Using Organs-on-a-Chip for Development of New Nanomedicine Diagnostics and Therapeutics”

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and Biomedical Engineering
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Organs-on-a-chip - Why are we doing this?

- Accelerating human clinical research by creating platforms for testing disease diagnostics and therapeutics (e.g. nanomedicine theragnostics)
- Simulating in-vivo model system with human cells (allowing drugs targeting human-specific signal transduction pathways) without the full complexity and human subjects concerns
- Creating an easier environment for testing and taking measurements (e.g. multi-modal imaging)
- Allowing for more rapid and safe testing of therapeutic interventions (e.g. nanotoxicity)
- Eventually allowing for joining of several organs-on-a-chip to simulate interactions of multiple body organs (e.g. allows for cell-cell signaling from cells of different organs)
- Allowing development and testing of one of more artificial, rather than human, body organs for transplantation (e.g. growing person-specific organs)
Some initial positive responses from NIH and the FDA about Organs-on-a-chip

"Drug toxicity is one of the most common reasons why promising compounds fail. We need to know which ones are safe and effective much earlier on in the process. This is an unprecedented opportunity to speed development of effective therapies, while saving time and money." – Francis S. Collins, M.D., Ph.D., director, National Institutes of Health (NIH News, September 16, 2011)

“it’s what we’re calling Human on a Chip. This is an ambitious project to create a tool that could revolutionize toxicology testing and it’s something I’m really excited to talk about. Scientists have relied largely on animal studies to determine if a drug is toxic before testing it in humans. And while animal testing is useful, it’s also expensive, time consuming, and has drawbacks. For example, it doesn’t always detect toxic effects specific to humans and doesn’t usually provide information about the role that genetic differences within human populations play in toxicity. It can also generate false alarms, showing an effect in animals that doesn’t predict an actual effect in people, which leads us to abandon promising new drugs. FDA is collaborating with DARPA, NIH, and the scientific community to spur innovation in this field by exploring how tools like Human on a Chip can be integrated into our development tool box to improve testing for toxicity and potentially reduce the need for animal testing”.

Dr. Jesse Goodman, Chief Scientist FDA
Tissue Chip for Drug Screening

NIH-DARPA-FDA Collaboration to Help Predict Drug Safety

Program Overview

The National Institutes of Health, in collaboration with the Defense Advanced Research Projects Agency (DARPA) and U.S. Food and Drug Administration, is leading an initiative to improve the process for predicting whether drugs will be safe in humans. The goal is to develop human tissue chips that accurately model the structure and function of human organs, such as the lung, liver and heart. Once developed, researchers can use the tissue chips to test drug candidates and help predict safety in human studies more rapidly and cost-effectively than current methods. More than 30 percent of promising medications have failed in human clinical trials because they are determined to be toxic despite promising pre-clinical studies in animal models. Tissue chips may enable scientists to predict more accurately the toxic effects of potential therapeutic candidates because they use human cells capable of mimicking an organ’s structure and function. The NIH Tissue Chip for Drug Screening initiative marks the first interagency collaboration launched by NIH’s newly created center, the National Center for Advancing Translational Sciences (NCATS). NIH’s Common Fund and National Institute of Neurological Disorders and Stroke led the trans-NIH effort to establish the program.

Improving Predictive Systems for Drug Safety

In July 2012, NIH issued 17 awards, 10 of which will support studies to develop 3-D cellular microsystems that represent a number of human organ systems. These bio-engineered devices will be functionally relevant and also accurately reflect the complexity of the tissue of origin, including genomic diversity, disease complexity and pharmacological response. The additional seven awards will explore the potential of stem and progenitor cells to differentiate into multiple cell types that represent the cellular architecture within organ systems. These could act as a source of cells to populate tissue chips.

In addition to focusing on organ systems, some of the awarded researchers will develop tissue microsystems that target specific health conditions, such as cardiovascular disease, cancer, degenerative arthritis and gastrointestinal disease.
Our work is the intersection of four technologies for development and testing of nanomedicine approaches to ductal breast cancer.
Our initial branching tree microfluidic chips

Culture of mammary epithelial cells in branched PDMS hemichannels

Fig. 3 Culture of mammary epithelial cells in branched PDMS hemichannels in the presence of laminin 111. HMT-3522 S1 cells were cultured in presence of laminin 111 (L) in complete microchannels or in hemichannels according to Fig. 2B. Laminin 111 was either coated and dried on PDMS (dry) or diluted in the H14 culture medium and dripped on the cell population at the time of plating (drip). (A) Bright field images. Individual round cells are indicated by arrowheads in a complete channel. Cell clumping in hemichannels with the drip method is indicated by the arrow. (B)–(D) Confocal analysis of DAPI-stained S1 cells in the terminal branch from a hemichannel coated with dried laminin 111. Maximal intensity projection of a z-stack taken at low magnification is shown together with tiled bright field micrographs of the hemichannel (B). Maximal intensity projection of a z-stack taken at high magnification (C) with orthogonal view at the level of the dotted line (D). (E) & (F) Confocal analysis of basal polarity marker α6 integrin (green) and apical polarity marker ZO-1 (red) in S1 monolayer located on a side wall of the hemichannel coated with dried laminin 111. Nuclei are counterstained in blue. Size bars, 20 mm (B) and 5 mm (C, E, F).

Making use of nano and cell measuring tools at Purdue University
Some Components of CTSI
“Bionanotechnology”

Nanosensing
(molecular and nanomechanical sensing of physiological parameters)

Nanomedical Systems Development

Nanochemistry
(nanoparticle synthesis, bioconjugation chemistry for peptide/antibody attachments, ...)

BioMEMS
Bio-MicroElectroMechanical Systems
(microfabrication, microfluidics, portable devices, ...)

BioCleanroom facility
(in development)
(production of sterile medical micro- and nanodevices)
In-vivo/organ-on-a-chip imaging

Nanosystems core design, construction, and characterization

Superparamagnetic iron oxide, quantum dots, chitosan
(Nanochemistry, TEM, XPS, AFM, confocal microscopy, flow cytometry)
Emily Haglund*, Mary-Margaret Seale*, Christy Cooper, Jaehong Key*/KIST

Therapeutics
Peptides, Drugs, In-situ drug manufacture, Nanotoxicity, Biodistribution
Trisha Eustaquio, Desiree White

In-vivo/organ-on-a-chip imaging

NIRF fluorescence imaging Magnetic Resonance Imaging
Jaehong Key*, KIST
Interactions Between Technologies for Development of Nanomedical Systems

Nanoparticle fabrication and quality control labs
- Nanochemistry
- Dynamic Light scattering sizing
- Zeta Potential
- Atomic Force Microscopy

Cell and intracellular targeting labs
- Flow cytometry
- High-throughput Imaging (laser opto-injection and ablation) cytometry
- Confocal (one- and multi-photon analysis)
- Super-resolution microscopy

Transient Gene Therapy ("gene drugs")
- Construction of therapeutic genes for specific biomedical applications
- Animal testing/comparative medicine
- Organ-on-a-chip

Nanomaterials biocompatibility labs
- Microscopy/image analysis/LEAP
- Gene expression microarray analyses

Biosensor Labs
- Biosensor molecular biology
- Results evaluated in targeting labs
Global Research Lab Program  “nano goes global”

Molecular Imaging and Nanomedicine for Theragnosis using Nano-Biomaterials


KPI: Kuiwon Choi (KIST)
FPI: James F. Leary (Purdue Univ.)
Potentials of SPIO (Superparamagnetic Iron Oxide) Nanoparticles

A. CONTRAST AGENT (Diagnosis)
- External magnetic field
- $H_2O$
- Induced field
- Tumor cell

B. DRUG DELIVERY (Therapy)
- Drug delivery
- Targeting ligand
- Core particle
- Drug release
- Coating material
- Tumor cell

C. HYPERTHERMIA (Therapy)
- External AC magnetic field
- Tumor cell

D. Physically Guided NPs (Diagnosis & Therapy)
- Strong Magnet
- Tumor cell
## Imaging Systems: Sensitivity and Spatial Resolution

<table>
<thead>
<tr>
<th>Imaging Technique</th>
<th>Spatial Resolution&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sensitivity&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Source of Imaging</th>
<th>Target</th>
<th>Tissue Penetrating Depth</th>
</tr>
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<tbody>
<tr>
<td>MRI</td>
<td>&gt; 7T, 25-300 µm Human 3T, 1mm</td>
<td>mM to µM (low)</td>
<td>Radiowave</td>
<td>Anatomical, physiological, molecular</td>
<td>No limit</td>
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<tr>
<td>CT</td>
<td>50-200 µm</td>
<td>not well characterized</td>
<td>X-ray</td>
<td>Anatomical, physiological</td>
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<td>PET</td>
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<td>No limit</td>
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<td>Optical fluorescence Imaging</td>
<td>In vivo, 2-3 mm In vitro, sub-µm</td>
<td>nM to pM (medium)</td>
<td>Visible or near-infrared light</td>
<td>Physiological, molecular</td>
<td>&lt; 1cm</td>
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<sup>a</sup> For high-resolution, small-animal imaging systems (Clinical imaging systems are different)

<sup>b</sup> Sensitivity of detecting probe relative to background
Why “multi-modality” in-vivo/organ-on-a-chip imaging?

- No single in-vivo imaging modality accomplishes everything we want the systems to do.

- We need to cover a range of sizes from centimeters (size of tumors) down to microns (cell-sized).
Potentials of optical/MR dual modality imaging

- MR Imaging
- NIRF Imaging
- Confocal Imaging

A Whole Body Imaging

Specific tumors

Nanoparticles in each tumor cell
Design of Multicomponent nanoparticles

Targeting ligands (for active targeting) (e.g. Antibodies, peptides, or aptamers)

Stealth effects (Polyethylene glycol, Dextran)

Therapeutic agents (Doxorubicin, Paclitaxel, siRNA)

Imaging agents (MR, CT, PET, Optical Imaging)

Figure 1. Schematic diagram of CSNRDARRC-HGC

SPIOs (4 nm) loaded HGC NPs (GS)

GC Conjugates → Self-assembled GC nanoparticle → Oleic acid capped SPIOs → SPIO loaded GC nanoparticle

SPIOs

15 nm

GS NPs

200 nm

200 nm
Multicomponent Nanoparticles for Optical/MR Dual-Modality Imaging

- Carrier
  (glycol chitosan nanoparticles)
- MR imaging agent
  (superparamagnetic iron oxide)
- NIRF dye
  (near infrared fluorescent Cy5.5)

MR Contrast Effect by Magnetic NPs

Agglomerated MNPs

\[ R_2 = \frac{64\pi}{135} \left( \mu_0 \gamma \mu_{sp} N_g \frac{L(x)}{4\pi} \right)^2 \frac{N_A C_a}{R_a D} \]

where
- \( \mu_{sp} \) is the magnetic moment of the elementary crystal,
- \( N_g \) is the number of nanoparticles in a single agglomerate,
- \( C_a \) is the agglomerate concentration,
- \( R_a \) is the radius of a single agglomerate, and
- \( D \) is diffusion coefficient of water

Coating effect of MNPs

\[ R_2 = \frac{1}{T_2} = \frac{\left( \frac{256 \pi^2 \gamma^2}{405} \right) V^* M_s^2 a^2}{D(1 + L/a)} \]

where
- \( V^* \) is the volume fraction of the magnetic core,
- \( M_s \) is the saturation magnetization of the NPs,
- \( a \) is the radius of the core, and
- \( L \) is the thickness of an surface coating

MR Contrast Effect by Magnetic NPs

Magnetic Properties of MNPs

Size & Surface Area-dependent MR properties of SPIOs

Aggregation of Ferromagnetic Materials by High Coercivity

\[ m_s = M_s \left( \frac{r-d}{r} \right)^3 \]

where
- \( m_s \) is the saturation magnetization,
- \( M_s \) is the saturation of bulk state,
- \( d \) is the thickness of the disordered surface layer

**T1 & T2 MR contrast agents**

<table>
<thead>
<tr>
<th><strong>T₁ contrast agents</strong> (Gadolinium)</th>
<th><strong>T₂ contrast agents</strong> (SPIO)</th>
</tr>
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<tbody>
<tr>
<td>Positive Contrast (White)</td>
<td>Negative Contrast (Black)</td>
</tr>
<tr>
<td>Detection limit (10⁻⁵ moles/l)</td>
<td>Detection limit (10⁻⁶ moles/l)</td>
</tr>
<tr>
<td>NSF by high toxicity (chelate compound), Very short circulation time (a few minutes)</td>
<td>Low toxicity</td>
</tr>
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## Imaging Systems

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MRI and NIRF imaging of multicomponent nanoparticles in tissue phantoms

(A) Water  HGC  GS  Water  Water  HGC  GS  Water

TE = 9 ms  TE = 72 ms

(B) ½ dilution

(C) ½ dilution
Near Infrared Fluorescence (NIRF) Imaging

Dual Reporter Imaging - High Resolution
Ex Vivo Applications

Field of View

The MI Lumina II Imaging System provides 5 fields of view.
In vivo and ex vivo of 10 nm SPIO loaded HGC

MBT2 cells implanted mice

Ex vivo NIRF Imaging
Ex vivo results means that most NPs were accumulated in cancer and liver. The accumulation in liver is a problem still remained. It might cause by large size or less flexibility of the NPs. However, when comparing current drugs available, it is still meaningful in terms of that the NPs were mostly accumulated in cancer.
Biodistribution of 10 nm SPIO loaded HGC
Nanocubes loaded CSNRDARRC-HGC

Reference: Kim et al., J AM Chem Soc., 2009
# Characterization

<table>
<thead>
<tr>
<th>Samples</th>
<th>β-cholanic acid (wt%) of GC</th>
<th>Nanocube Feed (µl) (Conc. 557 µg/µl)</th>
<th>Diameter (nm)</th>
<th>Polydispersity Index</th>
<th>Z-potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGC</td>
<td>12</td>
<td>-</td>
<td>288.7 ± 5.0</td>
<td>0.212 ± 0.017</td>
<td>9.76 ± 0.4</td>
</tr>
<tr>
<td>CSNRDARRC-HGC (C-HGC)</td>
<td>12</td>
<td>-</td>
<td>359.7 ± 2.5</td>
<td>0.195 ± 0.008</td>
<td>10.4 ± 2.5</td>
</tr>
<tr>
<td>CSNRDARRC-HGC-NC15 (C-HGC-NC)</td>
<td>12</td>
<td>15</td>
<td>481.8 ± 8.7</td>
<td>0.230 ± 0.041</td>
<td>32.3 ± 0.9</td>
</tr>
</tbody>
</table>

![Graphs showing intensity vs. diameter for HGC, C-HGC, and C-HGC-NC](image-url)
Size and shape of CSNRDARRC-HGC

Scale bar: 500 nm
Intra-cellular targeting of nanoparticles

(A) NP(-), K9TCC

(B) C-HGC, K9TCC, 2H

(C) HGC, K9TCC, 2H

(D) C-HGC, K9TCC, 4H

(E) HGC, K9TCC, 4H

(2hr and 4hr incubation)
BACKGROUND

Glycol Chitosan (GC)

A. DDS System

B. Dual-modality Optical and MR imaging

C. pH dependent binding GC NPs

Kim, JCR 2010

Lee, Bioconjug Chem, 2011

Crayton, ACS Nano, 2011
Z-potential of NPs and binding effects

Schematic Representation of z-potential

Highly positive NPs: non-specific binding

Highly negative NPs: No binding

Slightly negative NPs: specific binding

http://www.malverninstruments.fr
# Z-potential of C-HGC-NCs

<table>
<thead>
<tr>
<th>Samples</th>
<th>ζ-potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanowater (1 mL)</td>
<td>-0.745</td>
</tr>
<tr>
<td>Nanowater (1 mL) + C-HGC-NCs (15 µL)</td>
<td>26.7</td>
</tr>
<tr>
<td>Serum (1 mL)</td>
<td>-8.37</td>
</tr>
<tr>
<td>Serum (1 mL) + C-HGC-NCs (15 µL)</td>
<td>-5.17</td>
</tr>
<tr>
<td>Plasma (1 mL)</td>
<td>-4.91</td>
</tr>
<tr>
<td>Plasma (1 mL) + C-HGC-NCs (15 µL)</td>
<td>-2.5</td>
</tr>
</tbody>
</table>
Current Leary-Lab Collaborative Projects

Stem cells & Regenerative Medicine

Cancer, Cancer stem cells & rare Circulating Tumor Cells

Lab-on-a-Chip /MEMS/NEMS

Nanomedicine & Molecular Imaging

Current Technologies & Applications:
1. Breast-on-a-chip (ductal breast cancer)
2. Multimodal imaging for theragnostics
3. Dog bladder cancer
4. Dog B-cell lymphoma
5. Nanobarcoding
6. High-throughput imaging
7. Stem cells & Regenerative medicine
8. Space blood analyzer
9. CTC microcytometer
10. Pediatric blood analyzer
11. High-speed flow cytometry/Rare cell detection and isolation
12. Plant cell reprogramming
13. Nano-approaches to brain aneurisms
14. Nano-ophthalmology
15. Neuroscience applications of nanomedicine
# Leary Lab Team and Current Collaborators

## Nanochemistry
- **Don Bergstrom** (Purdue)

## Combinatorial chemistry/Drug Discovery
- David Gorenstein (UTMB)
- Xianbin Yang (UTMB)
- Andy Ellington (UT-Austin)

## Nanoparticle technology
- Nick Kotov (Univ. Michigan)
- Kinam Park (Purdue)
- Alex Wei (Purdue)

## Nanotoxicity studies
- **Debbie Knapp** (Purdue)
- James Klaunig (IU-SOM)

## MRI Imaging
- **Tom Talavage** (Purdue)
- Charles Bouman (Purdue)

## Mol. Imaging/Theranostics
- Kuiwon Choi (KIST)
- Ich Chan Kwon (KIST)
- Kwangmeyung Kim (KIST)

KIST=Korean Institute of Science and Technology (many others)

## Molecular Cytometry Facility
- **Director: James Leary**
- Lisa Reece (SVM) – flow cytometry/Magnetic cell sorting; tissue culture
- **Christy Cooper** (SVM) - bioanalytical chemistry, nanochemistry, XPS, AFM
- **Meggie Grafton** (BME) - BioMEMS
- **Emily Haglund** *(BME) – multilayered Qdots for ex-vivo nanomedicine
- **Mary-Margaret Seale-Goldsmith** *(BME) – multi-layered magnetic nanomedical systems
- Michael Zordan (BME) – prostate cancer, rare cell flow/image cytometry
- **Trisha Eustaquio** (BME) – gene silencing/therapy; interactive imaging
- **Jaehong Key** *(BME)-MRI imaging
- **Teimour Maleki**, PhD – micro- and nanofabrication; BioMEMS
- Desiree White (IBSC) mesoporous NPs for spinal cord injury

## Nanomedicine studies
- **Debbie Knapp** (Purdue-SVM)
- **Deepika Dhawan** (Purdue-SVM)
- **Sophie Lelievre** (Purdue-SVM)
- **Tarl Prow** *(U. Brisbane, Australia)

*RRecently graduated ** Former student

## X-ray Photon Spectroscopy
- **Dmitry Zemlyanov** (Purdue)

## High-Energy TEM
- **Eric Stach** (Purdue)
- **Dmitri Zakharov** (Purdue)

## Atomic Force Microscopy
- **Helen McNally** (Purdue)

## Magnetic Cell Sorting
- Paul Todd *(Techshot, Inc)
- Dave Kennedy (IKOtech, Inc)

## LEAP Interactive Imaging
- Fred Koller (Cyntellect, Inc.)

## BioMEMS/Microfluidics
- Kinam Park (Purdue)
- Steve Wereley (Purdue)
- Huw Summers (Swansea Univ, UK)

## Nano-Ophthalmology
- **Gerald Lutty** (Johns Hopkins)
- Robert Ritch *(Glaucoma Found.)*
- Marco Zarbin (NJ Med. School)
- Carlo Montemagno (U. Cincinnati)

*Funding from NIH, NASA, USDA, DOD Breast Cancer*
Thank you for your attention!