Detection of Mass, Growth Rate, and Stiffness of Single Adherent Cells Using Silicon Pedestal Sensors

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Co-Advisers: Rashid Bashir, Electrical and Computer Engineering/Bioengineering

Hyun Joon Kong, Chemical and Biomolecular Engineering

Key Research Aims and Goals

To study and characterize the mass of an individual cell.

Research Highlights and Results

- Recently, our group developed an improved MEMS resonator sensor that can be used to directly measure the biophysical properties, mass, and growth rate of single adherent cells (Fig. 1).
- Decoupling the relationship between the cell's dynamics and the apparent mass reported by the sensor is of utmost importance. Hydrogels with tunable stiffness and mass are used to achieve higher understanding of our measurement system.

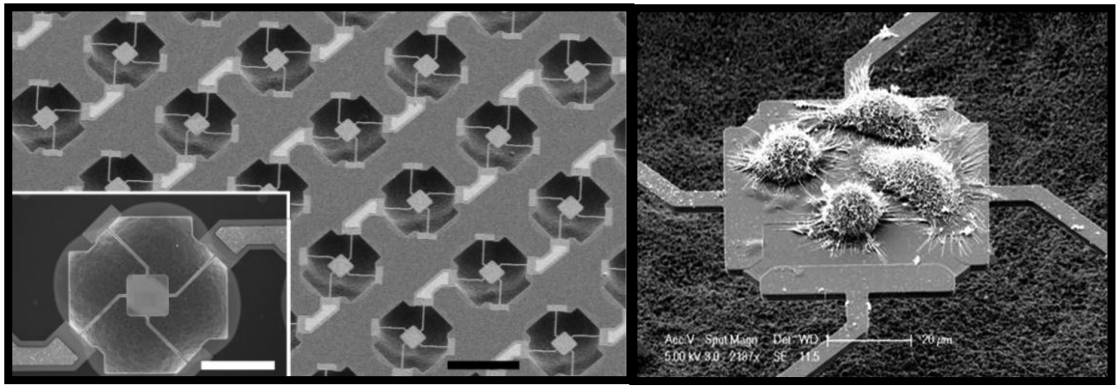

Fig 1 (left) SEM image showing a sensor array; an individual sensor is shown in the inset.[1] (right) HT-29 (Human Colon Cancer) cells are captured on a pedestal sensor.[1]

Future Research Plans

- To investigate how a neuron's mass changes with the rate of cell growth through the cell cycle. Such measurements have the potential of elucidating the cellular growth patterns.
- Explore the cell mass of different cell lines, before and after fixation. As the previous work has shown that the measured apparent mass in human colon cancer cells (HT29) is greater for fixed cells than for the corresponding live cell.[1] This demonstrated that the measured apparent mass is a function of the stiffness.

[1] K. Park, R. Bashir et al. Measurement of adherent cell mass and growth . PNAS. 2010;107:20691–6

Angiogenic Models for Blood Vessel Patterning and Tissue Engineering Applications

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Adviser: Rashid Bashir,
Electrical and Computer Engineering & Bioengineering

Key Research Aims and Goals

To understand the process of angiogenesis and be able to induce patterned blood vessel formation for tissue engineering and disease treatment.

Current Research Highlights and Results

- We have demonstrated a successful method to direct and control the organization and formation of neovessels in vivo. A stereolithography apparatus (SLA) was utilized for the rapid prototyping of a permeable, circular hydrogel 'stamp' made of PEGDA-MA with vertical channels to augment the transport of nutrients and oxygen within the circular stamp.
- Living cells that release angiogenic growth factors were encapsulated in the hydrogel, and a CAM assay was utilized to assess the ability to create patterns in the tissue vasculature based on localized release of growth factors through the channels.
- Neovascularization resulted in the patches with microchannel diameters of 300 and 500µm, demonstrating the successful formation of capillary-like vessels in a pre-determined pattern.

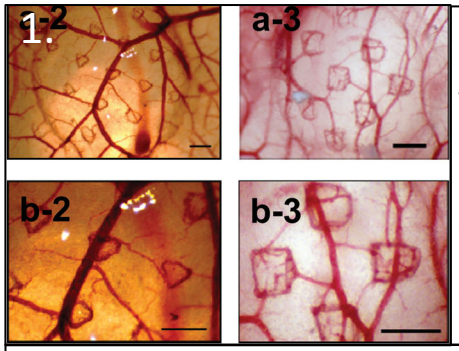
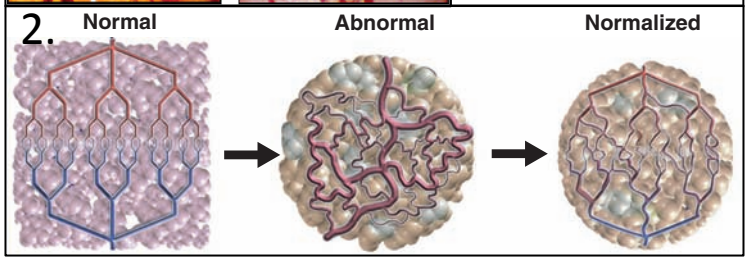


Fig 1: Neovessels formed in circular patterns under the patches with channel diameters of 300 and 500µm.[1]

Fig 2: Treatment of cancer will require the 'normalization' of the abnormal vasculature surrounding a tumor.[2]



Future Research Plans

- The stamp can be altered for the creation of neovessels in almost any organized 2D pattern on a physiological micrometer scale. This successful combination of SLA technology and cell biology will have implications in all aspects of tissue engineering, as the formation of blood vessels is critical to the survival of any tissue.
- The system is promising for diverse therapeutic angiogenesis as well as tissue regeneration applications. We plan to use this technology in the development of a therapeutic system for an in vivo myocardial rat infarct model as well as an in vivo window mesentery rat model.

[1] Jae Hyun Jeong, Vincent Chan, Chaenyung Cha, Pinar Zorlutuna, Casey Dyck, K. Jimmy Hsia, Rashid Bashir, and Hyunjoon Kong. **"Living" Microvascular Stamp for Patterning of Functional Neovessels; Orchestrated Control of Matrix Property and Geometry**. Adv. Mater. (2012) 24, 58–63

[2] Rakesh K. Jain. **Normalization of Tumor Vasculature: An Emerging Concept in Antiangiogenic Therapy**. Science (2005) 307 58-62

Cell Response to Geometrical and Pressurized Environment

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Co-Advisers: K. Jimmy Hsia, Mechanical Science and Engineering
Supriya Prasanth, Molecular and Cellular Biology

Key Research Aims and Goals

To understand the effect and cellular response to geometric cues and hydrostatic pressure.

Research Highlights and Results

- We have developed 2 pressurized incubation chambers. One chamber works together with a standard incubator and is capable of handling multiple samples. The other is a standalone single specimen incubation pressure chamber that can be mounted on a microscope for live imaging of cells in the pressurized environment.
- We have been characterizing a uniquely formed microstructure. The structure is a wavy surface that is formed using mismatched strains. We are capable of feature sizes ranging from hundreds of nanometers up to a couple micrometers.

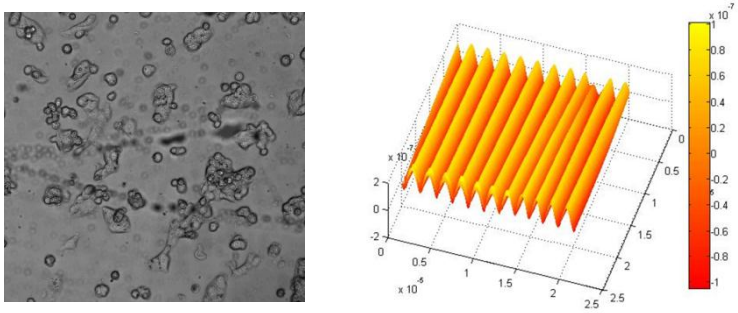


Fig 1 (a) Live image of HepG2 cells inside pressure chamber at 100 kPa, (b) AFM image of buckled surface with wavelength of about 2 micrometers and amplitude of about 200 nanometers

Future Research Plans

- Use the pressure chambers to study the molecular response of the cells and develop and understanding of the response that metastatic and non-metastatic cells have when exposed to hydrostatic pressure.
- Using the microstructure we hope to explore cellular response to geometrical dimension and shape (e.g. curvature). Explore how senescent and quiescent cells respond to changes in geometrical environment and to find out what is the molecular basis for this.

Alleviation of Sarcopenia Using Mechanical Strain-Induced Myogenic Stem Cells

Heather D. Huntsman, Kinesiology and Community Health
Co-Advisers: Marni Boppart, Kinesiology and Community Health
Hyun Joon Kong, Chemical and Biomolecular Engineering

Key Research Aims and Goals

Aim #1: To determine the optimal conditions for sythesizing myogenic stem cells *in vitro*

Aim #2: To determine the extent to which newly sythesized myogenic stem cells contribute to increased muscle fiber growth and function *in vivo*

Research Highlights and Results

- We have optimized conditions for isolating and mechanically stimulating Sca-1^{+}CD90^{+}CD45^{-} mesenchymal stem cells from skeletal muscle (mMSCs) of wild type and α7 integrin transgenic mice. While exposure to mechanical strain upregulates Sca-1 and CD90 on laminin, MSCs strongly enhance expression of myogenic factors, including myf-5 and myoD on gelatin [Fig. 1a].
- Our most recent work also demonstrates that Sca-1^{+}CD90^{+}CD45^{-} mMSCs expand in muscle as a result of eccentric exercise (mechanical strain *in vivo*), and these cells subsequently contribute to new fiber growth [Fig. 1b].

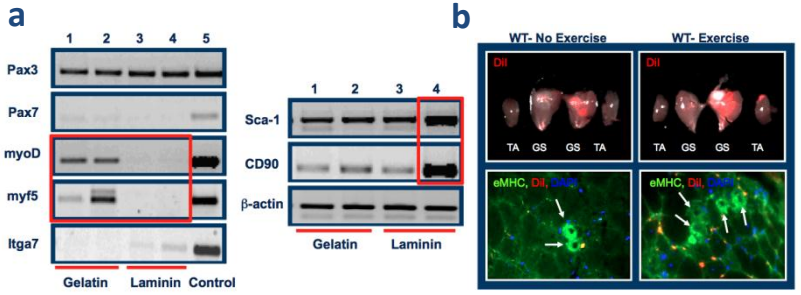


Fig 1 (a) Cells were seeded on bioflex membranes and following 5hrs of 10% biaxial strain cells were harvested and analyzed by RT-PCR for myogenic or stem cell markers. Lanes 2,4 strained; lanes 1,3 unstrained; lane 5 control C_2C_{12} cells. (b) Top- Gastrocnemus/soleus complex and tibialis anterior muscle imaged with dark box and demonstrates expansion of transplanted DiI labeled mMSCs in exercised muscle. Bottom- Immunofluorescence images showing increase in de novo fiber formation as labeled by eMHC positive myofibers.

Future Research Plans

- To isolate MSCs from other tissues, such as adipose tissue, and direct differentiation through mechanical cues. This would potentially help overcome the barrier of low cell yields, and increase the potential for autogolous transplantation stem cell therapies.
- To further explore the microenvironmental changes that occur following eccentric exercise, and use this knowledge to identify key signals in myogenic determination *in vivo*.
- The ultimate goal of this research is to better understand the regulation of the skeletal muscle microenvironment to subsequently develop novel therapeutic strategies to combat devastating losses in muscle mass and function that occur as a result of aging and/or disease.

A Microfluidics Approach to Probing Filopodial Development in Neurons

Anika Jain, Cell and Developmental Biology
Co-Advisers: Martha U. Gillette, Cell and Developmental Biology
Rashid Bashir, Micro & Nano-technology Lab.

Key Research Aims and Goals

To investigate the effects of substrate-bound and diffusive guidance cues on the development of dendritic filopodia and spines.

Research Highlights and Results

- We are exploiting the unique properties of polydimethylsiloxane (PDMS) microfluidic devices towards studying early neuronal development. Recently, our group demonstrated the efficacy of solvent-extraction as a PDMS-treatment protocol that enables low-density cultures of primary hippocampal neurons. [[1], Fig. 1a].
- We have used flow manipulations in closed-channel microdevices to generate stable and instructive gradients of substrate-bound cues, such as laminin and poly-L-lysine (PLL), to allow precise control over neuron development and network formation. [[2], Fig. 1b].

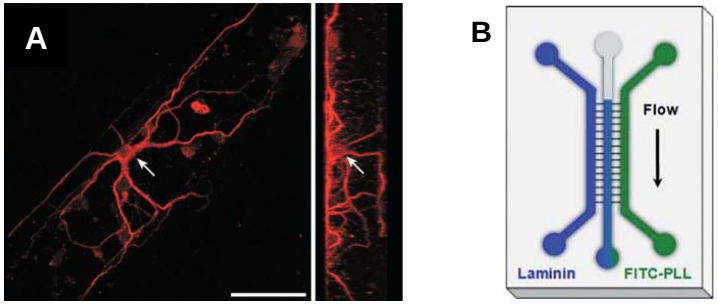


Fig 1 (a) Top-down and side views of a neuron in a microdevice at 8 days *in vitro*. Cell body (arrows) and neurites are labeled with neuronal marker MAP2. Scale bar: 50 µm. (b) Schematic of device design used for generation of substrate gradients. Three primary channels, each 200 µm wide and 45 µm high, communicate through narrow interconnects.

Future Research Plans

- These neuron culture and gradient generation techniques will be used to compartmentalize neuronal dendrites into fluidically isolated channels, in order to selectively stimulate only certain regions of the cell, with a high degree of spatio-temporal control.
- This will enable us to study filopodial dynamics during early neuronal development. We will specifically investigate the effects of substrate-bound cues (e.g., laminin and PLL), diffusive cues (semaphorin3A), chemical stimulation (glutamate) and cell-cell contact and signaling.

[1] Millet L, Stewart M, Sweedler J, Nuzzo R, Gillette M (2007) Microfluidic devices for culturing primary mammalian neurons at low densities. Lab Chip 7, 987-994.
[2] Millet L, Stewart M, Nuzzo R, Gillette M (2010) Guiding neuron development with planar surface gradients of substrate cues deposited using microfluidic devices. Lab Chip 10, 1525-1535.

Investigating Local Intrinsic Fluctuations in Cells

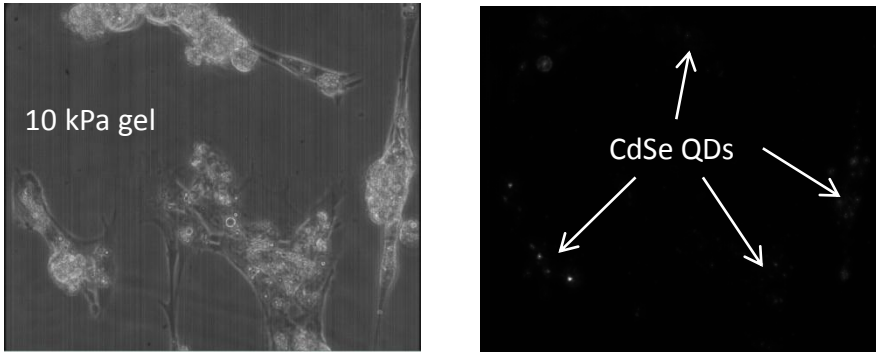
Samantha Knoll, Mechanical Science and Engineering
Adviser: Taher Saif, Mechanical Science and Engineering

Long Term Goals

- Gain an understanding of intracellular, local force fluctuations , apply it to help predict cell responses, and ultimately develop a diagnostic method for identifying pre-cancerous cells
- Implement modern optical and mechanical techniques in live cell experiments

Current Research

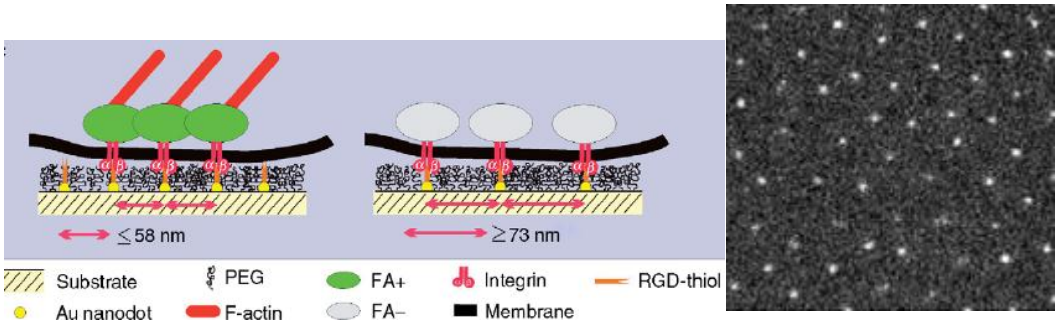
- Developing a method that utilizes CdSe quantum dot (QD) nanoparticles to assess local, natural cell fluctuations
- Investigating the frequency of natural cellular vibrations



A phase contrast image of fibroblast cells (left) and a fluorescent image of the same field of view (right) showing QDs fluorescing in regions where cells are located. The QDs were placed on the surface of the substrate prior to seeding the cells and are being used to track intrinsic, natural cell vibrations.

Future Research Plans

- Understand fluctuations related to focal adhesion complexes because that is how cells contact the substrate
- Utilize Joachim Spatz's group's method for uniform patterning of Au on hydrogels to ensure highly precise control of cell adhesion density [1, 2]
- Determine the effect of variable stiffness substrates on cell mechanosensitivity
- Apply Spatz's study that demonstrated nanopattern spacing affects focal adhesion of cells to develop a novel method for assessing cell fluctuations that incorporates nanomaterials [1, 2]



Gold (Au) nanostructures uniformly patterned on PDMS (right). The schematic (left) shows different focal adhesion efficiencies for osteoblasts at different Au nanodot distances. Understanding how nanoparticle spacing affects cell adhesion may relate to how frequently cells exert forces on the substrate. [[1], Figs. 7A and 12C]

[1] Geiger, Benjamin, Spatz, Joachim P., and Bershadsky, Alexander D. Environmental Sensing through Focal Adhesions. Nature Reviews. 2009; 10: 21-33.
[2] Spatz, Joachim P. and Geiger, Benjamin. Molecular Engineering of Cellular Environments: Cell Adhesion to Nano-Digital Surfaces. Methods in Cell Biology. 2007; 83: 89-111.

Exploring Cell:Cell and Cell:ECM Interactions with Photonic Crystal Biosensors

Erich A. Lidstone, Bioengineering PhD/MD Candidate

Co-Advisers: Brian T. Cunningham, Electrical and Computer Engineering, Bioengineering

Lawrence B. Schook, Veterinary Pathobiology, Animal Sciences

Key Research Aims and Goals

To investigate the role of cell attachment in disease progression and in therapeutic intervention

Research Highlights and Results

- Recently, I have demonstrated the first use of Photonic Crystal Enhanced Microscopy (PCEM) to examine cell growth, proliferation, movement, and apoptosis in a number of settings[1]. Figure 1 summarizes the new technique, and shows images generated by cells cultured on photonic crystal biosensors.
- Our sensors can be functionalized with various extracellular matrix proteins and signaling molecules to enhance or inhibit cell attachment. Fig. 1d shows a contractile cardiac myocyte in culture on a biosensor functionalized with collagen and fibronectin to facilitate cell attachment and maturation.

Future Research Plans

- Use photonic crystal enhanced microscopy to investigate tumor immunogenicity and immune surveillance of individual cancer cells in a label-free, high resolution study.
- Combining micro-contact printing and other functionalization techniques, optimize the culture microenvironment to prolong hematopoeitic stem cell survival and direct stem cell fate with increased specificity and yield.

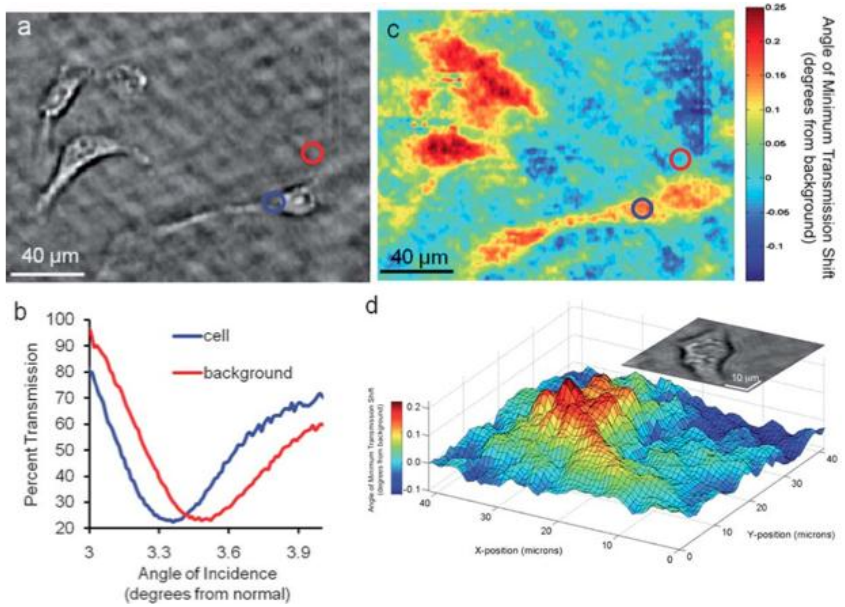


Fig. 1 Summary of Photonic Crystal Enhanced Microscopy. a) Bright field microscopy of HepG2/C3 cells shows cell spreading and morphology. b) Transmission intensity is plotted as a function of angle of incidence for individual pixels on (blue) and off (red) a cell. Pixel regions are highlighted in (a). c) A composite PCEM image, describes the angle of minimum transmission (AMT) as a function of position. Attachment proteins deposited on the biosensor by viable cells result in a reduced angle of minimum transmission shift. d) A surface plot of cardiomyocyte attachment at 24h obtained via PCEM corresponds with the morphology observed in bright field microscopy (inset).

[1] Lidstone EA, .., Schook LB, Bashir R, Cunningham BT. Label-free imaging of cell attachment with photonic crystal enhanced microscopy. *Analyst* 2011;136:3608-3615

MSC Differentiation across the Tendon-Bone Interface using CG Scaffolds

Laura Mozdzen, Chemical and Biomolecular Engineering
Co-Advisers: Brendan Harley, Chemical and Biomolecular Engineering
Amy Wagoner Johnson, Mechanical Science and Engineering

Key Research Aims and Goals

To create a functionalized 3-D scaffold which promotes the regeneration of the tendon-bone interface

Research Highlights and Results

- Our group has developed a method to produce 3-D, anisotropic collagen-GAG scaffolds.
- Recently, our group has shown that the combination of anisotropic Collagen-GAG scaffolds and growth factors influence tendon cell recruitment, alignment, and metabolic activity.[1]

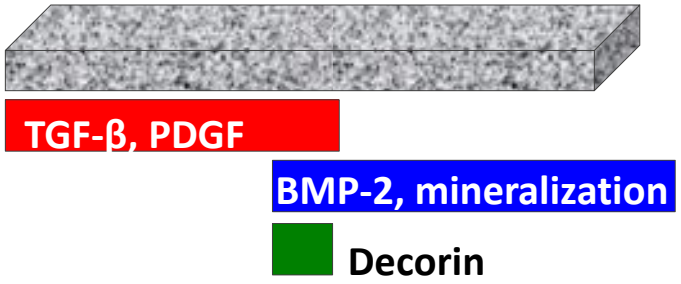


Fig 2: A sample pattern of combinations of growth factors and ECM proteins across the Tendon-Bone interface on a two dimensional collagen-GAG scaffold.

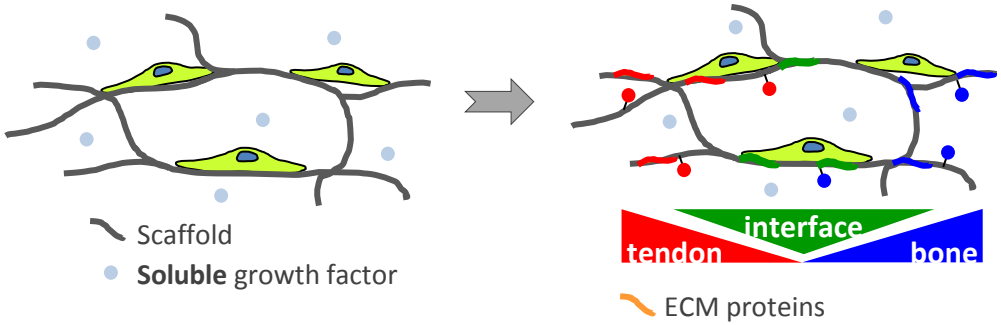


Fig 1: the addition of ECM proteins to a 3D scaffold to encourage cell differentiation across the Tendon-Bone Interface.

Future Research Plans

- Since growth factors and an anisotropic scaffold have already encouraged good tendon-bone differentiation, a combination of ECM proteins should have an additive effect. The combination of ECM proteins and growth factors will further encourage cell differentiation across the tendon-bone interface.
- Once the ECM proteins and growth factors are shown to successfully encourage a distinct tendon-bone interface on a two-dimensional collagen-GAG membrane, the same interface will be replicated in a 3D scaffold.

[1] Caliari, S., Harley, B. The effect of anisotropic collagen-GAG scaffolds and growth factor supplementation on tendon cell recruitment, alignment, and metabolic activity. Biomaterials. 2011;32:5330-40

Direct-Write Assembly of 3D Microperiodic Hydrogel Scaffolds for Human Embryonic Stem Cell Culture

Lucas Osterbur, Materials Science & Engineering

Co-Advisers: Jennifer Lewis, Materials Science and Engineering

Ralph Nuzzo, Chemistry

Key Research Aims and Goals

To develop a system for the culture and proliferation of human embryonic stem cells (hESCs) in a biocompatible, 3D environment as a closer mimic of biological systems compared to current 2D well plates.

Research Highlights and Results

- Development of a shear thinning hyaluronic acid gel that is compatible with the direct ink write fabrication system. The ink is UV curable through chemical modification and biocompatible. 3D scaffold feature sizes can be arbitrarily designed to fit project parameters.
- Initial cell studies with hESCs have shown the 3D hyaluronic acids scaffolds to be a promising system for proliferation of hESC cultures. Proliferation has continued for up to 14 days, with growth on scaffolds more pronounced than flat substrates.

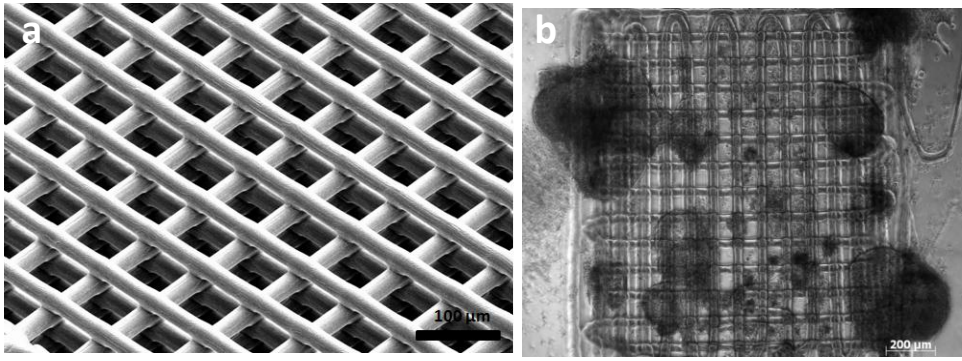


Fig 1 (a) Micrograph of cured hyaluronic scaffold with 30µm filament width and 6 total layers. (b) hESCs after 11 days in culture within a hyaluronic acid scaffold.

Future Research Plans

- The substrate stiffness which the hESCs are experiencing will be fully characterized. Through modification of the chemical synthesis, UV exposure intensity, and feature size, we will be able to examine cell responses to varying substrate stiffness and geometries.
- Expansion of the hyaluronic acid system to other cell types and differentiation pathways can push the technology into implantable designs. Current work with mesenchymal stem cells and chondrogenic repair is promising.
- Scaffolds are able to withstand a large degree of deformation before experiencing mechanical failure. Further exploration is warranted to investigate the possibility of injectable scaffolds systems.

In collaboration with: Yijie Geng[1], Rong Tong[2], Fei Wang[1], and Jianjun Cheng[2]

1. UIUC, Molecular and Cellular Biology; 2. UIUC, Materials Science and Engineering

Mechanotransduction at Cell-Cell Junction

Hamid Tabdili, Chemical and Biomolecular Engineering
Co-Advisers: Deborah Leckband, Chemical and Biomolecular Engineering
Ning Wang, Mechanical Science and Engineering, Peter Wang, Bioengineering

Key Research Aims and Goals

- We try to advance our understanding of mechano-transduction in biology, and pave the way towards our long-term goals of identifying targeted therapies for leaky blood vessels, and of enhancing tissue regeneration and wound healing. Our main goals are:
  - **To discover parameters that modulates cadherin mechanosensing mechanism**
  - **To identify the key cytoskeletal components and molecular pathways in cadherin-mediated mechanosensing**

Research Highlights and Results

- Although the role of cadherins in morphogenesis and different diseases such as cancer is extensively documented, the effect of mechanical forces on cadherin function and their effect on the mechanical properties of the cell remain unanswered. Although the central role of cadherin as an adhesive and an active mechanical link that regulates many biological behaviors is evident, the fundamental question that we are trying to address is whether cadherin, in a similar fashion to integrin, can feel their environment and proportionally respond in order to regulate cell function. Our preliminary confirmed our hypothesis that cadherin can act as mechanosensor. While we are trying to confirm the active mechanosensing capability of cadherin, our next step is to try to correlate signaling pathways which are involved in the cell's response to external forces, followed by studying the localization of different components at adherens junctions which is directed by tension
- Recently, our group demonstrated that cadherin complexes are tension sensors. With magnetic twisting cytometry (MTC), we showed cadherin junction reinforcement, in response to cadherin-specific, externally applied (exogenous) force. By using MTC and TFM I am investigating the role of alpha catenin in cadherin force sensing. I am also interested in the role of actin and other proteins in the cell-cell force transmission mechanism.

Future Research Plans

- **To visualize spatiotemporal changes in subcellular organization during mechanotransduction.**
- **To investigate the role signaling molecules such as RhoA and Src in cadherin force modulation.**

Effects of Mechanical Rigidity and Geometric Control on Colon Cancer Metastasis

Xin Tang, Mechanical Science and Engineering
Co-Advisers: Taher Saif, Mechanical Science and Engineering
Mark Kuhlenschmidt, Pathobiology

Key Research Aims and Goals

To understand the mechanism of coupled mechanical-elasticity cues and geometric cues on the onset of *in vitro* colon cancer metastasis.

Research Highlights and Results

- We have discovered that human colon carcinoma (HCT-8) cells consistently show *in vitro* metastasis-like phenotype (MLP) when cultured on substrates with appropriate mechanical stiffness (21-47 kPa), but not on very soft (1 kPa) and very stiff substrates (3.6 GPa) [[1, 2], Fig. 1a].

- We now have developed a novel micro-patterning technique to spatially confine the living cells adheison within pre-defined geometric zones on 2D polyacrylamide hydrogel substrates (Fig. 1b).

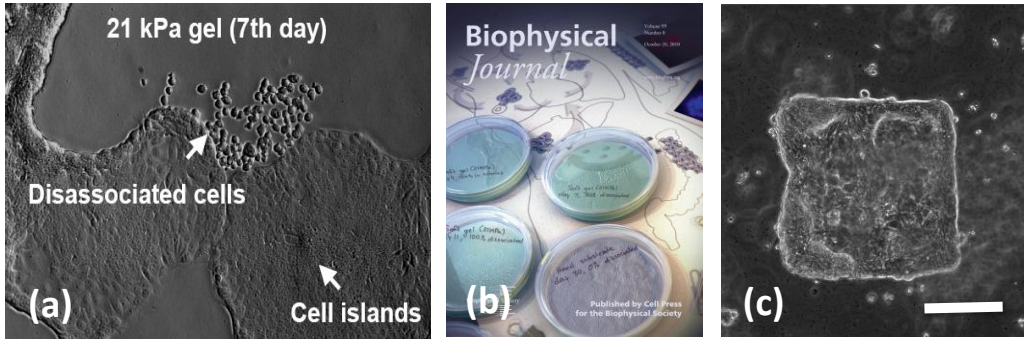


Fig 1 (a) HCT-8 cells cultured on intermediate stiffness gel substrates (E=21 kPa, coated with fibronectin) show metastatic-like phenotype (MLP) after 7 days culture; (b) The Biophysical Journal Coverpage of our results; (c) HCT-8 cells are cultured on micro-patterned fibronectin square (edge width: 500 µm) on 21 kPa gel substrate. Scale bar: 250 µm.

Future Research Plans

- The developed micro-patterning technique will enable us to study the coupled effect of geometric cues and rigidity cues on the onset of *in vitro* metastasis.
- Successful identification and mechanistic understanding of metastasis-triggering signals is critical for the design of novel anti-metastasis therapeutics.

[1] Xin Tang, Theresa Kuhlenschmidt, Jiaxi Zhou, Philip Bell, Fei Wang, Mark Kuhlenschmidt, Taher Saif. Mechanical Force Affects Expression of an In Vitro Metastasis-Like Phenotype in HCT- 8 Cells. Biophysical Journal. 2010;99:2460-9**. (Featured as Cover Article)**
[2] Xin Tang, Tony Cappa, Theresa Kuhlenschmidt, Mark Kuhlenschmidt, Taher Saif. Specific and Non-Specific Adhesion in Cancer Cells with Various Metastatic Potentials. Book Chapter of "Mechanobiology of Cell-Cell and Cell-Matrix Interactions", 2011. Springer Science+Business Media, LLC.

Fabrication and Characterization of Mineralized Multi-Compartment Collagen-GAG Scaffolds for Tissue Regeneration

Daniel Weisgerber, Material Science and Engineering
Co-Advisers: Brendan Harley, Chemical and Biomolecular Engineering
Michael Insana, Bioengineering

Key Research Aims and Goals

To develop a multi-compartment scaffold mimicking the native tendon-bone insertion site for tissue regeneration, and characterize these scaffolds using a variety of techniques including ultrasound elastography.

Research Highlights and Results

- Scaffolds containing two microstructurally and chemically distinct regions that mimic tendon and bone have been successfully fabricated using a liquid-phase cosynthesis technique and subsequent lyophilization (Fig. 1a) [1]. Scaffold microstructure, mineral content, permeability, mechanics, and bioactivity has been characterized.
- Ultrasound elastography has been successfully employed to determine the local strain behavior of these scaffolds in compression (Fig. 1b).

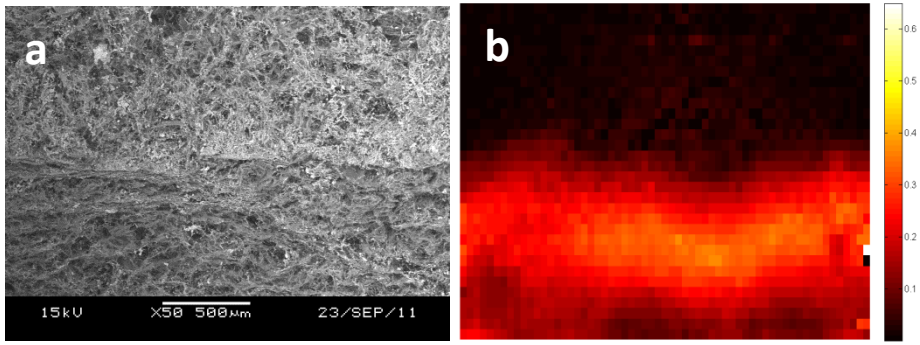


Fig 1 (a) An SEM image revealing the two distinct osseous (top) and tendinous (bottom) compartments, (b) a strain map obtained via ultrasound elastography indicating that the tendinous (bottom) compartment experiences the majority of the strain during compression (50% or 0.5 on scale bar) compared to the osseous (top) compartment.

Future Research Plans

- Incorporate pore anisotropy in the tendinous compartment to better mimic the aligned tendon structure found natively.
- Use ultrasound elastography to monitor local remodeling and the resulting changes in scaffold mechanics and permeability during *in vitro* cell culture.
- Apply micro- and nano-mechanical characterization techniques to evaluate transition zone between multi-compartment scaffolds (IGERT International Collaborator: M. Oyen, Cambridge University, UK)

[1] Harley BA, Lynn AK, Wissner-Gross Z, Bonfield W, Yannas IV, Gibson LJ (2010) Design of a multiphase osteochondral scaffold III: Fabrication of layered scaffolds with continuous interfaces. J Biomed Mater Res A 92 (3):1078-1093. doi:10.1002/jbm.a.32387

Metal Ion Responsive Sensing and Actuation in Live Cells via Delivery of Metal Ion-Specific DNAzyme-Nanoparticle Conjugates

Peiwen Wu, Department of Biochemistry
Advisor: Dr. Yi Lu, Department of Chemistry
Co-advisor: Dr. Peter Yingxiao Wang, Department of Bioengineering

Research Goals:

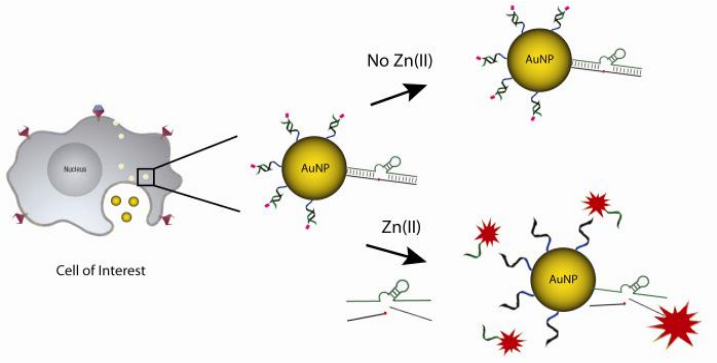
- Design and use of novel DNAzyme and metal nanoparticles to detect and image the dynamics of metal ion distribution inside live cancer cells and correlate it with cell mechanical response.
- Better understanding of cell mechanical properties as a function of disease state

Research Highlights and Results:

- Our group has used a rapidly screening technique called *in vitro* selection to select DNAzymes specific for a wide range of bioavailable metal ions (e.g., Pb^{2+}, Cd^{2+}, Ca^{2+}, Zn^{2+}, Cu^{2+}, Co^{2+}) from a large DNA library (up to 10^{15} sequences) and converted these DNAzymes into metal-ion specific sensors *in vitro* for environmental detection[1]. Such a development has significantly expanded the number of metal ions one can detect. Despite of such advancement, no report on using DNAzymes for cellular detection and actuation has been reported.
- To deliver DNAzymes into live cells, we've made DNAzyme-nanoparticle conjugates and tested its activity toward specific metal ion *in vitro*. It shows that the conjugates have good activity and stability.

Future Plans:

- Imaging and quantification of fluorescent signal upon delivery of the sensor into a cellular environment
- Study of the time-course of alterations in zinc homeostasis between normal prostate cells and prostate cancer cells
- Elucidation of the influence of zinc transportation on metastasis, including structure changes of cytoskeleton and cell membrane, changes in cell deformability and motility



[1]. Liu, J. W.; Cao, Z. H.; Lu, Y., Functional Nucleic Acid Sensors. *Chem Rev* **2009,** *109* (5), 1948-1998.

Optimal Gene Delivery In Human Fibroblasts and Embryonic Stem Cells

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Key Research Aims and Goals

To develop and optimize an efficient method for gene delivery into human embryonic stem cells to transiently overexpress genes to control stem cell fate.

Research Highlights and Results

- Recently, our group has developed a novel cationic helical peptide that has cell membrane interaction properties. I thus attempt to use this novel peptide to increase gene delivery efficiency into hESC and fibroblast cells.
- We were able to demonstrate gene transfer into IMR90 and hESC after a screening and modifications of the helical peptide PVBLG-8. (see figure 1) (hESC data not shown)

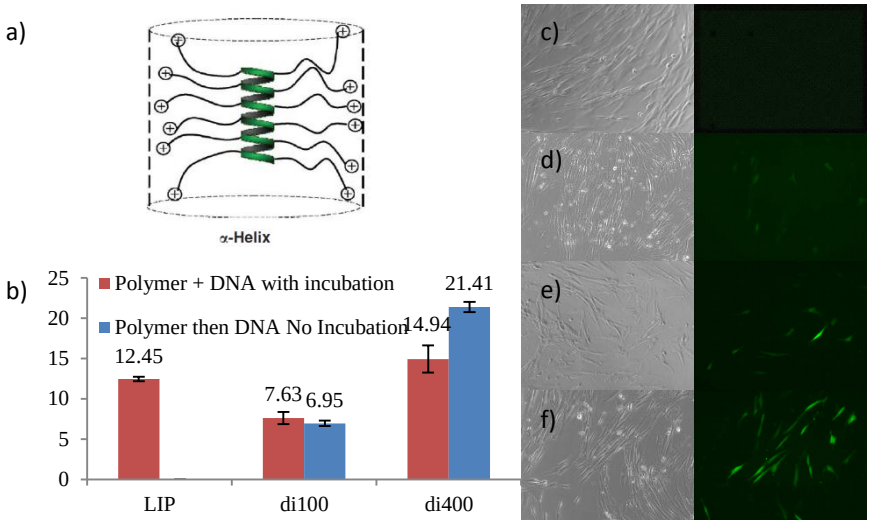

Figure Caption: a) Novel cationic helical peptide structure. b) Highest transfection efficiency obtained with polymers of GFP in IMR90 cells. Brightfield and fluorescence images of c) negative control, d) Lipofectamine 2000, e) diblock 100 and f) diblock 400.

Future Research Plans

- To study the mechanism of this novel peptide of its interaction with DNA/RNA and its ability to enter the cells
- Optimize and functionalize with new side chains or functional groups to increase gene delivery efficiency into hESC
- Use novel method to transiently overexpress key genes for highly efficient direct differentiation of hESC

[1] Lu, H.; Wang, J.; Bai, Y.; Lang, J. Liu, S. Lin, Y.; Cheng, J.* "Ionic Polypeptides with Unusual Helical Stability", *Nature Communications*, **2011**, 2, 206.