.

MATERIALS AND METHODS

Tissue Construct Preparation

TCs were prepared by neutralizing a sterilized acid solution of type I collagen (Sigma Chemical Co.) to desired concentrations with 10X phosphate buffered saline. Collagen concentration was varied (1.0 and 3.0 mg/ml) to create micro-structurally altered collagen ECMs, as described previously [1]. Just prior to polymerization, Swiss mouse 3T3 fibroblasts (American Type Culture Collection) were added at 4.0×10^4 /ml. The neutralized collagen solution with cells was polymerized in a "dog-bone" shape mold in a humidified environment at 37°C. Following polymerization, TCs were cultured in Dulbecco's modified Eagle's medium with 1.5 g/L $NaHCO_3$, 10% calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine at 37°C in a humidified environment of 5% CO_2 in air for 24 hours prior to micromechanical testing.

Micromechanical Test Procedure

Immediately prior to micromechanical testing, TCs were labeled with $DiOC_6(3)$ to facilitate discrimination of cells from the ECM. A combined confocal-mechanical experimental setup [2] was used to acquire 3D images of collagen ECM microstructure (using reflection microscopy) and cells living within the collagen ECM (using fluorescence microscopy), as specimens were incrementally loaded in tension (1.0 mm/min). The confocal-mechanical apparatus consisted of a Minimat 2000 miniature materials tester (Rheometric Scientific) combined with a BioRAD MRC 1024 Confocal. TCs were maintained in complete media at 37°C during mechanical testing. Confocal images were acquired at applied strain levels (ε_{app}) of 0.00, 0.02, 0.04, 0.06, 0.08 and 0.10. Images tracked a specific volume of approximately $150\times150\times10$ μm^3 representing a single cell and its ECM. Later, 3D images were collapsed to 2D projections for analysis.

Strain Analysis

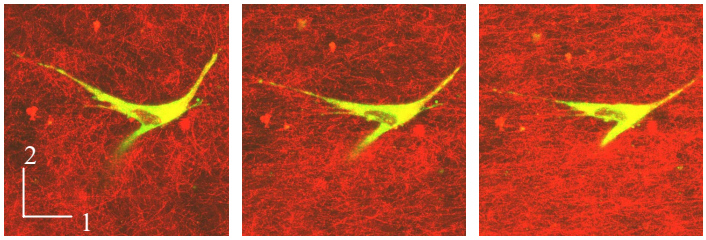
Incremental digital image correlation was then used to measure 2D tensorial strains in the microstructure of the collagen ECM from reflection images. ECM strains (ε_{ij}) were determined based on the deformation gradients (du_i/dx^o_j) using the full definition of the Lagrangian strain tensor (Equation 1).

$$\varepsilon_{ij} = \frac{1}{2}\left[\frac{du_i}{dx^o_j} + \frac{du_j}{dx^o_i} + \frac{du_m}{dx^o_i}\frac{du_m}{dx^o_j}\right] \quad (1)$$

At each applied strain level, cell shape was determined from fluorescence images by tracing software written in MATLAB (The Mathworks). Areas determined by tracings facilitated measurements of cell length, width, orientation, and area. Cellular deformations (changes in length, etc.) were non-dimensionalized as strains using a simplified form of Equation 1. Strains were measured and compared for cells in five TCs at collagen concentrations of both 1.0 & 3.0 mg/ml.

RESULTS AND DISCUSSION

TCs created with a collagen concentration of 3 mg/ml were characterized by a significantly higher fibril density and increased mechanical integrity (failure stress, linear modulus) compared to those created at a concentration of 1 mg/ml [1]. As mechanical loads were applied to all constructs, deformations were noted in both cells and the ECM (Figure 1). As noted previously [2], the ECM responded with collagen fibrils orienting in the loading direction.



$\varepsilon_{app} = 0.00$ $\varepsilon_{app} = 0.06$ $\varepsilon_{app} = 0.10$

Figure 1. Both the ECM (red) and cells (yellow) were deformed in response to applied mechanical loads. Tensile loads were applied in the 1-direction.

To determine the ECM-cell strain transfer properties of TCs, strains were transformed to a coordinate system relevant to the cell's orientation. As such, a local (x-y) coordinate system was defined with the x-axis was along the long axis of the cell (Figure 2). In the global (1-2) coordinate system, the 1-direction is defined as the direction of applied load.

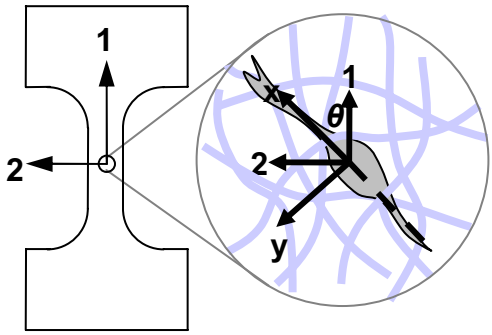


Figure 2. Definition of the local cell based coordinate system (x-y). The x-axis is along the length of the cell. The angle θ describes the orientation of the cell in relation to the global (1-2) coordinate system.

ECM strains were transformed to the x-y coordinate system based on the cell orientation (θ). Equation 2 provides the strain transformation equation used to determine the ECM strain in direction of the cell (ε_{xx}).

$$\varepsilon_{xx} = \left(\frac{\varepsilon_{11} + \varepsilon_{22}}{2}\right) + \left(\frac{\varepsilon_{11} - \varepsilon_{22}}{2}\right)\cos 2\theta + \varepsilon_{12} \sin 2\theta \qquad (2)$$

ECM microstructure dictated the strain transfer properties between the ECM and cells. Results showed that TCs with higher collagen fibril density transferred less strain to cells. At equivalent ECM strains, there is a larger amount of cell length strain for cells in 1.0 mg/ml collagen ECMs when compared to 3.0 mg/ml collagen ECMs (Figure 3). In comparing linear least-squares fits of the experimental data using the equation:

$$\varepsilon_L = m\varepsilon_{xx}\,, \qquad (3)$$

the slope of the least-squares line (m) decreased from 1.836±0.637 to 0.438±0.547 when collagen concentration increased from 1.0 to 3.0 mg/ml (p<0.05). A slope equal to unity denotes perfect strain transfer. Interestingly, when collagen ECMs are loaded in tension, significant contractions result in the transverse (2-direction) [1,2,3]. As noted in Figure 3, many cells, specifically those oriented in the transverse direction, were exposed to compressive ECM strains.

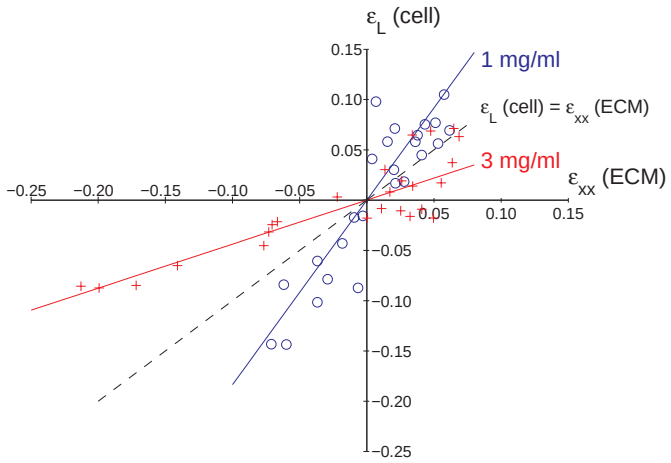


Figure 3. Cell length strains (ε_L) compared to strains measured in the ECM along the long axis of the cell (ε_{xx}).

CONCLUSIONS

Previously it was shown that ECM microstructure determines the stress-strain behavior of the ECM [1], the macro-micro level strain transfer within an ECM [3], and the micro-level strain response of the ECM [3]. For the first time it has been demonstrated that ECM microstructure determines the level of strain transferred from the ECM to resident cells. Specifically, ECMs with increased collagen fibril density transfer less strain to cells or in effect "shield" cells from ECM strains due to applied mechanical loads.

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