Intracellular NEMS

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Partners

**NEMS – MEMS technology**

**Cell biology and embryos**

**Biochemical functionalization**

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**UB - IBEC**

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Micro- and Nanotechnologies in Cell Biology

**Extracellular Tools**

**MEMS/Microsystems**

- Devices bigger than cells
- Technology based on semiconductor industries
  - High control of the dimensions
  - High control of geometries (3D devices)
  - A large variety of materials: silicon, silicon oxide, silicon nitride, gold, platinum, aluminum, chromium, titanium, polymers...
  - Sensors or actuators: mechanical, biochemical, electrical, magnetic, etc

**Intracellular Tools**

**Micro-Nanoparticles**

- Devices smaller than cells
- Produce by chemical synthesis
- They can be internalized inside cells
- Drug delivery
- Hyperthermia
- Image contrast

**NEMS/Nanosystems**

- NEMS/Nanosystems smaller than cells
- Technology based on semiconductor industries
- They can be internalized inside cells
Suspended NEMS for intracellular applications

High density of small devices in suspension

- Fabrication technology
- Manipulation
- Cell internalization
- Cell viability
- Interrogation / Measurement
- Applications
Is it possible to produce and manipulate NEMS in suspension as nanoparticles?

Semiconductor based technologies

- Defined by micro and nanolithography
- Fully release of devices by surface Micromachining (HF vapour)
Is it possible to produce and manipulate NEMS in suspension as nanoparticles?

- Collect nanodevices
- Disperse (alcohol, PBS, etc)
- High concentration
- Cleaning
- Sterilization
Is it possible to produce and manipulate NEMS in suspension as nanoparticles?

HF vapour surface micromachining

- High yield
- Simple and standard technology

- High limitation in materials
- Materials degradation
- Losses during manipulation

Si, Poly-Si, Au, Pt
Is it possible to produce and manipulate NEMS in suspension as nanoparticles?

HF vapour surface micromachining

- High yield
- Simple and standard technology
- High limitation in materials
- Loses during manipulation

New release technology
Mechanical release and collection

- Very high yield (with no losses during manipulation)
- Compatible with all materials
- No damage of biofunctionalization
- More complex technology

Patent: P201430864
Multifunctional microdevices

“Universal platform”
Is it possible to internalize NEMS inside living cells?

Lipofection: HeLa cells

Phagocytosis: human macrophages

Microinjection: Mouse Embryos

Nanomachining by Focused Ion Beam of a human macrophage with a internalized chip.
Is it possible to internalize NEMS inside living cells?

Polysilicon microparticle phagocyted by a macrophage
NEMS interact with living cells? Intracellular biochemical sensors

**Chemical functionalization**

Polysilicon

Surface activation

Amine modification

FDA derivatization

OH

OH

OH

Si NH₂

O

O

O

O

O

O

O

Polysilicon

**Funtionalized silicon microchips with diacetate of fluorescenine** (CFDA) interact with the cell cytoplasm in HeLa cells show intracellular ICCs with green fluorescence

Cell viability?

Interrogation / Measurement?

- Optical
- Magnetic
- Electrical
- RF
- Ultrasounds
- ...

No direct access
Interrogation / Measurement?

- Optical
- Magnetic
- Electrical
- RF
- Ultrasounds
- ...  

**In vitro applications**

In combination with confocal microscopy

*Confocal laser scanning microscopy (CLSM):*

- standard technology in biology
- high capabilities
- high resolution
- light sources
- powerful analysis
- some flexibility

**Resolució:** 200 nm

**Làsers:**

- Diode UV 405 nm
- Argó multilinial 458, 476, 488, 496 and 514 nm
- DPSS 561 nm
- Heli-Neó 594 nm
- Heli-Neó 633 nm
- Titani-Safir-IR MaiTai broadband (710-990 nm)
Single-cell Barcodes
Technology offers precise control of chip-geometry

Intracellular Barcodes for single cell tracking and labeling

Pentagonal bits
Start marker

Human macrophage

Single-cell Barcodes

Intracellular Barcodes for single cell tracking and labeling

Cell Viability

Daily movements of encoded macrophages during 10 days.
Direct tagging of mouse embryos

- Microinjection of the barcodes into the perivitelline space
- Embryo freezing and thawing

In vitro development of embryos microinjected with different types of polysilicon barcodes into their perivitelline space


Adhesion of barcodes to the embryo surface:
(A) Hatched blastocyst
(B) Corresponding empty zona pellucida,
Direct embryo tagging and identification

- WGA biofunctionalized barcodes (Immobilization of lectins using self-assembled monolayers)
- Attachment of barcodes to the ZP outer surface

Barcode release after blastocyst hatching

In vitro development of tagged embryos and identification process

**Assisted reproduction technologies**

- Barcode tagging of human oocytes and embryos to prevent mix-ups
- Identification of bovine embryos

Intracytoplasmic sham injection of tagged oocytes

Biofunctionalization of polysilicon barcodes


The Idea

- Chips made by standard semiconductor technologies
- Integrate a mechanical sensor and an optical transducer (Intracellular NOMS)
- Internalized inside living HeLa cells

Gómez-Martínez, R. et al., “Silicon chips detect intracellular pressure changes in living cells”
Nature Nanotechnology, Volume 8, Issue 7, July 2013, Pages 517-521
Design

The sensing principle

Small enough to be internalized by cells
Nanometric-thick mechanical layers

\[ D = \frac{E t_{memb}^3}{1-v^2 \cdot 12} \]

\[ \text{Displ}_{memb} = 0.00126 \frac{a^4}{D} P \]
Design

The transduction

Compatible with one of the standard techniques for cell biology studies

Confocal Laser Scanning Microscopy (CSLM)

*Fluorescence dyes*

Fabry-Perot Resonator

Reflected light
Intracellular silicon pressure sensor

Design

SEM image

Transmitted light image
Chip Fabrication

- Polysilicon as structural material (25 nm, 25 nm, 50 nm)
- Silicon oxide as sacrificial layer (100 nm, 300 nm, 30 nm)
  - Etching selectivity (RIE, HF vapors etching)
  - Polysilicon hermeticity

Sequence of steps for the fabrication of the chips.

SEM images of the fabricated chips.
Chip Characterization (Pressure)

Fluorescence microscopy

Fluorescence Dyes

Emission

Excitation

Fluorescence Dyes

Emission

λ

λ
The tilt

\[ \lambda = 514 \text{ nm} \]

\[ \lambda = 594 \text{ nm} \]
Chip internalization inside Human HeLa Cells

Lipofection: a technique used to introduce genetic material into a cell by means of liposomes, which are vesicles that can easily merge with the cell membrane since they are both made of a phospholipid bilayer.

Cell viability

Area ratio = ~10%  Volume ratio = ~0.2%

CellTracker Green
MitoTracker Red (bio-dyes)

Nile Red and the vital probe Calcein-AM (green)
MitoTracker red and the vital probe Calcein-AM (green) 24h
Mitochondrial potential-dependent probe DiOC
Experimental set-up

Pressure chamber / Cell culture chamber

Confocal Laser Scanning Microscope

Pressure calibration
Pressure transmission inside the cell

In air

Reflected intensity

Reflected intensity

Inside a cell

Reflected intensity %
Osmotic Shock

Chips inside a HeLa cell during an osmotic shock

Extrapolated $I$ for the minimum reflection of chips in the cytosol and inside the vacuole before and after an osmotic shock, showing a non-significant shift of the reflected spectrum after the shock.
Conclusions

- Intracellular NEMS could be powerful devices
  - Technology very flexible
  - Opens new applications areas
  - Really multifunctional (mechanical, chemical sensing, actuation, therapies...)
  - Combination of intra and extracellular NEMS
  - New interrogation principles

- Cell viability ... but how affects measured parameters?
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