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Cell migration studies at the interface of a dual chemical-mechanical gradient

Yuzhen Yang
Lehigh University

Yang, Yuzhen

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Cell Migration Studies at the Interface of a Dual Chemical-Mechanical Gradient

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Yuzhen Yang

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CERTIFICATE OF APPROVAL

This master thesis, submitted by Yuzhen Yang, is accepted in partial fulfillment of the requirement for the degree of Master of Science in Chemical Engineering.

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4/23/07
Research Advisor

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Faculty Advisor

Chairman of Department

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ABSTRACT

Cell migration is crucial in numerous physiological, histological and pathological processes, such as wound healing, response to inflammation and cancer metastasis. Researchers have shown that cell movement can be guided by a purely chemical gradient, which is also called chemotaxis. In recent years, accumulating evidence indicates that cell movement is regulated not only by chemical factors but also by mechanical parameters. Moreover, in living organisms, cell migration is directed by complex chemical and mechanical stimuli. Thus, understanding the cellular response to a combination of chemical and mechanical stimuli will be critical for *in vivo* applications. In this study, the main purpose is to understand whether a chemical or mechanical stimulus plays the major role in directing cell migration. A polyacrylamide (PAAM) hydrogel that exhibits chemical and mechanical gradients in opposite directions was synthesized. The chemical gradient was created by varying the collagen concentration and the mechanical gradient was introduced by changing the concentration of a bis-acrylamide cross-linker. The Young's modulus of the bulk PAAM hydrogel for different concentrations of bis-acrylamide was measured. Our results indicate that cell motility is guided by chemotaxis.

Chapter 1

INTRODUCTION

Cell migration is essential for many biological and physiological processes, including embryonic development, wound healing, angiogenesis, and the metastasis of tumor cells. In embryogenesis, cellular migration is considered a crucial factor for gastrulation and the development of the nervous system [1]. Leukocytes migrate into the infected area and mediate phagocytic during the inflammatory response [2]. Migration of fibroblasts and vascular endothelial cells play an essential role in the process of wound healing [3, 4]. In metastasis, tumor cells migrate from the initial position into the circulatory system and then migrate into other sites [5]. Finally, cell migration is crucial to many technological applications such as tissue engineering and implants [6]. Thus, understanding the underlying mechanisms that direct cell migration is very important in obtaining a comprehensive overview of cellular processes.

Earlier studies indicate that cells can sense their environment and adherent cells *in vivo* can show an active migration in a certain direction and at a particular speed only after stimulation [7, 8]. There are many kinds of stimuli that can affect the cell locomotion, such as phototaxis [9], galvanotaxis [1] and geotaxis [10]. Saranak et al [9] showed that the rhodopsins, which are the visual pigments in humans and other multicellular animals, can guide the fungus to move in the direction of light intensity, suggesting that the phototaxis influences migration. Erickson and coworkers [1] have discovered a remarkable example of galvanotaxis. They found that electric fields could influence the direction of migration, orientation, and shape of fibroblast cells. When the strength of the electric field lies between 1 and 10mV/mm, fibroblast cells migrated towards the cathode end of the field by extending lamellipodia in the direction of cathode end, indicating that the galvanotaxis has a pronounced effect on cell migration. Cellular elongation occurred when the strength of the field was increased to 400mV/mm or higher. Experimental measurements on detecting the average cell swimming direction under variable gravity have been studied by Noever et al [11]. The results showed that, for *Tetrahymena*, most cells moved

randomly under a low-gravity field while directed upward orientation was found more frequently in a high gravity field. This phenomenon indicates that gravity has an obvious effect on biological and cellular functions including cell migration.

Another major stimulus that can direct the cell migration is chemotaxis [12, 13, 14, 15]. Many cells are able to sense the presence of extracellular chemical signals and then guide their migration in the direction of the chemical concentration gradient. This process, called chemotaxis, has a role in various functions such as finding food in prokaryotes, forming multicellular structures in protozoans, tracing bacterial infections by leukocytes and embryogenesis in metazoans [16, 17, 18, 19]. For example, Ramsey and coworkers [16] discovered that in the presence of a uniform chemoattractant concentration, leukocytes move randomly over a long time scale. In contrast, leukocytes exhibit a continuous migration in the direction of a chemical concentration gradient, suggesting that chemotaxis evidently presents a directed signal to cell migration. Knapp and coworkers [12] found using the RGD (Arginine-Glycine-Aspartic acid) peptide sequence found in fibronectin useful in directing cell migration. A biphasic dependence on chemical concentration with an optimal concentration of about 10 nM was observed. Time-lapse video microscopy showed that the orientation of cell migration was up the chemical concentration gradient. Jeon et al [20] designed a microfabricated device that consisted of a network of microfluidic channels that can generate spatially and temporally controlled stable gradients of chemical concentration. When they placed human neutrophils within the microchannels, the cells exhibited strong directional migration toward increasing concentrations of interleukin-8 in linear gradients. Another example is the ability of developing axons to be guided to their final position by chemoattractive and chemorepulsive ligands [18]. These reports are some of the examples that clearly demonstrate that chemotaxis plays a very important role in regulating the cell orientation and cell migration.

Although most investigations have focused on chemical factors, in recent years, more and more evidence indicates that cells are able to orient and migrate in response to physical parameters such as substrate rigidity, mechanical stress and topographic features [7, 21, 22].

The cellular response to a mechanical gradient is called mechanotaxis or durotaxis. For instance, an *in vitro* study on testing the cellular response to mechanotaxis was done by putting fibroblast cells onto collagen-coated polyacrylamide substrata with a mechanical gradient, and the results showed that fibroblast cells can be guided by a purely mechanical gradient [7]. When cells move towards the high Young's modulus side of the substratum, the cells would extend their lamellipodia and move directly onto the rigid side of the substrate. In contrast, when cells migrate towards the low Young's modulus side, the cells would move up and down the mechanical interface but not cross over to the other side [7]. Wang et al [23] have shown that deformation of flexible hydrogel substrata also affects the spreading and retraction of lamellipodia which then regulates cell migration. Their conclusions were that both leading edge adhesions and trailing end adhesions contribute to transmit the mechanical forces of fibroblast cells to the flexible substrate. They reported that adhesion on the leading end contribute to force transduction while adhesions on the trailing end contribute to a force resistance when the cell moves. During the detachment process, they observed that the leading edge adhesions cannot be transferred while trailing end adhesions can distribute forces exerted by the moving cell. Therefore, the combination of mechanical interactions at the leading and trailing edges can direct cell migration. Similarly, Reinhart-King et al [24] studied mechanical force generation by endothelial cells on a RGD (Arginine-Glycine-Aspartic acid) -peptide-derivatized hydrogel Their studies showed that increasing the RGD concentration would enhance the cell spreading on the surface of the hydrogel substrata and cell areas increased linearly with the increasing peptide density. Additionally, Reinhart-King et al also found that mechanical forces exerted by endothelial cells are obvious at the ends of moving cells and are almost negligible under the center of cells which indicates that endothelial cells may use their lamellipodia to exert the mechanical force and propel cell migration continuously.

Other studies also suggest that cell migration can be guided by a purely mechanical gradient. For example, it has been demonstrated that during fibril switching, neutrophils cells can probe the tension in a three-dimensional extracellular matrix and preferentially migrate along the fibrils with highest tensile and rigidity. Cells sought the fibers under

maximum tension and migrated along the most stable fibers. Thus increased adhesion complexes would favor the migration of the cells along rigid, high-tension matrix fibrils. Stabilization of the orientation of the cell migration can also be achieved by increasing the rigidity of the fibrils [25]. Studies conducted by Wong et al [4] demonstrate that vascular smooth muscle cells (VSMCs) can detect and respond to mechanotaxis and preferentially adhere on surfaces that exhibit higher rigidity. However, cell migration was random when cells were cultured on a homogeneous surface. Moreover, experimental observations showed that the VSMC cells preferentially accumulate in the region of highest substrate stiffness when the VSMCs were cultured on radial-gradient-compliant substrata. Thus, mechanical interactions between a cell and its underlying substratum play a crucial role in modulating cell motility.

In order to clearly understand the wound healing process, the migration of cultured fibroblasts has been studied for many years [26, 27]. Fibroblast migration is a combination of several processes such as protrusion, adhesion, translocation, and detachment [6]. Fibroblasts have been shown to respond to physical signals such as substrate adhesivity, stiffness or tension [7, 28]. Wang and coworkers [7] found that when manually inducing the substratum deformation in the direction of pulling away the flexible substratum from the nucleus of cells, spreading of lamellipodia would occur for the fibroblast cells. In contrast, when pushing the flexible substrate towards the nucleus of cells, the fibroblasts started retracting. In another study, highly elongated cells were found both inside the microgrooved substrates and along the edge of substrata, one explanation for this phenomenon is that cells were looking for maximal mechanical stimulation. [29]. Because the detection of physical characteristics such as rigidity cannot be achieved through purely chemical means, the cell must invoke a contractile mechanism that probes the environment.

Lo et al [30] showed that myosin IIB might be involved in directing the cell migration from several ways. Their study pointed out that cells may be able to sense the mechanical signals by varying concentrations of myosin IIB. The fact that myosin IIB was found preferentially located on the most rigid fibers in the central region of the cell suggested that myosin IIB

may have the function in stabilizing the direction of cell migration. The phenomenon that the retraction of secondary protrusions was controlled by myosin IIB indicated that myosin IIB may contribute to the regulation cell shape during the cell movement.

Therefore, the mechanisms of cell migration are still unclear because cell migration *in vivo* is affected not only by chemotaxis but also by mechanotaxis and/or other stimuli. The measurement of cell movement based upon a purely chemical gradient or purely mechanical gradient is not representative of cell motility *in vivo*. Thus, understanding the cellular response to a combination of chemical and mechanical stimuli will offer a more comprehensive understanding of the cell migration.

The objective of this study is to determine whether cell migration is guided by chemotaxis or mechanotaxis. We chose polyacrylamide (PAAM) to create the mechanical gradient substrate for several reasons. First, their mechanical properties can be easily tuned over a wide ranage of elastic module [31]. Second, the surface of PAAM hydrogels is inert unless modified chemically. These hydrogels can be linked covalently to proteins and peptides to render them biocompatible. Third, they are optical transparent, enabling their use in microscopy [32]. Finally, the porosity of the hydrogel provides a more physiological environment in comparison to glass substrata or silicone membranes.

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Chapter 2

Materials and Methods

2.1 Materials

Glutaraldehyde (50% v/v solution, Electron Microscopy Sciences, Hatfield, PA), 3- APTES (3-Triethoxysilylpropylamine, Sigma-Aldrich Chemical Co., St. Louis, MO), acrylamide solution (40% v/v solution, Bio-Rad, Hercules, CA), TEMED (N,N,N`,N`-tetramethylethylenediamine, 0.1 g/ml, Bio-Rad), bis-acrylamide solution (2% v/v solution, Bio-Rad), ammonium persulfate (AP, 0.1 g/ml, Bio-Rad) were used as received.

2.2 Activation of Glass Cover Slips

Glass cover slips were activated to ensure a durable bond between the PAAM hydrogel and the glass surface. Glass cover slips (25mm in diameter, Fisher Scientific, Pittsburgh, PA) were passed briefly through an oxidizing flame to remove hydrocarbon residues. The cover slips were cooled, and then coated with a thin layer of 0.1N NaOH solution. Upon drying, a thin layer of 3- APTES was coated on the cover slips. The cover slips were immersed in an 8% glutaraldehyde solution. The cover slips were rinsed several times in DI (deionized)-water at room temperature and stored at 4°C for 24 hours until use.

2.3 Synthesis of Opposing Chemical and Mechanical gradient PAAM Substrata

In order to create an interface with a large difference in mechanical properties, we prepared two monomer solutions and varied the crosslinker concentration. Both solutions contained 0.8%v/v acrylamide. The concentration of the bis-acrylamide crosslinker was maintained at 0.2%v/v in one solution and 0.56%v/v in the other solution. DI water was added to maintain the volume of both monomer solutions at 10mL. 0.15%v/v of TEMED was added to both monomer solutions. 0.5%v/v of fluorescent rhodamine beads (0.5 μm Fluospheres®, carboxylate- modified, rhodamine conjugated microspheres, Molecular Probes Inc., Eugene, OR) were added to the monomer solution that contained 0.56%v/v bis-acrylamide. The bulk PAAM hydrogels formed using these volumes were used in Young's modulus measurements. PAAM hydrogels used in cell migration experiments were prepared from

monomer solutions where the total volume was scaled down from 10mL to 2.5mL while maintaining the ratio of all the individual components, with the exception of the fluorescent beads. In order to create an interface with sharp differences in mechanical and chemical properties, 20%v/v of FITC-collagen beads (Diameter: 2μm, Molecular Probes Inc.) were added into 100μl monomer solution that contained 0.2%v/v bis-acrylamide and 1%v/v of FITC-collagen beads (Diameter: 2μm, Molecular Probes Inc.) were added into 100μl monomer solution that contained 0.56%v/v bis-acrylamide. For the monomer solution that contained 0.2%v/v bis-acrylamide, 4%v/v of ammonium persulfate (0.1 g/ml, AP, Bio-Rad) was added as the initiator. For the monomer solution that contained 0.56%v/v bis-acrylamide, only 2%v/v of ammonium persulfate (0.1 g/ml, AP, Bio-Rad) was added.

10 μl drops of each solution were placed next to each other on an activated glass cover slip. In order to ensure a flat hydrogel surface, an 18mm cover slip was placed on top. The 18mm cover slip was coated with Rain-X and dried for 30 minutes before placing it on the monomer droplets. PAAM hydrogels that exhibited a sharp interface where one side of the interface was highly cross-linked with a low collagen concentration and the other side was lightly crosslinked with a high collagen concentration were obtained. After removing the top cover slip, the hydrogel was rinsed for 5 hrs in cold DI-water to remove any residual monomer and sterilized under UV light for 1 hr prior to cell culture.

2.4 Cell Culture

Balb 3T3 cells (Balb/3T3 clone A31, American Type Culture Collection, Manassas, VA) were incubated at 37°C and 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, California) supplemented with 10% bovine calf serum (Hyclone, Logan, UT) and 2% Penicillin-Streptomycin (Invitrogen). For cell motility measurements, Balb 3T3 fibroblast cells were plated on PAAM substrata at a cell density (~240000cells/cm^2). The cells used in cell motility measurements were maintained between passages 10-20. The samples were placed on an optical microscope (Nikon, Eclipse, TE2000-U, Ardmore, PA). The microscope is equipped with 10, 20 and 40X objectives, a motorized stage, and an environmental chamber maintained at 37°C and 5% CO_2. Phase-

contrast images of the cell and fluorescent images of the marker beads embedded in the substratum were analyzed using Image-Pro Plus imaging software (MediaCybernetics, Bethesda, MD).

2.5 Cell Migration Studies

Phase-contrast images of fibroblast cells and the fluorescent images of the marker beads were captured every 30 minutes up to 8hrs using time-lapse microscopy. Cells were chosen along the chemical and mechanical interface. Only cells that fit the criteria listed below were included in the analysis. Cells that died, underwent mitosis, or collided with each other were excluded. The mean-squared displacements of the centroid for each cell were calculated which was used to calculate the migration speed.

2.6 Young's Modulus Measurements

The Young's Modulus of the PAAM hydrogels was measured under different values of cross-linker concentration. The PAAM hydrogel was polymerized in a 15ml centrifuge tube to form a cylindrical sample with a diameter of ~1cm and length of ~10cm. By applying weights to the bottom of suspended PAAM sample and then measuring the changes in the length and cross-section of the sample upon a gradual increase in weight, the stress-strain relationship was obtained. The slope of the line is the Young's modulus of the bulk hydrogel.

The equation for calculating the Young's Modulus of the PAAM hydrogel is:

$$Young'sModulus = \frac{Stress}{Strain} = \frac{F/A}{\Delta L/L}$$

In the above equation, F is the weight applied to the bottom of the hydrogel; A is the across sectional area; L represents the original distance of two marked lines on the hydrogel and ΔL is the length changing of the hydrogel.

Chapter 3
RESULTS AND DISCUSSION

3.1 Synthesis of PAAM substrata

Images of polymerized PAAM substrata were taken using an optical microscope. Figure 3-1 (a) shows a sharp interface of a chemical gradient and Figure 3-1(b) shows a clear mechanical gradient interface from top to the bottom of the PAAM substratum. Figure 3-1(c) indicates that the interface of chemical gradient and mechanical gradient are at the same place. Figure 3-1(d) shows the spreading of fibroblast cells on the surface of the PAAM substrate. Rhodamine markers are indicated by red fluorescent dots and FITC-collagen by the green fluorescent dots. The Young's Modulus is ~60kPa for the region with a high concentration of rhodamine beads and ~30kPa for the region with a high concentration of FITC-collagen beads. Fibroblasts adhered to both side of the chemical/mechanical interface although cells preferentially adhered on the region that contained a high FITC-collagen concentration.

3.2 Young's Modulus testing for PAAM hydrogels

The values for Young's modulus for PAAM hydrogels synthesized with different cross-linker concentrations were obtained. These values provide information on the differences in rigidity on either side of the mechanical interface. All tests were conducted within 48h of hydrogel synthesis to prevent dehydration. Figures 3.2- 3.5 show the stress-strain curve at a cross-linker concentration of 0.04%, 0.2%, 0.56% and 0.72%v/v.

Table 3-1 contains the mean and standard deviation values of Young's Modulus for PAAM hydrogels with different cross-linker concentrations. The Young's Modulus for 0.04%, 0.2%, 0.56% and 0.72% v/v bis-acrylamide hydrogel is 8.15±0.56kPa, 30.37±0.97kPa, 55.75±2.55kPa and 58.85±1.35kPa respectively. The variance in the data is small, two major trends are apparent. There is a significant increase in Young's modulus with an increase in bis-acrylamide concentration. For example, the Young's Modulus at a bis-acrylamide concentration of 0.2%v/v is approximately four-fold higher in comparison to a bis-

concentration of 0.04%v/v and the Young's modulus for a PAAM hydrogel that contained 0.56%v/v bis-acrylamide hydrogel is approximately two-fold higher than a gel with a cross-linker concentration of 0.2%v/v. The Young's modulus values did not differ significantly for hydrogels that contained 0.56 and 0.72%v/v/ bis-acrylamide.

The Young's Modulus for 0.08%, 0.12%, 0.16%, 0.3% and 0.4% Bis-acrylamide hydrogel have also been tested. Figure 3-6 shows the plot of Young's modulus for PAAM hydrogels that have different cross-linker concentration. A linear relationship between cross-linker concentration and Young's modulus was observed up to a bis-acrylamide concentration of 0.56%. In the future, a continuous gradient in mechanical properties may be obtained by small changes in cross linker concentrations. Such hydrogels can recapitulate mechanical profiles *in vivo*.

3.3 Chemical Gradient Testing

The density of FITC-collagen beads was measured on both sides of the chemical/mechanical interface using fluorescence microscopy. For PAAM substrata that exhibit 30.37kPa/55.75kPa mechanical interface, the mean and standard deviation of the number of FITC-collagen beads was calculated and is shown in Table 3-2. Table 3-3 shows the mean and standard deviation of the number of FITC-collagen beads for the PAAM substrata that contain 55.75kPa/58.85kPa interface.

Overall, the density of FITC-collagen beads was found to be higher, in all cases, on the side of the interface with lower Young's modulus values. For example, in PAAM hydrogels that exhibit a 30.37kPa/55.75kPa mechanical gradient, the FITC-collagen bead density is approximately thirty-fold higher on the side of the interface with lower rigidity. In the case of a PAAM hydrogel that contains a 55.75kPa/58.85kPa mechanical interface, the collagen-conjugated bead density was approximately eight-fold higher on the side of the interface with low Young's modulus. In both cases, a sharp chemical gradient was observed at the same location as the mechanical gradient. In the future, measurements on collagen

concentration through radio-active isotope labeling will provide quantitative values on protein concentration.

3.4 Cell migration

The composite images of fibroblast cells and fluorescent beads were obtained using Image-Pro image software. A representative composite image is shown in Figure 3-7. Rhodamine markers are indicated by red fluorescent dots and FITC-collagen by the green fluorescent dots. The region with a high concentration of rhodamine beads has a Young's Modulus ~55.75kPa and the region without rhodamine beads has a Young's Modulus ~30.37kPa. The fibroblast cells are indicated by white dots. A sharp chemical/mechanical gradient was obtained on the surface of PAAM surfaces. These images demonstrate the presence of a dual chemical and mechanical gradient that are on either side of an interface and that fibroblasts preferentially adhere on regions enriched in FITC-collagen.

Cells that were in close proximity to the dual chemical-mechanical interface were chosen for observation and subsequent analysis. Overall, through multiple experiments, thirty eight cells were observed over a three hour time period with images taken every thirty minutes. Figure 3-8 is an example of one such cell adhered and spread close to the interface. The motion and direction of this cell was tracked. Figure 3-9 shows a series of images of this cell over 30 minute time intervals. These images show that this particular cell moved towards the high collagen region over 3 hours migration.

The mean squared displacement for the centroids of all cells cultured on a hydrogel with a 30.37kPa/55.75kPa mechanical interface was calculated and the results are shown in Table 3-4. Similar data for a hydrogel surface that contains a 55.75kPa/58.85kPa mechanical interface is shown in Table 3-5. Table 3-6 contains a summary of the displacement and direction of motion for all the cells analyzed.

The data provided in Table 3-6 indicate that more than 50% of the observed cells preferentially migrated towards the high collagen concentration side. Only 20% of cells moved towards the lower collagen concentration side, although these cells were originally adhered and spread on the low collagen concentration, and most likely are undergoing a random walk motion. . A possible explanation could be that the cells can not sense the chemical gradient when they are beyond a critical distance from the interface. Another 20% of the fibroblast cells moved towards or along the interface. A majority of these cells were adhered originally on the region with a high FITC-collagen concentration. It is of interest to note that these cells chose not to cross the interface.

Thus, all these results suggest that the chemical gradient may play the major role in directing the migration of fibroblast cells.

Chapter 4
CONCLUSIONS AND FUTURE DIRECTIONS

PAAM hydrogels that exhibited a sharp interface where one side of the interface was highly cross-linked with a low collagen concentration and the other side was lightly cross-linked with a high collagen concentration were synthesized. The chemical and mechanical interfaces were in perfect alignment. A significant increase in rigidity was observed in gels with varying bis-acrylamide concentration. An optical analysis of the chemical gradient showed that the collagen concentration was significantly higher in highly cross-linked regions of the surface. Migration data indicate that cells move preferentially towards regions of high collagen concentration. This may indicates that a chemical stimulus play a major role in directing fibroblast motility.

Although bulk testing of mechanical properties is a good predictor of the rigidity in the hydrogels, other techniques may be applied to obtain more information. Our preliminary measurements (data not shown) indicate a good correlation between the bulk test and the indentation method. Future studies will focus on direct measurements of protein concentration using radio-labeled proteins to provide quantitative values. Additionally, imaging focal adhesions for cells on either side of the interface may provide more clues to the cytoskeletal organization that promotes directed migration.

In vivo, gradients tend to be continuous. It will be interesting to determine whether the results shown in this study can be duplicated on continuous chemical and mechanical gradients.

APPENDIX A: FIGURES AND TABLES

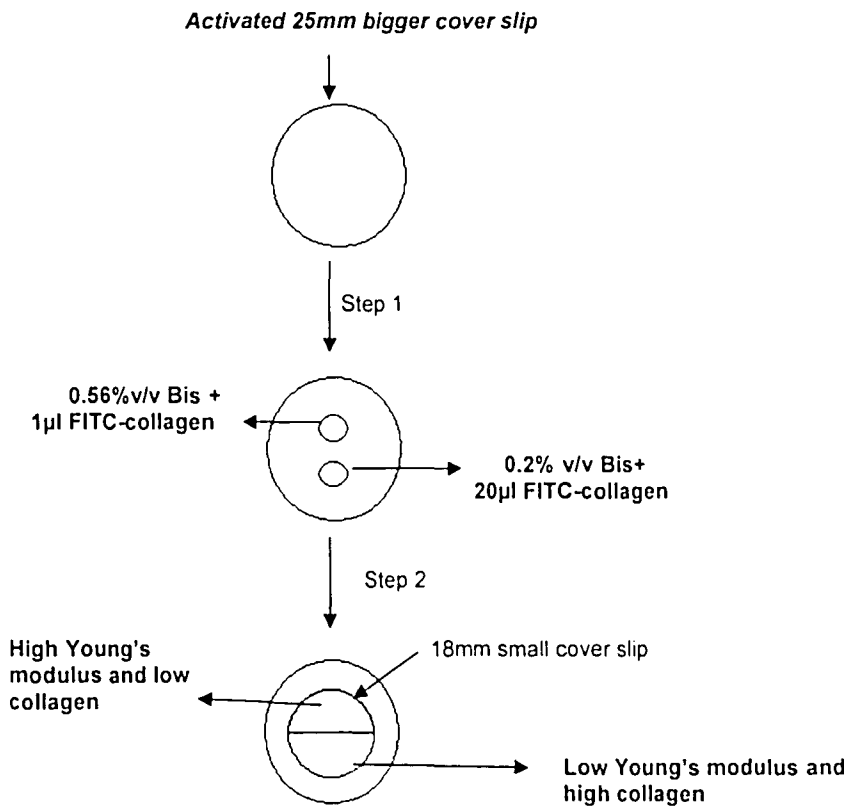


FIGURE 2-1. Schematic for synthesizing PAAM hydrogels with a sharp interface in chemical and mechanical properties.

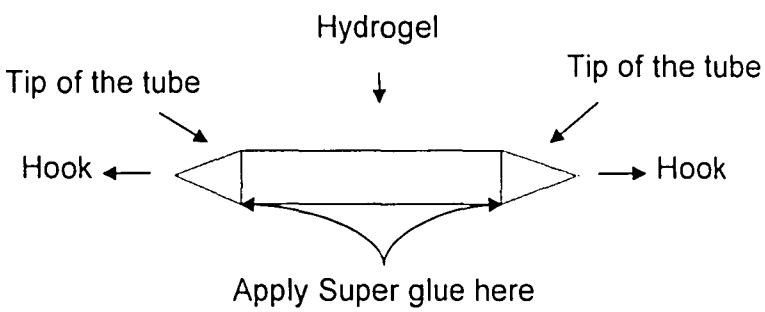


FIGURE 2-2. Schematic of sample preparation for Young's Modulus measurements.

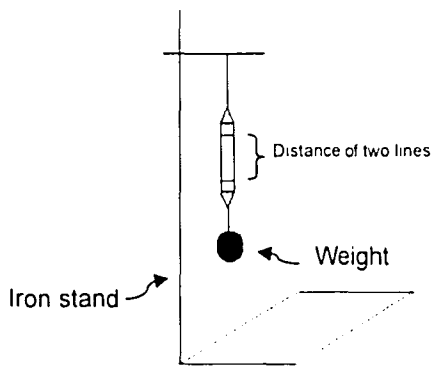


FIGURE 2-3. Schematic of measuring the Young's Modulus of PAAM hydrogels.

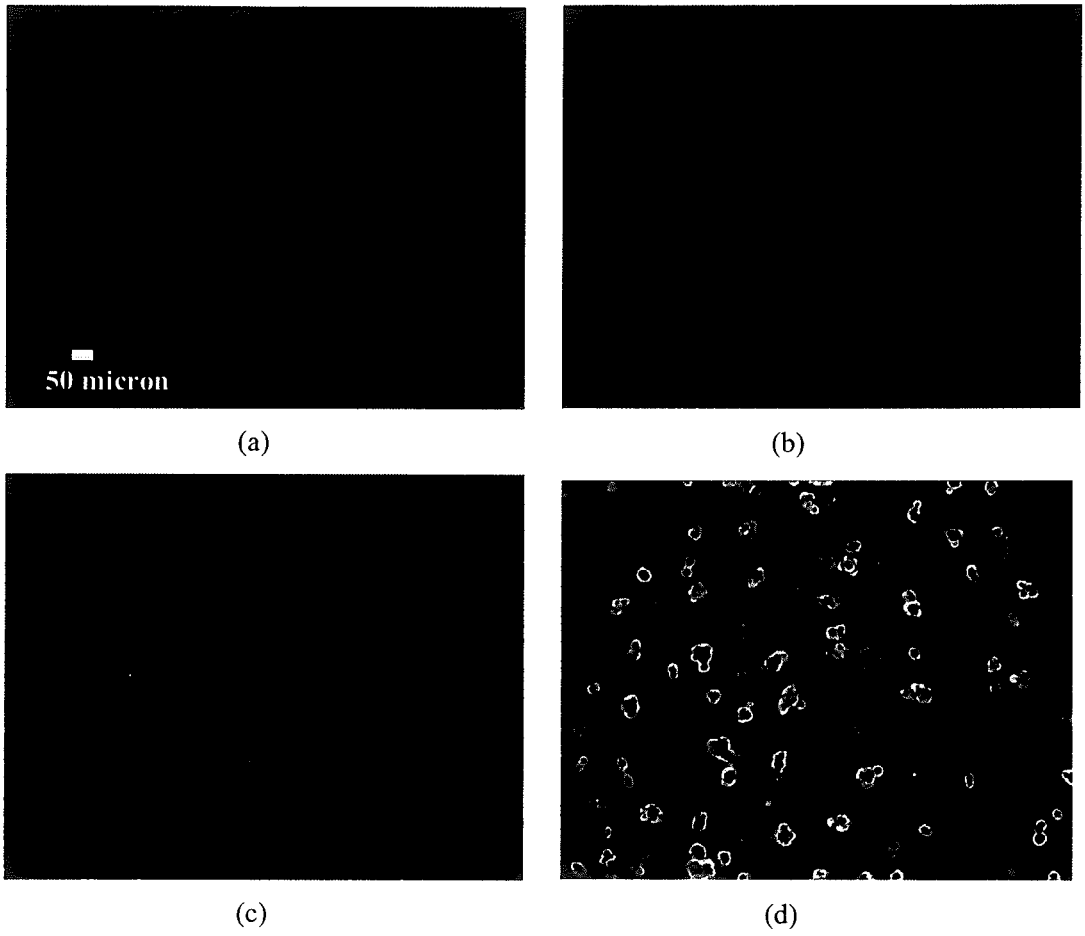


FIGURE 3-1. Images of a PAAM hydrogel (a) At the interface of the collagen gradient (b) At the interface of the Young's modulus gradient. (c) A composite image that demonstrates that the chemical and mechanical interface are aligned. (d) Balb/c 3T3 fibroblast cells adhered on the PAAM substratum. Scale bar: 50μm.

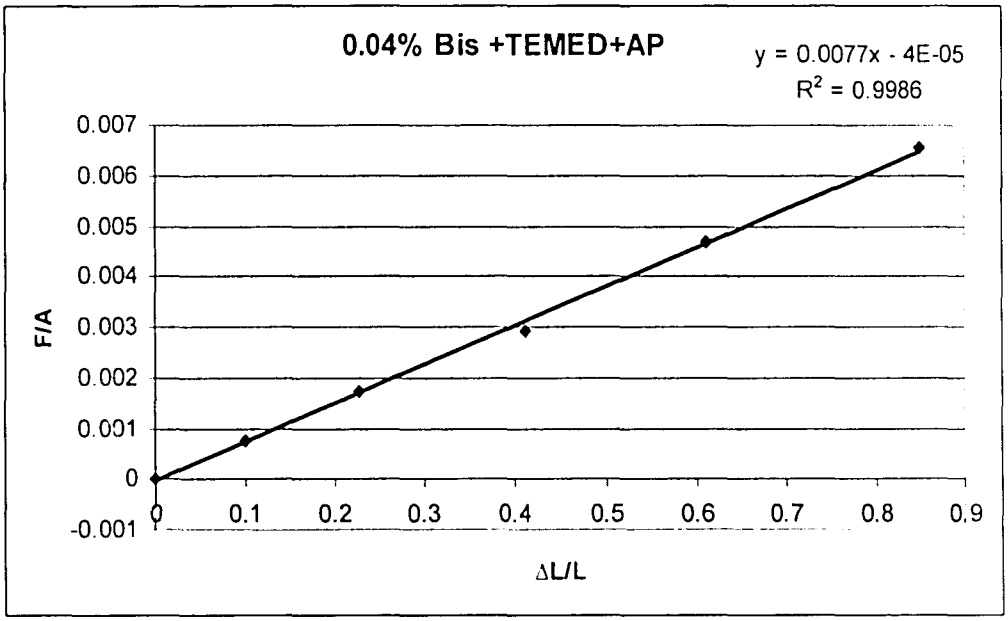


FIGURE 3-2 Stress versus strain for 0.04%v/v bis-acrylamide hydrogel.

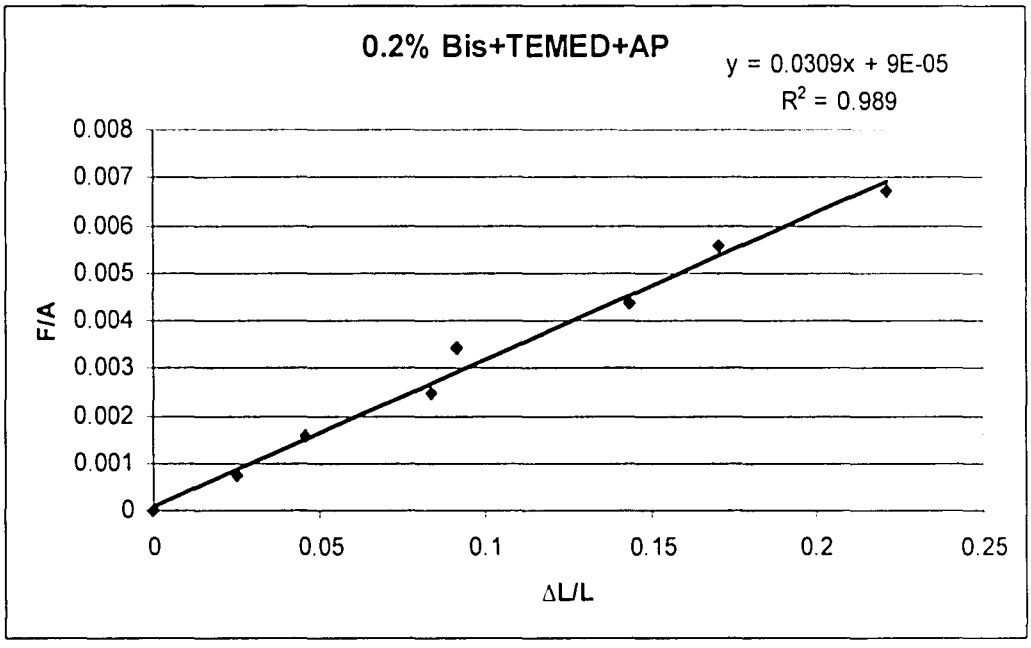


FIGURE 3-3 Stress versus strain for 0.2%v/v bis-acrylamide hydrogel.

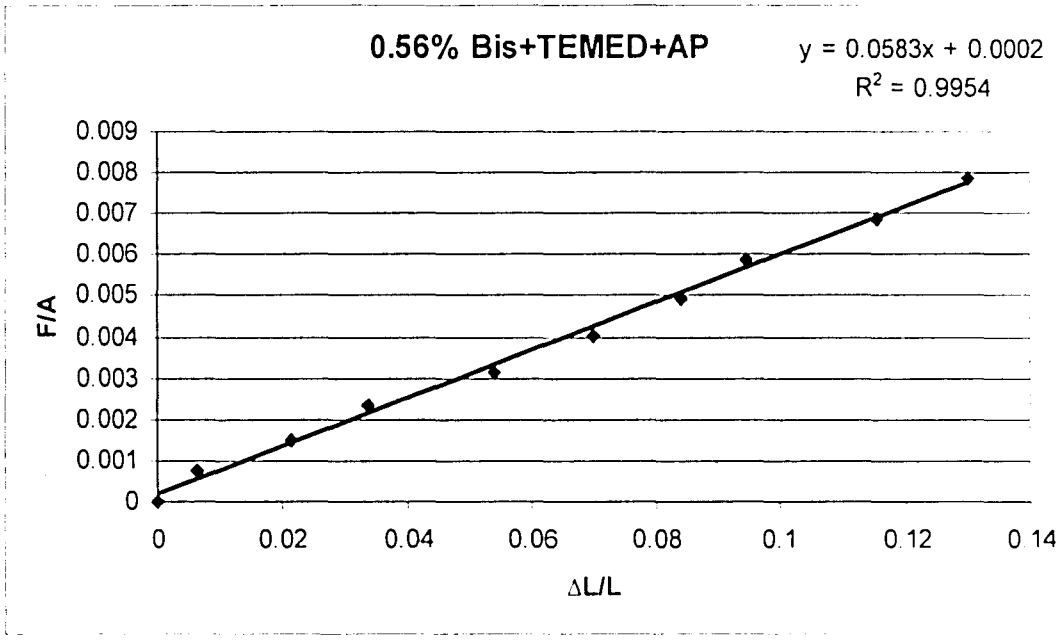


FIGURE 3-4 Stress versus strain for 0.56%v/v bis-acrylamide hydrogel.

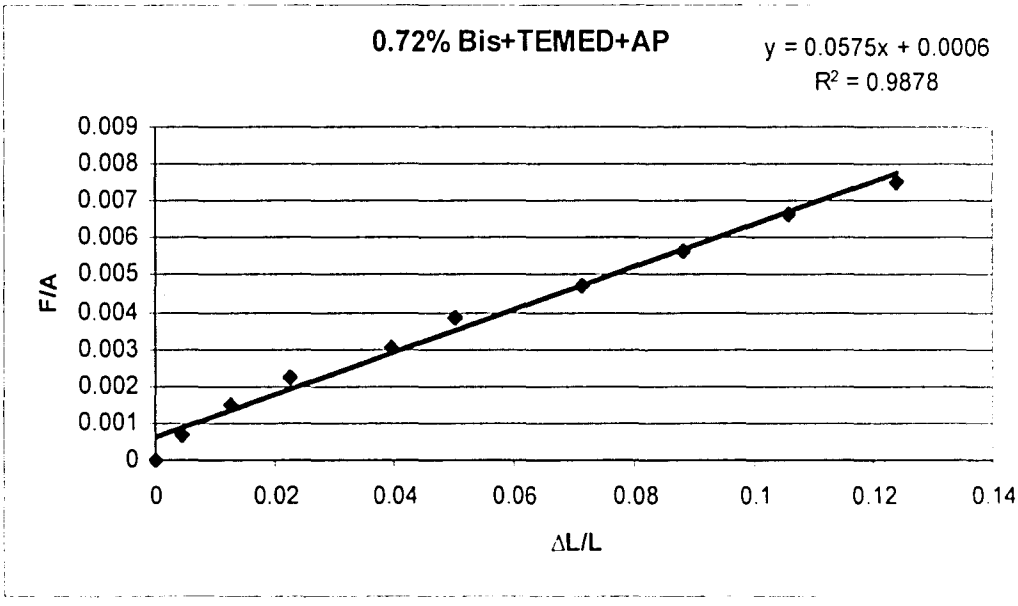


FIGURE 3-5 Stress versus strain for 0.72%v/v bis-acrylamide hydrogel.

Bis Concentration (%)	Young's Modulus (kPa)	
	Mean ± Std dev	Number of test samples
0.04	8.15 ± 0.56	4
0.2	30.37 ± 0.97	3
0.56	55.75 ± 2.55	3
0.72	58.85 ± 1.35	3

Table 3-1 Young's Modulus values for PAAM hydrogels for a range of bis-acrylamide concentrations.

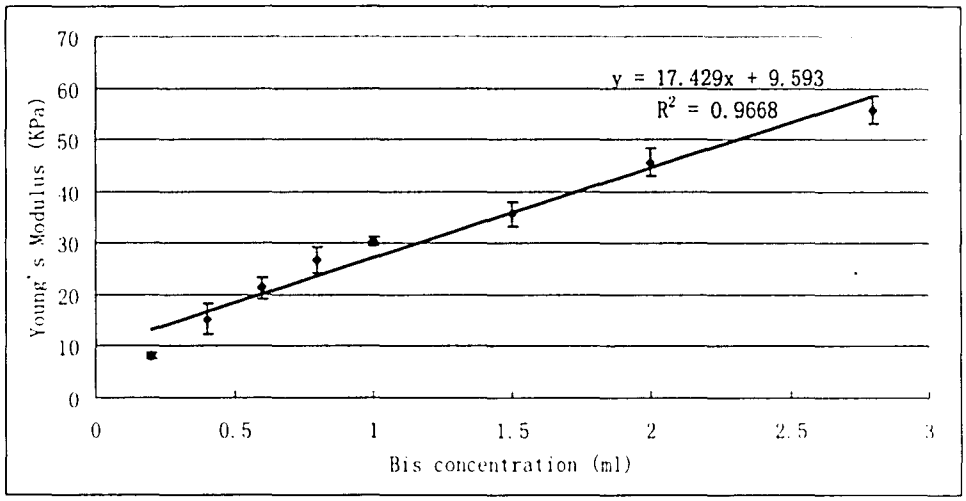


FIGURE 3-6. Young's Modulus values at varying bis-acrylamide concentrations

Sample Number		Number of FITC-Collagen Beads Mean ± Std dev	Number of regions tested
1	High	1176 ± 185	10
	Low	60 ± 27	10
2	High	1820 ± 317	10
	Low	48 ± 18	10
3	High	2001 ± 140	10
	Low	35 ± 16	10

Table 3-2 FITC-collagen beads density on PAAM substrata that exhibit a 30.37kPa/55.75kPa mechanical interface.

Sample Number		Number of FITC-Collagen Beads Mean ± Std dev	Number of regions tested
1	High	712 ± 90	10
	Low	109 ± 25	10
2	High	1620 ± 71	10
	Low	239 ± 69	10
3	High	1609 ± 115	10
	Low	180 ± 38	10

Table 3-3 FITC-collagen beads density on PAAM substrata that exhibit a 55.75kPa/58.85kPa mechanical interface.

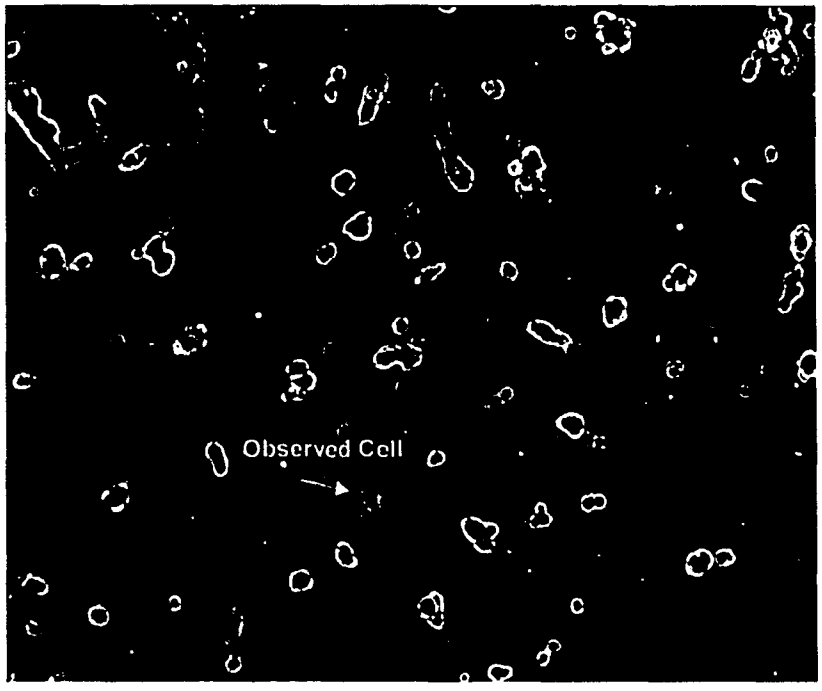


FIGURE 3-7. Cell migration on a dual chemical-mechanical gradient surface. Images were recorded with simultaneous phase and fluorescence microscopy. Scale bar: 50 μm.

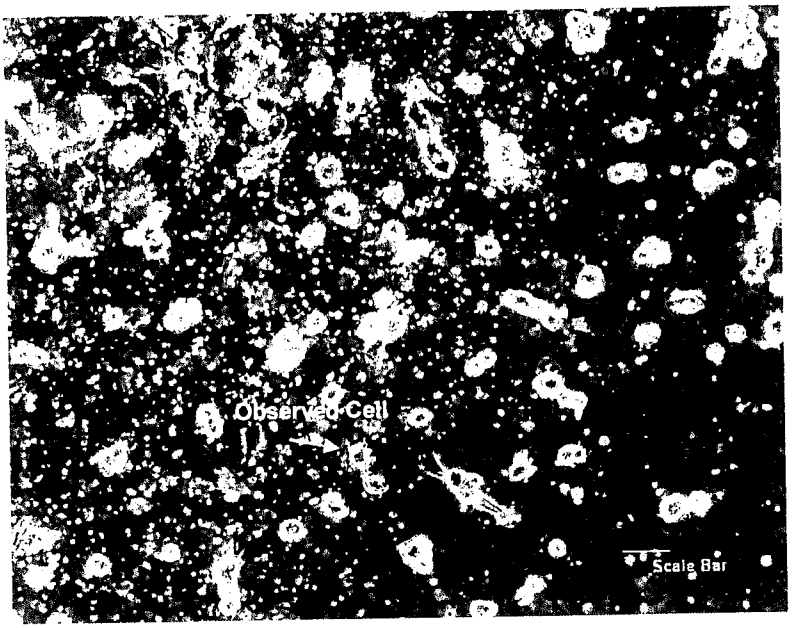


FIGURE 3-7. Cell migration on a dual chemical-mechanical gradient surface. Images were recorded with simultaneous phase and fluorescence microscopy. Scale bar: 50 μm.

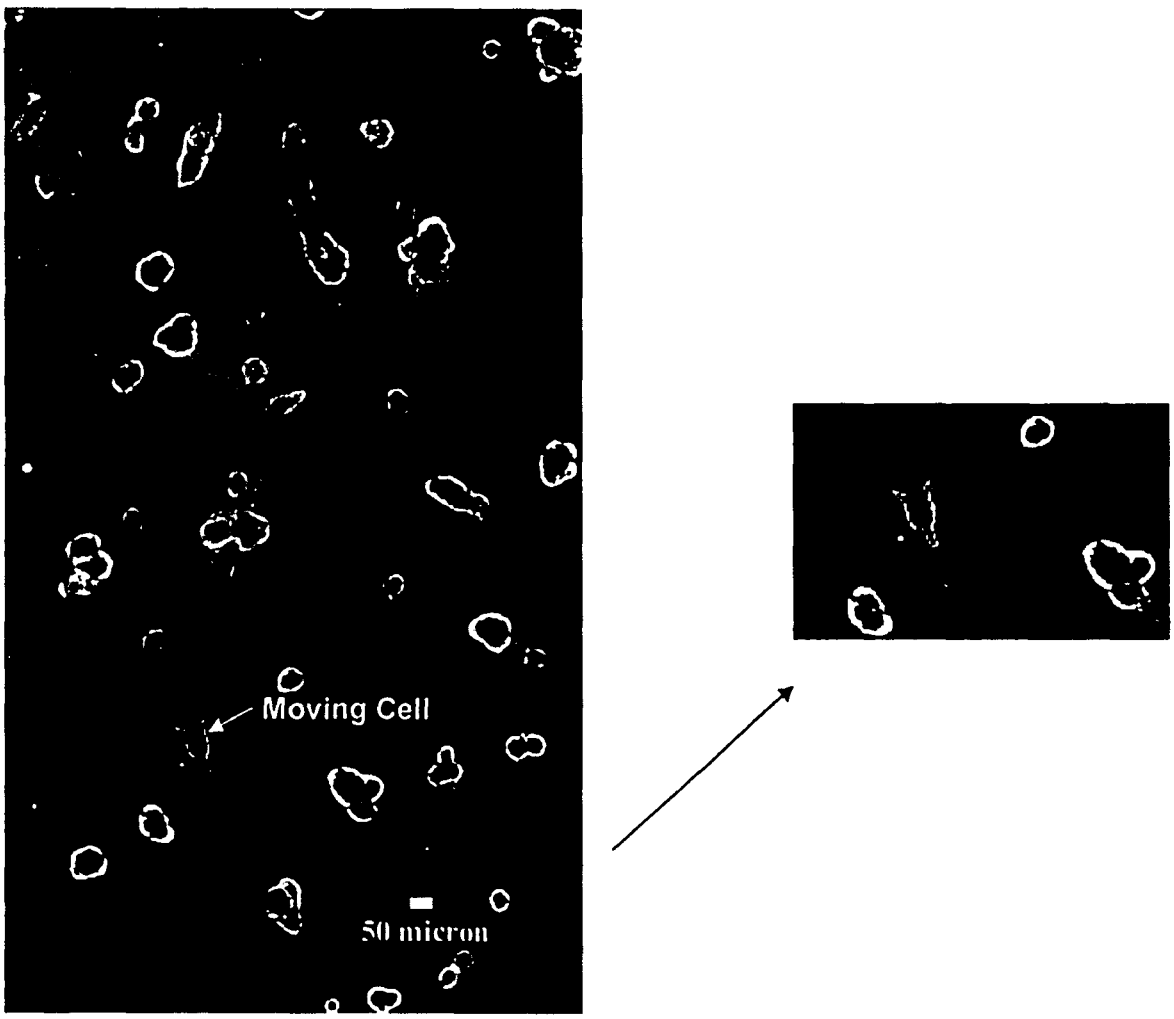
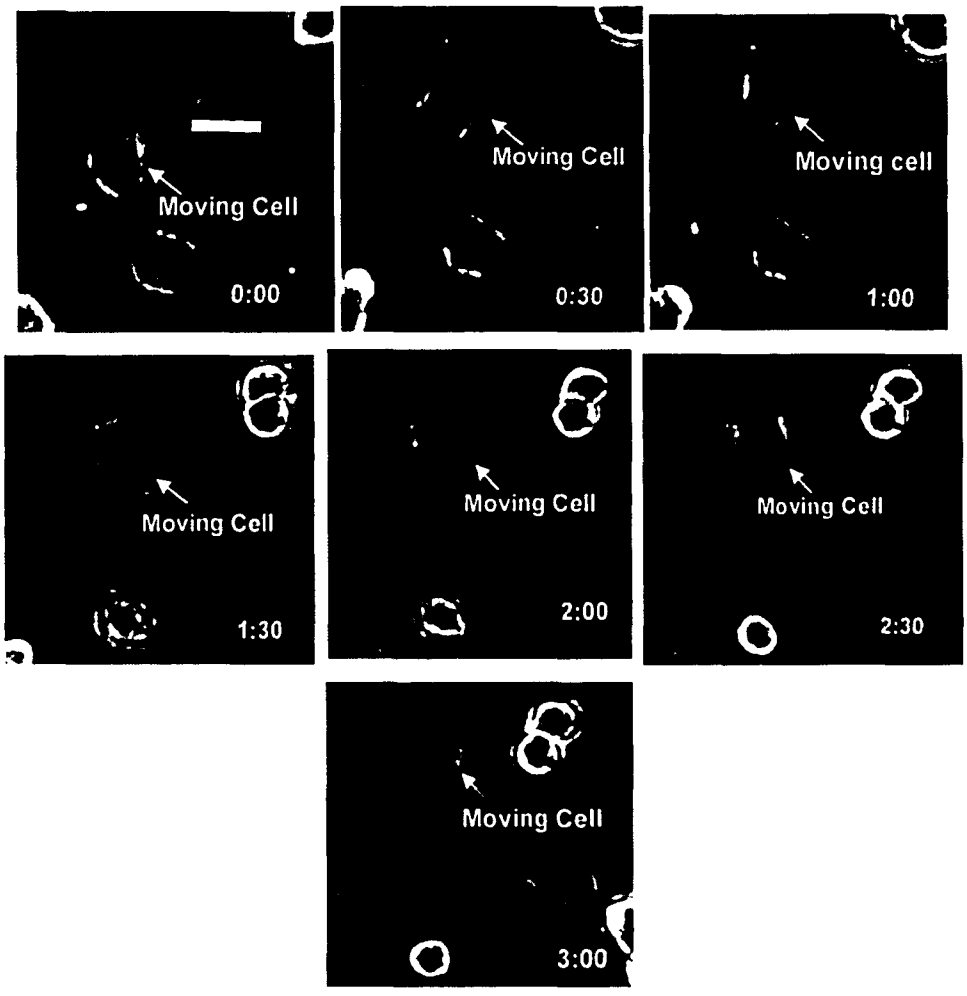


FIGURE 3-8 Composite pictures of FITC-collagen beads and fibroblast cells. The cell highlighted with a yellow circle was observed for cell migration. Scale bar: 50 μm.



(a)

Figure 3-9 (a) Cell migration towards the high collagen region. Images were captured every 30 minutes. Scale bar: 20 μm.

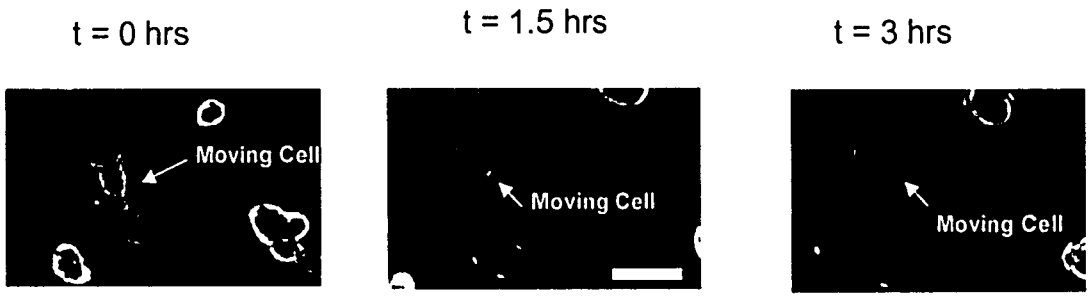


Figure 3-9 (b) Composite pictures of FITC-collagen beads and a moving cell. Scale bar: 20 μm.

	Number of observed cells	Mean ± Std.dev of displacement (μm)
Moving from low collagen concentration to high collagen concentration	11	31.2 ± 40.0
Moving from high collagen concentration to low collagen concentration	4	41.19 ± 18.55
Moving along the interface on the side that has low collagen	2	18.13 ± 15.41
Moving along the interface on the side that has high collagen	2	49.48 ± 12.17

Table 3-4 Displacement and direction of motion for cells on a PAAM hydrogel surface that exhibits a 30.37kPa/55.72kPa interface.

	Number of observed cells	Mean ± Std.dev of displacement (μm)
Moving from low collagen concentration to high collagen concentration	9	58.26 ± 86.94
Moving from high collagen concentration to low collagen concentration	4	83.97 ± 71.5
Moving along the interface on the side that has low collagen	1	39.0 ± 0
Moving along the interface on the side that has high collagen	3	47.87 ± 19.89

Table 3-5 Displacement and direction of motion for cells on a PAAM hydrogel surface that exhibits a 55.72kPa/58.85kPa interface.

	Young's Modulus 30.37/55.75kPa	Young's Modulus 55.75/58.85kPa
Motion towards high collagen concentration	11	9
Motion towards low collagen concentration	4	4
Moving towards or along the interface	5	4
Moving randomly or stay	2	0
Total	21	17

Table 3-6. A summary of the direction of cell migration.

VITA

Yuzhen Yang
124 W.4th Street
Bethlehem, PA 18015
484-716-5373

Education

Tsinghua University, Beijing, China, 2000-2004
B.S. in Chemical Engineering, July 2004
Overall GPA: 3.2
Lehigh University, Bethlehem, Pennsylvania, 2005-2007
M.S. in Chemical Engineering, May 2007 (anticipated)
Overall GPA: 3.95

Research Experience

Cell Migration Studies at the Interface of a Dual Chemical-Mechanical Gradient. --- Lehigh University, 2006.

Poly(2-hydroxyethyl acrylate-co-ethylene glycol dimethacylate) monoliths grafted with thermoresponsive polymer. --- Tsinghua University, 2004.

END OF TITLE