Nano and Microtechnologies of hybrid bioelectronic systems
Bio MEMS

Nano and Microtechnologies of hybrid bioelectronic systems
Applications (I) : Bio-sensors

- bio-capsule (Desai)
- Bacteria sensor (Craighead)

Nano and Microtechnologies of hybrid bioelectronic systems

Figure 3. Atomic force microscopic image of antibody-stamped silicon surface. AFM was operated in the tapping mode and the height scale posted on the right side of the image.
Applications (II) : Cell biology

Cell biology
Effect of restricted cell extension:
• Adhesive islands
• Cell shape determines survival
• What determines cell’s death?

Geometric Control of Cell Life and Death
Christopher S. Chen, Milan Mrksich, Sui Huang, George M. Whitesides, Donald E. Ingber*

Nano and Microtechnologies of hybrid bioelectronic systems
Applications (II): Cell biology

Albert Folch
Intercell signaling, multiple cell cultures

Nano and Microtechnologies of hybrid bioelectronic systems
Applications (III)

Neuronal networks

- How networks form?
- How do they signal?
- Role of different elements
- To learn how to build artificial systems
- Biologically inspired algorithms

• Effective recording
• Controlled network

Nano and Microtechnologies of hybrid bioelectronic systems
Outline

• Applications (General)
• Bio-fouling
• Approaches
  • Micro-contact printing/micro fluidic
    • Proteins
    • SAMs
  • Non-fouling coatings
  • Nano-topography
• Neural recording, Lab on a chip
• Summary

Nano and Microtechnologies of hybrid bioelectronic systems
Cell Adhesion (I)

Two general classes of cell adhesion mechanisms:

- Nonspecific attachment (the hydrophobic effect).
- Specific protein-cell interactions.
Approaches (IV) : Non-Fouling Coatings


Nano and Microtechnologies of hybrid bioelectronic systems
Cell Patterning Approaches

- Direct protein lithography
- Micro-contact printing/micro fluidics
  - Proteins
  - SAMs
- Dry lithography
- Patterned polymers
- Temperature sensitive polymers
- Nano-topography
- Electric-field
- Wells
Photolithography

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (I) : Direct Protein Patterning

Poly-d-lysine

Too harsh for many biological molecules

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (II) : Soft lithography

Poly-dimethylsiloxane

1) Pour PDMS over Master
2) Cure at 65 °C for ~4 h

Peel off from the Master

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (II) : Soft lithography

Plasma treatment to modify the surface

1 μm resolution

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (II.1) : protein stamping

Hippocampal neuron on poly-lysine printed surfaces
http://www.wadsworth.org/divisions/nervous/nanobio/DG03.htm

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (II.1) : protein stamping

- Adhesive (FN) and non-adhesive islands.
- More cells die on small disks compared with un-patterned substrates.

Whitesides

Nano and Microtechnologies of hybrid bioelectronic systems
Nano and Microtechnologies of hybrid bioelectronic systems

Approaches (II.1) : protein stamping

- Total area (ECM growth factors) or contact area (focal adhesion formation)
- Similar contact area, different overall dimensions

Whitesides
Approaches (II.1): protein patterning

Silicon substrates
OTS stamping, rinse in DETA
LRM55 (astroglial cell line)

OTS: Octadecyltrichlorosilane (hydrophobic)
DETA: N-[3-(trimethoxysilyl)propyl]diethylenetriamine (hydrophilic)

Craighead

http://www.hgc.cornell.edu/neupostr/celindex.htm

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (II.2): Self-Assembled Monolayers - SAMs

Well define surface chemistry

Terminal Groups
(CH$_3$, OH, COOH, CF$_3$, etc.)

Anchor Groups
(thiol, silane, etc.)

Body (CH$_2$)$_n$

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (II.2): SAM stamping

Alkanethiol $\text{CH}_3(\text{CH}_2)_{n-1}\text{SH}$
- Selective affinity to Gold
- Hydrophobic (not inert to protein/cell attachment)
- Adsorption from a solution

More exotic terminal group molecules
- Blocking protein adsorption (ethylene glycol)
- Dynamic substrates
Approaches (II.2): SAM stamping

Tri(ethylene glycol) "Standard" for non fouling
Hexadecanethiol (Fouling)


Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (II.2) : SAM stamping

Poly(methylene)  Tri(ethylene glycol)

Day 1 Day 2 Day 3 Day 6

Day 8 Day 9 Day 12 Day 19

Day 21 Day 25

Milan Mrksich

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (II.2) : SAMs

Dynamic substrates

- With RGD peptide
- Supports cell adhesion
- Not affected by electrical potential

- With RGD peptide
- Supports cell adhesion
- Affected by electrical potential

Patterning with PDMS mask

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (II.2) : SAM stamping

- Dynamics
- 2 cell types

Nano and Microtechnologies of hybrid bioelectronic systems
Active SAMs

Nano and Microtechnologies of hybrid bioelectronic systems
Active SAMs

Onset of migration

Initially inert

Fouling after CV

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (III): Dry “Lift-off”

- Parylene deposition
- Lithography
- RIE
- Protein adsorption
- Peeling
Approaches (III) : Dry “Lift-off”

- Parylene film
- Low contamination (compared to PDMS)

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (III) : Dry “Lift-off”

PDMS stencil

A. Folch

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (IV) : Non-Fouling Coatings

Poly(ethylene glycol) - PEG
H-(O-CH2-CH2)n-OH

Dramatic reduction of protein adsorption to synthetic surfaces
Approaches (IV): Non-Fouling Coatings (for cell capsules)

- Silicon: stability, pore uniformity
- Optimal pore size
- Nutrient availability
- PEG coating to prevent clogging
Approaches (IV): Non-Fouling Coatings (for cell capsules)

Polysilicon
Silicon nitride

Holes in silicon
Through SiO₂ mask

Dry thermal oxide

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (IV): Non-Fouling Coatings (for cell capsules)

- Glucose and Oxygen should move freely (<3.5 nm)
- Insulin transport through pores
- Antibodies (large) should be prevented from crossing
- Clogging - PEG
Approaches (IV)  : Non-Fouling Coatings - pp4G

Plasma polymerized tetruglyme
CH₃-(O-CH₂-CH₂)₄-O-CH₃

• Non - fouling
• Plasma polymerization: excellent adhesion and durability
• Patterning with Photolithography

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (IV)
: Non-Fouling Coatings - pp4G

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (IV)
: Non-Fouling Coatings - pp4G

Bovine Aortic Endothelial Cells

Methylobacterium extorquens AM1

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (IV)
: Non-Fouling Coatings - pNIPAM

Poly(N-isopropylacrylamide)

- Temperature sensitive polymer
  - $T < 31^\circ C$ hydrophilic
  - $T > 31^\circ C$ hydrophobic
- Plasma polymerization
- Micro heaters

Nano and Microtechnologies of hybrid bioelectronic systems
How would you design the micro-fabricated substrate?

Nano and Microtechnologies of hybrid bioelectronic systems
Approaches (IV): Non-Fouling Coatings – pNIPAM Heaters

- Substrate Material: auto fluorescence, heat conductivity
- Heater design: low voltage, low power
- Passivation/metallization: durability in solutions

Nano and Microtechnologies of hybrid bioelectronic systems
Nano and Microtechnologies of hybrid bioelectronic systems

Approaches (IV): pNIPAM and heaters

Figure 4. (a) Micro-heater array. (b) FITC-anti-BSA pattern. (c) TRITC-goat-IgG pattern
Nano and Microtechnologies of hybrid bioelectronic systems

Approaches (IV): Non-Fouling Coatings - pNIPAM and heaters
Approaches (V) : Nanotopography

- Silicon pillars
- Silicon grass
- Carbon nanotubes

Nano and Microtechnologies of hybrid bioelectronic systems
References

- Yousaf MN, Houseman BT, Mrksich M, Using electroactive substrates to pattern the attachment of two different cell populations, P NATL ACAD SCI USA 98 (11): 5992-5996 MAY 22 2001

Nano and Microtechnologies of hybrid bioelectronic systems