Nanomechanical Properties of Structured Biopolymer Networks

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Abstract:

The process by which nanoscale motor proteins operate in time and space to generate the force needed to complete cell division is not well understood. The current study aimed to better understand the forces that are generated during cell division on the length scale of whole cells. This top-down approach can give insight into the forces that are generated by the nanoscale motor proteins during cell division. Sea urchin cells were encapsulated in a hydrogel to be characterized using three dimensional traction force microscopy (3D TFM). The forces that a cell exerts can be quantified based on the deformations in the hydrogel. To identify the optimal matrix for 3D TFM, several hydrogels were tested with cell viability studies and magnetic tweezers-based microrheology. Of three potential hydrogels, a collagen-based one was found to be the most promising.

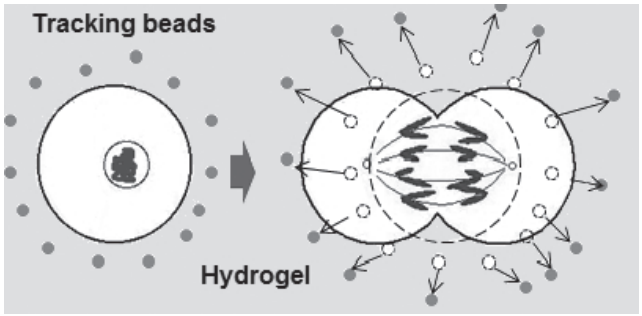

Figure 1: Model of how 3D TFM is used to relate displacements to forces

Introduction:

The interactions between cells and their environment have become recognized as important in many biological processes, including cell proliferation and differentiation [1, 2]. This study aims to apply a modified form of traction force microscopy (TFM) in order to understand the effect of mechanical confinement on cell division. By tracking the displacements of fluorescent beads in the gel, forces that are generated during cell division can be determined (Figure 1) [3]. Sea urchin embryos were used as a model system due to the predictable timing of first division, large size (~ 50 μm), and availability at the laboratory. Hydrogels, the primary transducer of force in TFM, were characterized to determine compatibility for use in TFM.

Experimental Procedure:

Cell Viability Tests. Peptide-based, hyaluronan-based, and collagen-based hydrogels were selected as potential candidates for use in TFM. A Live/Dead sperm viability kit (Invitrogen™) was used to test for sea urchin embryo viability in each gel. To initiate spawning, KCl was injected into the sea urchins. Release of gold fluid signifies eggs, while a white fluid signifies sperm. Each fluid is collected and combined in artificial seawater (ASW) to initiate fertilization. The fertilization envelope is removed by passing embryos through a 53 μm mesh. Approximately 50 μL of the embryos are added to each sample of gel, prepared at concentrations of 25% gel in ASW. Propidium iodide from the Live/Dead kit was added to the cells as directed. Each sample was imaged under a scanning laser confocal microscope and cells with a permeabilized membrane (and thus dead) fluoresced red. Cells were also visually inspected for division within the expected period of 90 minutes.

Mechanical Tests. Gels were prepared at concentrations of 25%, 33%, 50% and 75% gel in ASW. Approximately 0.1 μL of magnetic beads was mixed into each gel. The gels were then flowed into glass cover slide flow-cells. Samples were tested under magnetic tweezers to characterize viscoelasticity. Known forces were applied to the gels by a permanent magnet in the vertical direction. The displacements of the magnetic beads were tracked based on the changing diffraction patterns around the magnetic beads.

Results and Conclusions:

Confocal images of the propidium iodide-stained sea urchin cells were taken and compared to a control population of cells in ASW. The peptide gel showed large amounts of red staining, indicating no cells remained viable within the gel. This was likely due to the very low pH of this gel (~ 2.0). The hyaluronan gel showed no red staining, but images showed no cell division, even after 120 minutes, indicating that the cells, while alive, were not healthy in this gel. The collagen gel performed best: no red staining was observed and cells also divided normally (Figure 2). Thus, mechanical testing was performed on the collagen gels only.

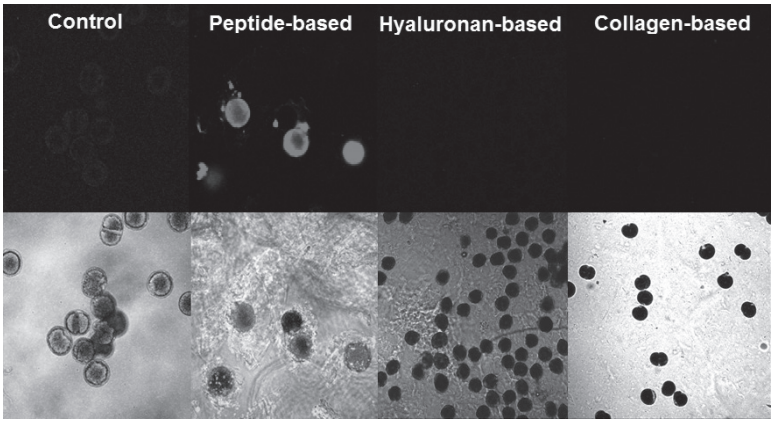

Figure 2: Cell viability data for three different hydrogels.

Data from the magnetic tweezers were used to characterize the collagen gel elasticity. A plot of the displacement versus the force indicated that at short times, the displacements of the magnetic beads are directly proportional to the applied force, as expected for a linear elastic material. This linearity also holds over a range of gel concentrations (Figure 3). By taking the inverse slope of this plot, the stiffness of each gel concentration can be determined in pN/nm (Figure 4). The stiffness increases with increased gel concentration, indicating that the gel stiffness is tunable. This is expected, because as the concentration of gel increases, the polymer content increases, allowing more cross-linking to occur. By varying the concentrations from 25% to 75% gel in ASW, the stiffness increased by a factor of ~ seven.

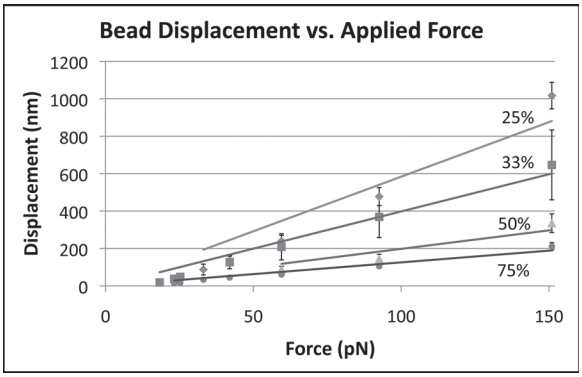

Figure 3: Results of magnetic tweezer studies for different collagen hydrogel concentrations.

Future Work:

The next step in this project is to implement the collagen gel in TFM. Fluorescent beads will be embedded in the gel along with the cells. Stacks of images will be taken with a confocal microscope and assembled into 3D images as the cells divide. A tracking algorithm will monitor the displacements of thousands of beads in the gel. In addition, further work can be performed on other cells such as neural and cancer cells.

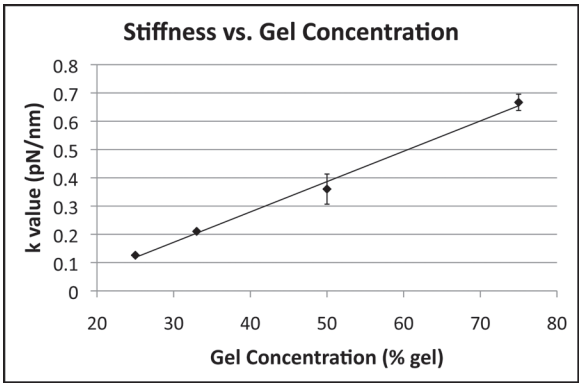

Figure 4: Tunable stiffness of collagen hydrogel over a range of gel concentration.

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