Un biochip per elettrostimolazione cellulare

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Bertinoro, 24/11/2005
Outline

• Introduction & background

• A biochip for genetic manipulation of single-cells
  – The single cell electroporation biochip
  – Electrical models of the biochip
  – Monitoring cell-electrode adhesion

• Other related activities
  – ISFET-based DNA sensors
  – EGFET-based DNA sensors

• Conclusions
• **Introduction & background**

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Introduction & background

• *Biotechnology means any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.*
  
  (definition by the UN Convention on Biological Diversity)

• Biotechnology is nothing really new
  
  – First farmers selected crops (peas, barley, wheat) in Fertile Crescent in 8000 B.C.
  
  – Insulin produced from genetically modified bacteria *Escherichia Coli* since 1978 (Genentech)
Introduction & background

• More and more Biotech drugs are blockbusters
  – An example: Epogen (Erythropoietin) introduced by AMGEN in 1989
  – World sales of EPO: 10B$ in 2004

• Is gene therapy behind the corner?
  – Control disease-causing genes on a person-to-person basis

• For a genetic-related disease, need to understand
  – Has gene XXX a role?
  – What if it is suppressed?
  – What if over-expressed?
  – …and so on…
Introduction & background

• Our aim: developing innovative microelectronics tools for molecular biology

• A field under growing interest
  – Microfluidics
  – DNA identification (DNA microarrays)
  – Quantitative assays (ELISA, RT-PCR, Q-PCR, …)
  – Single cell manipulation
  – Recording of electrical activity of living cells
  – Proteomics (protein microarrays)
  – Implanted devices (not just pacemakers...)
  – …
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The single cell electroporation biochip

• A theoretical experiment…
  – You are a biologist studying the function of a gene implied in Alzheimer disease
  – Your models are rat neurons growing in vitro
  – The gene must be activated only in this neuron
The single cell electroporation biochip

- Several methods are available to force the expression of a given gene in cultured cells
  - Chemical
  - Viral
  - Mechanical
- All of these work in a population of cells
  - Results averaged over the population
  - Large spread of statistical data
  - Unable to modify a single cell in a culture
The single cell electroporation biochip

- Realize an array of microelectrodes on a silicon biochip
- Cultivate cells on chip surface
- Apply a voltage to one electrode
  - The voltage is transferred to the cell growing above the electrode
  - Temporary pores open in the cell membrane (electroporation)
  - Molecules in solution enter the cell cytoplasm
  - The membrane reseals
The single cell electroporation biochip

• A GFP (Green Fluorescent Protein)-encoding plasmid is inserted into a single CHO cell (el. 22)

• After 24h, the selected cell divided: both daughter cells express the GFP
The single cell electroporation biochip

- Any pattern can be realized in the same way
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Electrical model of the biochip

- An electrical model is needed in order to
  - Design effective and non invasive electroporation stimuli
  - Allow reliable operation of the device

\[ (1-\alpha)Z_{el} + \alpha Z_{el} + Z_{cell} \]

Electrical model of the biochip

- Electrode impedance
  - Charge transfer resistance $R_t$
  - Double layer capacitance $C_e$
  - Constant phase element $C_{pe}$
  (electrochemical system nonlinearities)

$L. \text{Bandiera, et al, IEDM, 2007.}$

\[ Z_e(f) = R_t + rac{1}{j \omega C_e} + rac{1}{1 + j \omega C_{pe}} \]

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$L. \text{Bandiera, et al, IEDM, 2007.}$

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Electrical model of the biochip

- To study for example what happens to the potential on the cell membrane when it is not exactly above the electrode we need a distributed model.

- Spatially Distributed Transfer Network approach
  - The electrode - electrolyte - cell system is divided in a grid.
  - Each grid branch is described by an impedance whose value is derived from the concentrated model.

- Similar to finite element methods (FEM) but no linearization of equations is needed (not at this stage).
Electrical model of the biochip

- How is distributed the voltage above the electrode?

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Monitoring cell-electrode adhesion

• Some questions
  – Is it possible to detect the presence of a cell by using electrical measurements only?
  – Is it possible to quantitatively evaluate the quality of cell adhesion?

• Use EIS (Electrochemical Spectroscopy Impedance) measurements over a limited frequency rage

Monitoring cell-electrode adhesion

- Impedance @66kHz for different electrodes of the same chip

\[ \Delta Z = \frac{Z_{\text{with cell}} - Z_{\text{without cell}}}{Z_{\text{without cell}}} \]

- If \( \Delta Z > 20\% \), we can assume that a cell is adhering to the electrode

Monitoring cell-electrode adhesion

- Changing the stimulation voltage changes the stimulus effect on the membrane.

No change in morphology
High cell viability

Morphology change
Low cell viability

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Other related activities

• Fluorescence-based DNA microarrays are used to understand the expression of many genes at once

• Drawbacks of microarrays:
  – Not reusable
  – No information on hybridization kinetics
  – Low signal-to-noise ratio
  – Expensive

• DNA molecule carries an intrinsic negative charge
  – Can we develop an all-electrical system to quantify gene expression?
ISFET-based DNA sensor

- First implementation: ISFET
  - Ion-Sensitive Field-Effect-Transistor
  - Basically, a MOSFET without the metal gate
  - Gate oxide ($\text{SiO}_2$-$\text{Si}_3\text{N}_4$ stack) is exposed to solution

Ag/AgCl reference electrode
ISFET-based DNA sensor

- Step 1: depose (positively charged) poly-L-lysine (PLL) on gate oxide
- $Q_{PLL} = (1.5\pm0.65)\times10^{-4}\text{C/m}^2$
ISFET-based DNA sensor

- STEP 2: deposit probe (known) DNA
- DNA carries a negative charge → $V_{TH}$ shifts rightward

![Graph showing changes in $V_{TH}$ during PLL adsorption, Probe DNA adsorption, and DNA denaturation with respective charges of $-34 \times 10^{-4} \text{C/m}^2$, $-18 \times 10^{-4} \text{C/m}^2$, and $1.5 \times 10^{-4} \text{C/m}^2$.]

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ISFET-based DNA sensor

- Step 3: deposit target (unknown sequence) DNA
  - Matching $\rightarrow$ $V_{TH}$ shift
  - Non matching $\rightarrow$ nothing happens

\[
C_{b,cDNA}(t) = C_{sat} \cdot (1 - e^{-a \cdot t})
\]

\[
C_{sat} = 20 \text{[pmol/cm}^2\text{]}
\]

\[
a = 5 \cdot 10^{-3} \text{[s}^{-1}\text{]}
\]
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EGFET-based DNA sensor

- ISFET are
  - Very sensitive to solution and to process conditions
  - Expensive devices (~CMOS process flow in small batches)

- move to simpler devices: EGFET
  - Extended Gate FET

Solution

Gold microelectrode

Ag/AgCl reference electrode
EGFET-based DNA sensor

- Thiol-modified DNA adsorption onto gold microelectrode
- $V_{TH}$ increases due to DNA negative charge

$V_{solution} = 0$ (GND)
$V_B = V_S = -2$
$V_D = V_S + 100 \text{mV}$

$V_g [V]$
$Id [A]$
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• Innovative tools for biological applications
  – Electrical stimulation if living (cultured) cells
  – Electrical detection of DNA sequence

• Our research interest
  – HW/SW design
  – Electrical modeling
  – Reliability

• Work in close collaboration with biology and nanoscience
  – A stimulating and challenging interdisciplinary environment!
  – CIVEN, Fisiology Dept., CRIBI, FBK, VIMM, Biosilab, Columbia Univ.