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Single Cell-Mediated Collagen Reorganization in 3D Matrices

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Abstract—**Cells use cytoskeletally-generated force to adhere, migrate and remodel their environment. While cellular forces generated by cells plated on 2D substrates is well-studied, much less is known about the forces generated by cells in 3D matrices, which more closely mimic the *in vivo* environment. Here, an approach to characterize cellular forces in 3D using confocal reflectance microscopy is presented. Remodeling of collagen fibrils due to the forces exerted by embedded cells was imaged in real-time as cells adhere to and contract the matrix. We implemented this approach in conjunction with 2D Traction Force Microscopy to compare cytoskeletally-mediated forces of cells in 3D collagen matrices to forces exerted by cells on 2D collagen-coated hydrogel substrates. Our results indicate that confocal reflectance microscopy of collagen fibrils can provide semi-quantitative information regarding cellular force in 3D matrices, and that the actin cytoskeleton plays a similar role in regulating cell contractility in both 2D and 3D microenvironments.**

I. Introduction

CELLULAR force generation is becoming increasingly appreciated as a critical regulator of the healthy and diseased state. Notably, changes in cellular force generation have been linked to the progression of disease [1]. For instance, it has been shown that malignant cells exhibit altered force magnitude and distribution compared to non-malignant cells [2-3]. Additionally, it has been demonstrated that vascular smooth muscle cells on matrices mimicking the changes in mechanical properties occurring during intimal hyperplasia alter their polarity and migratory behavior [4]. In our own lab, we have shown that endothelial cell forces and polarity are altered on matrices mimicking the stiffness of tumor tissue [5-7]. These results demonstrate that force generation may be linked to disease progression.

The majority of studies investigating cellular force generation have been performed on cells plated on 2D, planar substrates or arrays of force-sensing microneedles [8-10]. Using these techniques, cells adhere only to the matrix on their basal-surface. While these studies have provided valuable information regarding the mechanism by which cells generate force against their extracellular environment in both health and disease, a greater understanding of cellular force generation in 3D matrices is required to translate these findings into more physiologically-relevant environments such as those which occur *in vivo*.

Here, we demonstrate the use of confocal reflectance microscopy to image cell-mediated collagen reorganization surrounding cells embedded in 3D matrices. As cells exert force against the collagen, the individual fibers are reorganized permitting direct visualization of the strains created by the cells [11]. We use this to compare force generation of cells in 3D to cells on 2D substrates measured using Traction Force Microscopy.

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II. Materials and Methods

A. Cell Culture

MDA-MB-231, highly metastatic breast cancer epithelial cells, were cultured in minimum essential medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin (Invitrogen).

B. Collagen Gel Fabrication

Rat tail type I collagen was isolated and reconstituted in 0.1% acetic acid at 10 mg/mL. After neutralization with 1 N NaOH, the appropriate volume of MDA-MB-231 cell suspension was gently mixed into the collagen solution.

C. Microscopy and Traction Force Microscopy

Traction Force Microscopy (TFM) of cells plated on collagen-coated polyacrylamide gels was performed as previously described using the LIBTRC software package developed by Micah Dembo at Boston University [12-14].

Confocal Microscopy was performed on a Zeiss 710 confocal microscope, where collagen is imaged using 488 nm laser light illuminated through a 80/20 dichroic mirror, reflected off of the collagen fibrils and detected through a 488 nm emission filter.

III. Results

A. Imaging of single cell contraction

While a number of methods are available to measure forces exerted by cells on 2D substrates, less is known about force generation of cells embedded in 3D matrices. To investigate the forces exerted by cells in 3D matrices, MDA-MB-231 cells were embedded in collagen matrices and the collagen fibril architecture was imaged using confocal reflectance microscopy (Figure 1A). As cells exert force against the collagen matrix, the fibrils are rearranged, and

this reorganization can be visualized in real-time using time-lapse confocal reflectance microscopy.

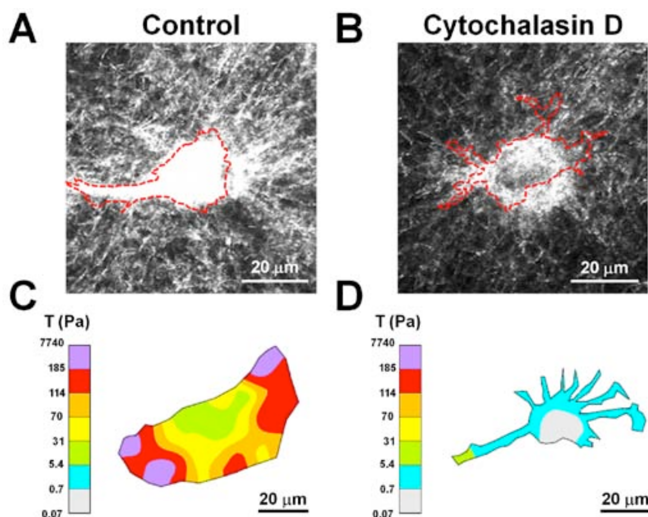

Figure 1. A. Confocal reflectance image of an untreated MDAMB231 cell in 1.5 mg/mL type I collagen. B. Confocal reflectance image of an MDAMB231 cell treated with 5 uM cytochalasin D in 1.5 mg/mL type I collagen. C. TFM map of control MDAMB231 cell on 5 kPa PA gel. D. TFM map of MDAMB231 cell treated with 5 uM cytochalasin D on 5 kPa PA gel.

B. Single cell-mediated collagen organization is inhibited by disruption of the cytoskeleton.

To further investigate single cell-mediated collagen reorganization as a metric of cell force generation, we investigated the effects of cytochalasin, an actin disrupting agent, on the ability of single cells to reorganize collagen fibrils. Upon addition of cytochalasin, collagen remodeling is greatly inhibited (Figure 1B). To compare the effect of cytochalasin on force generation by cells in 2D substrates to cells in 3D matrices, Traction Force Microscopy was used (Figure 1 C, D). Note that cellular force generation in 2D was also disrupted. These results indicate that the actin cytoskeleton plays a critical role in cellular force generation in cell embedded in 3D matrices and plated on 2D substrates.

IV. Discussion

While methods to investigate cellular traction generation in 2D are established, few methods exist to investigate cellular force in 3D [10]. Recently, the first study describing the quantification of 3D forces was published [15]. While this study is landmark because it is the first to quantify forces of cells in 3D matrices, the method is limited in its ability to fully recapitulate the *in vivo* environment. It requires the use of a synthetic matrix which is homogenous and well-characterized [16] and is significantly different in their properties as compared to physiological matrices. Therefore, it is difficult to translate these findings to cellular force generation in vivo. Here, we describe steps to understanding cellular forces in physiological matrices and the role of the cytoskeleton in mediating this force generation.

Our data indicate that parallels exist between force generation of cells in 2D and 3D matrices. There is recent evidence to suggest the nature of focal adhesions and their role in cell migration differ from 2D to 3D environments [17]. Continued investigation of the parallels and differences between 2D and 3D behaviors is necessary to determine the cellular elements that contribute to cellular mechanosensing in physiological environments.

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