CORE P: Cell-Based Profiling of Polymeric Biomaterials

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Rutgers University

Visualizing the Interactions of Cells and Biomaterials
High Resolution Imaging for Regenerative Medicine and Tissue Engineering

You are cordially invited to view our exhibit at the biennial NJCBM Symposium, Hyatt Regency New Brunswick, October 29-31

Sign up for demonstrations and learn about our:
• State of the art instruments
• 2D and 3D imaging capabilities
• Knowledgeable service staff
• Commitment to furthering your research

FROM MACRO TO MICRO
BIOMATERIALS TO CELLS
WOUND HEALING TO DRUG DELIVERY
STEM CELLS TO REGENERATIVE MEDICINE

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Visit us on the web at www.confocal.rutgers.edu

RESBIO
CORE P: High Content Imaging for Cell-Biomaterials Profiling

- Introduction
  - Motivation for High Content and High Throughput Imaging of Cells on Biomaterials

- Overview of Core Imaging Resources
  - Imaging and Profiling Tool-Box
  - Cell Reporter Libraries

- Applications
  - Stem Cell Lineage Profiling on Biomaterials
  - Decoding Cell Fates on High Throughput Platforms
  - Biomaterials for Tissue Regeneration & Cancer Management

- Facility Outreach and Training
Multiphoton Microscopy: High Resolution Imaging Methodology

- 2-Photon Absorption
  - Fluorophore excitation is restricted to the plane of focus.
  - Volume bleaching is eliminated.
  - Less scattering leads to higher penetration depth

- **Femtosecond pulsed laser**
- **100 fs pulse; 100 MHz**

**References**
- The Combinatorial-Computational Method for Biomaterials Optimization
- Integrated Technologies for Polymeric Biomaterials funded by NIH EB001046
Multiphoton Imaging for Composite Profiling of Microstructure and Fluororeporter Cell Organization within Porous Polymer Scaffolds

Maximum intensity projection image of poly(DTE carbonate) porous scaffold microstructure using MPM imaging technique. 10x objective was used, and z depth=250µm

Maximum intensity projection MPM images of GFP-fibroblast on porous scaffolds of 30% poly(DTE carbonate)/70%poly(DTO carbonate) blends. 20x objective; z depth=98.71µm
High Magnification Multiphoton Microscopy of Cellular Adhesion to Fibers of an Electrospun Polymer Mesh:
SaOS2/pGFP-actin cells on electrospun pDTEc fibers

Fiber Spacing/Width: Supercellular Scale
Fiber Spacing/Width: Subcellular Scale
Core P : Cell Based Profiling of Biomaterials

Rationale:
The field of biomaterials science is challenged by the lack of objective, quantifiable biological metrics of cell responses.

Goals:
To develop a cell-based “materials bio-profiling tool box”:
- Genetically engineered fluorescent reporter cells
- Fluorescent imaging and related image processing algorithms
- Semi-empirical modeling methods to correlate cell descriptors to biomaterials.

Apply the tool-box to elucidate cell-material interactions that govern selected, key cell functional fates on biomaterials.
High Content Imaging of Cells on Biomaterials

Discrimination of cell responsiveness to biomaterials with incrementally engineered surface properties, and to large arrays of combinatorially synthesized or processed biomaterials

Polymer with charge and PEG

Poly(DTE carbonate)

• Cell descriptors can capture the heterogeneity of cell response to the same biomaterial or across systematically engineered biomaterials.

• Living cell based descriptors are not skewed by cell adhesive responses and allow dynamic reporting.

• Descriptors convey information about the organization of “cytoskeleton”, as integrators of the outside-in signaling.

Cell morphology is altered on biomaterials with varying chemistry:
Challenge: Predicting Cell Functions

Treiser et al., Biotechniques 43: 361, 2007
Toolbox for Profiling Cell-Biomaterial Interactions

CELLS (Stem Cells)

Substrate

Image-Based Morphometric Analysis of Cells at Early Time Points

Soluble Cues

24 hrs → 1-2 weeks

Functional Output at late Time Points

Fat

Bone

Multi-Dimensional Scaling

Computation of “High-Content Descriptors” Based on Nuclear and Cytoskeletal Protein Morphologies and Spatial Distributions

Prediction of long-term behavior in response to different substrates or soluble factors based on early “High-content” Descriptors
Modeling Methods to Determine Material Responsive Descriptors

Decision tree identifying descriptor sensitive to PEG

- Clumpiness
  - < 0.105
    - PEG Present
  - ≥ 0.105
    - No PEG Present

PEG Present

Iodine
- Present
  - Poly(85% DTE-co-15% PEG1k carbonate)
  - 0.0797
- Not Present
  - Poly(I2 DTE carbonate)

0.1549

0.1088
Application 1: Parsing Biomaterial Effects on Stem Cell Lineage Commitment

% Cell Positive

Polymer Fast Blue Average

Polymer Oil Red Average

- The Combinatorial-Computational Method for Biomaterials Optimization
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Cell Morphometric Descriptors Can be Used to Model Fates of Mixed Populations

Predictions:
Bone Lineage

Entire descriptor dimensional space is converted to three dimensions
The hypothesis is that each path leads to different cellular fates
Two classes of cell fates were observed using a non-supervised, automated process.
Two paths predicting bone and fate lineage differentiation identified.
Reference point indicates cells which have not grown or differentiated appreciably
Tools: Multi-functional imaging

Application 2: High Throughput Platforms for Cell-Biomaterials Interactions

- Spin coating of selected polymers
- Polymer-coated coverslip
- Sample fabrication platform

Confocal/Multiphoton imaging facility

- Cell growth screening
- Surface marker expression screening
- Telomerase mRNA expression screening
- High content Nuclear protein imaging

Gradient fabrication platform

Sample fabrication platform

 Increasing Temperature
- HOT
- COLD

Screening abnormal transformation of hMSCs
**Tools: Multi-functional imaging**

**Application 2: High Throughput Platforms for Cell-Biomaterials Interactions**

**Hydrophobicity: DTO>DTE**

- Rapid screening & High resolution
  - Programming for motor-driven automatic xy scanning
  - Confocal-multiphoton imaging

- **Screening speed:** 2~5s/image
- **Throughput:** 720-1,800 frames/h

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- The Combinatorial-Computational Method for Biomaterials Optimization
- Integrated Technologies for Polymeric Biomaterials • funded by NIH EB001046 •
Rapid Screening Reveals Insights about Cell Adhesion in Response to Local Surface Roughness…
Rapid Screening Reveals Insights about Cell Adhesion in Response to Local Surface Roughness…

![Graph showing cell number normalized to cells on DTE against specific gradient steepness of roughness (10^-6/mm).]

- 70DTE/30DTO
- 50DTE/50DTO
- 30DTE/70DTO
High-content imaging based descriptors inform about cell adhesive responses on complex polymer substrates

Saos-2 GFP-farnesylation (10x) on 70%p(DTEc)/30%p(DTOc) blend

- Ras-farnesyl residue
- Plasma membrane
- Adhesion
- Growth
- Death
- Cytoskeleton organization

Gray bar - Cell spreading (μm²)

<table>
<thead>
<tr>
<th></th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
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</table>

White bar - Farnesylated spots normalized to cell area (μm²)

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“Zoom-In” to derive high content cell descriptors governed by biomaterial composition and surface roughness
Tool Box: Cell Reporters for study of cell-biomaterial interaction (in collaboration with Area M)

Fluororeporter cells: genetically engineered cells with fluorescence sensor, reporting site-specific, time-dependent changes in cell response to biomaterials.

**Change of cell behaviors**

**Cell**

**Input variables**

Biomaterial Surface
- Chemical, physical, & morphological parameters
- 2D, gradient, & 3D formats

**Visual report by fluorescent sensor (ex. GFP-actin)**

Different biomaterial surfaces

Treiser et al., Biotechniques, 2007.
GFP: Green fluorescent protein
## Green Fluorescence Protein (GFP)- reporters

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<th>Function</th>
<th>Plasmid</th>
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<td>Cell spreading</td>
<td>pEGFP-F (Farnesylation)</td>
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<tr>
<td>Oncogenic Ras signaling</td>
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<tr>
<td>Proliferation</td>
<td>pECFP-Nuc, pEGFP-RecA-NLS</td>
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<td>Apoptosis</td>
<td>pCaspase3-Sensor, pEGFP-GAPDH,</td>
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<tr>
<td>Cytoskeletal Organization</td>
<td>pEGFP-Actin, pEGFP-Tub, pEGFP-α-actinin</td>
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<td>Focal Adhesions</td>
<td>pEGFP-α5-integrin, pEGFP-paxillin, pGFP-vinculin</td>
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<td>Signaling Mechanism</td>
<td>pEGFP-rhoA, pEGFP-cdc42, pEGFP-rac1,pEGFP-rac, pEGFP-rac,</td>
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<td>pEGFP-rac (T17N), pEGFP-rac (Q61L), pEGFP-STAT1</td>
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<tr>
<td>Cell-Cell Adhesions</td>
<td>pEGFP-ECAD, pEGFP-NCAD</td>
</tr>
</tbody>
</table>

**GOALS:** To develop a cell-based “material bio-profiling tool box”.

1. Genetically engineered fluororeporter cells
2. Imaging and related image processing algorithms
3. Semi-empirical modeling methods to predict cell responses to biomaterials, supported by experimental, mechanistic studies
Dynamic Real-time Resolution of Cell Descriptors:
Example: Cell cytoskeletal remodeling on biomaterials: GFP-Actin

Dying cell
(Cell death induction)

Live cell

60 minute after induction
Live tracking of programmed cell death on biomaterials: Apoptotic nuclear translocation of GFP-GAPDH

- TCPS: Tissue culture plate
- *p<0.05 between the connected groups
- †p<0.05 vs. all the other groups
## Impact of RESBIO Resources

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<th>Collaborating Investigator</th>
<th>Affiliation &amp; Grant Source</th>
<th>Projects</th>
<th>Nature of Resource Impact</th>
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<td><strong>Andres Garcia</strong></td>
<td>Bioengineering, Georgia Institute of Technology, NIH NIBIB R01</td>
<td>Parsing cell behaviors on mixed ligand based biomaterials</td>
<td>Identification of intracellular descriptors on complex substrates</td>
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<tr>
<td><strong>Bonnie Firestein</strong></td>
<td>Neuroscience, Rutgers, NSF</td>
<td>Role of NOS1AP in forebrain development</td>
<td>Quantitative Imaging of subcellular structures (dendritic spines) in neural cells</td>
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<td><strong>Sangeeta Bhatia</strong></td>
<td>Bioengineering, MIT, NIH NIDDK RO1</td>
<td>Stem cell differentiation on matrix microarrays.</td>
<td>Correlation of cell descriptors to cell differentiation</td>
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<td><strong>Treena Arinzeh</strong></td>
<td>Biomedical Engineering, NJIT; NSF PECASE</td>
<td>Stem cells on polymer scaffolds</td>
<td>Imaging of cells in 3-D</td>
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<td><strong>Brett Hoover</strong></td>
<td>Cleveland Clinic Tissue Engineering Center</td>
<td>Application of Colonyze tissue colony morphometrics</td>
<td>Benchmarking of RESBIO resources against clinical tissue morphometrics.</td>
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<td><strong>Michael Sefton</strong></td>
<td>Chemical Engineering, U. Toronto</td>
<td>Biological profiling of polymeric materials</td>
<td>Imaging and biological profiling of materials</td>
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<td><strong>Rami Mangoubi</strong></td>
<td>Draper Laboratories NIH R01EB006161</td>
<td>Dynamic Image Analysis of Human Embryonic Stem Cell Pluripotency</td>
<td>Data sets for complementary modeling</td>
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Follow-up

• Facility tours
• Group Demos
• Training
• Short Course
  – Introduction to high resolution imaging of cell-biomaterials interactions: Confocal/Multiphoton Microscopy
  – Applications for cells in 3-D scaffolds
  – High Content Imaging and Modeling for Strategic Cell Functions