BioMEMS For Disease Detection and Treatment

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- Post-docs – Jikou Zhou, Craig Steeves
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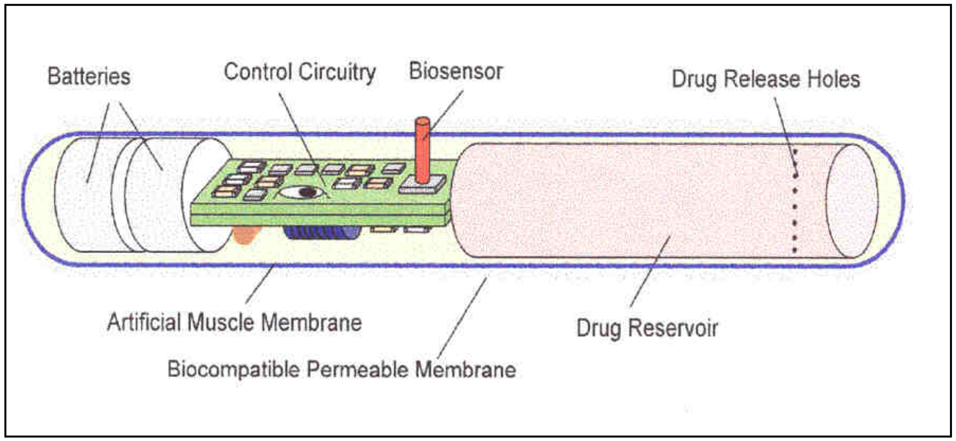
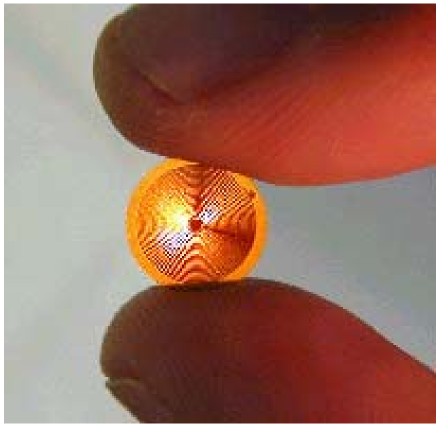
Introduction to BioMEMS Systems

- BioMEMS structures are micron-scale devices that are used in biomedical or biological applications
- At this scale, a wide range of devices are being made (e.g. pressure sensors, drug delivery systems, and cantilever detection systems)
- Explosive growth in emerging markets – civilian and military applications expected to reach multi-billion dollar levels

Drug Delivery System



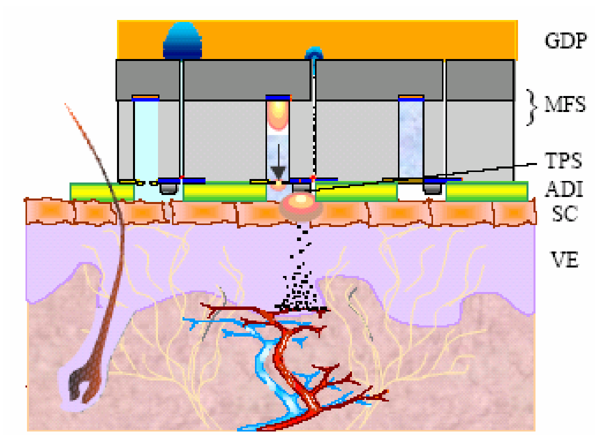
Implantable Blood Pressure Sensor



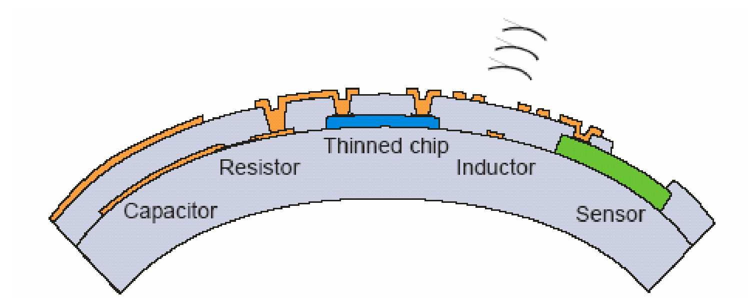
Motivation for Research on BioMEMS

- BioMEMS has the potential to produce many of the important biomedical devices
- Implantable and non-implantable systems may be used for disease detection or treatment

Dermal Patch BioMEMS

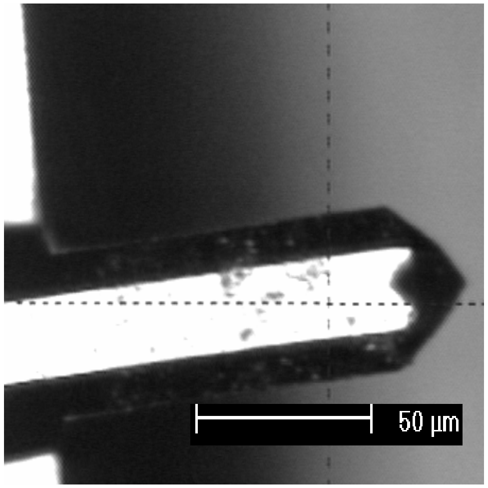


Flexible BioMEMS



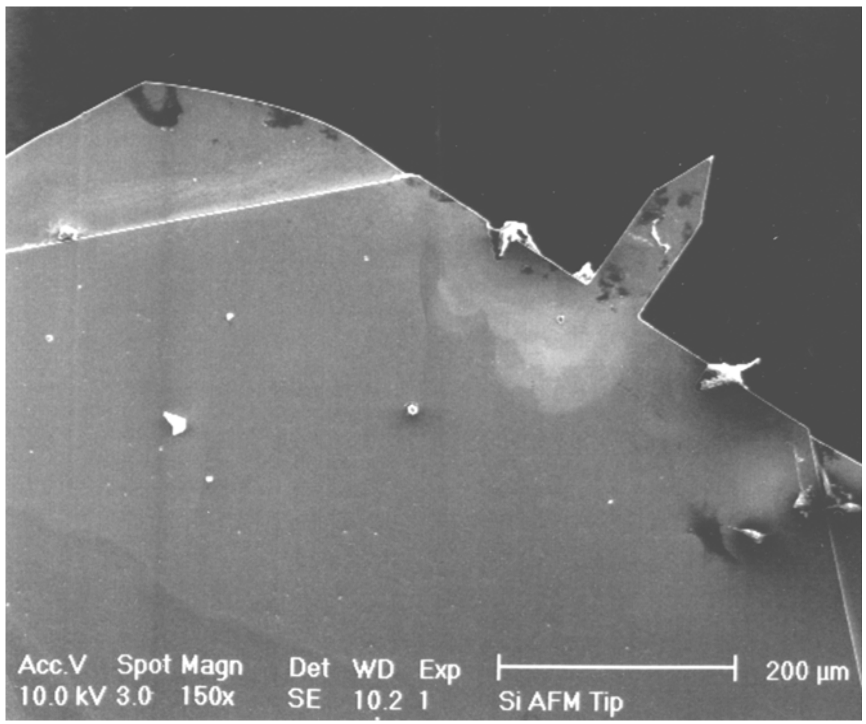
A FEW METHODS FOR DETECTING CANCER

- View under a microscope at high magnification
- Use a biochemical assay to reveal cells
- External imaging system, e.g. MRI
- Use a bioMEMS cell detector e.g. a cantilevered MEMS structure

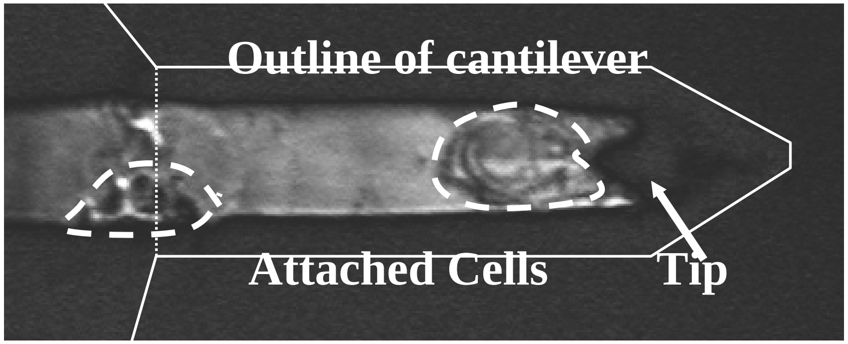


Single HOS Cell on Si Cantilever in AFM

Single cell on Si Cantilever



CELL DETECTION ON CANTILEVER



Cantilever No. 17

Initial Frequency: 263.36 KHz

Spring constant: 44.86 N/m

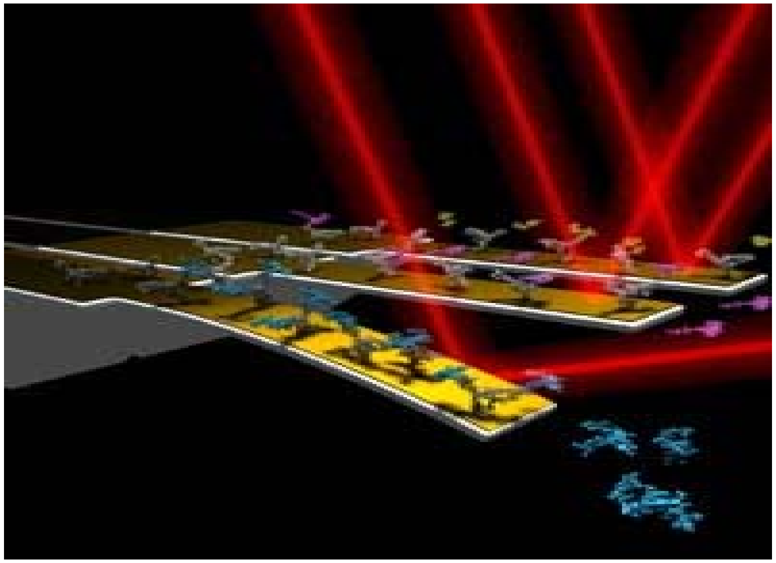
Final Frequency: 261.59 KHz

Difference: 1.77 KHz

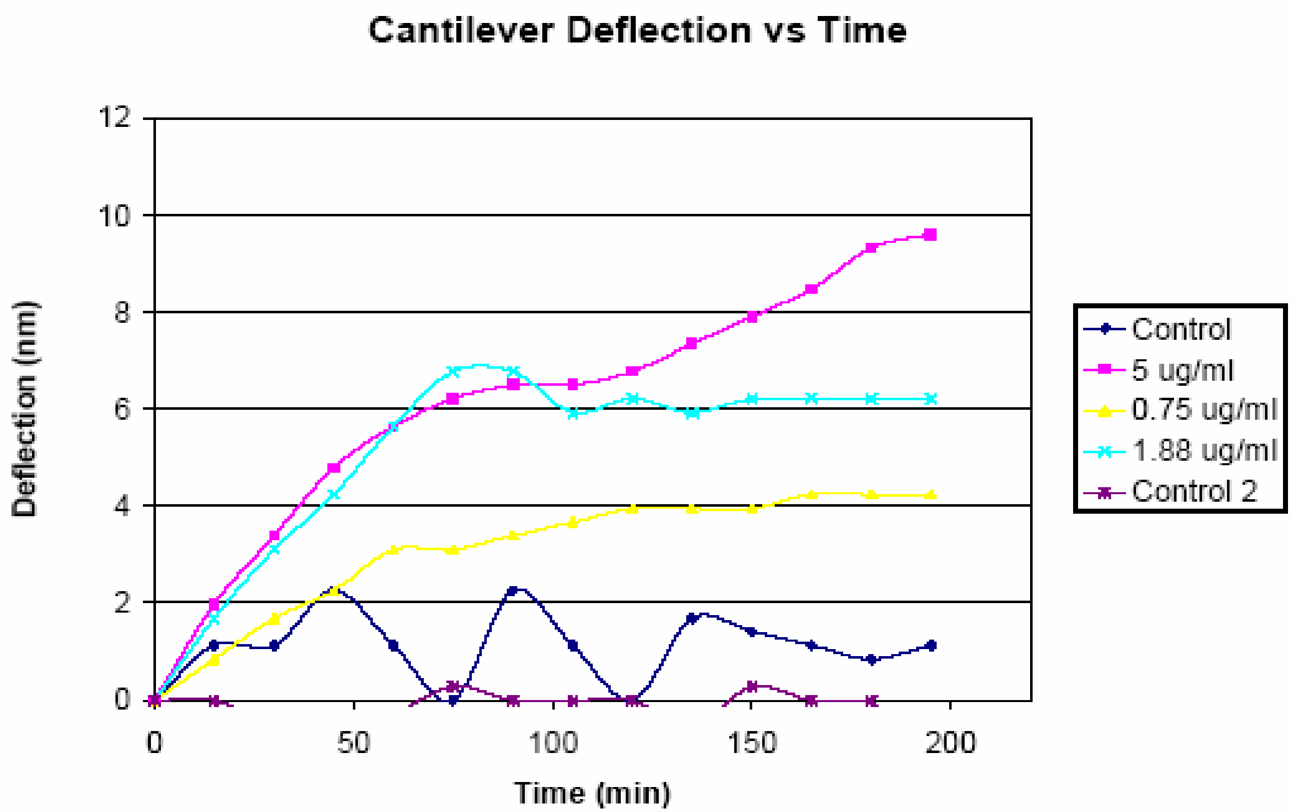
- Cantilever shows the presence of two cells
  - one attached near the tip, the other is at the base of the cantilever

Antibody/Antigen Interactions

- Antibody/antigen interactions cause surface stresses to develop
- These surface stresses are the result of new conformations of molecular structures at the surface
- Interactions between Vimentin antibodies and antigens gives rise to surface stress and cantilever deflection



Cantilever Deflection data



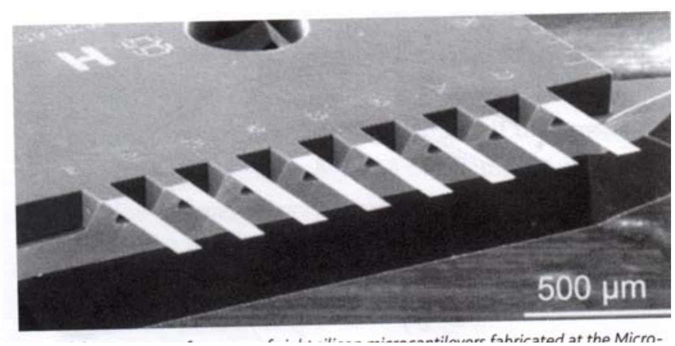
THE FUTURE OF CANTILEVERED BIOMEMS STRUCTURES – BIOMOLECULAR DETECTION

- Research will lead to future cantilevered bioMEMS structures
- Devices may be resonating devices for improved sensitivity
- However, non-resonating devices can also be used
- Multifunctional structures emerging with multiple cantilevers

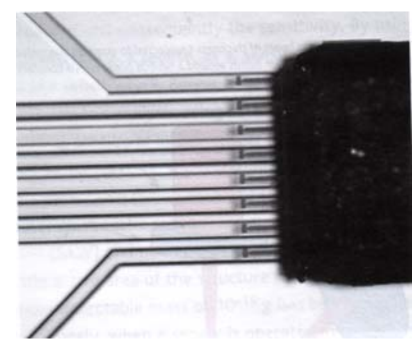
Functionalized Cantilever

- DNA
- Folic Acid
- Antibodies

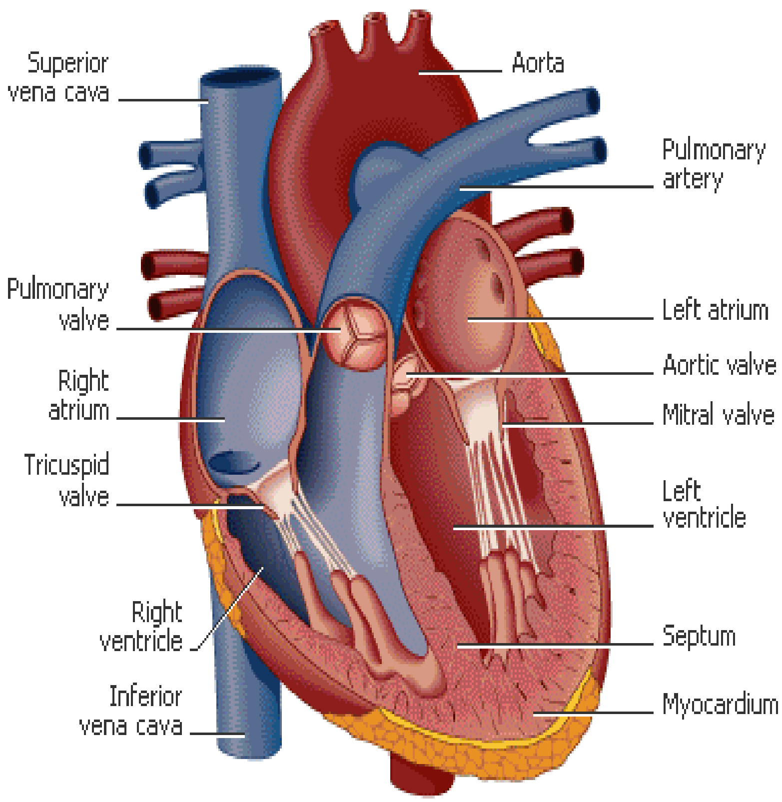
Microcantilever Array



Packaging

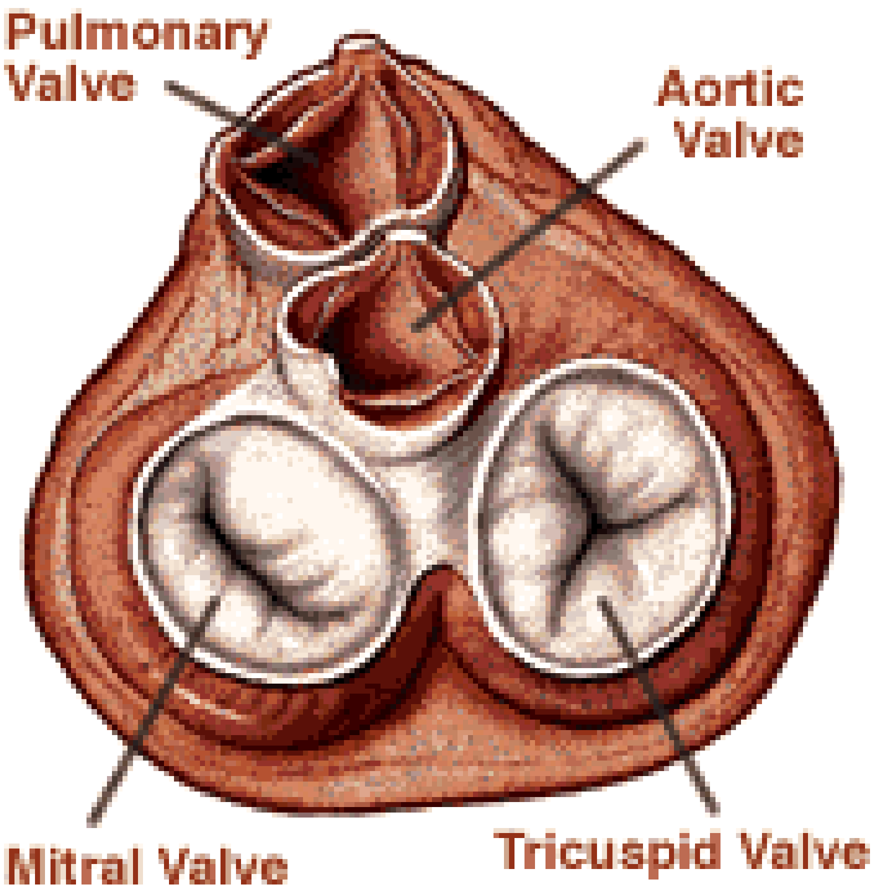


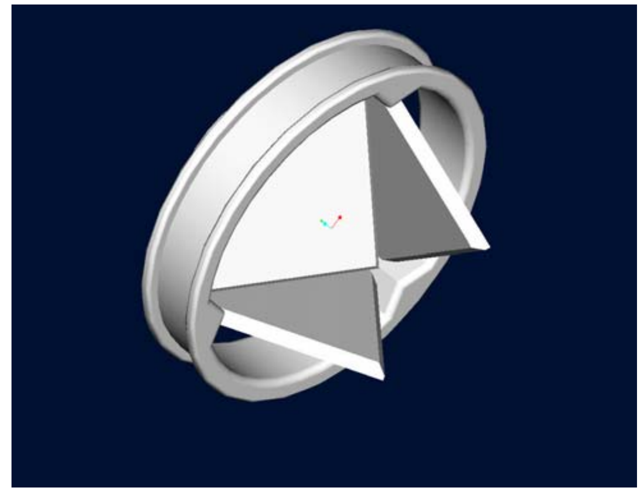
The Heart and Cardiovascular Implants

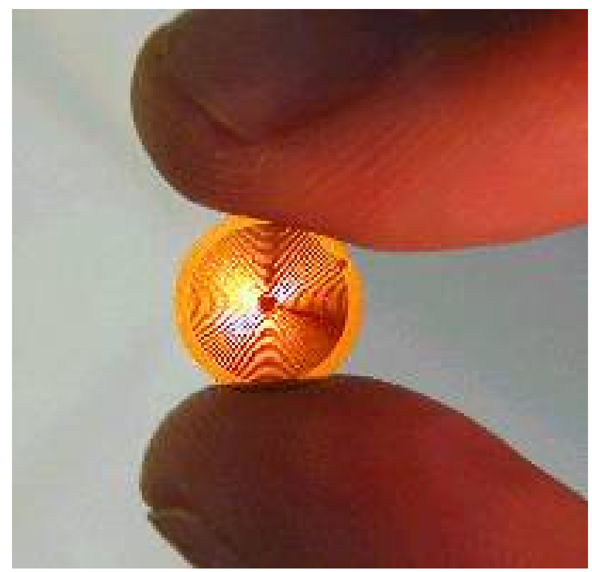


- The heart is a pump that sends oxygenated blood throughout the body
- It consists of four chambers(R/L ventricle/atrium) and four valves(tricuspid, pulmonary, mitral, and aortic)

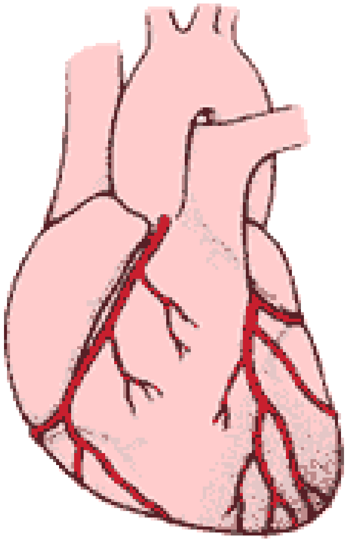
MEMS-Enhanced Trileaflet Valve



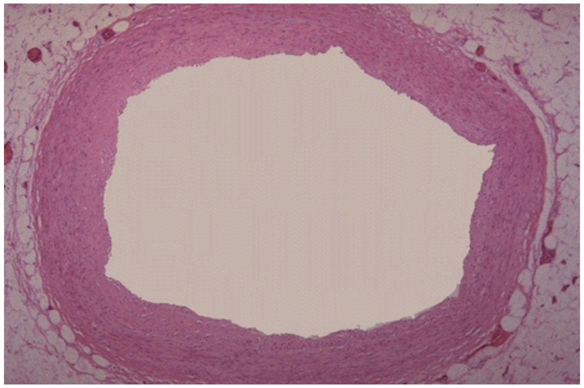


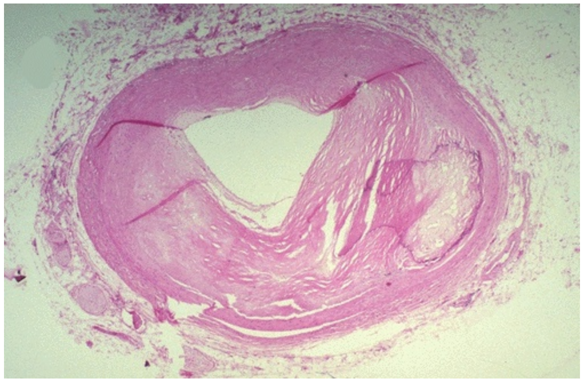


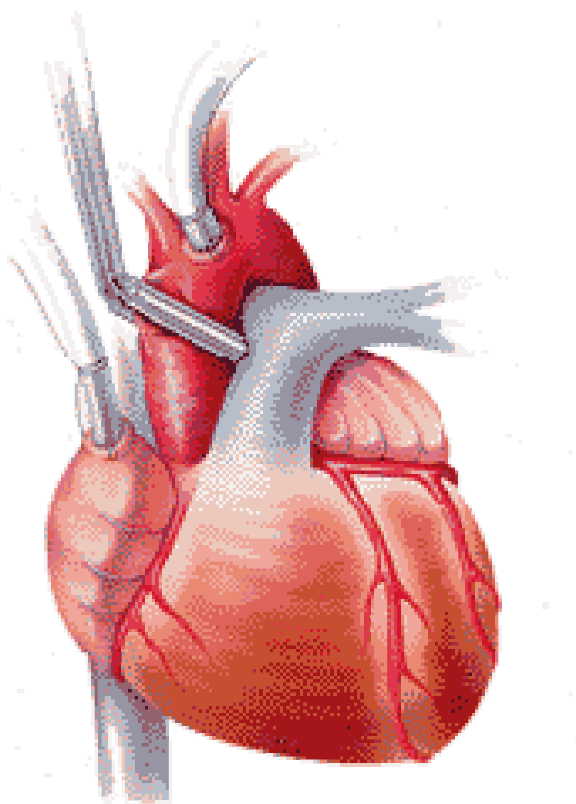
Atherosclerosis

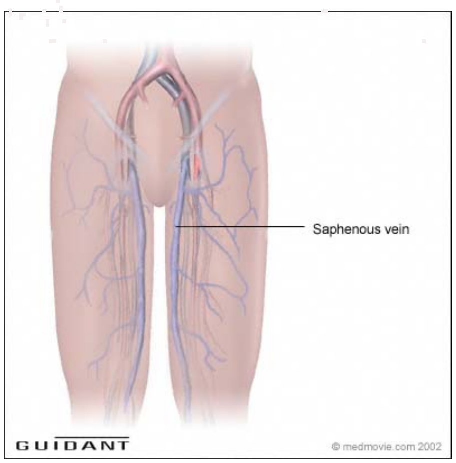


- Atherosclerosis is the hardening and narrowing of blood vessels caused by buildup of plaque
- Plaque is made up of cholesterol, calcium, and other blood components that stick to the vessel walls
- When plaque bursts, blood tends to clot, thus creating more blockage









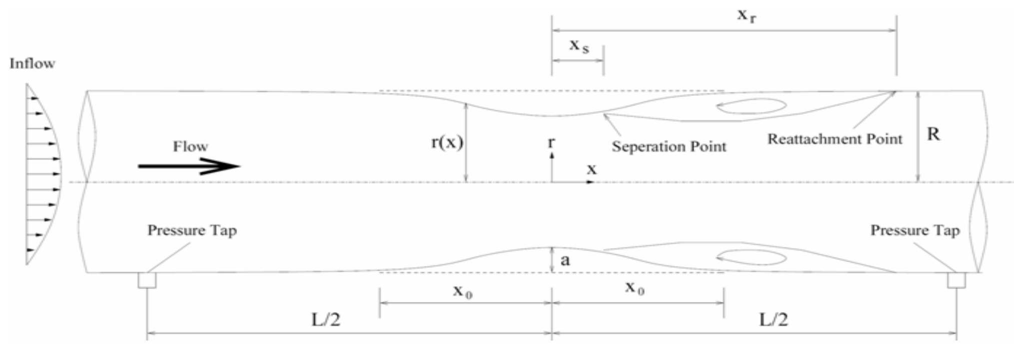
Bypass Surgery

- There are over 300,000 bypasses performed each year
- In a bypass, blood vessels from other parts of the body are used to “bypass” the stenosis
  - Often, the saphenous vein from the thigh is used, as it is quite long
- Unfortunately, 20-30% of bypasses become restenosed within 10 years of surgery

Determining Stenosis

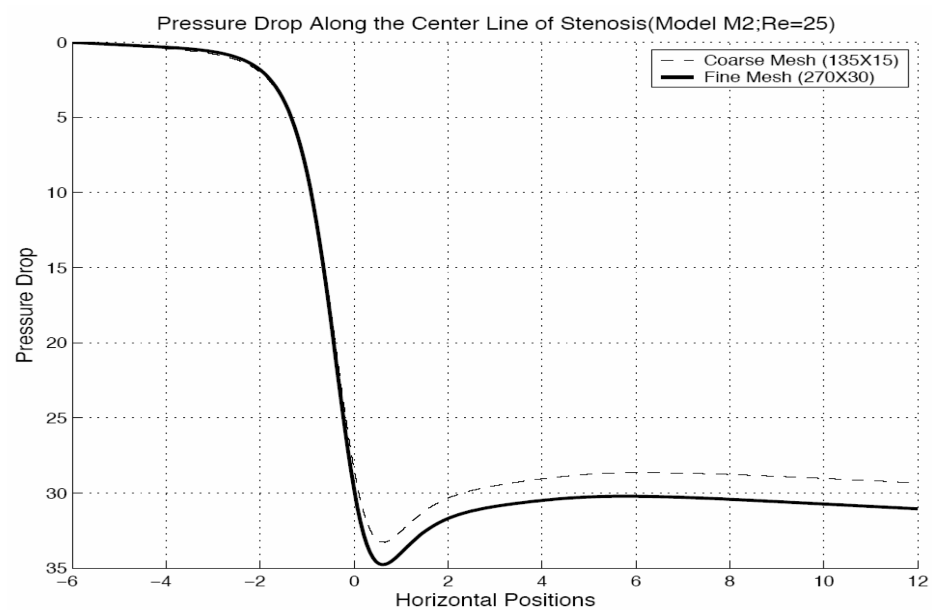
- Degree of stenosis is the percent decrease in area
- There are several ways in which a stenosis affects fluid flow, and by studying these effects, stenosis may be predicted
- Pressure losses, localized increased velocity, lower flow rates are effects of stenosis

Effects of stenosis

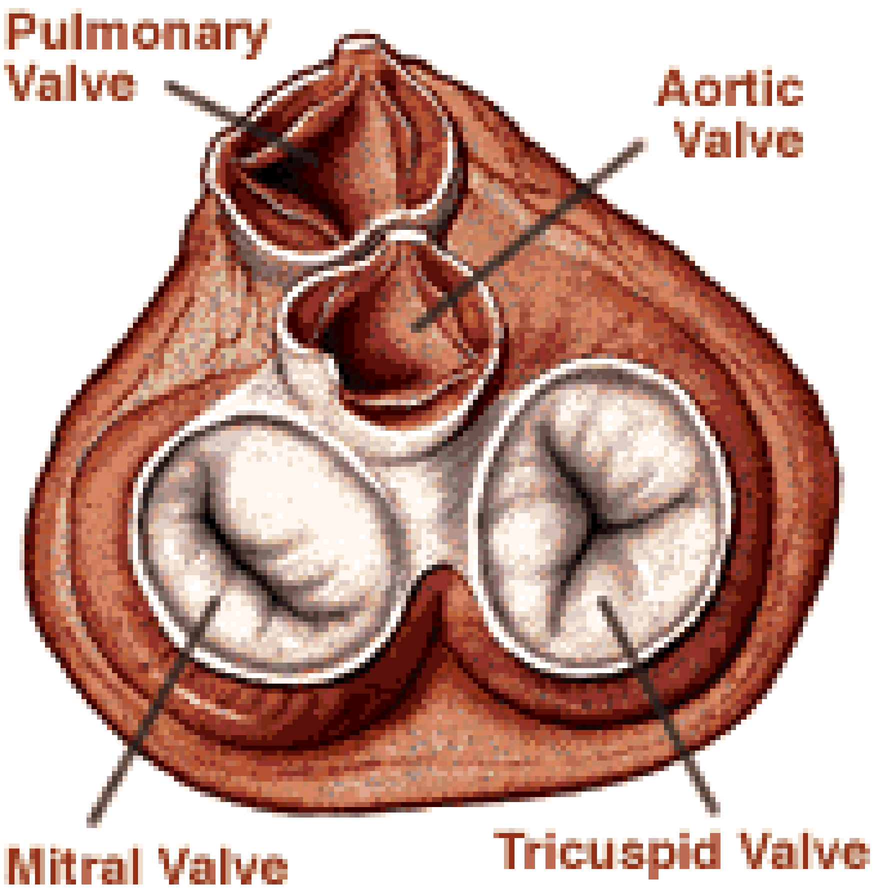


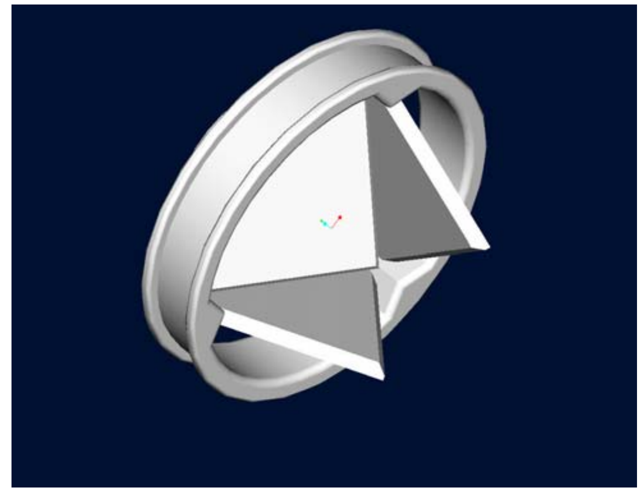
-Pressure drop due to localized velocity increase

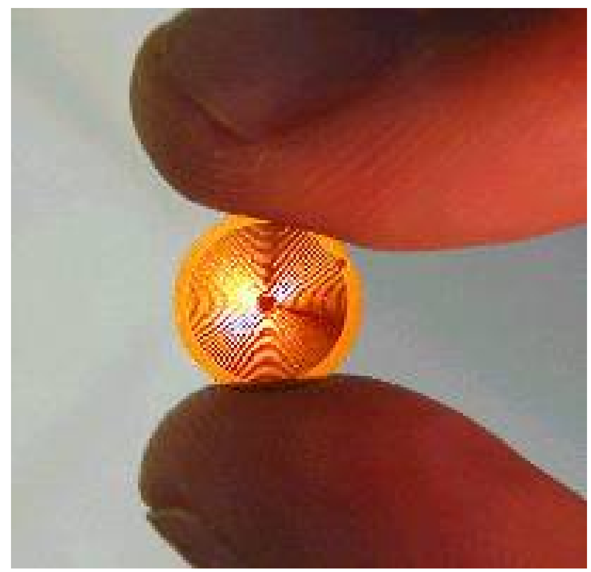
-Some pressure recovery after stenosis



MEMS-Enhanced Trileaflet Valve

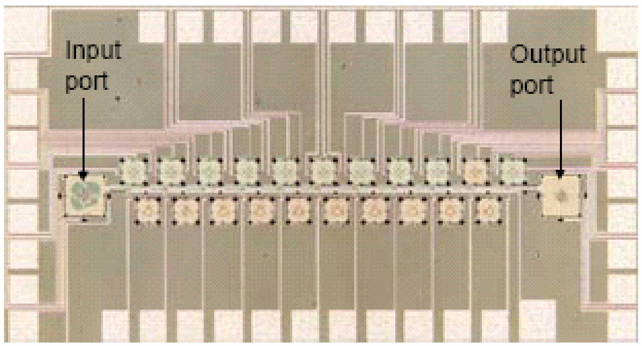






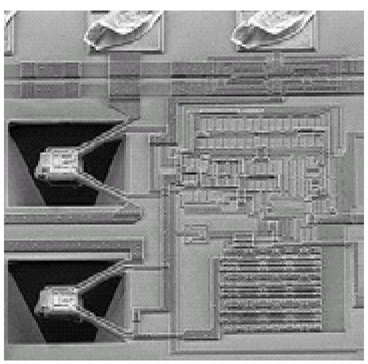
Some Microfluidics/BioMEMS Devices

Fluidics + Pressure Sensing

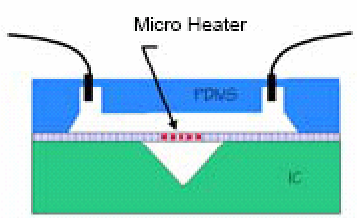


C.-M. Ho (UCLA/MAE), Y.C. Tai (Caltech)

Heater + Circuits + Fluidics



Heater + Circuits

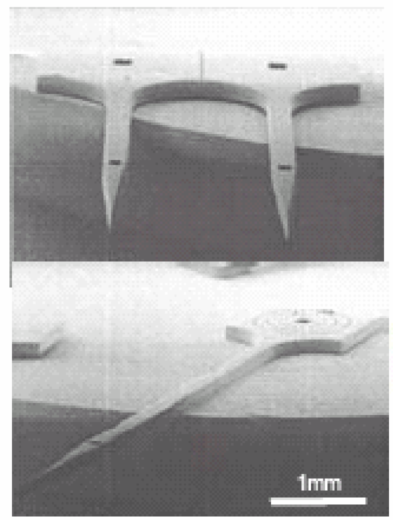


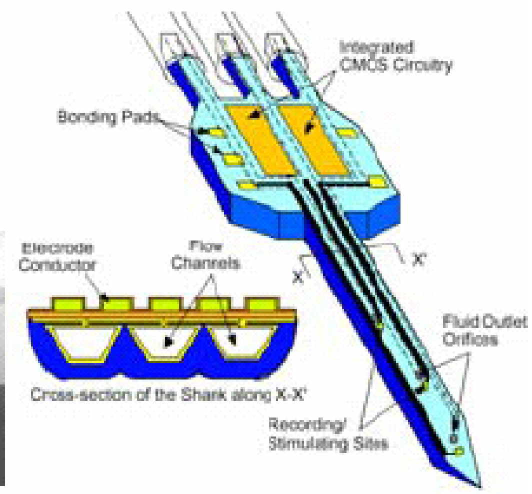
Heater + PDMS Fluidics

⇒ Addressable Micro-reactor

Photo courtesy of M. Gaitan and L. Locascio, NIST

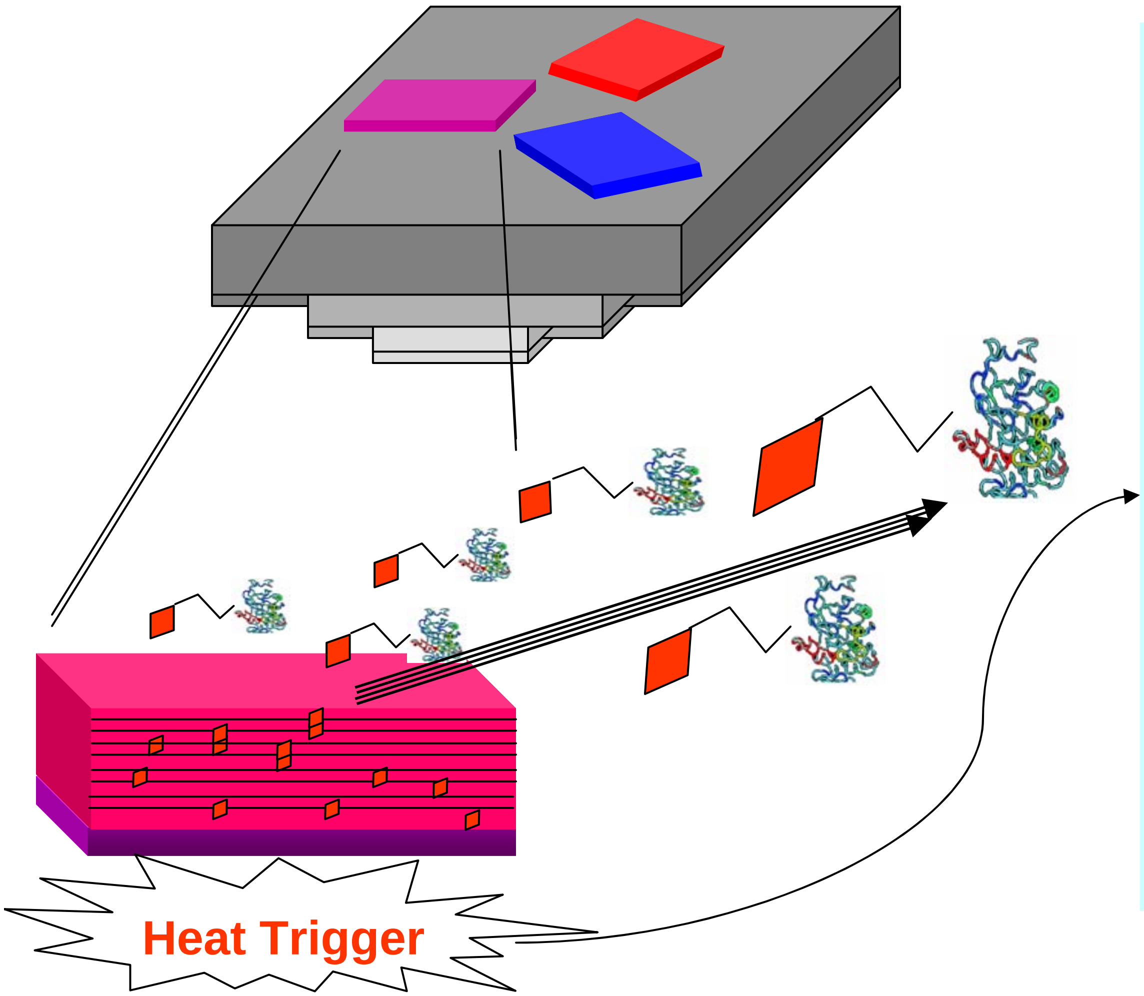
Drug Delivery Platforms





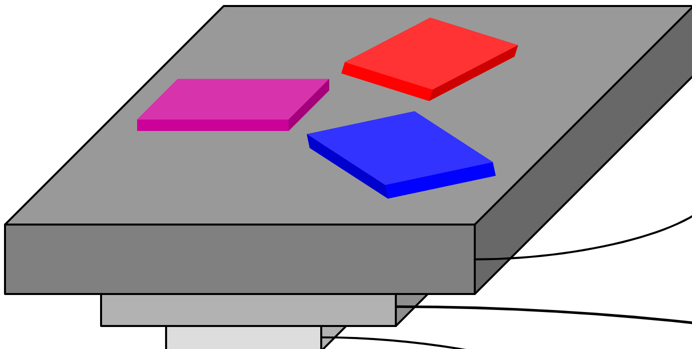
Photos courtesy of N. Talbot and A. Pisano, UC Berkeley
Diagram courtesy of K. Wise, U. Michigan.

Drug Delivery by Resistive Heating



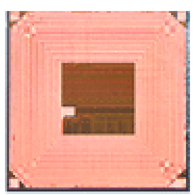
- Hydrogels sit on metallic plates
- Current running through plates heat plates
- Temperature controlled by current
- Current controlled by open/closed switch programming

Electrical Components



3) Battery

1) RFID Tag

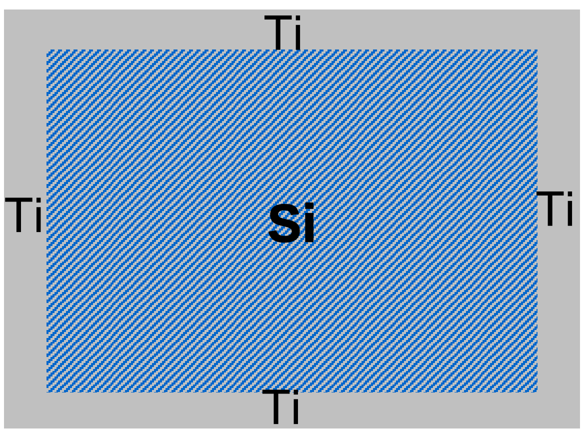


2) Microprocessor

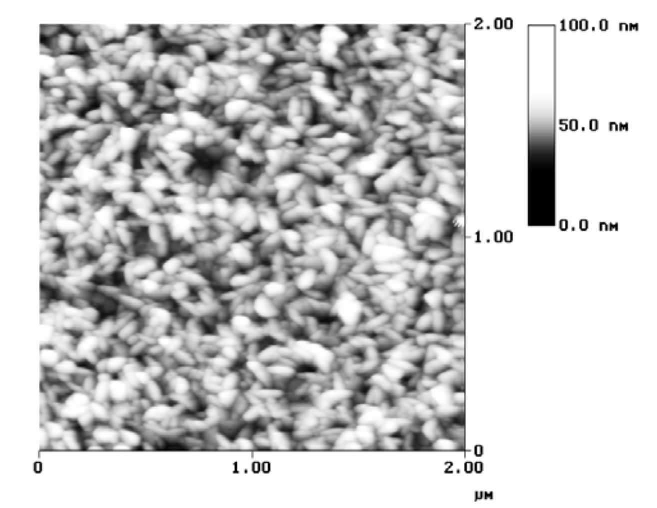
Biocompatibility of Silicon MEMS SystemS

- Si is not the most biocompatible material
- Can be made biocompatible through the use of polymeric or Ti coatings.
- Polymeric coatings used on Si drug release systems.
- Ti coating approaches are also being developed.

Coated BioMEMS Structure

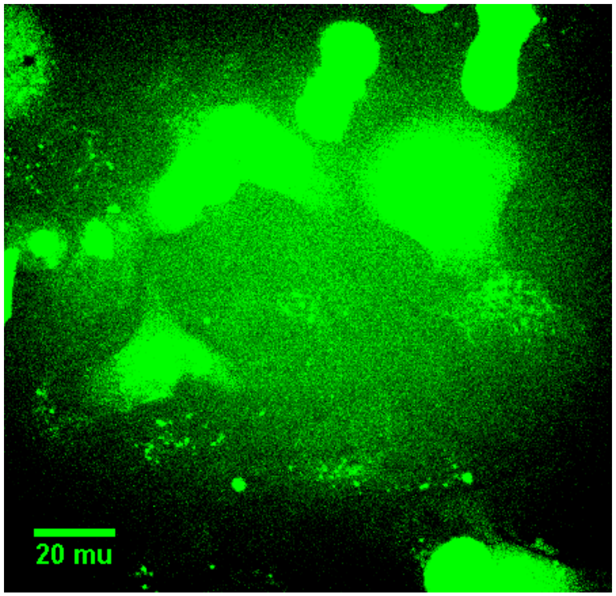


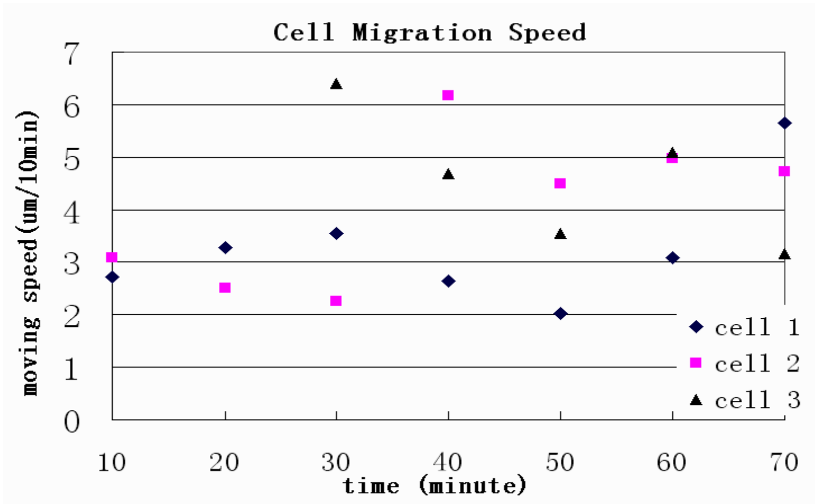
500 nm Ti Layer on Si



Live Imaging of Cell Dynamic and Adhesion Using *In-Situ* Confocal Microscopy

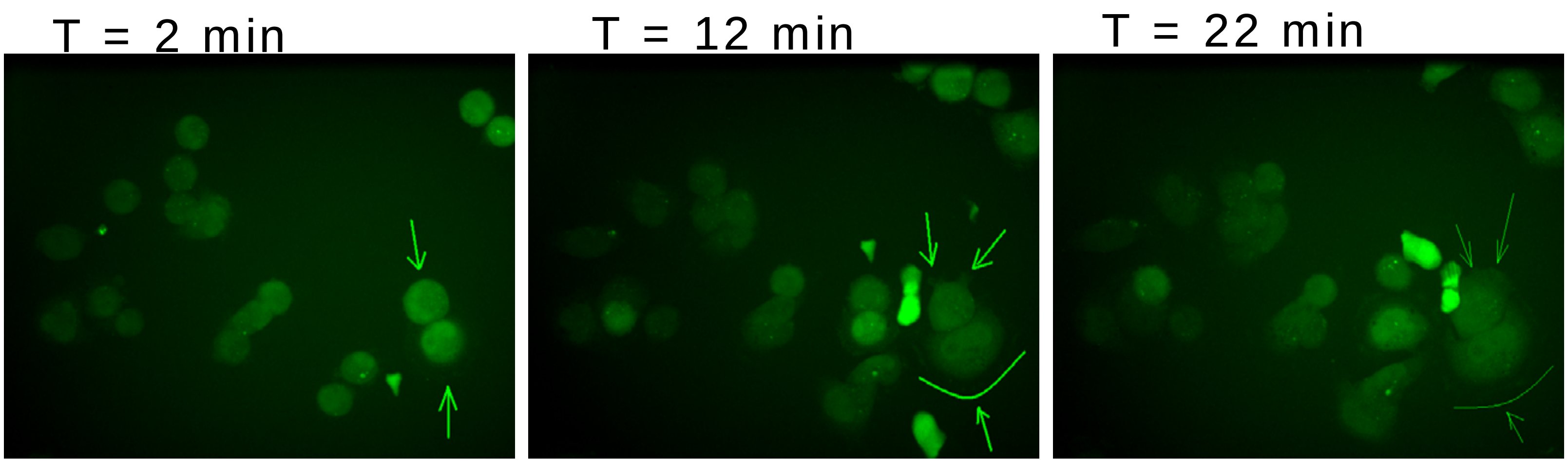
- Cell migration speed and adhesion may reflect cell age and disease
- May be used in BioMEMS devices to detect disease
  - Our work explored HOS cells on PDMS
  - Nishiya et al (2005) bound paxillin to the α_4 integrin subunit inhibits adhesion-dependent lamellipodium formation. A significant decrease in migration speed of hamster ovary cells drops from 22 μm/h to 8 μm/h





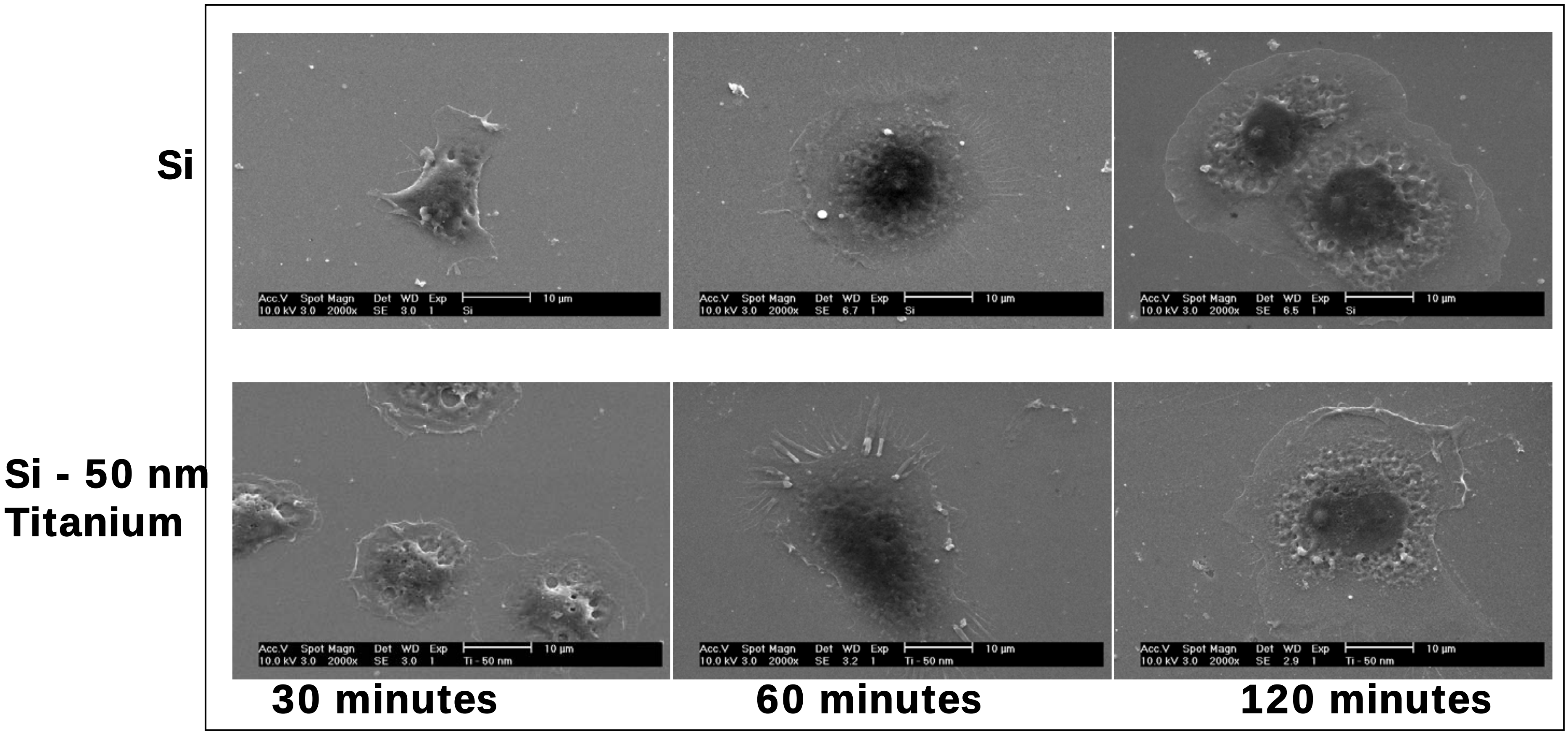
Live Imaging of Cell Adhesion Process

- Cell migration is a complex but regulated process
  - Involving the continuous formation and disassembly of adhesions
  - Adhesion formation takes place at the leading edge of lamellipodium, whereas disassembly occurs both at the cell rear and at the base of lamellipodium (Webb, 2004)

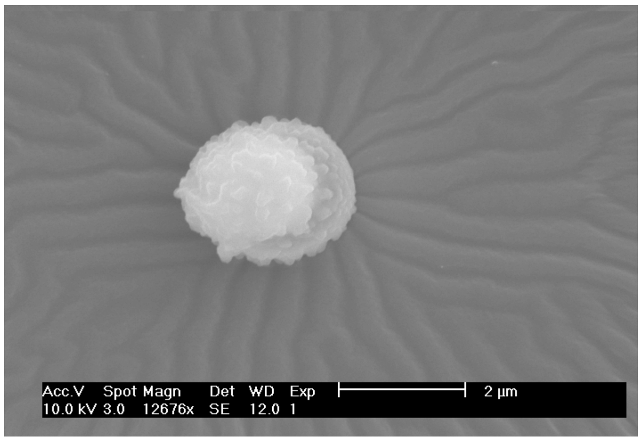


SURFACE CHEMISTRY – CELL SPREADING

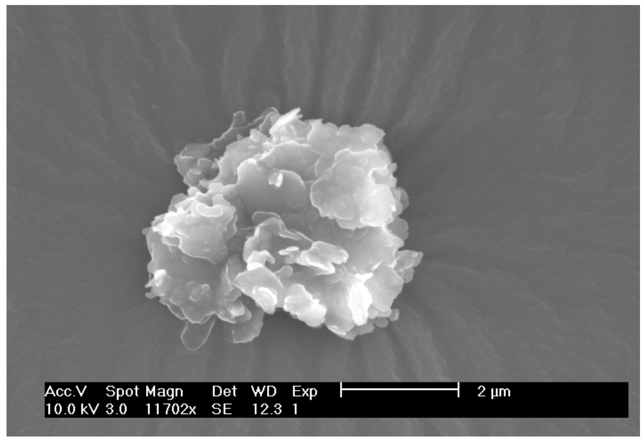
HOS Cells



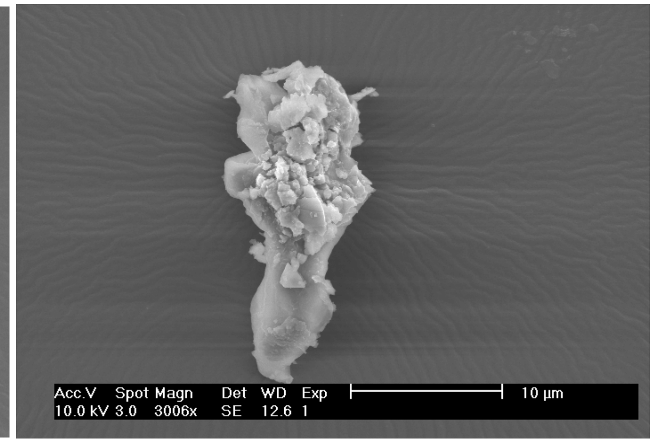
HOS Cell Spreading on Smooth PDMS Surface: Single Cell



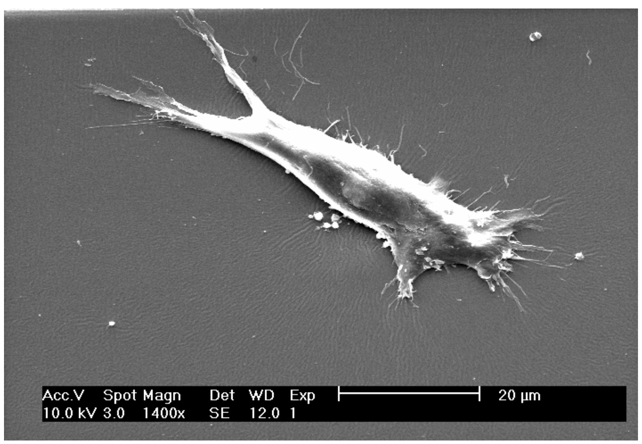
3-hour



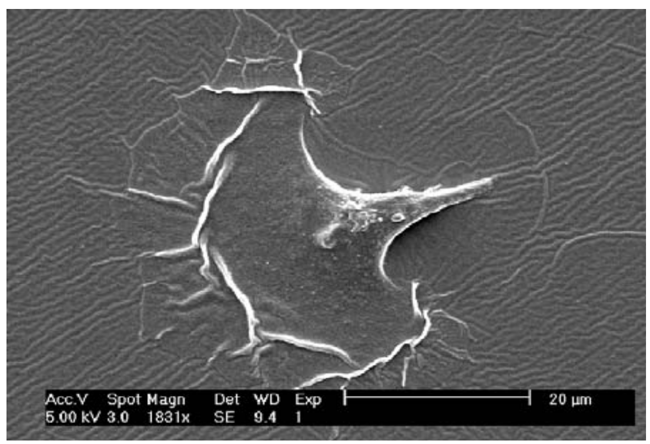
4-hour



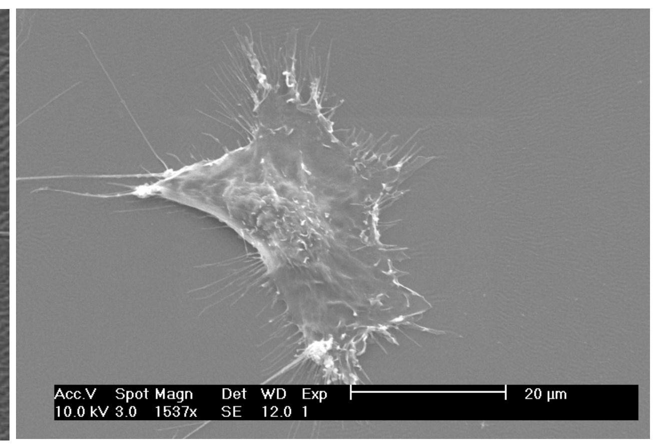
6-hour



2-day



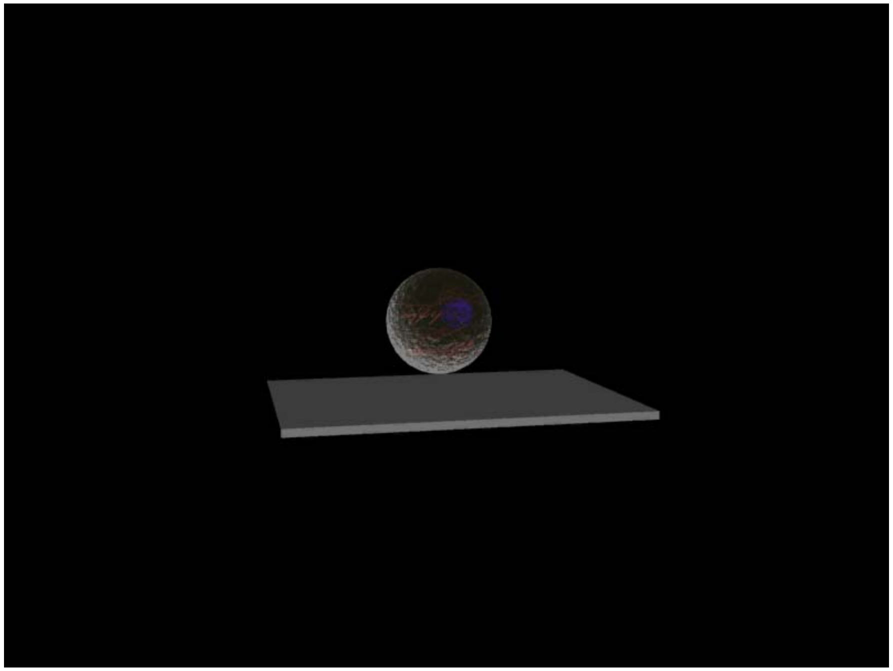
3-day



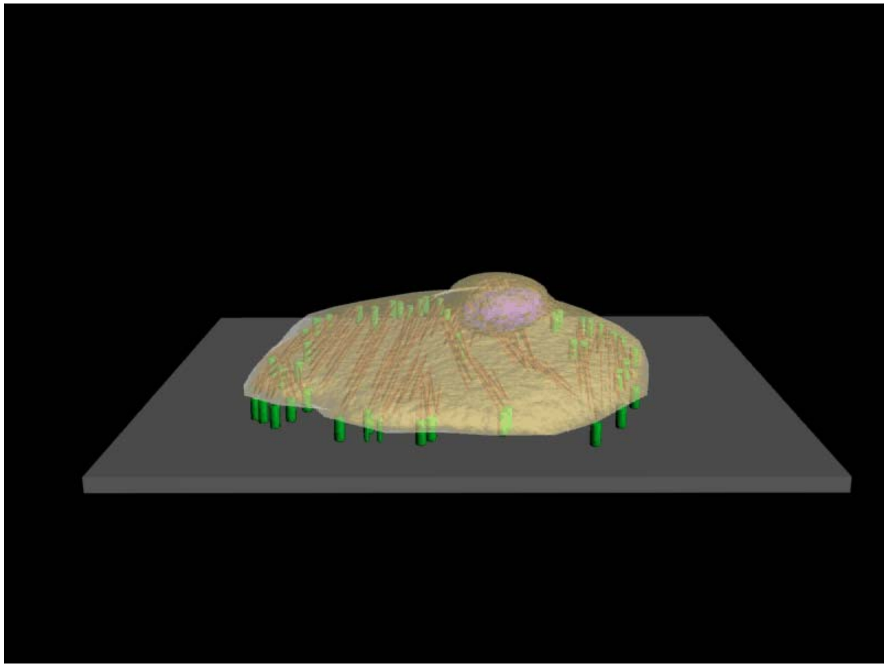
5-day

Cell Attachment on PS/Ti Surfaces

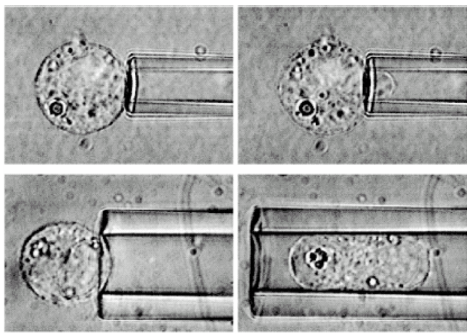
Cell Spreading on PS/Ti Surface



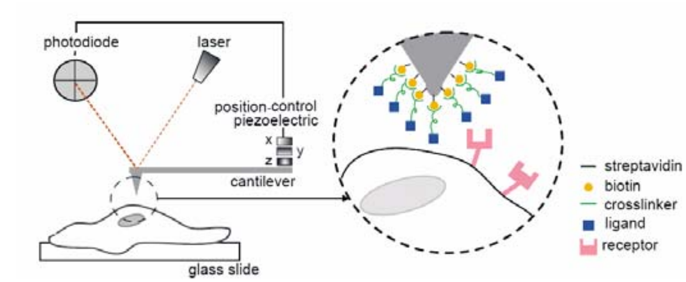
3D View of Attached Cell



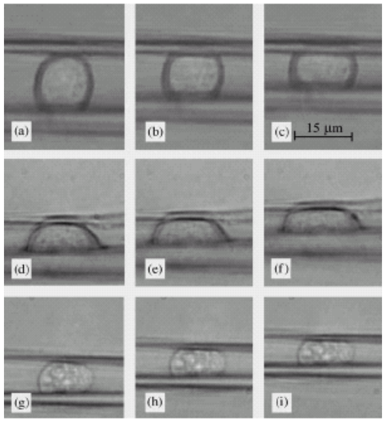
Potential Approaches for the Study of Cell Deformation and Adhesion



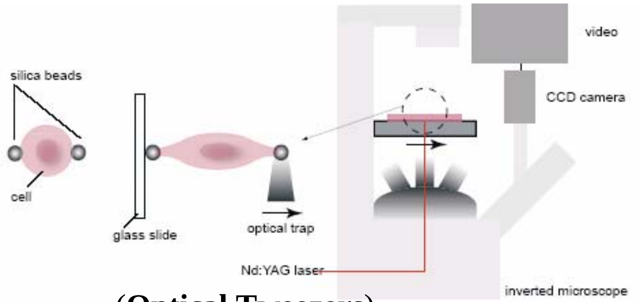
(**Micropipette Aspiration**)



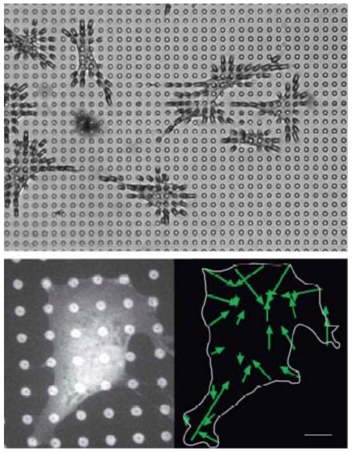
(**Atomic Force Microscopy**)



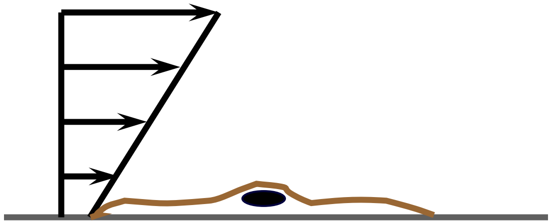
(**Microplate Compression**)



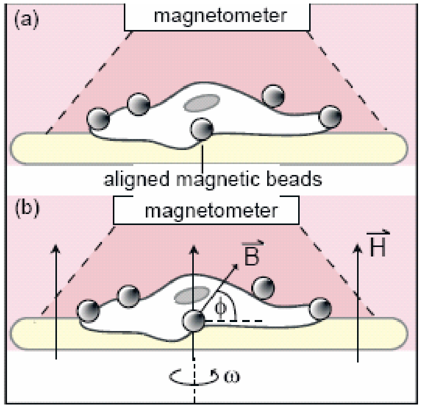
(**Optical Tweezers**)



(**Microfabricated Post Array Detector**)



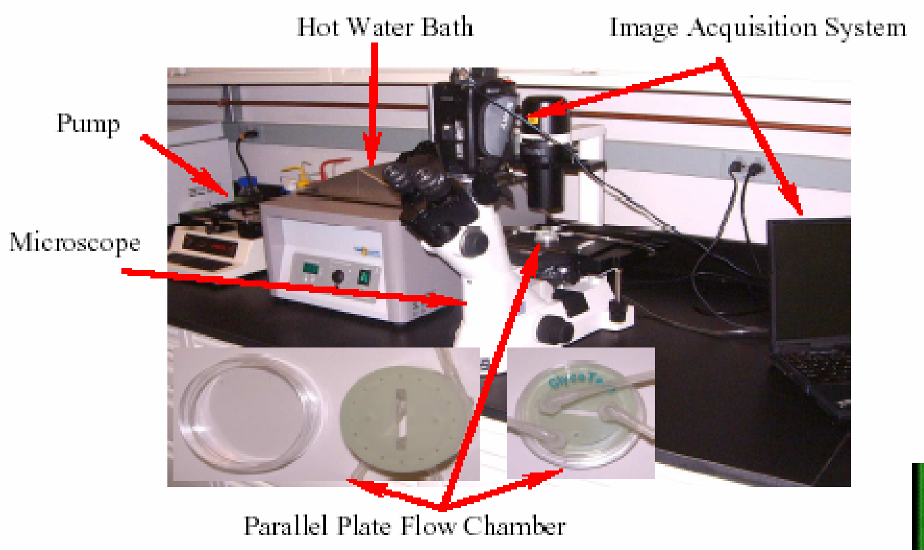
(**Shear Assay**: Current Study)

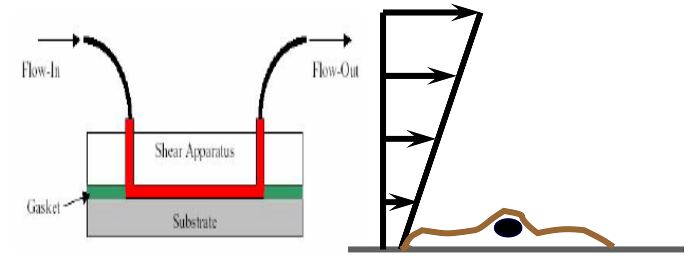


(**Magnetic Twisting Cytometry**)

Shear Assay Experiment

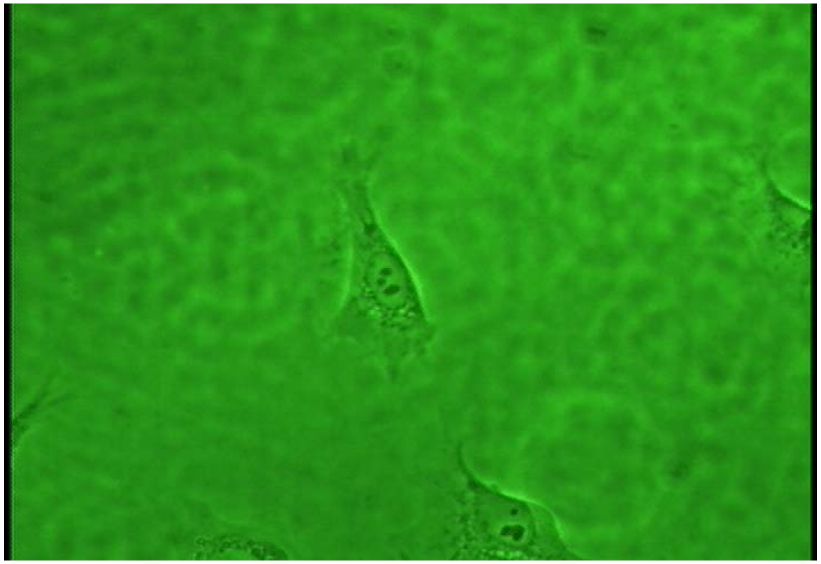
- Measurement of the interfacial strength



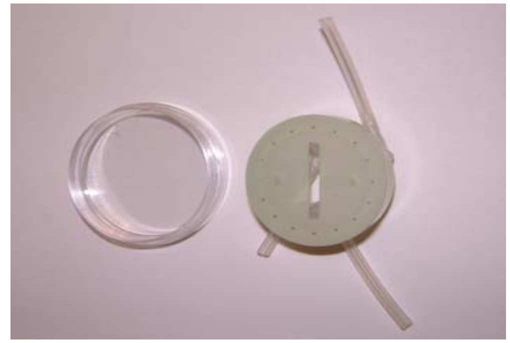


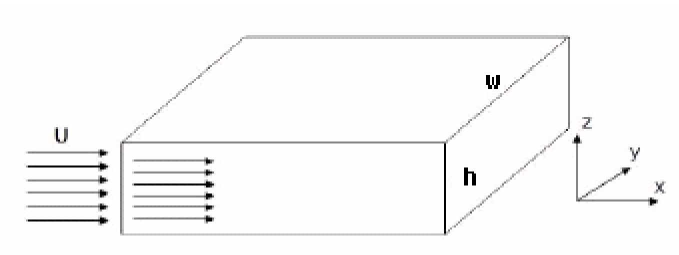
  - Syringe pump: controllable flow rate
  - Flow chamber: build up flow region
  - CCD: capture cell detachment under shear flow
  - Water bath:

    keep temperature (37 ^{o}C)



Fluid Flow Through Micro-Channel





- Flow chamber:
  2.5mm width
  20.5mm length
  0.254mm height

- Analytical consideration
  incompressible,
  Newtonian fluid,
  laminar,
  typical channel flow.
- Width >> height
  Fully-developed duct flow is simplified to 2D parallel flow

$$\tau_w = \frac{6\mu Q}{wh^2}$$

Shear Assay Results

Shear Stress at detachment for 2 Day HOS cultures

Determined as wall shear stress given by:

$$\tau = \frac{6Q\mu}{wh^2}$$

Material	Adhesion Strength (Pa)
Polystyrene	70
Ti-Coated Polystyrene	81
Silicon	82
Ti-Coated Silicon	104

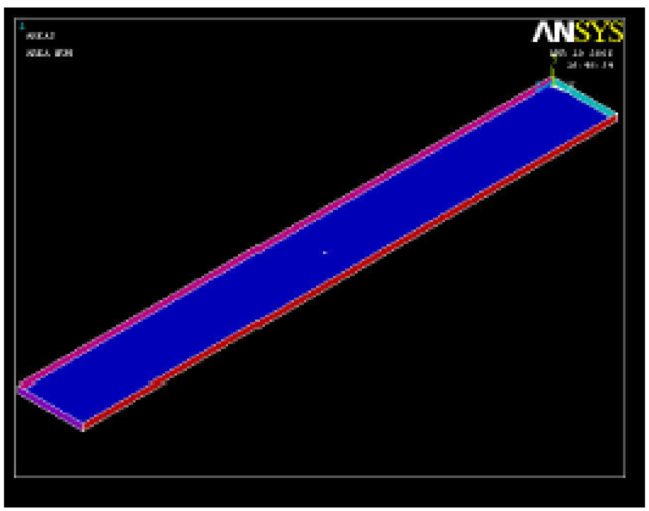
CFD Simulation

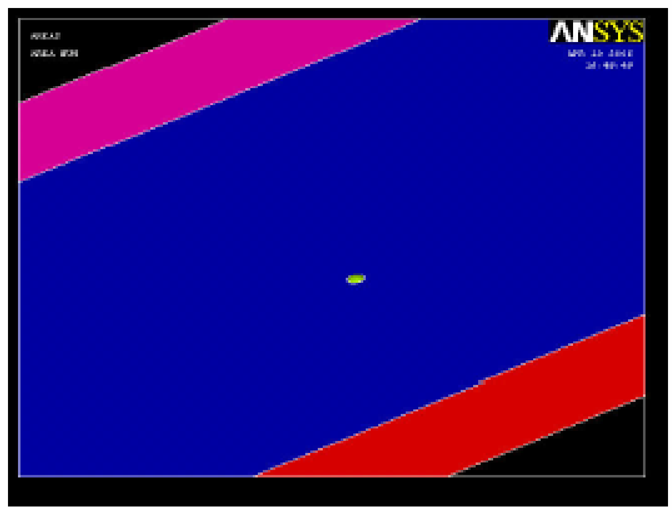
- Fluid mechanics modeling

  Computational Fluid Dynamics (CFD)

  →

  Detailed Information on Flow Field





Theoretical Basis

- Knudsen number $Kn = \frac{\lambda}{L}$

  Navier-Stokes equation: continuous flow

- Mean fre

  For gas: $\lambda = \frac{1}{\sqrt{2}nd^2} = \frac{m}{\sqrt{2}\pi\rho d^2} = \frac{kT}{\sqrt{2}\pi d^2}$

  1 atm, 20^{o}C, air λ ~ 68 nm

  distance between liquid molecules << gas λ

- Current microfluidic devices can be modeled using Navier-Stokes equations in this regime
  (Tabeling, 2001)

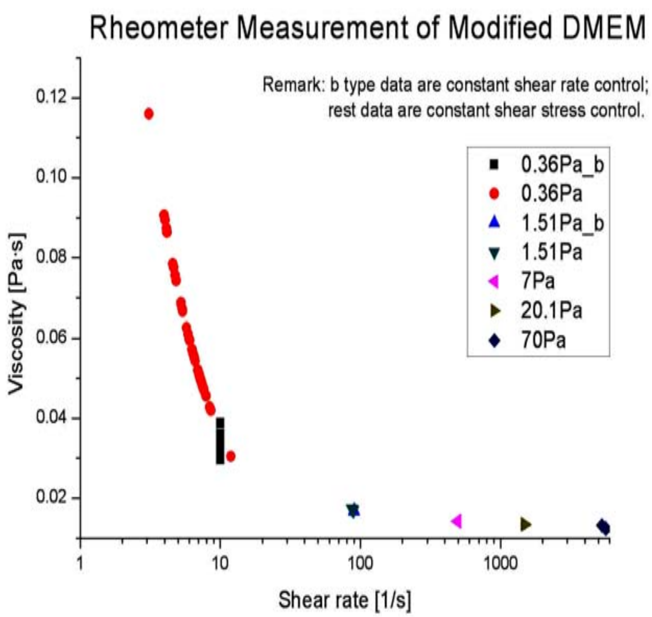
Fluid Property Measurement

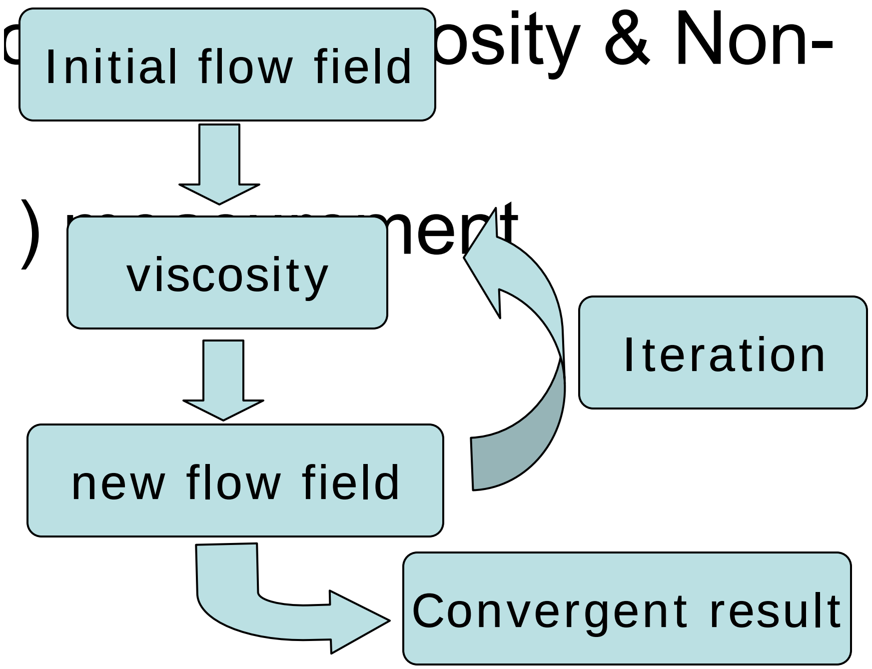
- Modified Dulbecco's Modified Eagle's Medium (DMEM)

  60% Methylcellulose + 40%DMEM

  osity & Non-

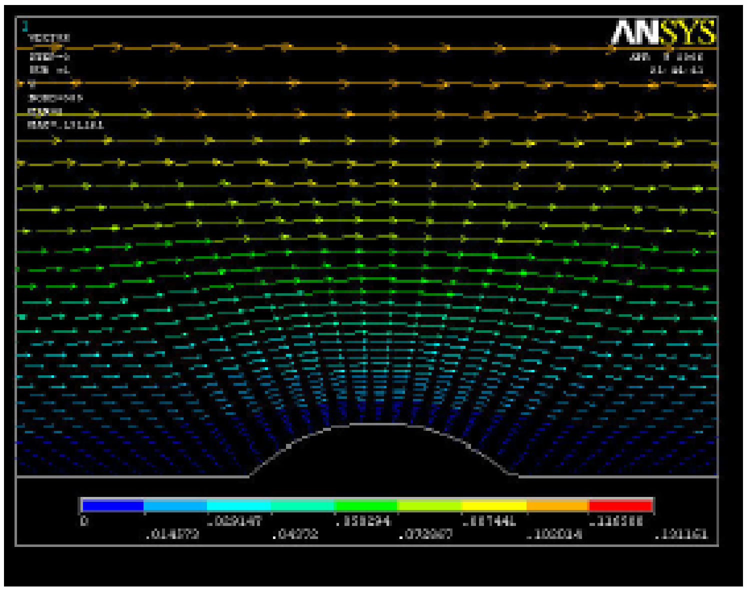
)



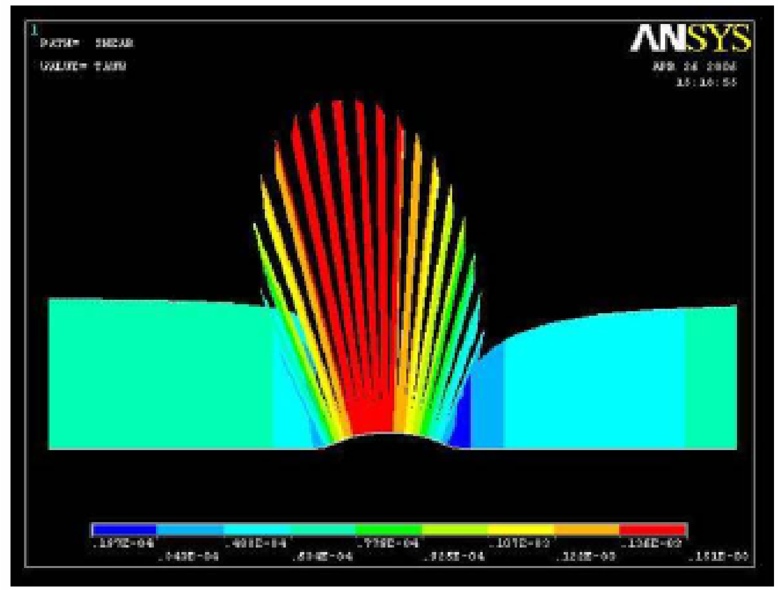


CFD Simulation (2D Check)

Flow Field (Velocity Vector)



Wall Shear Stress Distribution



- Ansys CFX (finite volume method)
  flow rate: 200ml/hour (0.0875 m/s^-1), constant viscosity: 0.0334 Pa·s
  For fully developed flat region:
    Analytical wall shear stress: 69.03Pa
    CFX wall shear stress: 69.08Pa

Cell 3D Modeling

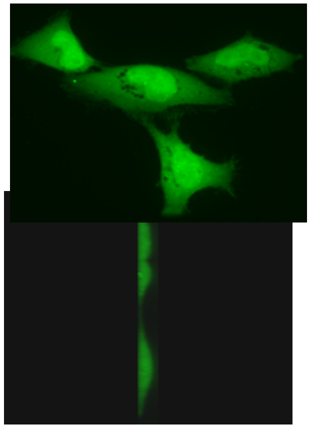
Confocal measurements:

- Cell adhesion area:
  effective adhesion circle
- Cell morphology:
  spline fit curve

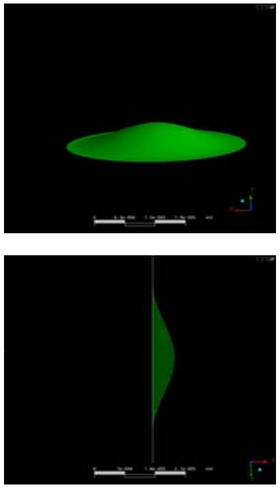
2Day HOS cells culture on Silicon

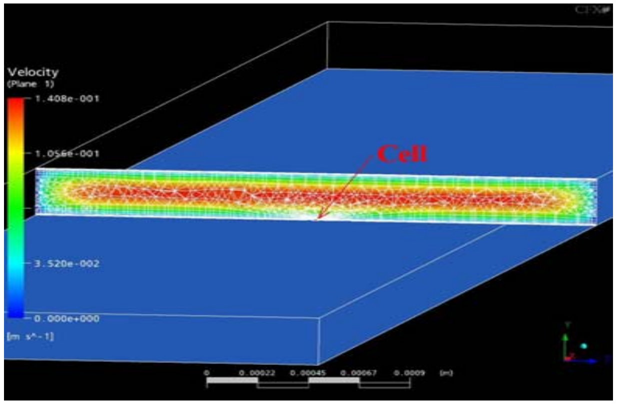
- Average adhesion area:
  $A=1087.5\ \mu m^2$
  radius of effective adhesion circle:
  $r=18.6\ \mu m$
- Average maximum height:
  $H= 6.6\ \mu m$
  modeling of cell morphology:
  spline fit curve based on r & H

Real cell



Model cell

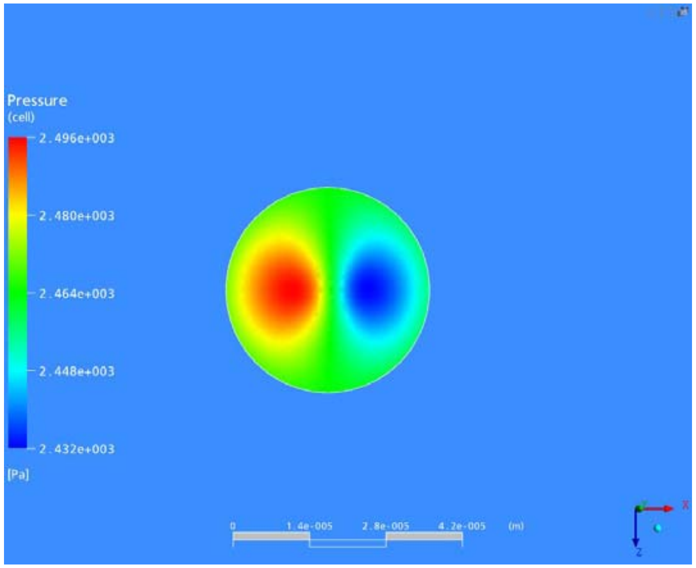




Shear Force on Cell Surface

Pressure Drag Distribution

- 2Day HOS on Ti-coated Silicon

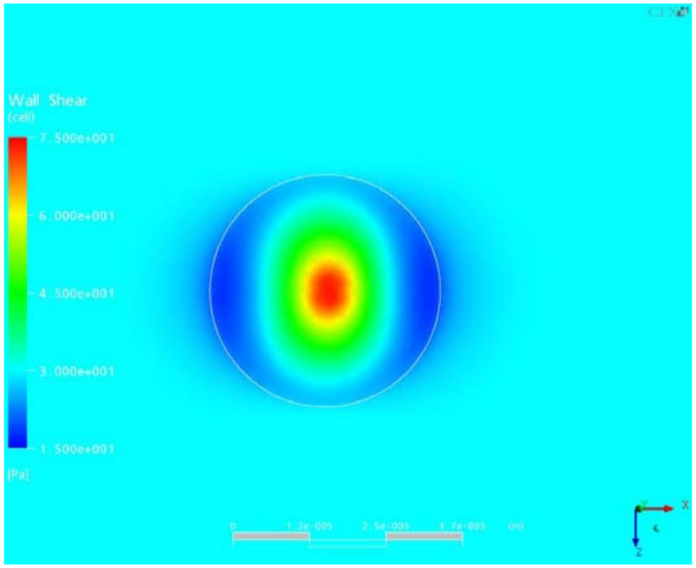


upstream face: 2496 Pa

downstream face: 2432 Pa

(relevant pressure)

Wall Shear Stress Distribution

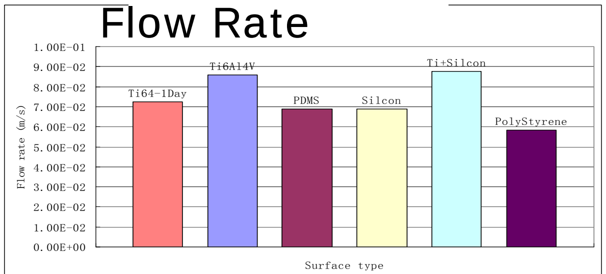


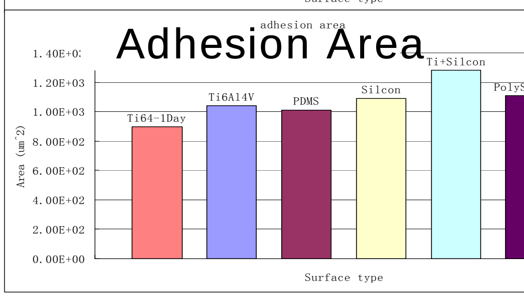
foot point: 17.8 Pa

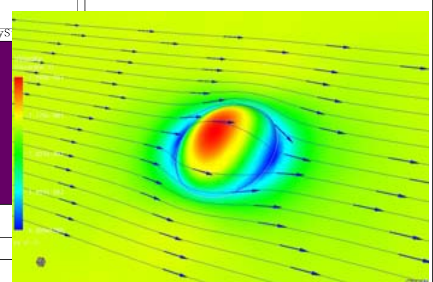
Peak point: 73.7 Pa

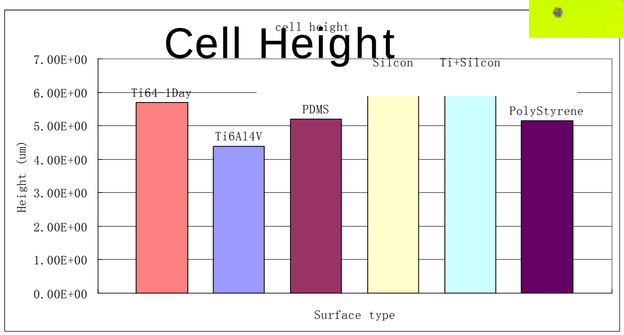
Forces on Cell Surfaces

Experiments

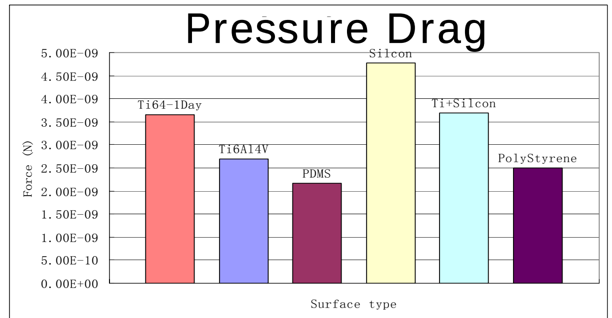


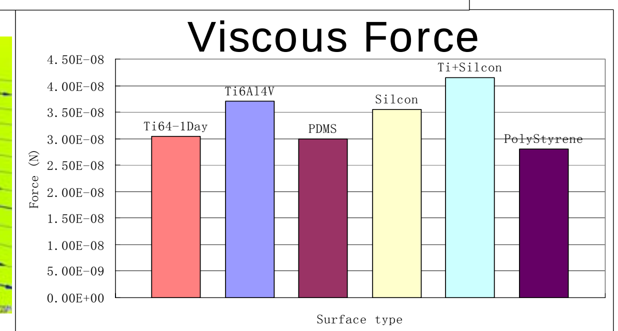


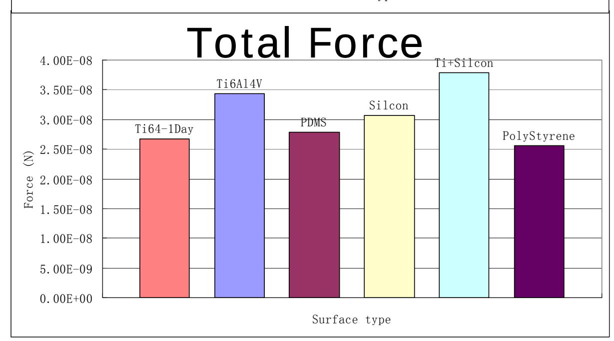




CFD Results

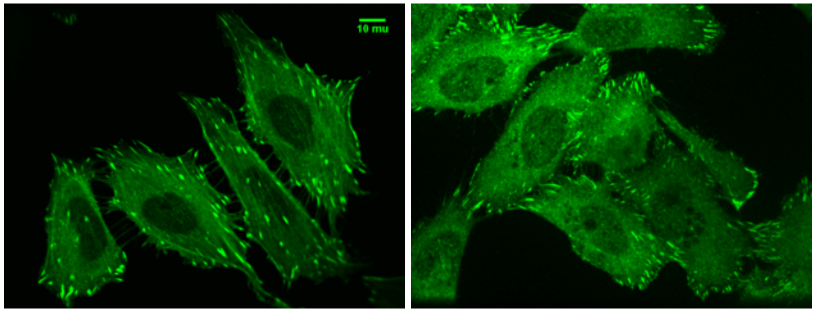


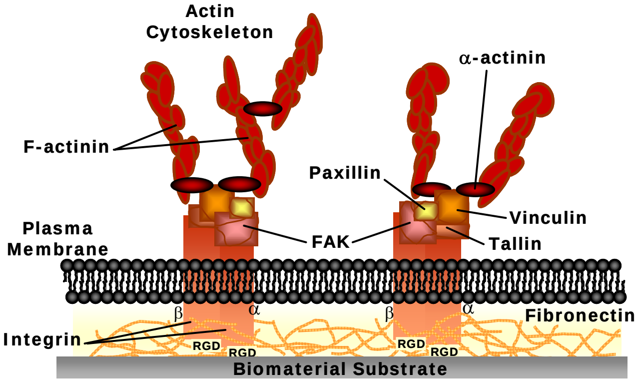




Insights Into The Adhesion Strength

- IF staining (vinculin)
  - Focal adhesion spots per cell: ~43
  - Spots area: ~1.8 μm^2
- Integrin based contact in cell matrix adhesion
  - Cytoskeleton —> integrin receptor —> fibronectin ligand —> extracellular matrix
  - Receptor-ligand binding is realized largely as noncovalent bonds, specifically hydrogen bonds. *(Zhu etc al. 2000)*
  - The energy of hydrogen bond is ~0.2-0.5 eV





Insight Into The Adhesion Strength

- Bond modeling (Hookean springs)

  Mechanical force ➔ ligand-receptor bond deformation

  - Check with CFD results (with typical modeling parpameters)
    – Receptor-ligand bond density: $6X10^9$ molecules/cm^2
      - IF results: FA area ~43*1.8=77.4 μm^2

      ➔ $N_{bond\ per\ cell}$~$4.6X10^3$

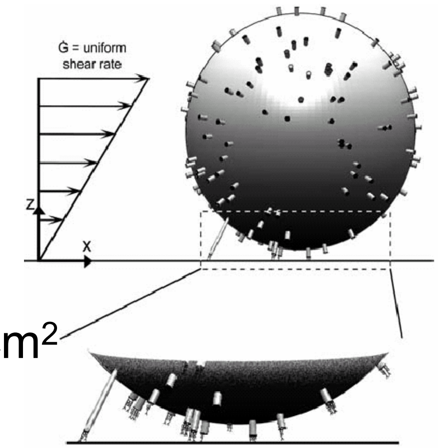
    – Spring elastic constant: k=0.25 dyne/cm
      - Shear assay simulation:

      ➔ F_{bond} ~ 7.6 pN $\qquad F_{total} = \int \tau dA \approx 3.5 \times 10^{-8} N$

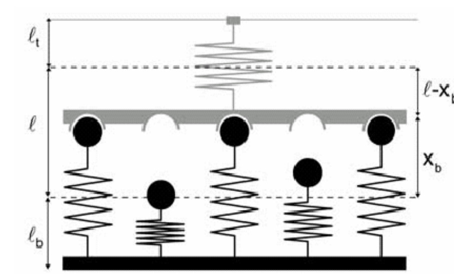
      ➔ L_{break} ~ 30.4 nm

    – Bond length: 10~30.4 nm
    – Ligand-receptor bond energy: E_{bond}~ 0.64 eV

$$E_{bond} = \int_{10}^{30.4nm} kldl$$



Caputo and Hammer (2005)



Erdmann and Schwarz (2006)

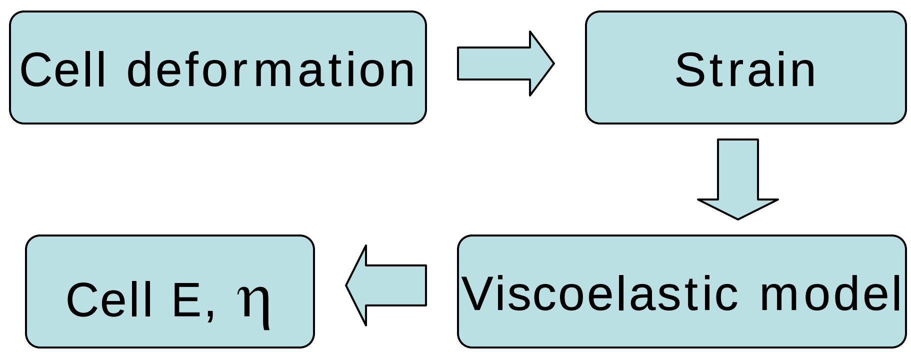
Cell Viscoelastic Properties

- Shear assay measurements can also be used to measure cell viscoelasticity in a non-invasive manner
- Track characteristics of point motion

  simplify three points as elements for estimation

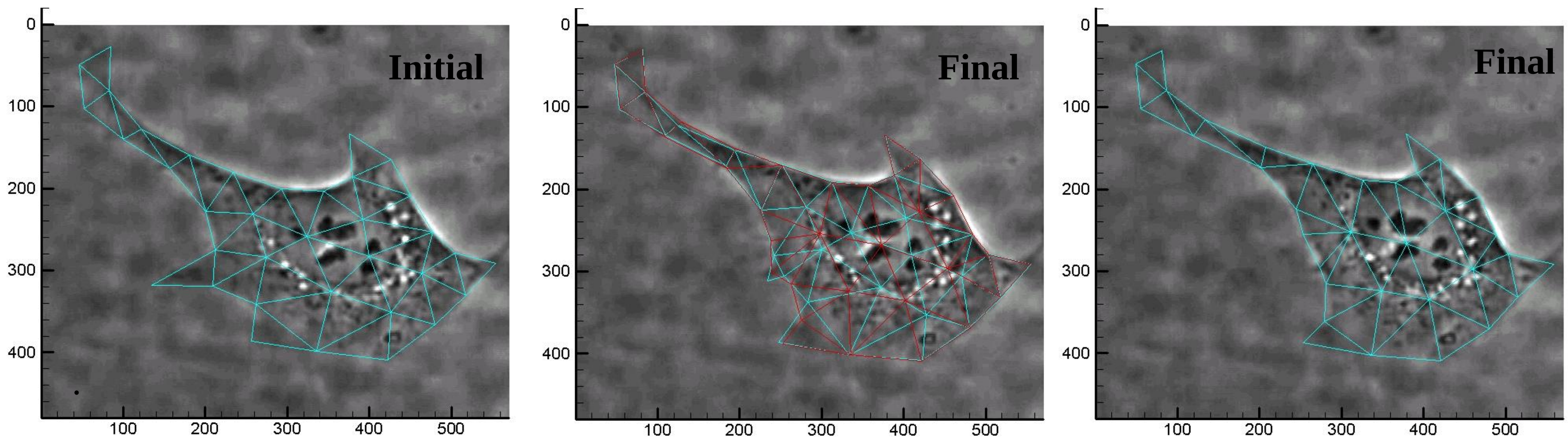
  use linear polynomials as displacement functions

Shear assay video

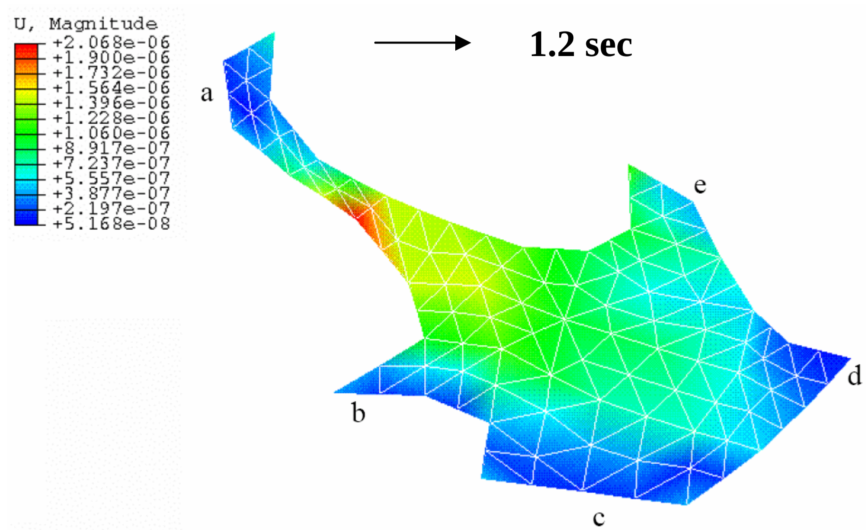


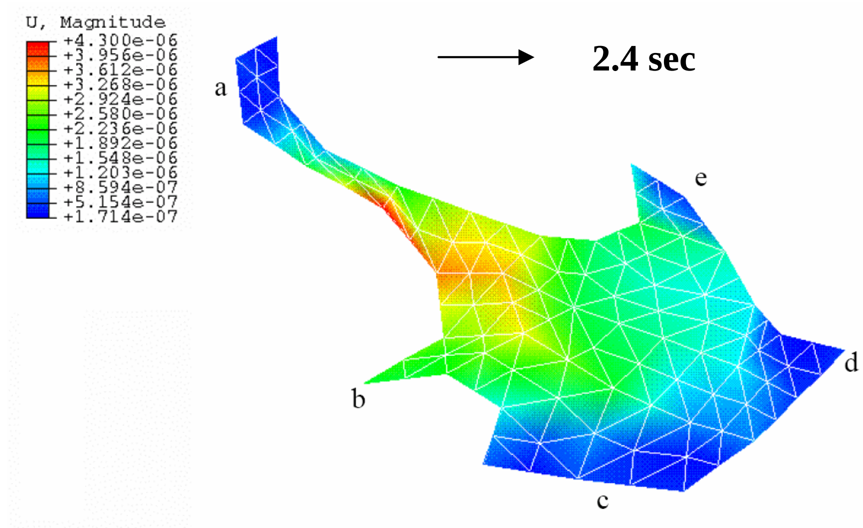
Digital Image Correlation

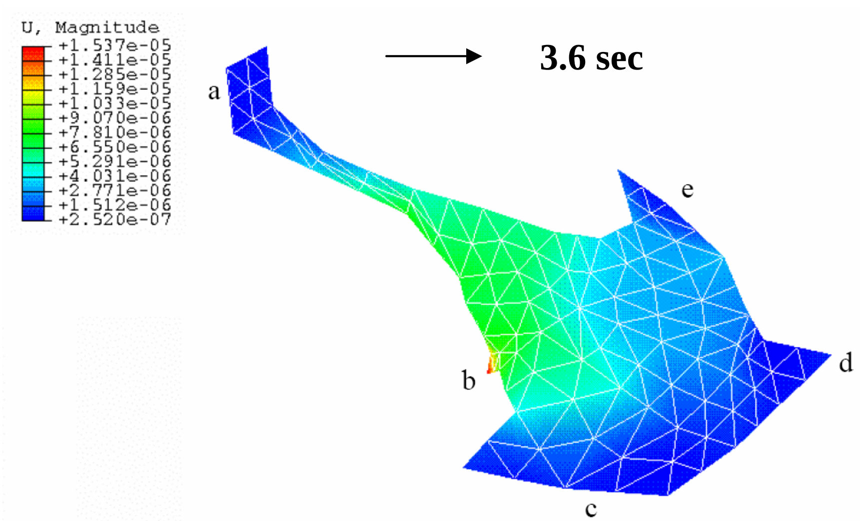
- Global Digital Image Correlation (GDIC) can be effectively utilized to characterize the cell deformation pattern by sequential correlating the images recorded during the assay shear test.
- The deformation mapping between these two images is obtained by a multi-variable minimization which conducted on a constrained system determined by the mesh
- Due to the severe deformations experienced by the cell during the assay test, a remeshing step is required to preserve the mesh quality



Cellular Displacement Subjected to Shear Flow



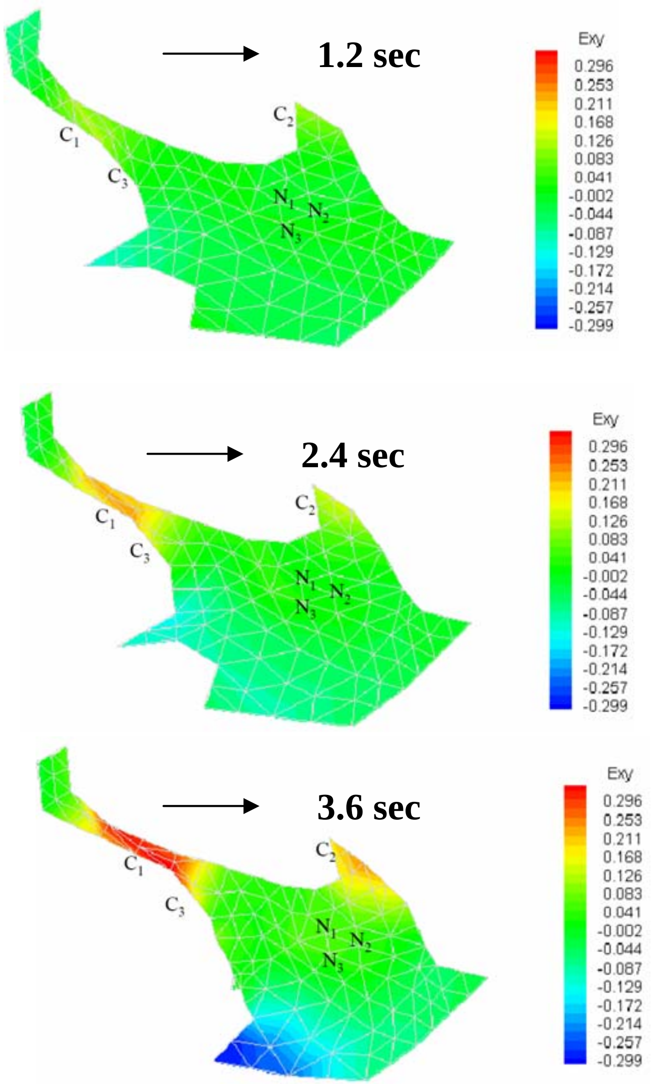


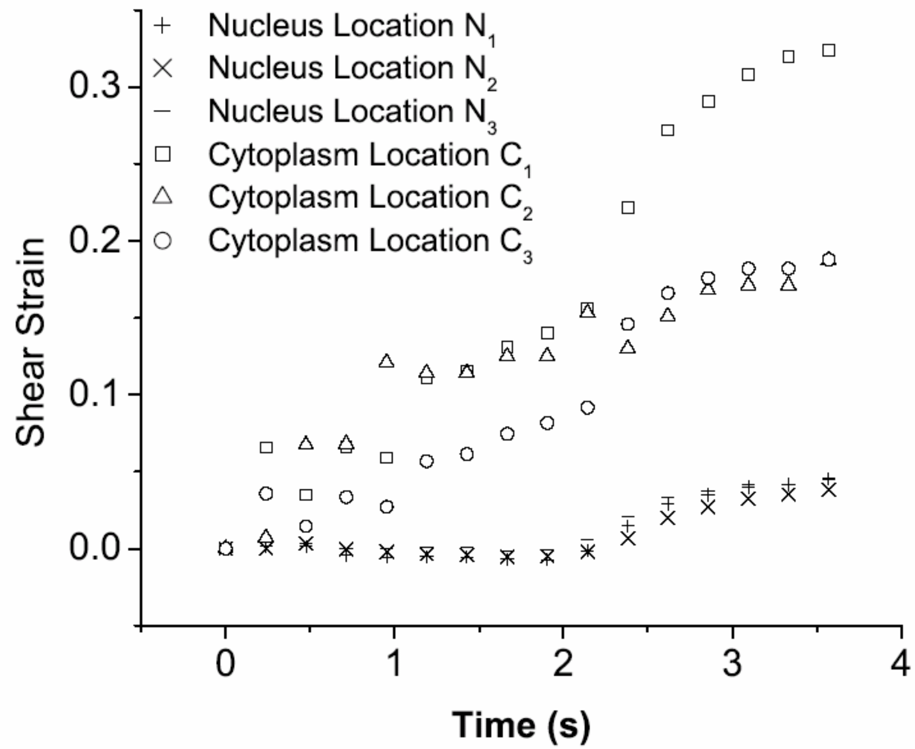


Higher mobility was observed at the rear edge (region b), compared to the front edge subjected to shear flow

displacement.avi

Cellular Strain Subjected to Shear Flow

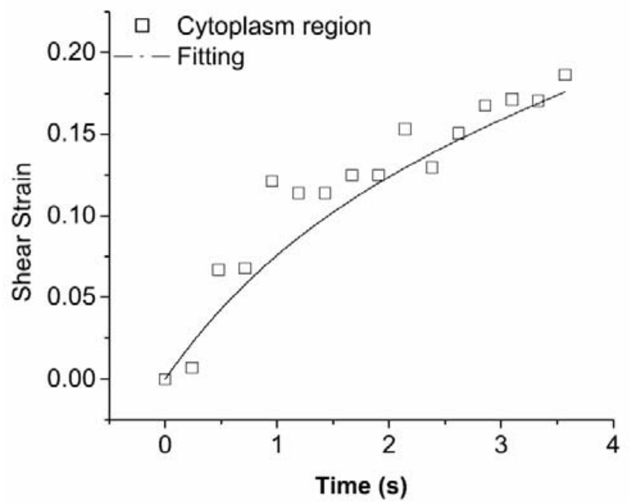


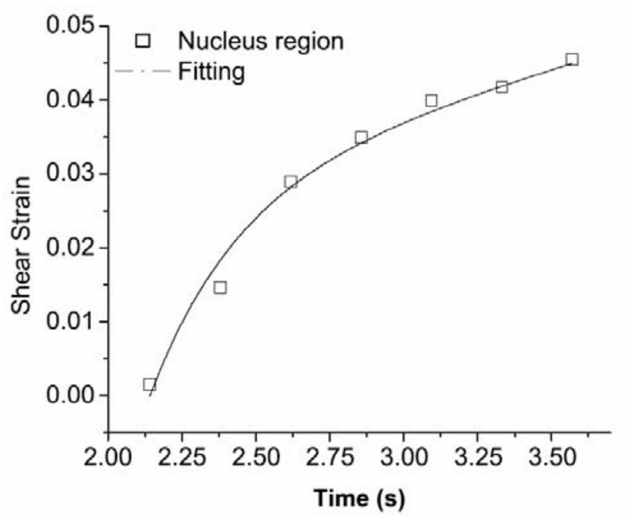


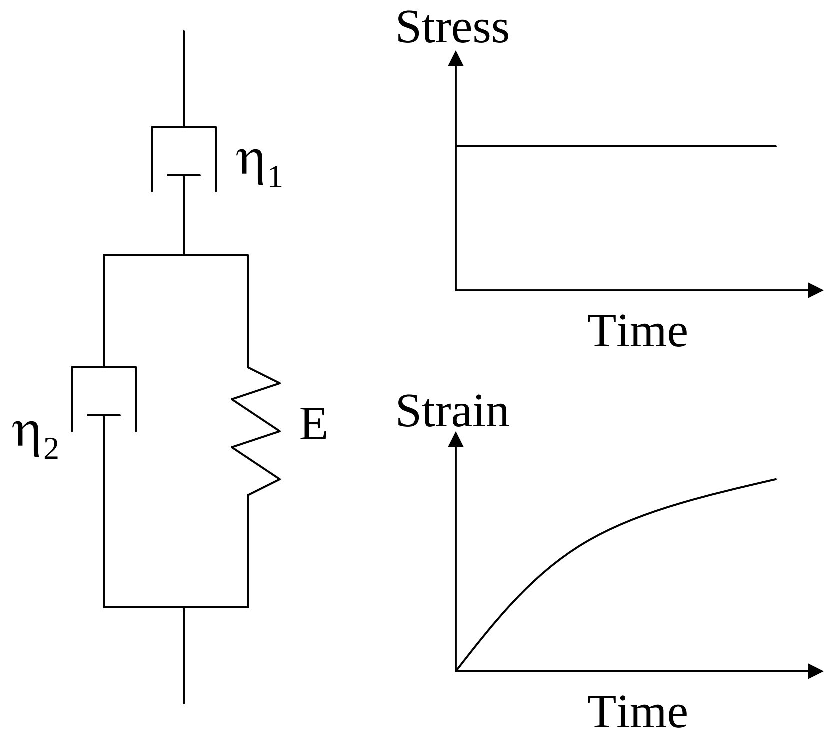
The shear strains in cytoplasm increased more significantly than those obtained in the nucleus during the shear assay experiment

shearstrain.avi

Viscoelastic Modeling







$$\epsilon = \frac{\sigma}{E}[1 - exp(\frac{-t}{\tau})] + \frac{\sigma}{\eta_1}t$$

Comparison of Moduli and Viscosities

AFM results (Mathur et al, 2000)

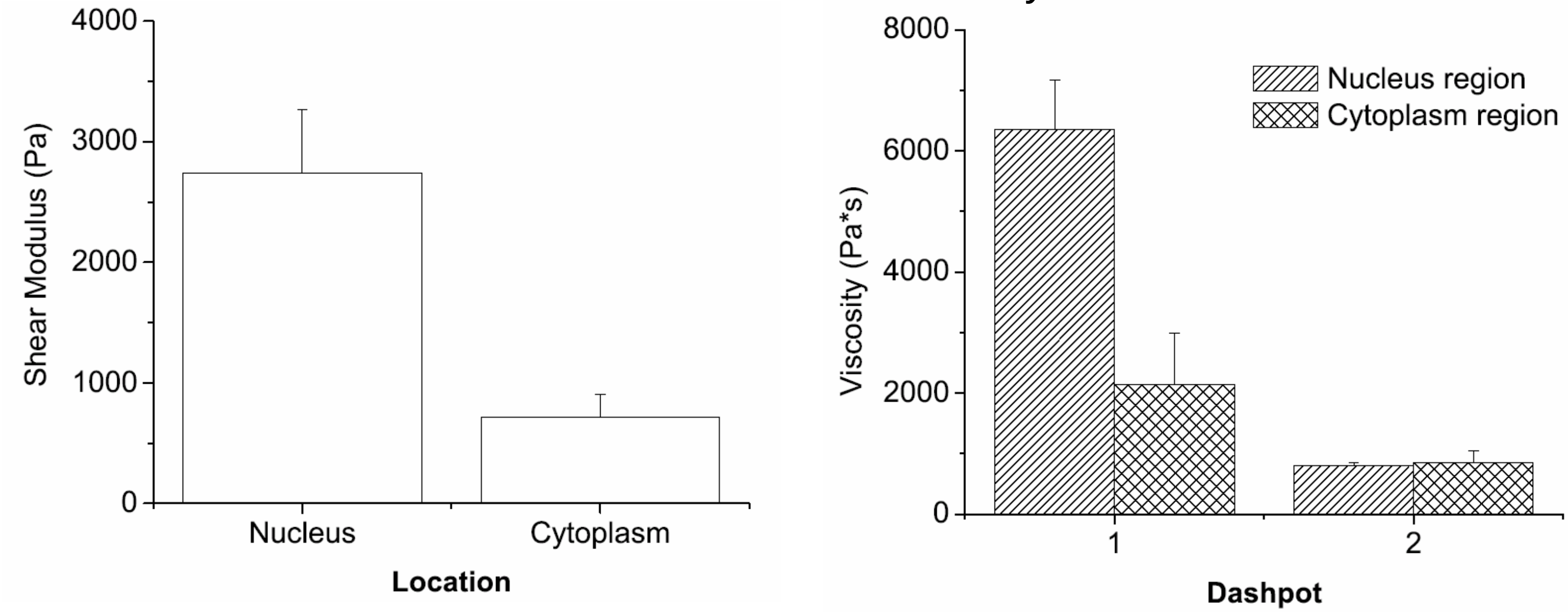
Young's modulus of vein endothelial cell

Nucleus: 7220 Pa   Cell body: 2970 Pa

Micropipette results (Guilak et al, 2000) Chondrocyte

Nuclear E: 1500 Pa   Nuclear viscosity: 5000 Pa·s

Whole E: 500 Pa   Whole viscosity: 2000 Pa·s



The fact that the nucleus is more rigid than the cytoplasm can explain why the nucleus deforms less than the cells when subjected to shear flow in the current study, or when the substrate is stretched.

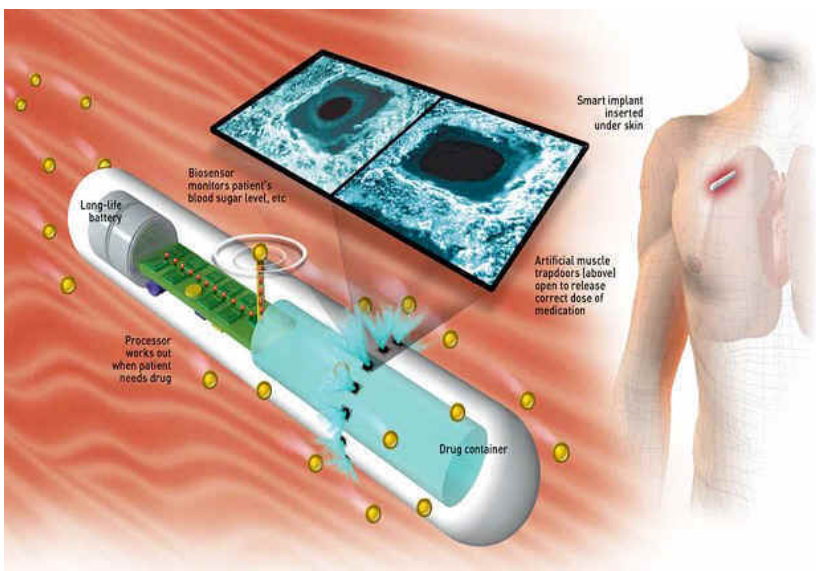
Potential Applications of Cell Mechanical Properties

- There are several scenarios in which the mechanical properties of biological materials are important
- Some examples include
  - Accidental biomechanics in which deformation can occur in cells, tissue and organs
  - Ageing in which the mechanical properties of cells, tissue and organs change with time due to a range of biological/biochemical processes
  - The mechano-transduction of biological cells
  - Movement of blood cells through capillaries and blood vessels
  - BioMEMs for disease detection and treatment….

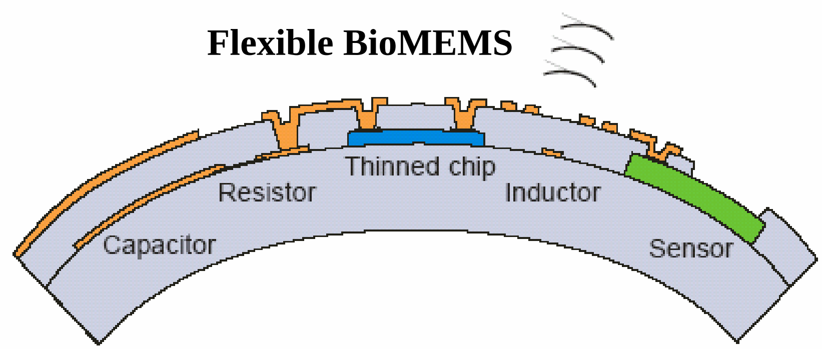
Bio-MEMS Meets Soft Materials

- Ongoing efforts are focused the development of BioMEMS for both implantable and non-implantable systems
  - Shear assay BioMEMS device – application of cell mechanical properties
  - Implantable drug delivery systems – localized cancer treatment (micro-fabrication + in-vivo/in-vitro expts + modeling)

Drug delivery systems



Flexible BioMEMS

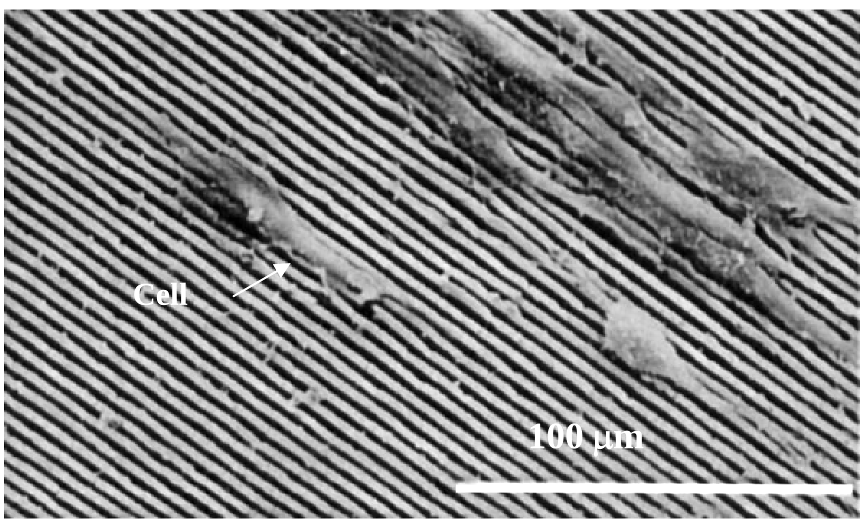


Biocompatible and mechanical flexible electronics are going to emerge as the new generation of Bio-MEMS.

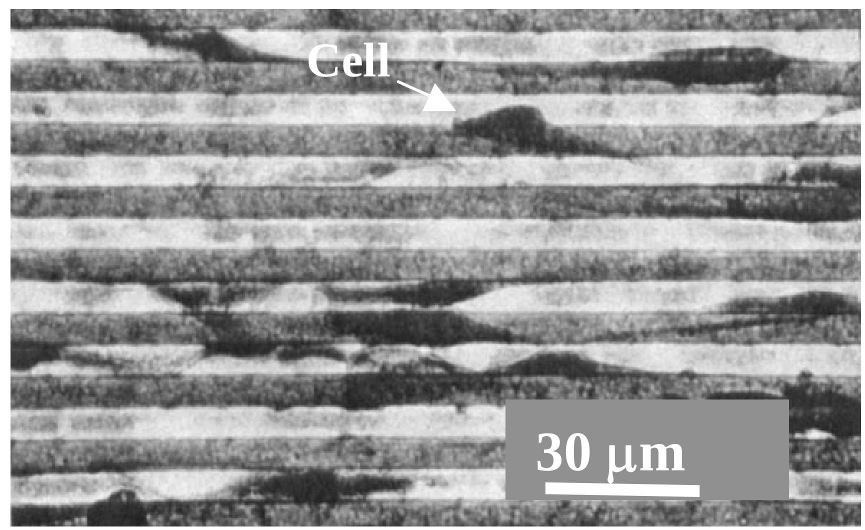
Micro-Groove Geometry and Cell/Surface Interactions

- Cells can undergo contact guidance when in contact with micro-grooved geometries
- This depends on the size of the grooves relative to the size of the cells
- Contact guidance has implications for wound healing and scar tissue formation

2 μm Micro-Grooves



12 μm Micro-Grooves



Microgrooves for Studying Contact Guidance

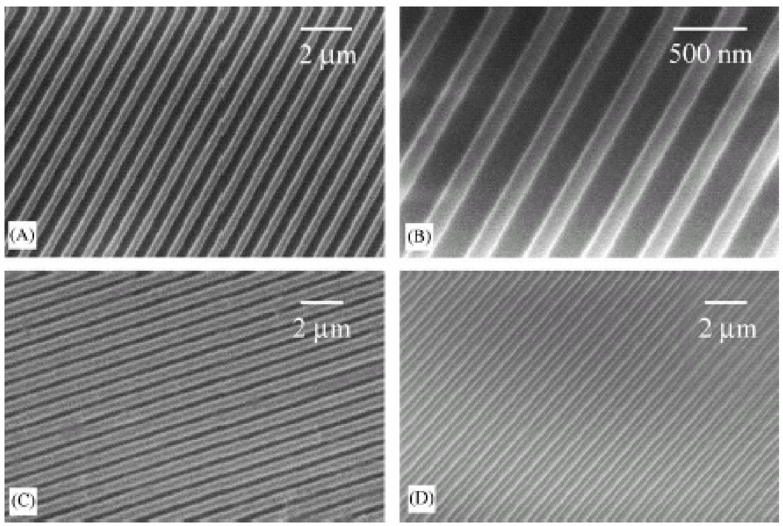


Fig. 1. Scanning electron micrographs of (A and B) nano-imprinted gratins on PMMA coating on SiO_2 wafer, (C) PDMS nanopatterned by replica molding and (D) collagen coated PDMS with nanopattern. Bar = 2 µm for A, C and D, bar = 500 nm for B.

Evelyn K.F. Yim, et al. *Biomaterials* (2005)

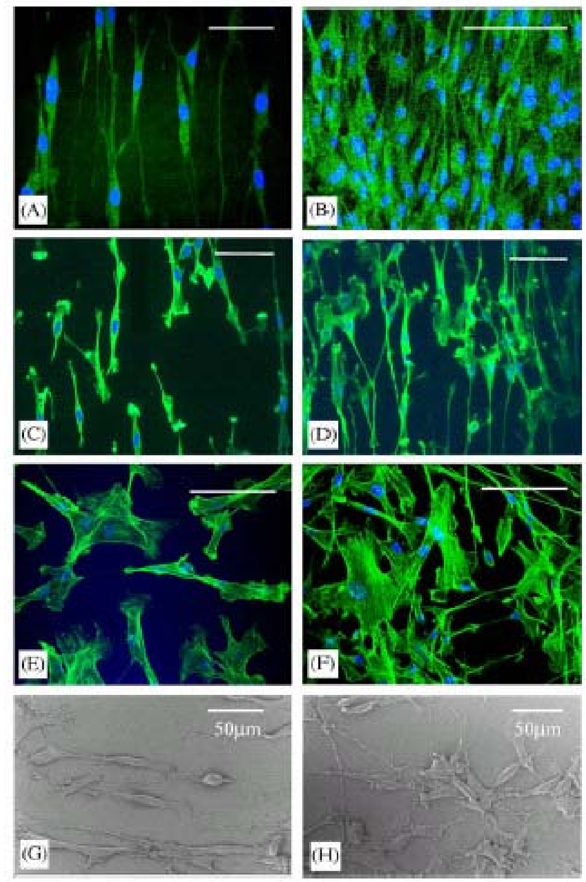


Fig. 2. Confocal micrographs of F-actin stained SMC on (A) nano-imprinted PMMA at low cell density, (B) nano-imprinted PMMA at high cell density, (C) nanopatterned PDMS at low cell density, (D) nano-patterned PDMS at high cell density, (E) non-patterned PMMA and (F) glass cover slip. Scanning electron micrographs of SMC cultured on (G) nano-imprinted gratings on PMMA coated on SiO_2 wafer and (H) non-patterned PMMA coated on SiO_2 wafer. Bar = 50 µm for all except (B) Bar = 100 µm.

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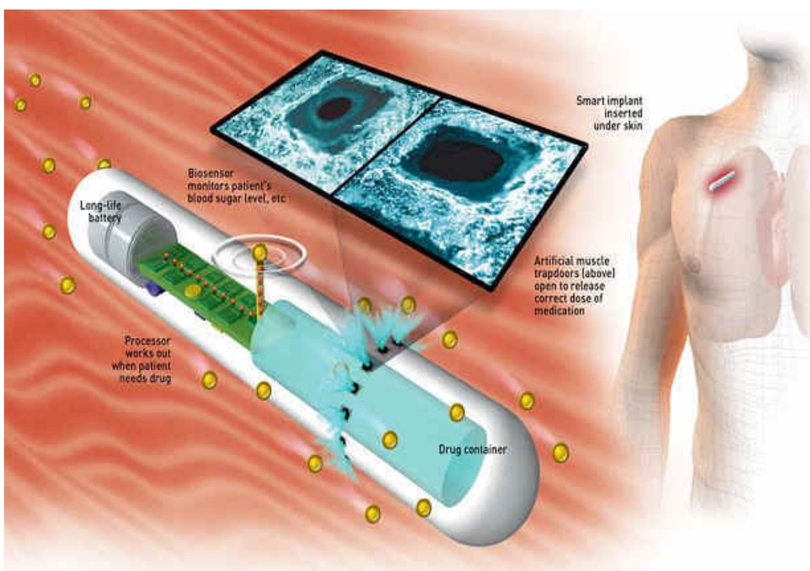
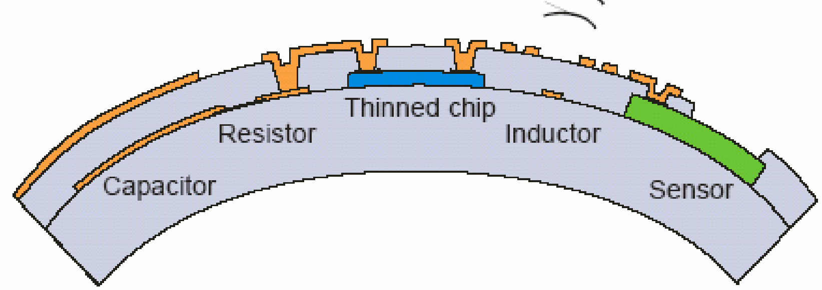


Flexible BioMEMS



Biocompatible and mechanical flexible electronics are going to emerge as the new generation of Bio-MEMS.

Summary and Concluding Remarks

- This class presents an introduction to implantable and non-implantable BioMEMS
- Cantilevered BioMEMS were shown to have the potential for biochemical/cellular detection
- Cardiovascular BioMEMS explored for the detection of stenosis – fluidics and sensing
- Cytoactive Ti coatings and microgrooves suggested for the design of biocompatible BioMEMS surfaces
- Examples of integration presented for implantable BioMEMS – diseased cell detection & treatment
- We welcome your involvement in the program…

THANK YOU!