

# Investigation of the Dynamics of Magnetic Nanoparticles for Targeting and Probing the Microenvironment of Cancerous Cells and Tissues

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Co-Advisers: Stephen A. Boppart, Department of Electrical and Computer Engineering, Bioengineering and Medicine

K. Jimmy Hsia, Department of Mechanical Science and Engineering, and Bioengineering

## Objective

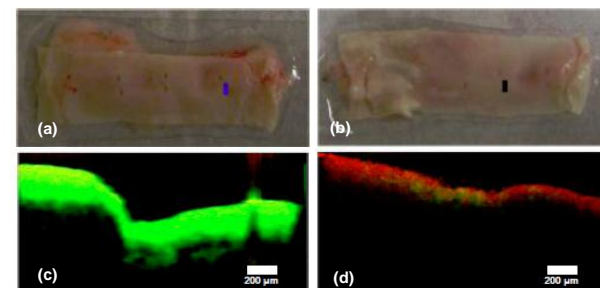
Magnetic nanoparticles can be perturbed by an external magnetic field and the resultant displacements can be optically measured with nano-scale accuracy to provide not only dynamic contrast in imaging but to also assess the biomechanical properties of the microenvironment. The objective of this project is to study the dynamics and targeting of these magnetic agents to cancerous cells and tissues under different flow conditions and magnetic field modulations.

## Research Highlights

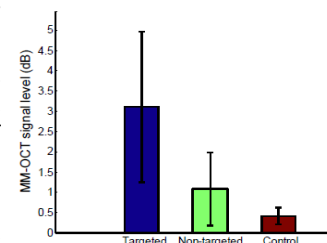
- Dependency of the magneto-motive signal on magnetic particle concentration and microenvironment stiffness was investigated using magneto-motive optical coherence tomography (MM-OCT).
- Microspheres containing magnetic nanoparticles were functionalized to target the  $\alpha_v\beta_3$  integrin that are overexpressed during angiogenesis. We demonstrated targeting of these microspheres by perfusing blood vessels overexpressing the  $\alpha_v\beta_3$  integrin in a custom designed flow chamber at physiologically relevant pulsatile flow rates and showed that modulating these magnetic particles by external magnetic fields can significantly enhance the contrast.

## Future Research

- Developing a catheter-based MM-OCT imaging system that can enable *in vivo* tracking and visualization of magnetic particles.



Rabbit aorta segments overexpressing the  $\alpha_v\beta_3$  integrin perfused with (a) targeted and (b) non-targeted microspheres. The blue and black colored regions correspond to the MM-OCT cross-sectional images shown in (c) and (d) respectively. The increase in contrast shown in (c) compared to (d) demonstrates successful targeting under flow conditions and the ability of MM-OCT to detect the presence of the microspheres



MM-OCT signal values between targeted, non-targeted, and control specimens

# Mechanism of Mechanical Microenvironmental Control of Cancer Metastasis

**M. Yakut Ali, Mechanical Science & Engineering**

Co-Advisers: Taher Saif, Mechanical Science and Engineering

Mark Kuhlenschmidt, Pathobiology

## Objectives

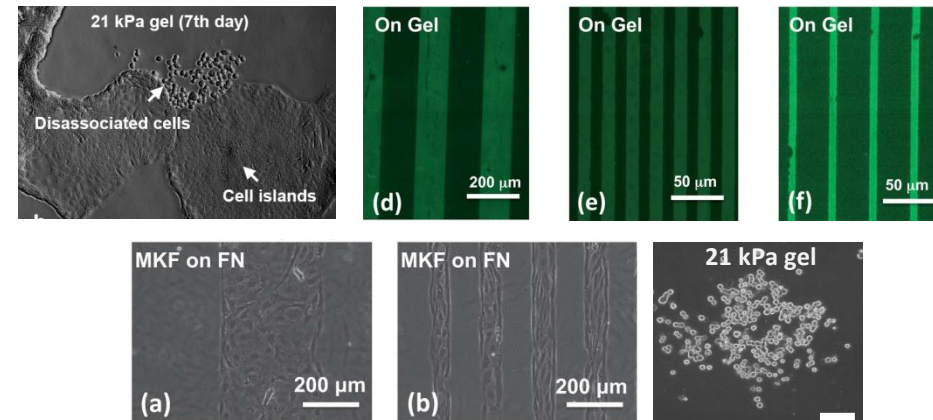
- To understand the effect of coupled mechanical cues and geometric cues on the onset of 2D in vitro colon cancer metastasis.
- Investigate the role of dimensionality (2D vs 3D) and biophysical environment on colon cancer cell behavior using a 3D cell culture model.

## Research Highlights

- Recently, our group has shown that human colon carcinoma (HCT-8) cells show metastasis like phenotype (MLP) in vitro when cultured on appropriate intermediate mechanical stiffness (21-47 kPa) substrates, but not on very soft (1 kPa) and very stiff substrates (3.6 GPa) [1], Fig. 1a].
- Development of a novel micropatterning technique to spatially confine cell adhesion molecules (e.g Fibronectin, Laminin and Collagen I) on 2D polyacrylamide hydrogel substrates and consequently obtain precisely defined cell culture [2](Fig. 1b).
- Demonstration that the metastasis like phenotype of HCT-8 cells is independent of adhesion sub-system [3].

## Future Research

- As the behavior of colon cancer cell is known in 2D cell culture model, a well characterized gelatin methacrylate hydrogels model will be developed to study the influence of matrix stiffness in 3D which resembles more closely the in vivo condition.
- Successful identification and understanding of metastasis-triggering signals is critical for the design of novel anti-metastasis therapeutics and hence for meeting the grand challenge of treating cancer successfully.



**Fig 1** Top (a) HCT-8 cells cultured on intermediate stiffness gel substrates ( $E=21$  kPa, coated with fibronectin) show metastatic-like phenotype (MLP), (d-f) FN patterns on PA gels Bottom (a-b) Cells are cultured on patterned fibronectin on 21 kPa gel substrates. (c) HCT-8 cells show MLP on E-cadherin coated 20 kPa gels as well. Scale bar: 100  $\mu$ m.

[1] Tang X, ..Kuhlenschmidt MS, Saif TA et al. Biophysical Journal 2010. [2] Tang X, Ali MY, Saif TA. Soft Matter 2012.

[3] Ali MY, Saif TA. Submitted for publication.

# Tissue-Engineered Cancer Construct for Studies of Nanobiomaterial Transport

**Ross DeVolder, Chemical & Biomolecular Engineering**

Co-Advisers: Hyunjoon Kong, Chemical and Biomolecular Engineering

K. Jimmy Hsia, Mechanical Science and Engineering

## Objective

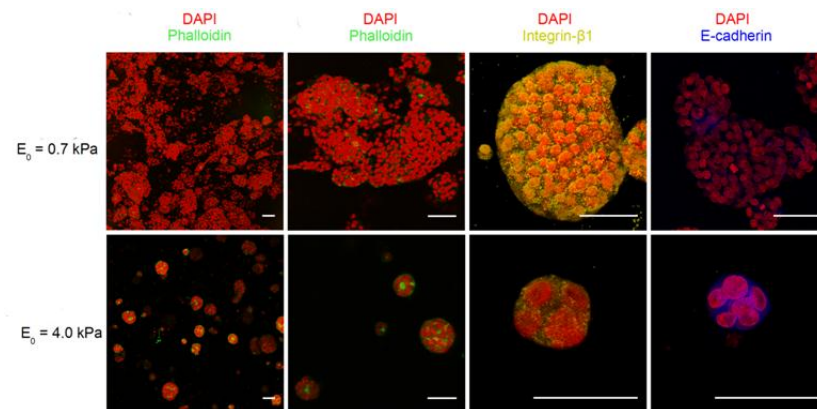
To uncover the role of cancer tissue's intercellular organizations in the intracellular uptake of nano-sized diagnostic and drug carriers using engineered 3D synthetic cancer tissue

## Research Highlights

- We successfully assembled a cell-encapsulating hydrogel with controlled stiffness independent of gel permeability.
- We have demonstrated that the softer hydrogel elevates malignancy of cancer cells encapsulated in the 3D gel matrix.
- We have demonstrated that the softer hydrogel promotes proangiogenic activity of cancer cells.
- The results were reported in Biomaterials Journal.

## Future Research

- We plan to test the response of tumor spheroids created in the softer matrix to nanocarriers of cancer drugs.
- We propose that this support should greatly expedite the development of the next generation of nanobiomedical tools to improve the quality of cancer detection and treatments.



Effects of hydrogel stiffness, quantified with an elastic modulus ( $E_0$ ) on proliferation,  $\beta_1$  integrin expression and E-cadherin expression of hepatocarcinoma spheroids

## Label-Free Electronic Detection of Cancer Biomarkers Using Silicon Nanowire Arrays

Brian Dorvel, Biophysics

Co-Advisers: Rashid Bashir, Electrical Engineering; Sue Clare, Indiana University School of Medicine

### Objective

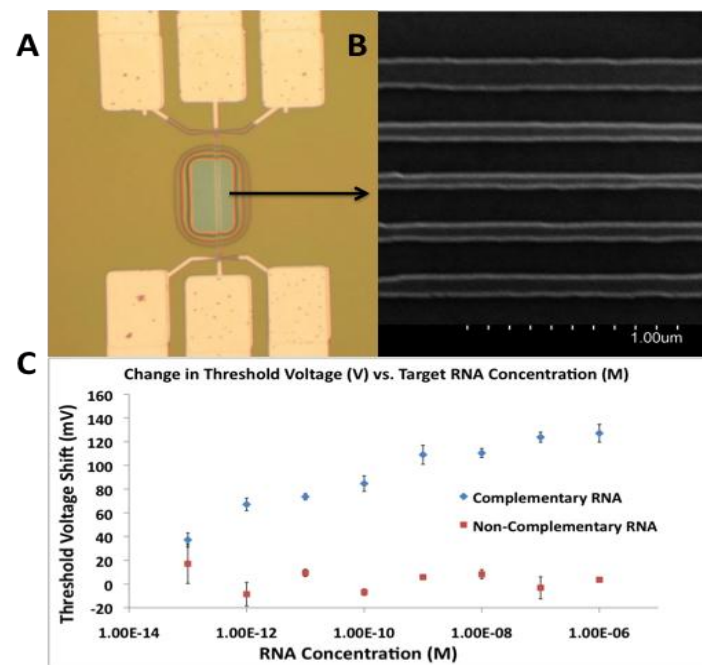
Silicon nanowires have been successful in detecting proteins and nucleic acids, such as RNA and DNA, down to femtomolar levels by utilizing the intrinsic charge of the biomolecule as the sensing moiety. By using a CMOS-compatible silicon nanowire fabrication process we aim to detect phosphorylated HER2 protein structures commonly upregulated in breast cancer, as well as relevant microRNA's specific to the cancer.

### Research Highlights

- We have fabricated 50nm wide silicon nanowires with high stability in aqueous solutions
- Detection of complementary pairing down to 100fM in solution has been accomplished, with minimal binding of negative control

### Future Research

- The final project aim is to take individual cells or very small cell volumes and measure the contents for the cancer biomarkers of interest
- By minimizing the sample volume and maximizing the sensitivity and throughput, cancers may be detected earlier with minimally invasive procedures



An example of a released device is shown in (A), with an arrow indicating where the nanowires are. An SEM image of the 50nm nanowires is in (B), with a graph of the device response to complementary and non-complementary RNA in (C).



# Label-Free Identification of Cancer Cells using DNA Aptamers and Laser-Based Biosensors

**William Goldslag, Electrical and Computer Engineering**

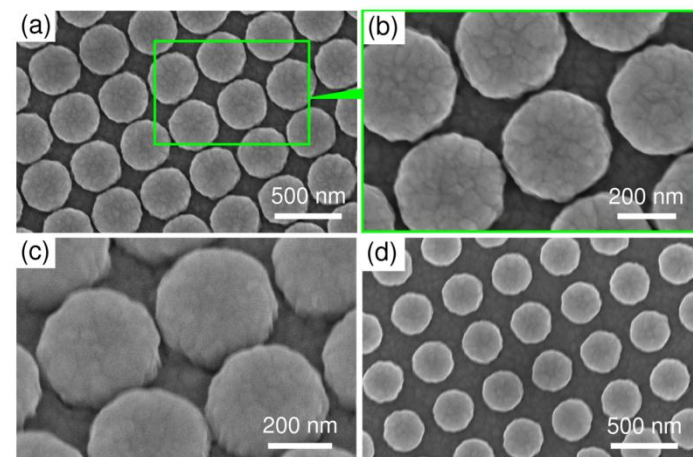
Co-Advisers: Brian T. Cunningham, Electrical and Computer Engineering; Yi Lu, Chemistry

**Objective:** To develop a biosensor for early detection and classification of cancer based on profiling the expression of cancer cell biomarkers.

**Motivation:** Personalized chemotherapy is an unmet challenge in cancer treatment. Many modern cancer drugs work by targeting particular cancer biomarkers expressed on cell membranes. As biomarker expression levels vary from person to person, so do individual responses to treatments. A diagnostic tool under development capable of measuring expression of multiple cancer biomarkers will replace a lengthy and dangerous trial-and-error approach currently used for determining drug effectiveness. Furthermore, the ability to track levels of biomarker expression over the course of treatment will enable doctors to timely adjust medication dosages, thereby enhancing treatment effectiveness.

## Research Highlights:

- Developed a fabrication protocol of SERS sensors using gold, rather than silver, for improved sensor biocompatibility and reduced background noise.



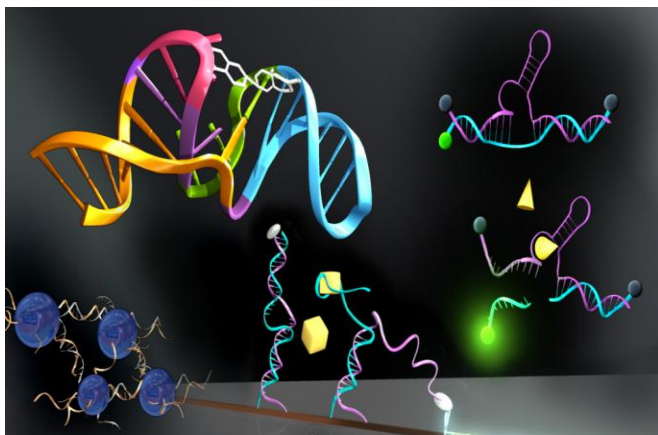
SEM images of Ag-coated nanodome array SERS substrates<sup>1</sup>

- Developed a surface chemistry protocol for immobilizing DNA aptamers
- Studied substrate wetting properties and dramatically improved analyte detection levels via better surface wetting.
- Demonstrated robust label-free detection of single-stranded immobilized aptamers.

## Future research:

- Demonstrate differentiation of an immobilized aptamer layer before/after exposure to its specific recognition target.
- Characterize sensitivity of substrate to the molecular makeup of aptamers.
- Characterize dose/response due to exposure to target molecules.
- Perform detection and quantification of unknown concentrations of biomarker molecules taken from cancer cell supernatant.

<sup>1</sup>C.J. Choi et. al. *Nanotech.* 21, 415301 (2010), <sup>2</sup>J. Liu, Z. Cao, Y. Lu, *Chem. Rev.* 109, 1948–1998 (2009).



Nucleic-acid based sensors, artist's rendition<sup>2</sup>

## Developing Novel Therapeutics for Prostate Cancer

**Michael Gregory, Biochemistry**

Stephen G Sligar, Chemistry, Biochemistry, College of Medicine; Brian T Cunningham, Electrical and Computer Engineering; Paul J Hergenrother, Chemistry and Biochemistry

### Objective

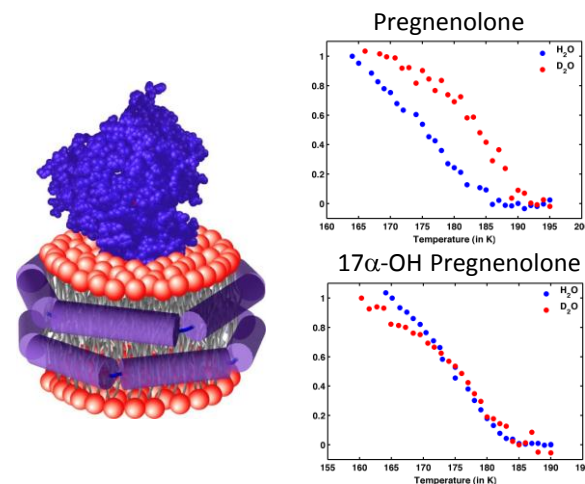
Prostate cancer (PC) is the most commonly diagnosed malignant neoplasm among the American male population and represents the second most common cancer among men worldwide. Recently, CYP17A1 has been identified as a potent target for inhibition in the treatment of advanced prostate cancer. By solubilizing this membrane protein using the Nanodisc system and thus rendering it amenable to interrogation by spectroscopic and surface based analytical platforms, we seek to identify mechanism based inhibitors for this and other steroidogenic P450 enzymes.

### Research Highlights

- A high-pressure instrument has been adapted to interface with BIND biosensors, permitting investigation of protein-protein interactions at up to 3 kbar.
- Detailed resonance Raman investigations have been conducted that have identified the the alcohol on C-17 of hydroxylated substrates forms a hydrogen bond with the oxy-complex of CYP17.
- Cryoradiolysis of the CYP17 oxy-complex has been employed to successfully generate the peroxo-ferric intermediate, and the decay kinetics of this species in  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  buffer systems was found to be isotopically sensitive in the presence of pregnenolone, but not  $17\alpha$ -hydroxypregnenolone, suggesting involvement of a novel reaction intermediate in CYP17 catalyzed C-C lyase chemistry.

### Future Research

- Future research will focus on further adapting the BIND biosensor to high pressure studies of protein-protein interactions, as well as continuing our commitment to interrogation of the key reaction intermediates that give rise to CYP17's unique C-C lyase activity that represents the first committed step of androgen formation.



The above figures show a kinetic solvent isotope effect during annealing of the peroxo-ferric intermediate in the presence of pregnenolone, but not in the presence of  $17\alpha$ -hydroxypregnenolone (right), and CYP17 incorporated into a Nanodisc (left), a membrane mimetic that enables rigorous biophysical interrogations of membrane bound proteins.

# Quantifying Molecular Crosstalk During Breast Cancer Progression Using 3D Cell Culture Models with Nano-microstructured Detail

**Sarah Holton, Bioengineering**

Co-Advisers: Rohit Bhargava, Bioengineering

Ann Nardulli, Molecular and Integrative Physiology

## Objective

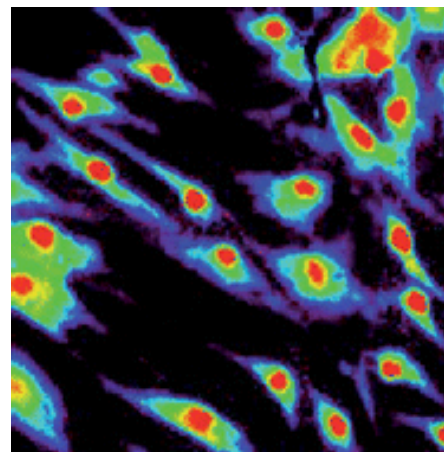
To develop label-free and multiplexed labeled methods to record molecular cross-talk between epithelial cells and fibroblasts during early breast cancer progression. Novel three-dimensional cell co-culture models and nanoprobe will be used to understand the dynamics of paracrine signaling events that lead to lethal tumors.

## Research Highlights

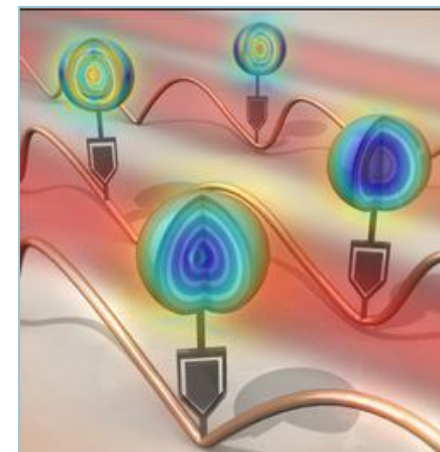
- Developed a three-dimensional cell co-culture model for breast cancer progression
- Analysis of co-culture model using chemical imaging
- Using chemical imaging and gene expression analysis to determine the effects of estrogen and tamoxifen on epithelial cells grown in 3D culture
- Determine appropriate targets for nanoparticle conjugation

## Future Research

- Integrating nano-LAMPs into molecular profiling of stromal-epithelial crosstalk in 3D co-culture models
- Collaborate with physicians at Mills Breast Cancer Institute to detect biomarkers of disease in human samples using nanoprobe



ATR FT-IR was used to determine chemical changes in cancer-associated fibroblasts



nanoLAMPs will be able to detect and enhance chemical signals from analytes occurring at very low concentrations

## Nanophotonic Sensor Integrated Microfluidic Imaging Platform for Studying Cancer Cell Mechanobiology in Metastasis

**Austin Hsiao, Bioengineering Department**

Co-Advisers: Logan Liu, Electrical and Computer Engineering

Yingxiao Wang, Bioengineering

### Objective

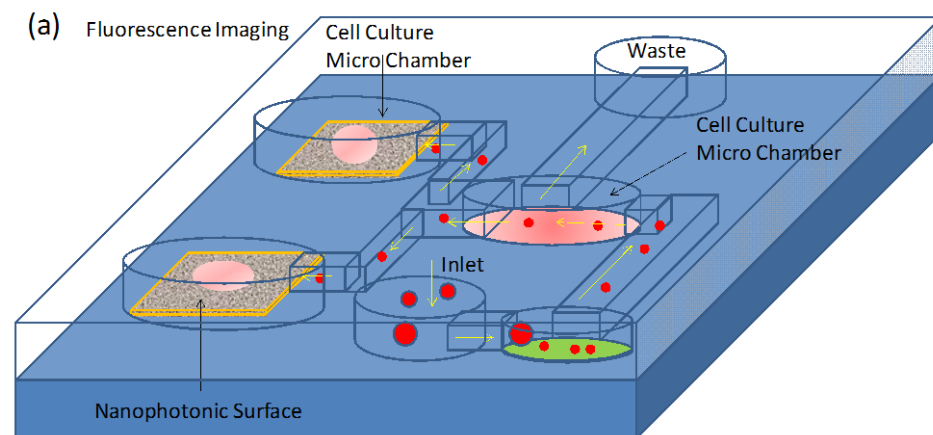
The mechanical properties of cancer cell are known to be critical in metastasis process, however the molecular level mechano-biology mechanisms of cancer metastasis are to be further discovered. We are creating an ex-vivo nanotechnology platform able to simulate the physiological environment for cancer cell growth and development and image molecular and cellular responses and investigate various mechano-biological aspects in cancer metastasis with high efficiency.

### Research Highlights

- Fabrication of Microfluidic platform for metastatic breast cancer cell culture and observation
- Fabrication of patterned nanophotonic substrates for integration with the microfluidic platform
- Preliminary 3D confocal images of GFP transfected metastatic breast cancer cells
- Fabrication of New colormetric nanopore sensor for cell attachment and imaging

### Future Research

- Integration of nanophotonic substrates with microfluidic platform
- Imaging of cancerous cell attachment on the colormetric nanopore sensor



A schematic of an integrated nanoplasmmonic imaging platform with microfluidic cell culturing chamber.



## Cell Stiffness as a Tool to Distinguish Cancerous Cells from Healthy Cells

**Katrina Keller, Department of Bioengineering**

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

Supriya Prasanth, Department of Cell and Developmental Biology

### Objective

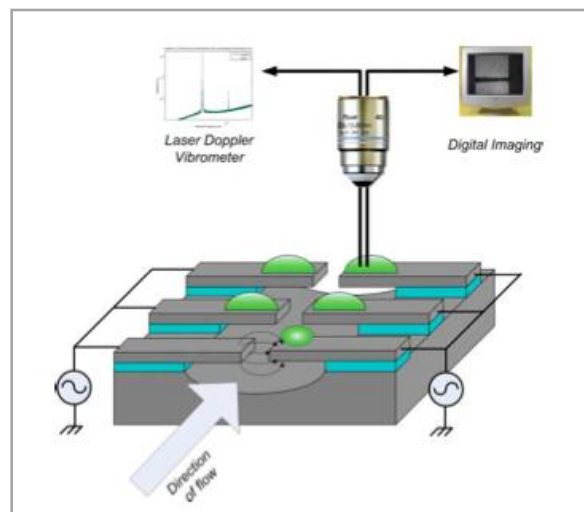
This project will focus on characterizing the stiffness of different kinds of cells, with a focus on cancerous cells compared to their non-cancerous analogs using a MEMS platform.

### Research Highlights

- Build the sensors
- Characterize different stiffness measurements for different cell types
- Compare cells of specific organs to cancer cells in those organs

### Future Research

- This project will eventually encompass the capture of circulating tumor cells (CTCs) from whole blood samples with the MEMS platform and subsequent measurement of stiffness and growth.
- This technology will be a powerful diagnostic tool to determine if CTCs are present and where they originated.



**Fig. 1** Diagram of a living cantilever array. Cells are captured from suspension and immobilized on the cantilever. The mass of the cell is then measured using the resonance frequency shift of the cantilever.

K. Park, et al, Lab Chip, 2008

## Click Chemistry for Nanomedicine Cancer Targeting

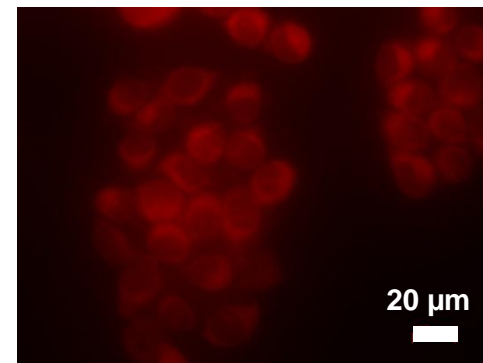
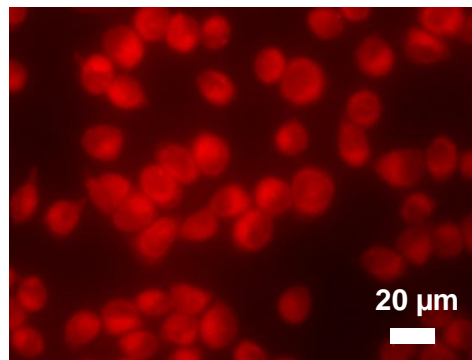
Vahid Mirshafiee, Department of Chemical and Biomolecular Engineering  
Co-Advisers: Mary L. Kraft, Department of Chemical and Biomolecular Engineering;  
Jianjun Cheng, Department of Materials Science and Engineering

### Objective

- The goal of this research is to develop a new cancer-targeting strategy through spontaneous, reagent-free “click chemistry” for cancer-specific drug delivery and cancer treatment.

### Research Highlights

- Active targeting of functionalized nanoparticles (NPs) to the metabolically labeled cancer cells.
- Higher accumulation of NPs in tumors.
- Increased in vivo efficacy and lower immune response.



Labeling live Hela cells using click chemistry

### Future Research

- By incorporation of controlled chemistry and engineering principles; I will develop a novel drug delivery system and cancer targeting strategy which can lead to a promising in vivo cancer therapy method.
- Although cancer targeting has been attempted for over 100 years, but there is no strategy that allows successful cancer targeting as of today. I believe this step-wise cancer targeting strategy is highly likely achievable in vivo and could be applied for diagnostic and imaging applications in cancer research too.

# Precisely Size Controlled Drug-silica Nanoconjugate for Cancer Therapy

Li Tang, Department of Materials Science and Engineering

Co-Advisers: Jianjun Cheng, Department of Materials Science and Engineering

Timothy M. Fan, Department of Veterinary Clinical Medicine

## Objective

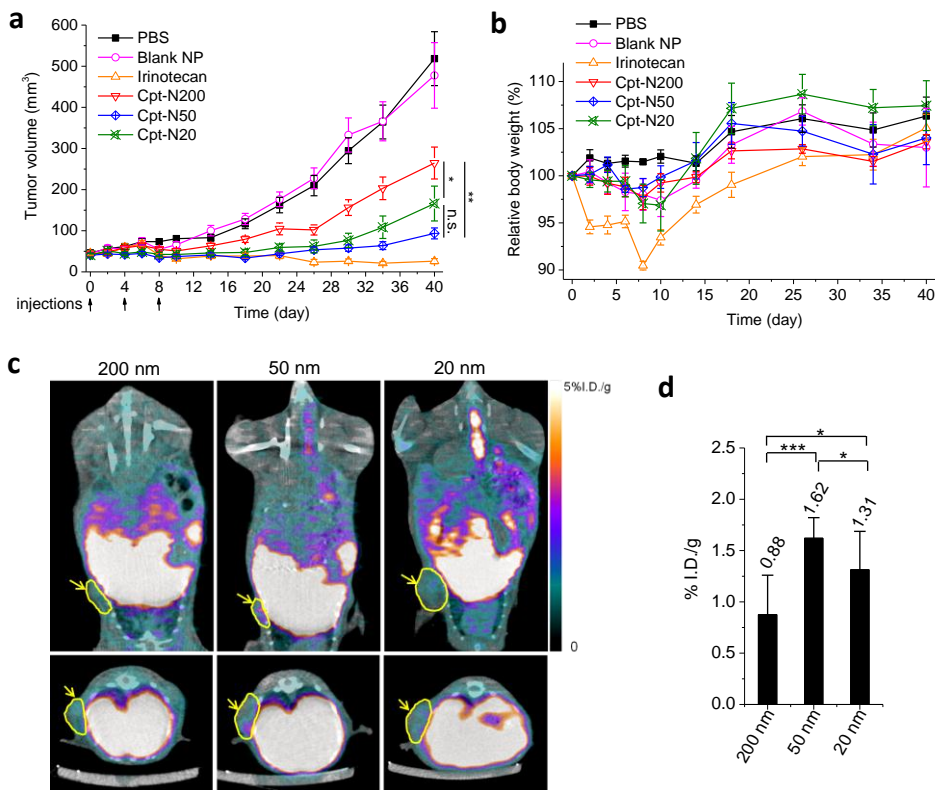
Drug delivery nanomedicines, in the size range of 1-200 nm, have attracted much interest in the past 2-3 decades as alternative modalities for cancer treatment. The size of these drug delivery vehicles has been strongly correlated with their *in vivo* biodistribution, penetration in tumor tissue, and intracellular trafficking. It potentially has significant impact on their antitumor efficacy. We aim for developing a clinic relevant, size-controlled drug delivery system to study and understand the size effect of nanomedicine in biological systems.

## Research Highlights

- We studied the size effect on *in vivo* anticancer efficacy and identify the optimal nanoparticle (NP) size (50 nm) for the most efficient tumor reduction in xenograft MCF-7 tumor model (Figure 1a, b).
- We also studied the size effect on *in vivo* biodistribution using athymic nude mice bearing MCF-7 tumor and found enhanced tumor accumulation were observed when the size of NP was  $\leq 50$  nm (Figure 1c, d).

## Future Research

- We will evaluate the efficacy of preventing tumor metastasis using the size-controlled drug-silica nanoconjugates in murine 4T1 tumor model and study the size effect.
- Study the toxicity of silica NP as a systemic drug delivery system and investigate the long term clearance of the silica NP from the treated mice using radio labeling method.



**Figure 1.** **a**, *In vivo* antitumor efficacy study in athymic nude mice bearing xenograft MCF-7 tumors; **b**, The body weight of mice were monitored during the whole study to evaluate if any acute toxicity caused by the treatments; **c**, *In vivo* biodistribution studies in athymic nude mice bearing xenograft MCF-7 tumors; **d**, Mice were euthanized 24 hours post injection. Tumors were collected and measured for radioactivity by  $\gamma$ -counter to determine the accumulation of silica NPs.

# Investigating Mechanoenvironment Change in Tumor Growth using Multimodal Contrast Agents

**Yue Wang, Department of Bioengineering**

Co-Advisers: Michael Insana, Bioengineering

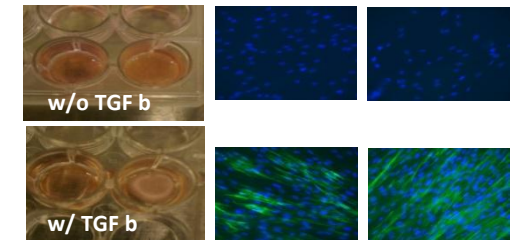
Stephen Boppart, Bioengineering and Electrical and Computer Engineering

## Objective

The tumor microenvironment is mechanically modified during cancer progression. Imaging of tumor mechanical environment will provide new information for early cancer diagnosis. The goal of this proposal is to more clearly understand the contrast mechanisms of elasticity images in terms of cellular activities that drive cancer. Two imaging modalities-ultrasound and OCT-are used with magnetic nanoparticles as contrast agents.

## Research Highlights

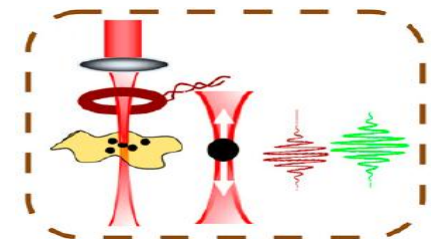
- Develop elasticity imaging techniques using magnetic nanoparticles and an magnetic source to measure local mechanical response
- 3-D cell co-culture system and animal model of breast cancer formation



3D cell co-cultures (left) and Immunofluorescence (IF) staining w/wo the exposure of cancer growth factor

## Future Research

- Identify the change in ECM when breast cancer develops; correlate the biological change with our elasticity imaging results; explore the contrast mechanisms in different scales using different imaging modalities
- Our research helps us understand the changes in mechanoenvironment caused by cancer. By understanding the contrast mechanisms physicians can more accurately evaluate cancer progression and treatment effectiveness through clinical elasticity images.



Magnetomotive displacements of magnetic nanoparticles inside a tissue sample