Designing Nano-Engineered Substrates to Probe Cell Organization, Motion and Traction Forces

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Abstract:

The mechanical forces involved in interactions between cancer cells and their environment are poorly understood and may present new diagnostic and treatment opportunities. The present study aimed to develop a technique to measure the traction forces cancerous cells exert on their environment. We developed deformable gels with specific and adjustable stiffnesses. The cell traction forces were quantified by the gel deformation. This study has led to the successful creation of gels and the development of a technique to characterize the gel elastic spring constant, and a method for measuring cell response to different mechanical environments. Preliminary results show the gels have an elastic response, characterized by a reasonably consistent spring constant. Additionally, results demonstrate a correlation between the bone cancer cell attachment and substrate stiffness.

Introduction:

Mechanical forces play an essential role in a variety of cell processes and are often altered in diseased tissue [1]. Cancerous tissues are often diagnosed by their abnormal stiffness, indicating cancer cells may have abnormal mechanical properties on a cellular level. This study aimed to create a technique to increase the understanding of cancer cell mechanics by measuring the forces that cells exert on their substrates, and studying the effects of different mechanical environments on cells. This device will measure cancer cell traction forces during mitosis, which is important for cancer proliferation, and during cell locomotion, which is necessary for metastasis [2].

The technique used a deformable polyacrylamide gel with embedded fluorescent beads. Gel deformation was related to the applied force, and was detected by bead displacement (Figure 1). Specifically, this study focused on measuring the mechanical properties of the gel and characterizing the cancer cells' responses to different gel stiffnesses.

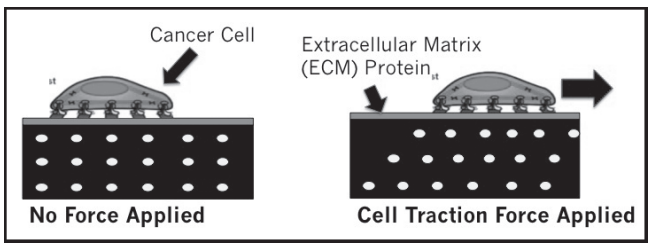

Figure 1: Schematic of substrate for cell traction force microscopy.

Experimental Procedure:

The polyacrylamide gel solutions were made using a previously described procedure [3]. Ten microliters of the gel solution were sandwiched between a hydrophobic glass slide and a silanized cover glass. After the gel polymerized, the glass slide was removed and the gel was ready for use.

For gel stiffness characterization, a 230 pascal modulus gel was made according to standard protocols [3]. Acryloyl-X conjugated biotinylated bovine serum albumin was added to the gel solution to promote attachment of streptavadin-conjugated magnetic beads to the gel surface. The magnetic beads were added to the polymerized gels and magnetic forces ranging from 0.0 to 60 piconewtons were applied to the beads. The bead displacement was calculated from the diffraction patterns of the beads, observed via microscopy.

U2OS (bone cancer cells), H460 (lung cancer cells) and HeLa (cervical cancer cells) were plated onto tissue culture treated plastic, and the fraction of cells attached was measured at multiple times. Cell morphologies were analyzed after twenty hours of incubation. Next, gels with 230 pascal, 2.8 kilopascal and 16.3 kilopascal moduli were made. Acryloyl-X conjugated fibronectin was added to the gel solution at 10% volume fraction to promote cell attachment. After gel polymerization, an ultraviolet germicidal lamp was employed to sterilize the gels. U2OS cells were plated onto the gels, tissue culture treated plastic, and untreated coverglasses. After twenty hours, cell morphology was analyzed.

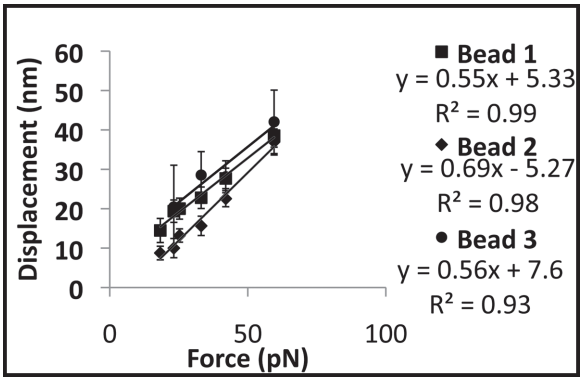

Figure 2: Results of gel modulus characterization.

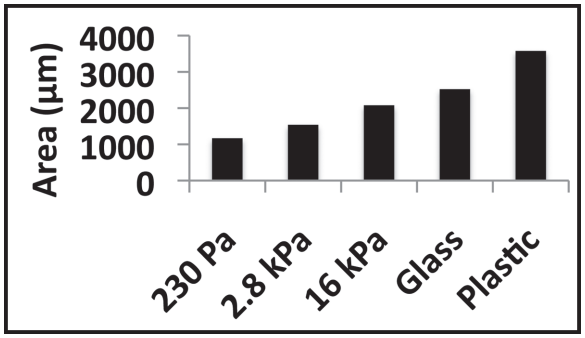

Figure 4: Correlation between mean U2OS cell area and substrate stiffness.

Results and Conclusions:

The data from the magnetic bead displacement tests were used to characterize the gel moduli. A linear regression was used to fit a plot of mean magnetic bead displacement versus force (Figure 2). Preliminary results show a linear relationship between force and displacement, signifying an elastic response from the gel. The spring constant of the gel was given by the inverse of the slope and was calculated to be 1.68 ± 0.20 piconewton/nanometer.

Assuming constant bead contact area for each trial, this result indicates that gel modulus was fairly consistent and uniform from gel to gel (13% variation). It is critical that the gel moduli are consistent and predictable, to ensure the accuracy of the cell traction force measurements.

As cells attach, they begin to spread and become more irregularly shaped. We used the mean area and roundness ($4 \cdot \text{area}/(\pi \cdot \text{major\_axis}^2)$) of the cells to quantify the degree of attachment to gels. We chose to use U2OS cells to plate onto gels because results showed that U2OS cells had the greatest area and the lowest roundness of the three cell types, which would allow us to see a greater range of results.

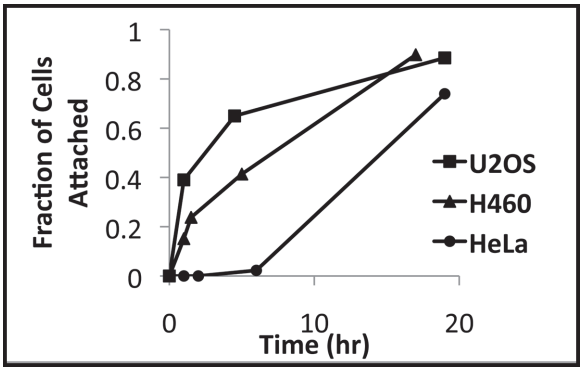

Figure 3: Results of cell attachment assay for three cell types.

Additionally, the U2OS cells had the highest rate of cell attachment (Figure 3). In tests of the effects of substrate stiffness on U2OS cells, we saw a strong positive correlation between gel stiffness and cell area (Figure 3). There was no obvious correlation between substrate stiffness and cell roundness (Figure 4).

Future Work:

Next steps include continuing gel modulus characterization by measuring the gel deformation field using the displacement of embedded beads. Additionally, future work includes determining the cell response to various mechanical environments using H460, HeLa, and other cancer cell lines. Cell traction force measurements have recently begun. These measurements will focus on the effects of substrate stiffness on mitosis and cell motility, and potentially increasing the understanding of the role of cell mechanics in cancer growth and metastasis.

Acknowledgements:

Thank you to the National Nanotechnology Infrastructure Network Research Experience for Undergraduates Program and National Science Foundation for funding my research. Special thanks to Dr. d T. Valentine, Ryan Meschewski, Jun Lin, Angela Berenstein, other Valentine lab members, and our collaborators.

References:

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[2] Silletti et al. Tumor Cell Motility and Metastasis: Autocrine Motility Factor as an Example of Ecto/Exoenzyme Cytokines. Pathology and Oncology Research (1997) 3(3), 230-254.

[3] Aratyn-Schaus et al. Preparation of compliant matrices for modulating and quantifying cellular contraction. Publication pending.