Nanotools for mechanobiology: substrates, probes and microscopes

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The role of forces in biology
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Forces are involved in our relationship with the environment: *Every living being interacts with its ambient with 6 senses:*

- Sight
- Smell
- Taste
- Hearing
- Touch
- Proprioception

Well known G-protein coupled receptors such as Rhodopsin (*discovered in 1878*)

Mechanoreceptors
The role of forces in biology

Many cellular process are dominated by forces. E.g.

- DNA separation in cellular mitosis
- Stem cell differentiation
- Viral spreading
- Cell adhesion and migration
- Cytoskeleton organization
- Embryo formation
- ...

A simple and straightforward way to control and understand how force control the process at a cellular level is to design proper substrate.
Neuronal precursors (NP) obtained with Stromal Cell-Derived Inducing Activity protocol (SDIA)

NP plated on nanopatterned substrates (NS)

NS are produced on PDMS by soft lithography and characterized with AFM and force spectroscopy
The enhancement depends on substrate Young modulus.

These points have different geometry but same “effective” Young modulus.

The most of the difference is already present few hours after plating.
Stem cell differentiation

Adhesion data correlates with differentiation data

Confocal fluorescent images of CHO-K1 cells. (A) A 43-pN TGT surface. (B) A 56-pN TGT surface. Actin in green and vinculin in red. Images were obtained after 2-hour cell plating.

Bright green lines in (B) are stress fibers that terminate in focal adhesion complexes marked in red.
Force intensity and *direction* triggers cytoskeleton formation

MEF adhere to NWs with filopodia, grow suspended and are not pinched by NWS.

**Max F:**
- **1nN**
- **4nN**
- **3nN**

![Graphs showing force intensity and direction](image-url)
Force intensity and direction triggers cytoskeleton formation

Wires are extremely resistant to longitudinal forces
But extremely compliant to deflection...

High aspect ratio silicon nanowires control fibroblast adhesion and cytoskeleton organization
It is well known that gratings can stimulate neurites growth and alignment.

Here neonatal opossum neuronal precursor are grown on patterned PDMS substrates.

Orientation, number and length of neurites are significantly increased.
DRG neurons are grown on suspended graphene periodic substrates microfabricated at IOM

Neurites align along suspended graphene although no topographic cues are present!

Lines:
We excluded mechanical cues, and we hypothesizes the modulation in substrate electrical conductivity

Control fibroblasts do not align
The role of forces in biology

Many important diseases involve forces.

E.g.

- Malaria
- Osteoporosis
- Heart failure
- Cancer
- Pain – (mechanotransduction)
OocYTE MECHANICAL SORTING FOR IVF

ZP is a multilayered structure which undergoes morphological and mechanical changes during maturation.

We followed ZP mechanical evolution as a score to monitor maturation and select the competent oocyte for IVF.

Oocite ZP Young modulus can be used to identify the maturation phase and correlates with pregnancy outcome.

Oocite ZP Young modulus can be used to predict oocite degradation 1 to 2 hours before degradation becomes visible at the microscope.

Oocite ageing
Oocyte mechanical sorting for IVF

AFM indentation provides information limited to the ZP

To investigate the mechanical behavior of the whole cells we fabricated flat and large cantilevers for:
- stress-relaxation
- creep measurements
Many-cell/tissue force spectroscopy

Single cell force spectroscopy suffer from severe drawback in term of Statistics
Proper cell adhesion and growth on the cantilever.
Our large and flat cantilever can address also these issues.

HEK 293A
Ongoing & future projects: 1. Ca++ imaging

Mechanotransduction:

**AFM** for controlled mechanical stimulation

**AND**

**Calcium Imaging** to monitor meccanosensitive channels

*Calcium wave is stimulated*

*The stimulation can be localized or wide depending on the tip*
Ongoing & future projects: 2. FluidFM

- FluidFM nanosyringe
- FluidFM nanopipette
- FluidFM micropipette

Single cell adhesion, Spotting, Colloidal spectroscopy, Single bacteria adhesion, Nano-printing, Nano-injection, Nano-extraction, Cell isolation
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