BioMEMS for Instrumenting and Controlling the Single Cell

John P. Wikswo

IEEE 2004 EMBS Conference, September 2004
Workshop on Microanalytical Devices for Bioprocessing
The rate at which DNA sequences began accumulating was exponential.

- ~13 million sequence entries in GenBank
- Nearly 13 billion bases from ~50,000 species

Rapid DNA sequencing invented
Human Genome Project begun

Year

GB


14,000,000
12,000,000
10,000,000
8,000,000
6,000,000
4,000,000
2,000,000
0

2002: 22,318,883


Courtesy of Mark Boguski

National Library of Medicine
- $10^9$ s: Aging
- $10^8$ s: Survival with CHF
- $10^7$ s: Bone healing
- $10^6$ s: Small wound healing
- $10^5$ s: Atrial remodeling with AF
- $10^4$ s
- $10^3$ s: Cell proliferation; DNA replication
- $10^2$ s: Protein synthesis
- $10^1$ s: Allosteric enzyme control; life with VF
- $10^0$ s: Heartbeat
- $10^{-1}$ s: Glycolosis
- $10^{-2}$ s: Oxidative phosphorylation in mitochondria
- $10^{-3}$ s
- $10^{-4}$ s: Intracellular diffusion, enzymatic reactions
- $10^{-5}$ s
- $10^{-6}$ s: Receptor-ligand, enzyme-substrate reactions
- $10^{-7}$ s
- $10^{-8}$ s
- $10^{-9}$ s: Ion channel gating
The Catch

• Modeling of a single mammalian cell may require 100,000 *dynamic* variables and equations

• Cell-cell interactions are critical to system function

• $10^9$ interacting cells in some organs

• Cell signaling is a highly *DYNAMIC*, multi-pathway process

• Many of the interactions are non-linear

• **The data don’t yet exist to drive the models**

• Hence we need to *experiment*…
The Grand Challenge

There are no technologies that allow the measurement of a **hundred**, time dependent, intracellular variables in a **single** cell (and their correlation with cellular signaling and metabolic dynamics), or between groups of different cells.
Instrumenting and Controlling The Single Cell

VIIBRE Goal

Develop devices, algorithms, and measurement techniques that will allow us to instrument and control single cells and small populations of cells and thereby explore the complexities of quantitative, experimental systems biology
What do we need to study cellular dynamics?

- Multiple, fast sensors
- Intra- and extracellular actuators for controlled perturbations
- Openers (Mutation, siRNA, drugs) for the internal feedback loops
- System algorithms and models that allow you to close and stabilize the external feedback loop

...
# Sizes, Volumes, Time Constants

<table>
<thead>
<tr>
<th>X</th>
<th>V, m³</th>
<th>V</th>
<th>Tau&lt;sub&gt;Diff&lt;/sub&gt;</th>
<th>Example</th>
<th>N</th>
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</thead>
<tbody>
<tr>
<td>1 m</td>
<td>1</td>
<td>1000 L</td>
<td>10⁹ s</td>
<td>Animal, bioreactor</td>
<td>100</td>
</tr>
<tr>
<td>10 cm</td>
<td>10⁻³</td>
<td>1 L</td>
<td>10⁷ s</td>
<td>Organ, bioreactor</td>
<td>100</td>
</tr>
<tr>
<td>1 cm</td>
<td>10⁻⁶</td>
<td>1 mL</td>
<td>10⁵ s = 1 day</td>
<td>Tissue, cell culture</td>
<td>10</td>
</tr>
<tr>
<td>1 mm</td>
<td>10⁻⁹</td>
<td>1 uL</td>
<td>10³ s</td>
<td>µenviron, well plate</td>
<td>10</td>
</tr>
<tr>
<td>100 µm</td>
<td>10⁻¹²</td>
<td>1 nL</td>
<td>10 s</td>
<td>Cell-cell signaling</td>
<td>5</td>
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<tr>
<td>10 µm</td>
<td>10⁻¹⁵</td>
<td>1 pL</td>
<td>0.1 s</td>
<td>Cell</td>
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<tr>
<td>1 µm</td>
<td>10⁻¹⁸</td>
<td>1 fL</td>
<td>1 ms</td>
<td>Subspace</td>
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<tr>
<td>100 nm</td>
<td>10⁻²¹</td>
<td>1 aL</td>
<td>10 µs</td>
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<tr>
<td>10 nm</td>
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<td>100 ns</td>
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</tr>
<tr>
<td>1 nm</td>
<td>10⁻²⁷</td>
<td>1 npL</td>
<td>1 ns</td>
<td>Ion channel</td>
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</tbody>
</table>
High-Content Toxicology Screening Using Massively Parallel, Multi-Phasic Cellular Biological Activity Detectors MP²-CBAD

F Baudenbacher, R Balcarcel, D Cliffel, S Eklund, I Ges, O McGuinness, A Prokop, R Reiserer, D Schaffer, M Stremler, R Thompson, A Werdich, and JP Wikswo

Vanderbilt Institute for Integrative Biosystems Research and Education (VIIBRE)
Edgewood Chemical and Biological Center (SBCCOM / ECBC)
Objective

• Develop cell-based, fast-response metabolic sensing arrays for detection and discrimination of toxins or for use in drug screening efforts.

• Use massively parallel arrays of devices with multiple sensors and cell lines, subnanoliter volumes, and active microfluidics for rapid response and closed loop control of the extracellular space!

• Massively Parallel, Multi-Phasic Cellular Biological Activity Detector (MP²-CBAD)
MP²-CBAD Discrimination

Discrimination Matrix

Sensor Array

Toxin

Cell Types
- HeLa
- NB
- HepG2

Output

pH  DO  Glc  Lac  CO₂  NADH

NanoPhysiometer
Simplified Metabolic Network

- Glucose
- Glycolysis
- NADH
- Oxidative Phosphorylation
- TCA Cycle
- NADPH Oxidase
- Lactate
- Acidification
- CO₂
- Heat
- Oxygen

### Chemical Equations

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Products</th>
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</thead>
<tbody>
<tr>
<td>Glucose + 2 ADP + 2 NAD⁺</td>
<td>2 Pyruvate + 2 ATP + 2 NADH</td>
</tr>
<tr>
<td>Pyruvate + NADH</td>
<td>Lactate + NAD⁺</td>
</tr>
<tr>
<td>Pyruvate + CoA + FAD + GDP + 3 NAD⁺ + NAD(P)⁺</td>
<td>3 CO₂ + FADH₂ + GTP + 3 NADH + NAD(P)H</td>
</tr>
<tr>
<td>0.5 O₂ + 3 ADP + NADH</td>
<td>3 ATP + NAD⁺</td>
</tr>
<tr>
<td>0.5 O₂ + 2 ADP + FADH₂</td>
<td>2 ATP + FAD</td>
</tr>
</tbody>
</table>

- Robert Balcarcel
- Franz Baudenbacher
- David Cliffel
- Ales Prokop
- Owen McGuinness
- John Wikswo
Objective

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• Massively Parallel, Multi-Phasic Cellular Biological Activity Detector (MP²-CBAD)
Cell-Based Biosensor as Generalized Toxicity Sensor

• We do not measure the toxin itself. We are measuring the impact of the toxin on cell physiology by probing cell functions!
  – Metabolic pathways
  – Signaling pathways
  – Electrical excitability
  – Cell-to-cell communication ..........

→ **Intrinsic amplification**
MP$^2$-CBAD Discrimination

- Simultaneous monitoring of *multiple metabolic signals*
- Characteristic response in a *conditioned* environment
- Characteristic responses of cellular *phenotypes* to toxins
- Characteristic reaction *kinetics* of metabolic pathways
Discrimination: Simultaneous monitoring of multiple metabolic signals

- **Infuse DNP**
  - Heat production
  - CO₂ production
  - O₂ Consumption
  - Glucose uptake
  - Lactate release

- **Infuse Cyanide**
  - Heat production
  - CO₂ Production
  - O₂ consumption
  - Glucose uptake
  - Lactate release
The well size determines the bandwidth

- Microliter – 10-100 seconds
  Modified Cytosensor MicroPhysiometer

- SubNanoliter – 10-100 milliseconds
  Vanderbilt NanoPhysiometer
Microliter – 10-100 seconds
Modified Cytosensor MicroPhysiometer
David Cliffel, Sven Eklund et al

MicroPhysiometer: Modified sensor head

Multicell Metabolism and Signaling

MicroPhysiometer measurement of the change in glucose, lactate, and oxygen concentrations and acidification rate in response to a 720 s treatment of CHO cells with 20 mM fluoride.
Macrophages upon exposure to ~15 mM alamethicin

Alamethicin is an antibiotic that creates membrane pores

- **Glucose Consumption Rate**
  - **Glucose** concentration decreases upon exposure to alamethicin.

- **Oxygen Consumption Rate**
  - **Oxygen** concentration decreases upon exposure to alamethicin.

- **Lactate Production Rate**
  - **Lactate** concentration decreases upon exposure to alamethicin.

- **Acidification Rate**
  - **Acidification** rate increases upon exposure to alamethicin.
The Next Steps

- Inverse sensor model
- Inverse metabolic network model
- Additional metabolic parameters
- Apply experiments, models and analysis to examine the blocking or enhancing of metabolic pathways
The well size determines the bandwidth

- **Microliter** – 10-100 seconds
  - Modified Cytosensor MicroPhysiometer

- **SubNanoliters** – 10-100 **milliseconds**
  - Vanderbilt NanoPhysiometer
### Physical and Biological Time Constants, Seconds

<table>
<thead>
<tr>
<th>Process</th>
<th>Time Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixing time to homogenize liquid in a large-scale bioreactor (10-100 m³)</td>
<td>$10^4 - 10^8$</td>
</tr>
<tr>
<td>90% liquid volume exchange in a continuous reactor</td>
<td>$10^5 - 10^6$</td>
</tr>
<tr>
<td>Oxygen transfer (forced not free diffusion)</td>
<td>$10^2 - 10^3$</td>
</tr>
<tr>
<td>Heat transfer (forced convection)</td>
<td>$10^3 - 10^4$</td>
</tr>
<tr>
<td>Cell proliferation, DNA replication</td>
<td>$10^2 - 10^4$</td>
</tr>
<tr>
<td>Response to environmental changes (temperature, oxygen)</td>
<td>$10^3 - 10^4$</td>
</tr>
<tr>
<td>Messenger RNA synthesis</td>
<td>$10^3 - 10^4$</td>
</tr>
<tr>
<td>Translocation of substances into cells (active transport)</td>
<td>$10^1 - 10^3$</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>$10^1 - 10^2$</td>
</tr>
<tr>
<td>Allosteric control of enzyme action</td>
<td>1</td>
</tr>
<tr>
<td><strong>Glycolysis</strong></td>
<td>$10^{-1} - 10^{-2}$</td>
</tr>
<tr>
<td><strong>Oxidative phosphorylation in mitochondria</strong></td>
<td>$10^{-2}$</td>
</tr>
<tr>
<td>Intracellular quiescent mass &amp; heat transfer (dimension 10⁻⁵ m)</td>
<td>$10^{-5} - 10^{-3}$</td>
</tr>
<tr>
<td><strong>Enzymatic reaction and turnover</strong></td>
<td>$10^{-6} - 10^{-3}$</td>
</tr>
<tr>
<td>Bonding between enzyme &amp; substrate, inhibitor</td>
<td>$10^{-6}$</td>
</tr>
<tr>
<td><strong>Receptor-ligand interaction</strong></td>
<td>$10^{-6}$</td>
</tr>
</tbody>
</table>

Lactate Diffusion Times

Linear Dimension, microns

Diffusion Time, seconds

Volume, liters

10^{-15} 10^{-10} 10^{-5} 1 10^5

10^{-6} 10^{-3} 10^{-1} 1 10^6

10^{-10} 10^{-4} 10^2 10^6

Smaller = much faster

mL = 3 \times 10^4 \text{ sec}

\mu L = 300 \text{ sec}

nL = 3 \text{ sec}

pL = 30 \text{ msec}
Rationale for Dynamical Cellular BioMEMS: What do we gain by small and fast?

- Wide measurement bandwidth, *i.e.*, good response to high frequencies, is required to track fast cellular events
  - Stable control of fast systems requires high bandwidth
  - Small is the best way to beat the time for diffusional mixing in large-scale assays
  - Electrochemical sensitivity is scale-invariant; frequency response improves as size is decreased
  - Reduced reagent volumes for rapid injection
  - Detect fast, direct response rather than slow secondary responses
  - Decreased mixing times for mass and heat transfer
  - Can titrate toxin exposure quickly and with feedback to avoid desensitization and other suprathreshold effects
- Small allows strong electric fields at low voltages
- Cell can serve as its own control
- Calibration of each cell with standard chemical stimuli prior to agent exposure
- Monitor known, small (N=1?) number of cells in each nanoculture
- Many nanocultures within a single device, so a single-chip array of NanoBioReactors and NanoPhysiometers, in parallel, in series, and with redundancy is ideal for high-content screening and for statistical reliability
- Small lets one look at individual cellular events rather than ensemble averages, avoiding the small group of cells that dominates the average of a large population
- Physiology at *The Speed of Life*
PDMS Soft Lithography

(A) Photoresist
   Substrate

(C) 

(D) Elastomer

(B) Mask

(E)
The Multianalyte NanoPhysiometer (MNP) will serve as a platform for studying, one at a time, large numbers of single cells. Upon activation, we will measure pH, O, $V_m$, [Ca], lactate, glucose, Q-Dot binding.
Microelectrodes to measure extracellular potentials and stimulate cells

Optical fiber array to measure propagating calcium waves in a single cardiomyocyte

Field Stimulation of a Single Adult Cardiomyocyte
A Werdich, E Lima, F Baudenbacher
Arrhythmogenic effects of CaMKII in a mouse model of cardiac hypertrophy
F. Baudenbacher, E. Lima, A. Werdich
Slow, Calcium-Induced Contractions
A Werdich, E Lima, F Baudenbacher
Microfabricated pH Electrodes
I. Ges, B. Ivanov, F Baudenbacher

A) pH electrodes
B) pH calibration
C) Reference electrode
D) Calibration device
E) Temporal response to a 1 pH step change.
F) and G) Stop-flow acidification for A9L HD2 fibroblasts and M3 WT4 CHO cells

Ges et al., Submitted for publication
Nanophysiometer Modeling
Mark Stremler

• 3D computational model:

Sensor:
- 10 µm wide, 100 µm long
- Zero concentration at surface
- Sensor flux proportional to current

Channel Walls:
- No transport
- Zero velocity condition

Inlet Flow:
- Specified flowrate, velocity profile
- Specified concentrations
- Upstream diffusion allowed

- Possible device flow and sensing scenarios:

<table>
<thead>
<tr>
<th>Flow</th>
<th>Sensing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>Continuous</td>
</tr>
<tr>
<td>Intermittent</td>
<td>Intermittent</td>
</tr>
</tbody>
</table>
Statistical Analysis

- Correlations of protein expression and dynamical state
- Effective (minimal) metabolic and signaling model
  - Metabolic Flux Analysis is primarily steady state
  - Dynamic measurements require dynamic network models
    - Accumulation and depletion of intracellular stores in short times
    - Enzyme concentrations fixed in the intermediate time period
  - Inverse analysis of exact models is intractable, so effective or minimal models are required
- Dynamic network analysis
OBJECTIVE: Identify the mechanisms for intraislet signaling in glucose-stimulated insulin release

METHODS: Trap an intact pancreatic in a microfluidic channel that allows independent control of the glucose levels on opposite sides of the islet. Apply glucose to one side of the islet and look for calcium release on the opposite side.

Spatially Restricted Glucose Produces Spatially Restricted Calcium Oscillations

**OBJECTIVE:** Study the mechanisms of cellular chemotaxis, motility, metastasis, and angiogenesis.

**PARTICIPANTS:** F Baudenbacher, C Black, C Chung, S Gruver, R Haselton, W Hofmeister, N Kassebaum, C Lin, L Matrisian, L McCawley, M Miga, K Parker, R Richardson, A Richmond, R Roselli, P Russ, J Sai, E Schutyser, M Stremler, G Walker, A Weaver, J Wikswo

**METHODS:** Develop and use gradient migration chambers, traction-force beds-of-nails, and migration bioreactors to study the response of immune, cancer and endothelial cells to chemokine gradients.
Harvard Gradient Mixer for Chemotaxis Studies

Jeon et al., *Langmuir* 16: 8311-6
VIIBRE Gradient Mixer for Studying Cancer Chemotaxis
G Walker, J Sai, A Richmond, C Chung, J Wikswo
Gradient linearity depends upon flow rate

Mutated cells don’t crawl
Flow rate affects cell trajectory
Shear Force Modeling
Mark Stremler

- Contours of velocity magnitude:
  - No-slip velocity condition on walls and cell

- Fluid force on cell:
  - Pressure distribution
  - Shear stress

Axial shear force provides a measure of lateral chemotactic force

Works in Progress
Johns Hopkins PDMS Needles for Traction Force Microscopy

VIIBRE Beds-of-Nails
Kweku Addae-Mensah, Nicholas Kassebaum, Lisa McCawley, John Wikswo
Bioreactors for Intelligent Tissue Microenvironments

Micro provides a platform for Nano
Conventional Transwell Plate Co-Culture
Manuela Martins-Green – UC Riverside

- Keratinocytes
- hDermal fibroblasts
- Upper layer collagen
- hMVEC
- Lower layer collagen
- PET membrane with pores
- Medium
- Upper chamber
- Lower chamber
Perfused MT-NBR with multiple trapping sieves, capable of generating nutrient gradients
F. Baudenbacher, A. Prokop, D. Schaffer, et al

NanoBioreactor
F. Baudenbacher, D. Schaffer, A. Prokop
Instrumented Bioreactors

- Biofilm
- 1-D flow
- 2-D flow and perfusion
NanoPore Filters for Perfused Tissue Microenvironments
OBJECTIVE: Use microfabricated picocalorimeters to measure the heat from single-cell metabolism, droplet evaporation, and protein denaturation.

METHODS: A custom-fabricated picocalorimeter has a sensitivity of 6 V/W, a 1 ms response time, 80 nV of noise, and a power sensitivity of 14 nW/Hz$^{1/2}$. It can detect with ~100:1 S/N the evaporation of a 100 pL drop of water in < 1 sec. We will use a picoliter injector to add urea to a droplet of concentrated protein and measure the incremental heat of denaturation.

Mobility of Protozoa through Microchannels
W.Wang, L.Shor, E.LeBoeuf, D.Kosson, J. Wikswo

*Keronopsis* sp. squeezes through 20 mm constriction.

*MOVIE Euplotes vannus* entering a 20 x 20 mm channel (40 sec).
The Payoff

• The simultaneous measurement of the dynamics of a hundred intracellular variables will allow an unprecedented advance in our understanding of the response of living cells to pharmaceuticals, cellular or environmental toxins, CBW agents, and the drugs that are used for toxin prophylaxis and treatment.

• The general application of ICSC technology will support the development of new drugs, the screening for unwanted drug side effects, and the assessment of yet-unknown effects of environmental toxins.
<table>
<thead>
<tr>
<th>X</th>
<th>V, m³</th>
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<td>1 m</td>
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<td>1000 L</td>
<td>10^9 s</td>
<td>Animal, bioreactor</td>
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<tr>
<td>10 cm</td>
<td>10^{-3}</td>
<td>1 L</td>
<td>10^7 s</td>
<td>Organ, bioreactor</td>
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<tr>
<td>1 cm</td>
<td>10^{-6}</td>
<td>1 mL</td>
<td>10^5 s = 1 day</td>
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<td>100 um</td>
<td>10^{-12}</td>
<td>1 nL</td>
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<td>10^{-15}</td>
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<td>10 us</td>
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<td>10^{-24}</td>
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<td>Protein</td>
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<td>1 npL</td>
<td>1 ns</td>
<td>Ion channel</td>
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</tbody>
</table>
Diseases and Organ Systems

- Asthma
- Cancer
- Diabetes
- Heart disease
  - Vascular
  - Myocardial
  - Electrical
- Infection
- Toxins
  - Bacterial
  - Environmental
  - CBW