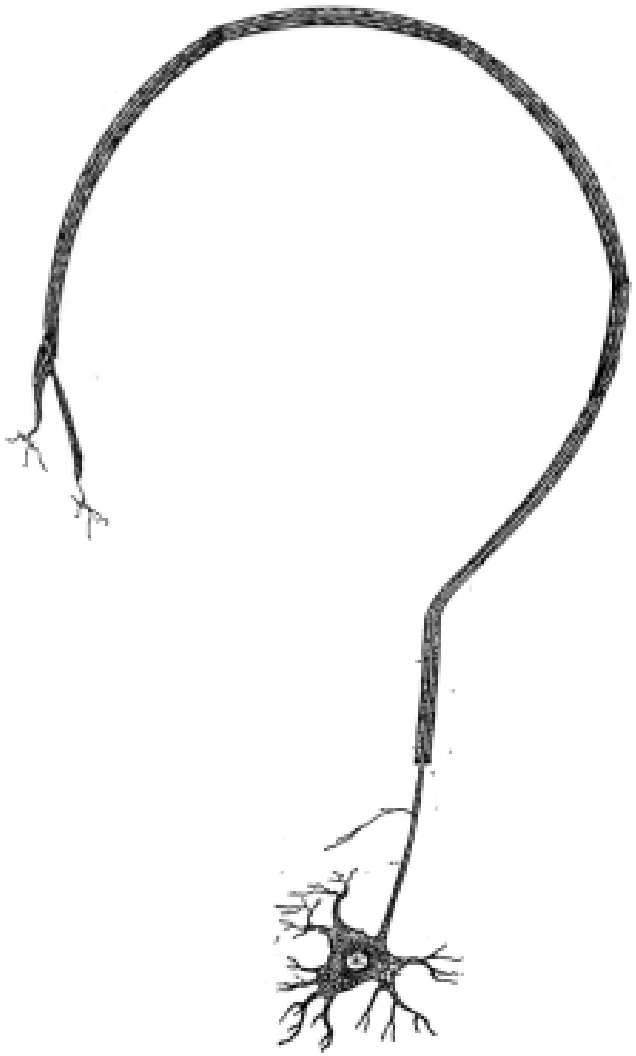
Istituto Officina dei Materiali

Nanotools for mechanobiology: substrates, probes and microscopes

Marco Lazzarino
CNR-IOM Basovizza Trieste Italy

Workshop *"**Nanotechnology and nanoApplication**"*
5-6 February 2020, Institut "Jožef Stefan", Ljubljana, Slovenia

The role of forces in biology



The role of forces in biology

Forces are inveolved in our relationship with the environment:
Every living being interacts with its ambient with 6 senses:

- Sight
- Smell
- Taste

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

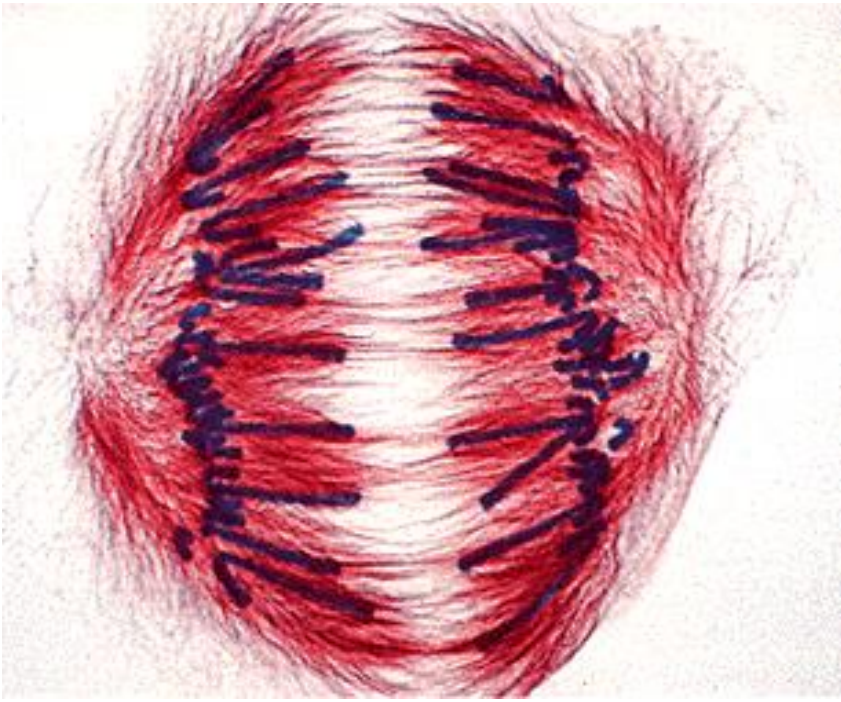
- Hearing
- Touch
- Proprioception

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 ans straightforward way to control and understand how force control the process at a cellular level is to design proper substrate

Stem cell differentiation

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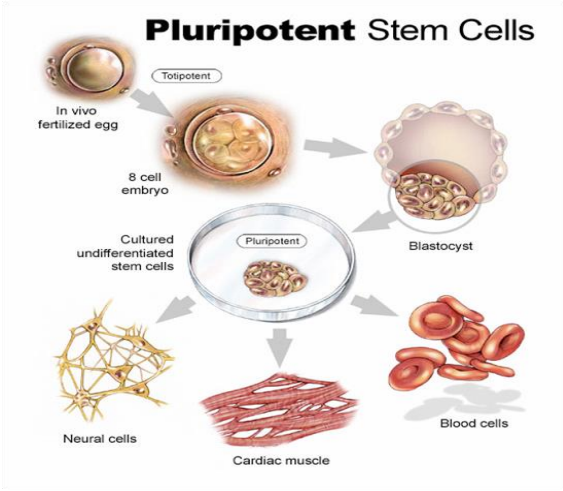
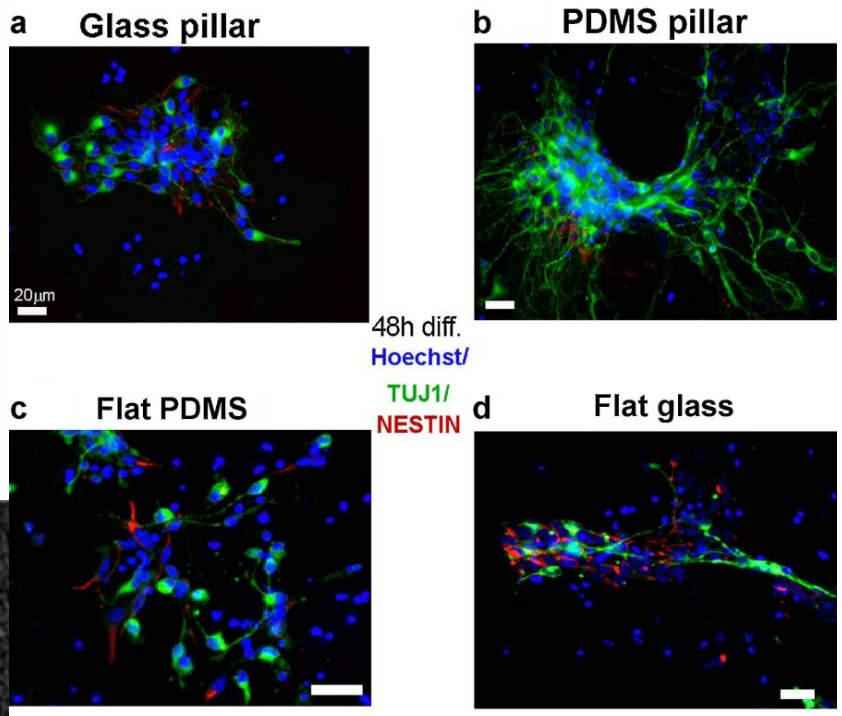
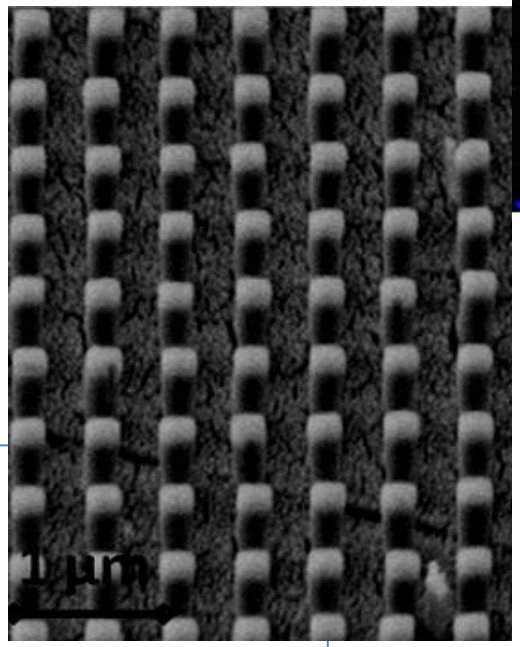
Neuronal precursors (NP) obtained with Stromal Cell-Derived Inducing Activity protocol (SDIA)

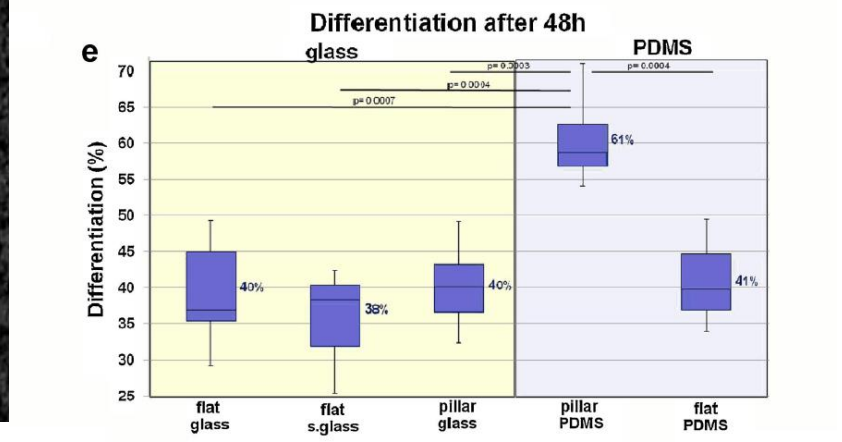
NP plated on nanopatterned substrates (NS)

NS are produced on PDMS by soft lithography and characterized with AFM and force spectroscopy









ARTICLE

Acceleration of Neuronal Precursors Differentiation Induced by Substrate Nanotopography

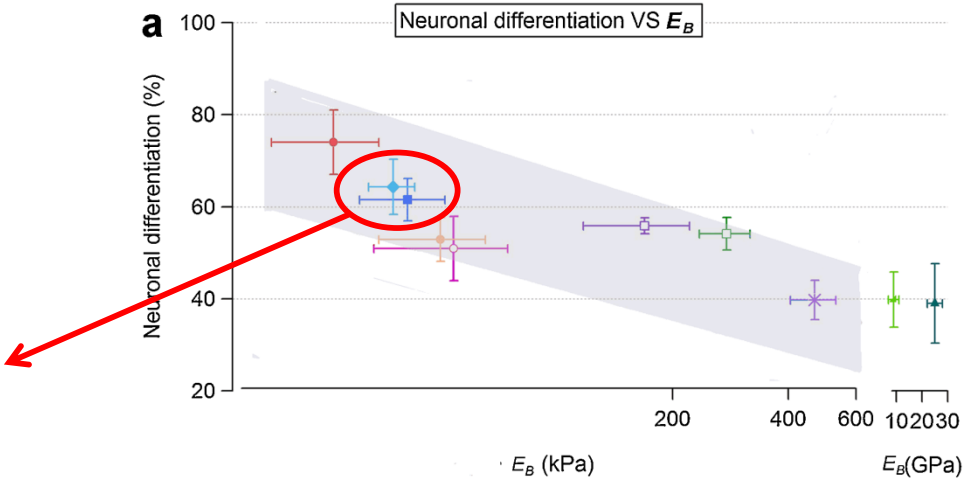
Elisa Migliorini,[1,2] Gianluca Grenci,[1] Jelena Ban,[3] Alessandro Pozzato,[1] Massimo Tormen,[1,2] Marco Lazzarino,[1,2] Vincent Torre,[3,4] Maria Elisabetta Ruaro[3,5]

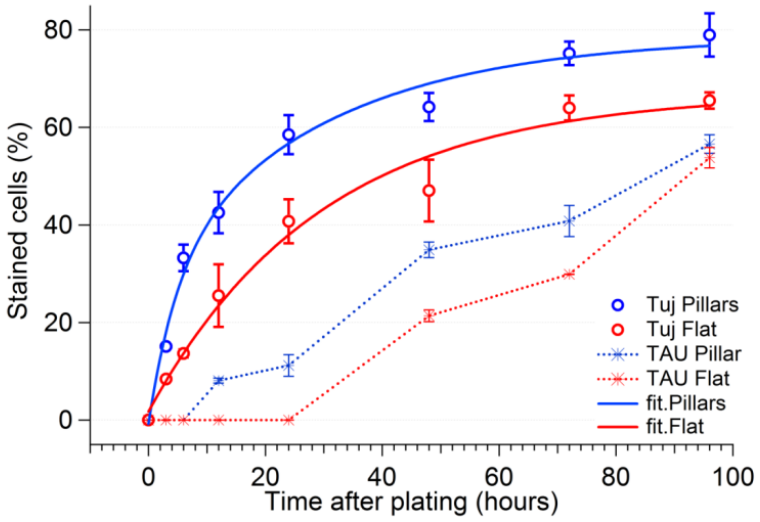
Stem cell differentiation

The enhancement depends on substrate Young modulus

These point have different geometry but same “effective” young modulus

The most of the difference is already present few hours after plating





ARTICLE

BIOTECHNOLOGY AND BIOENGINEERING

Nanomechanics Controls Neuronal Precursors Adhesion and Differentiation

Elisa Migliorini,[1,2] Jelena Ban,[3] Gianluca Grenci,[1] Laura Andolfi,[2] Alessandro Pozzato,[1] Massimo Tormen,[1] Vincent Torre,[3,4] Marco Lazzarino[1,2]

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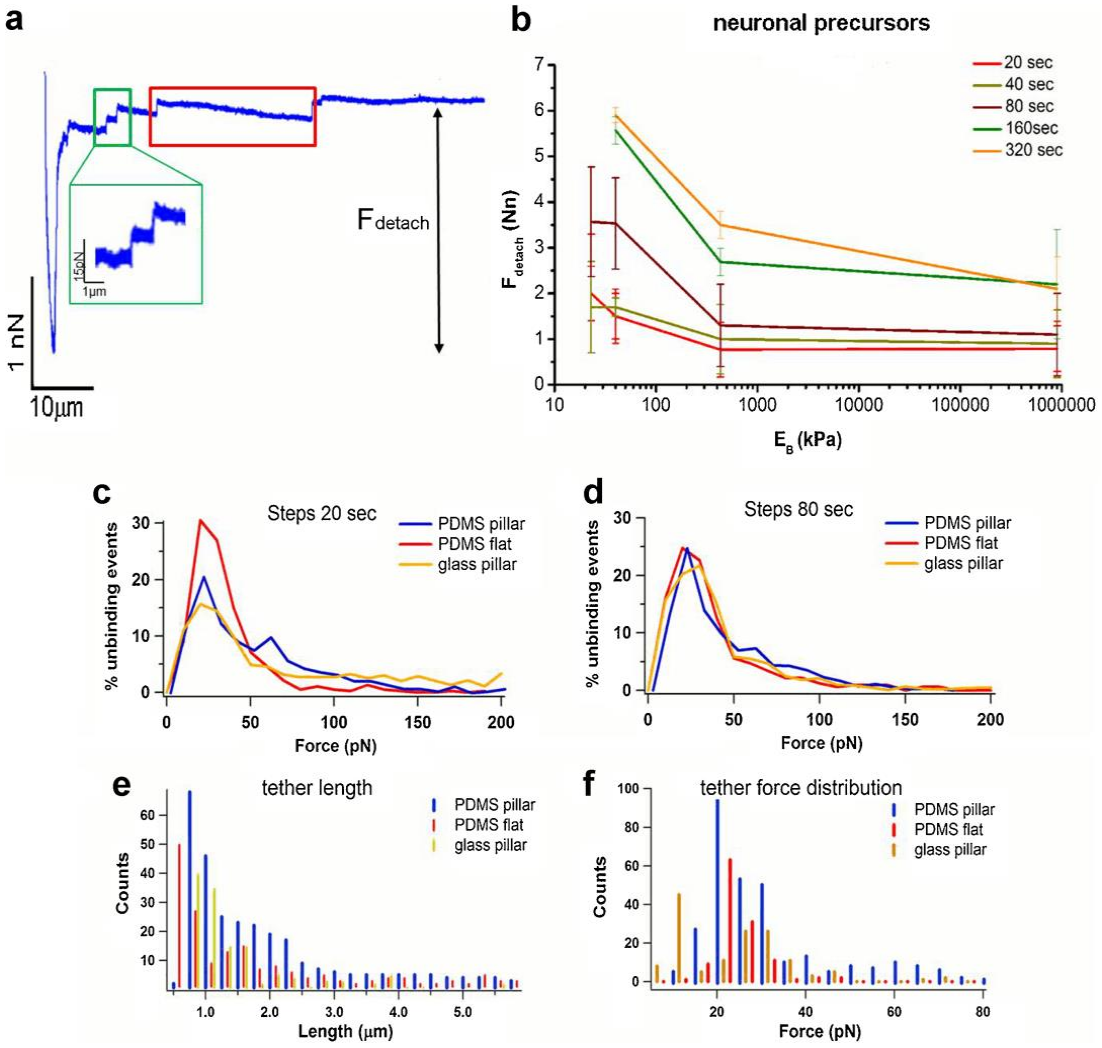
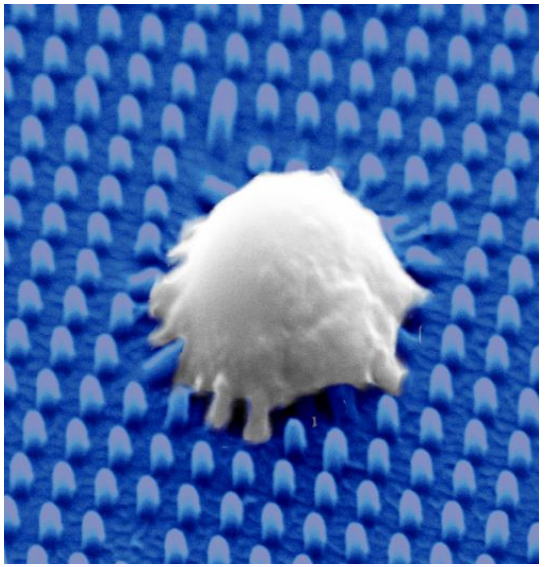
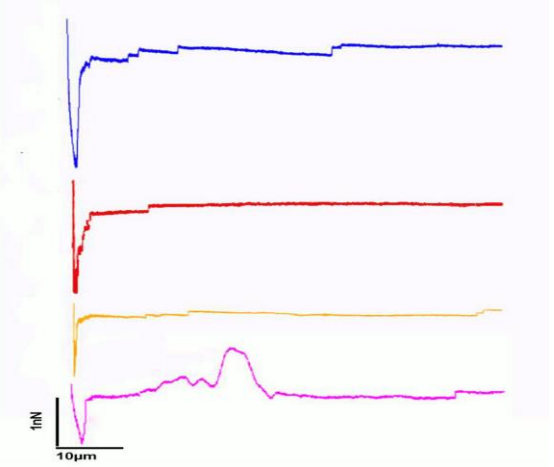
Stem cell differentiation







Adhesion data correlates with differentiation data

E, Migliorini, et. al. Biotech and Bioeng. 2013

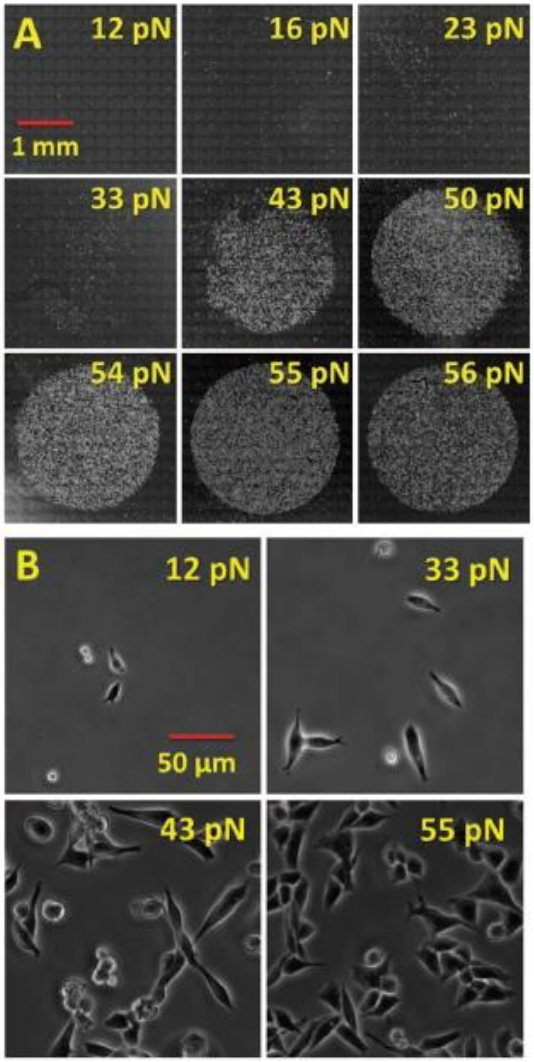
Force intensity and *direction* triggers cytoskeleton formation

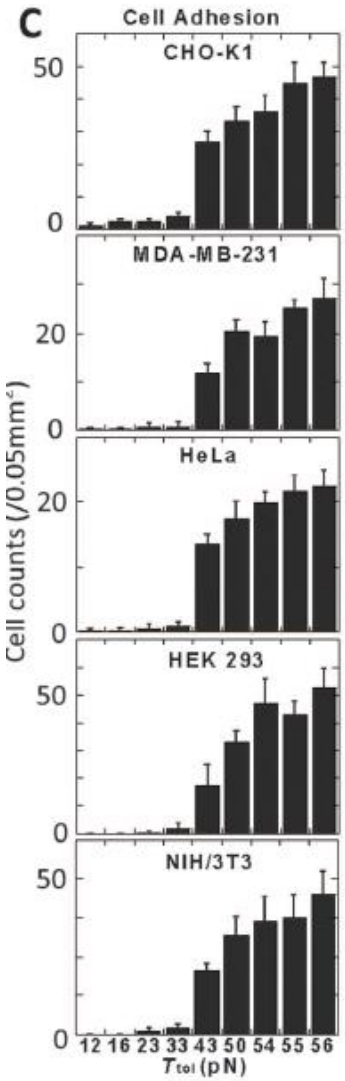
Science
AAAS

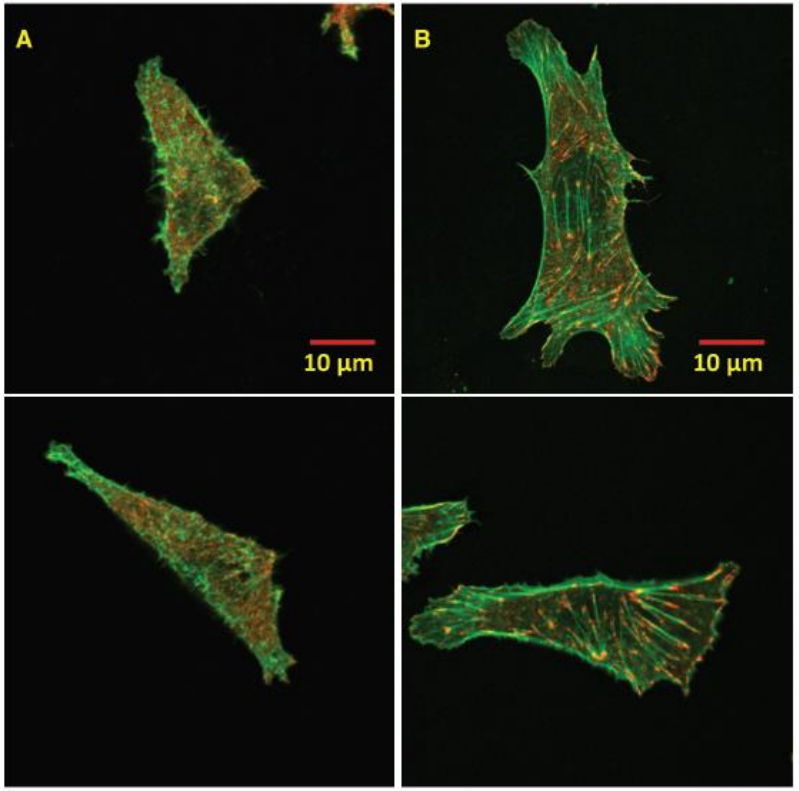
Defining Single Molecular Forces Required to Activate Integrin and Notch Signaling

Xuefeng Wang and Taekjip Ha

Science **340**, 991 (2013);
DOI: 10.1126/science.1231041



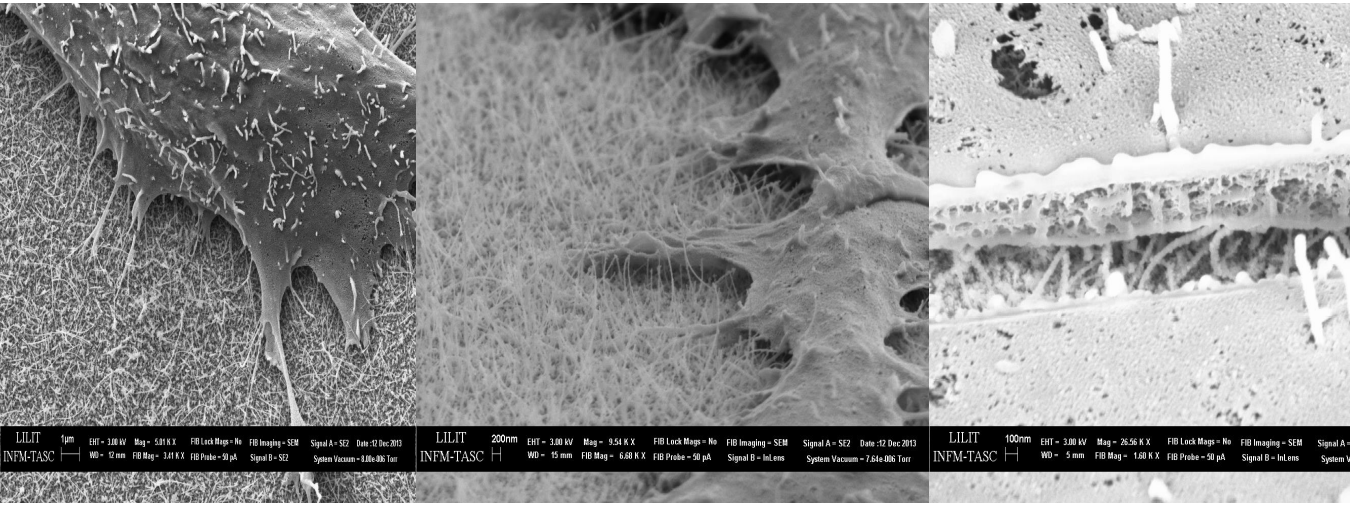




Confocal fluorescent images of CHO-K1 cells. (A) A 43-pN TGT surface. (B) A 56-pN TGT surface. Actin in green and vinculin in red. Images were obtained after 2-hour cell plating.

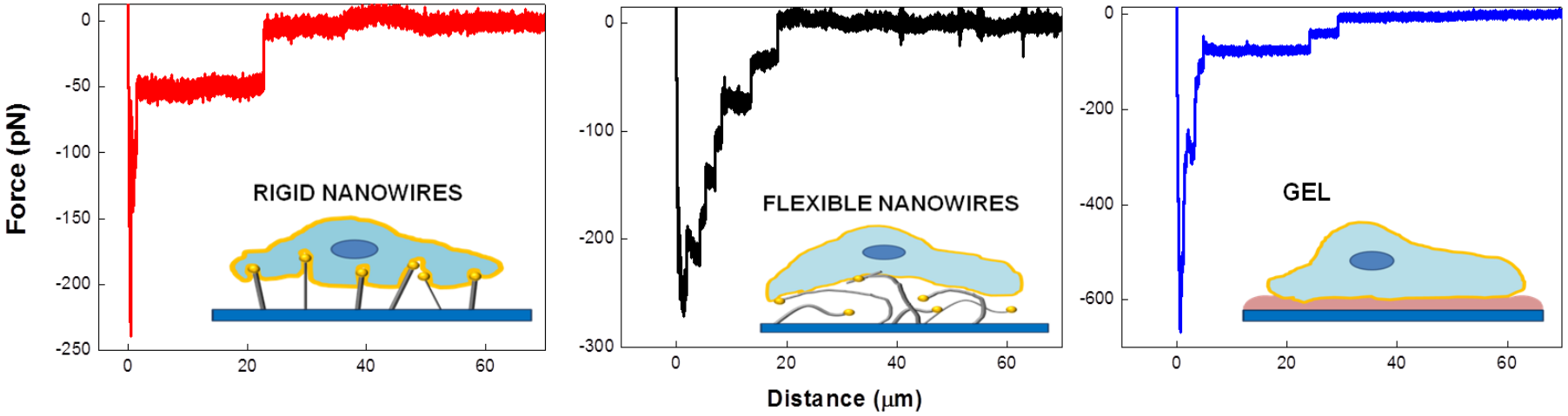
Bright green lines in (B) are stress fibers that terminate in focal adhesion complexes marked in red.

Force intensity and *direction* triggers cytoskeleton formation



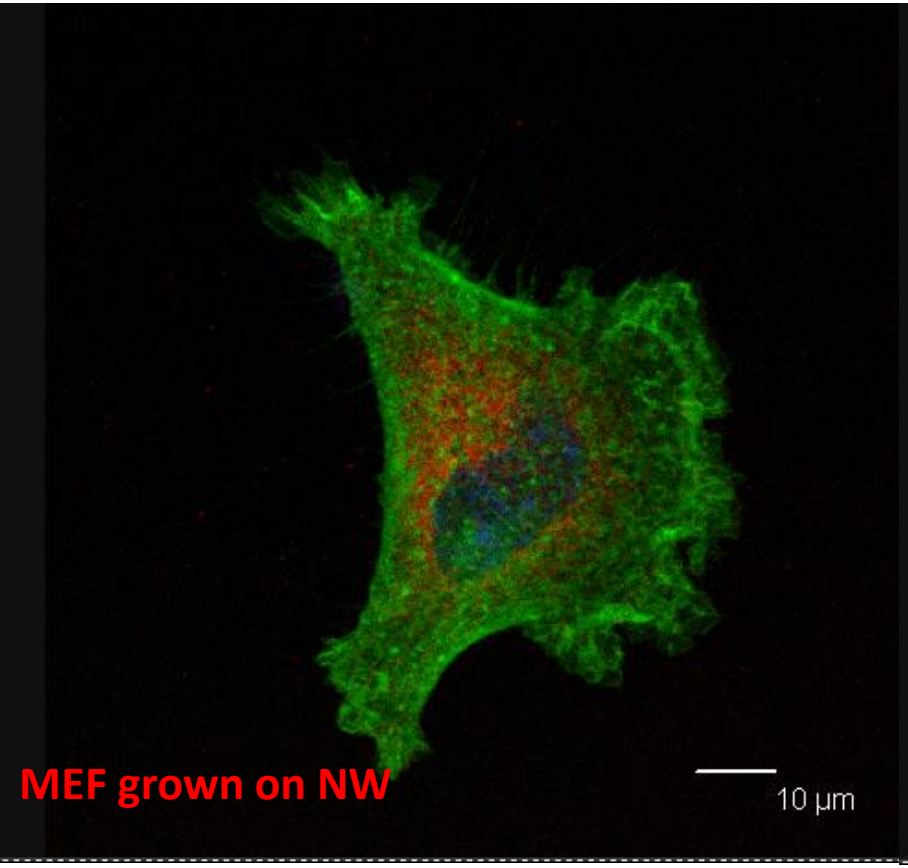
MEF adhere to NWs with filopodia, grow suspended and are not pinched by NWS

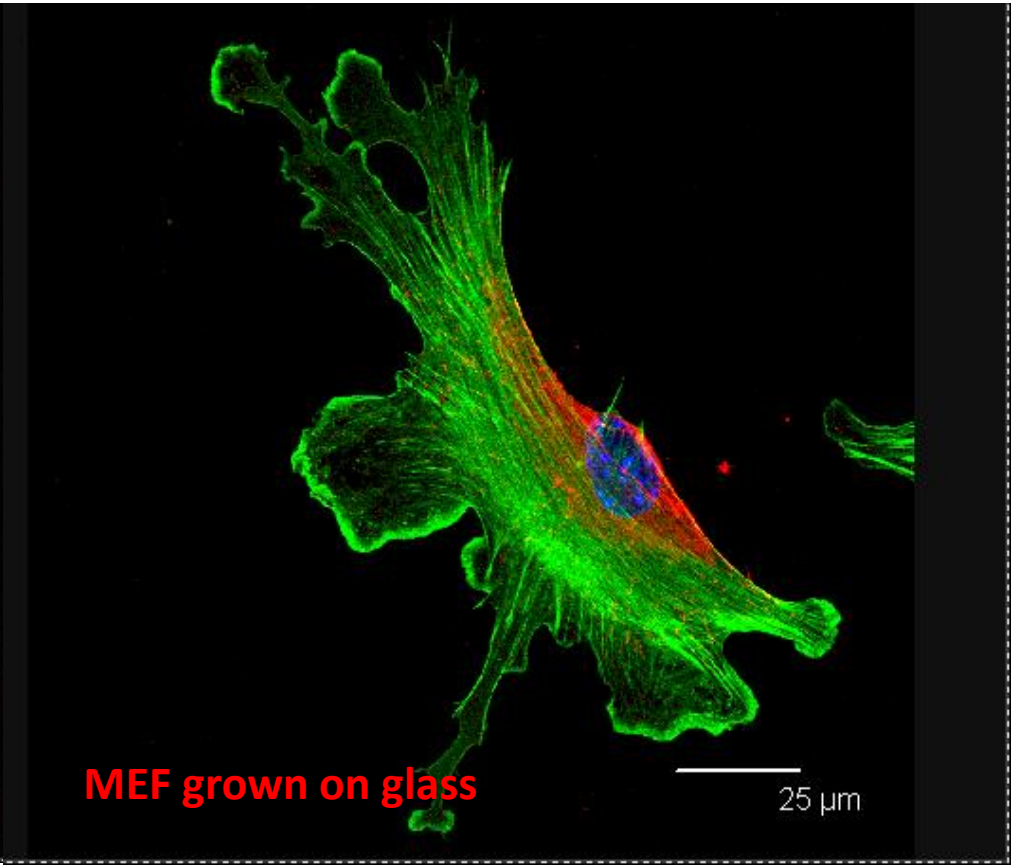
Max F: **1nN** **4nN** **3nN**



Nanotechnology **28** (2017) 155102 (9pp)

Force intensity and *direction* triggers cytoskeleton formation





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

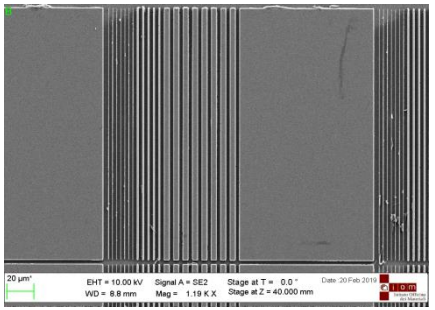
IOP Publishing Nanotechnology
Nanotechnology **28** (2017) 155102 (9pp) https://doi.org/10.1088/1361-6528/aa5f3a

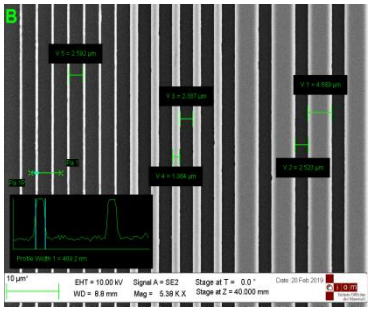
High aspect ratio silicon nanowires control fibroblast adhesion and cytoskeleton organization

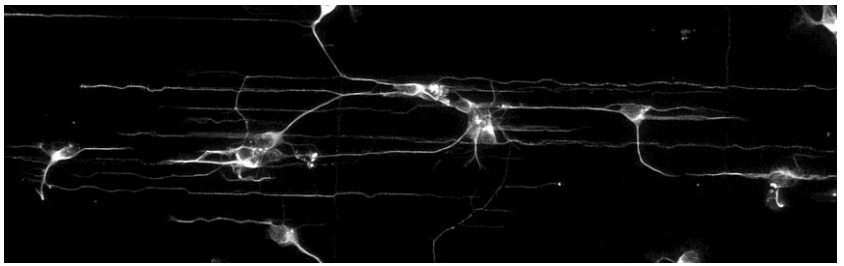
Laura Andolfi[1], Anna Murello[1,4], Damiano Cassese[1,5], Jelena Ban[2,3], Simone Dal Zilio[1] and Marco Lazzarino[1]

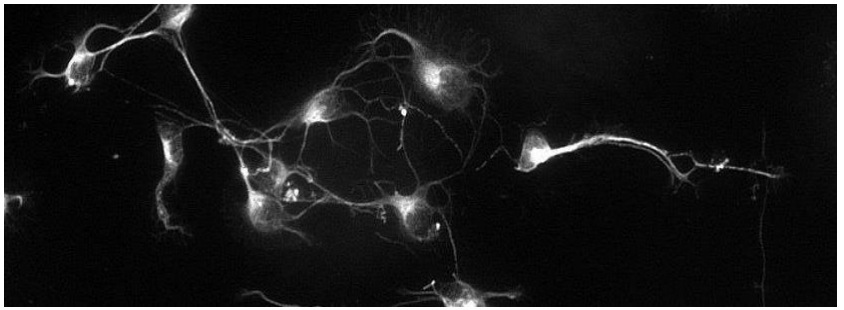
[1] Istituto Officina dei Materiali, Consiglio Nazionale delle Ricerche (IOM-CNR) Basovizza, Area Science Park, I-34149 Trieste, Italy
[2] International School for Advanced Studies (SISSA), Via Bonomea, 265, I-34136 Trieste, Italy
[3] Department of Biotechnology, University of Rijeka, Radmile Matejčić 2, 51000 Rijeka, Croatia

Cell alignment on patterned substrates





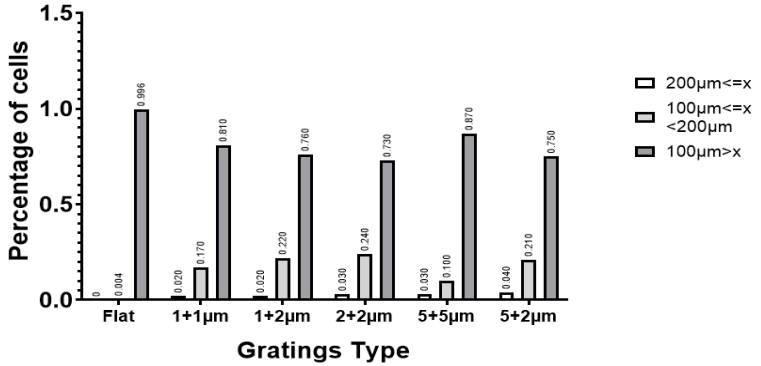




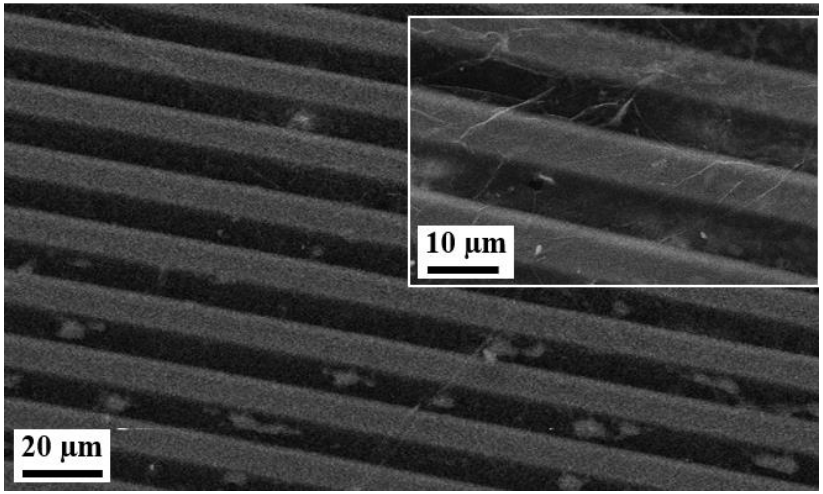
It is well known that gratings can stimulate neurites growth and alignment

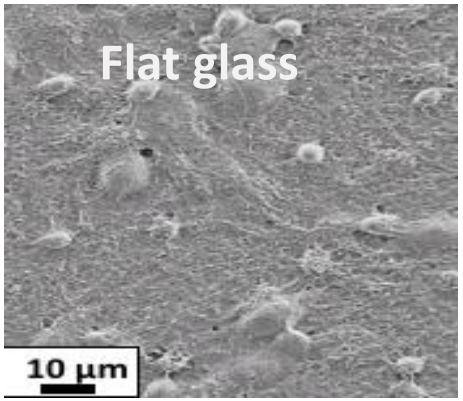
Here neonatal opossum neuronal precursor are grown on patterned PDMS substrates

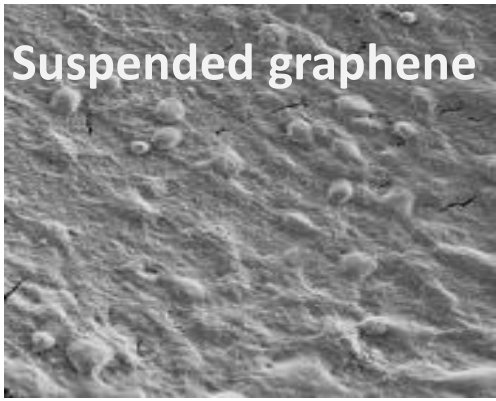
Orientation, number and length of neurites are significantly increased

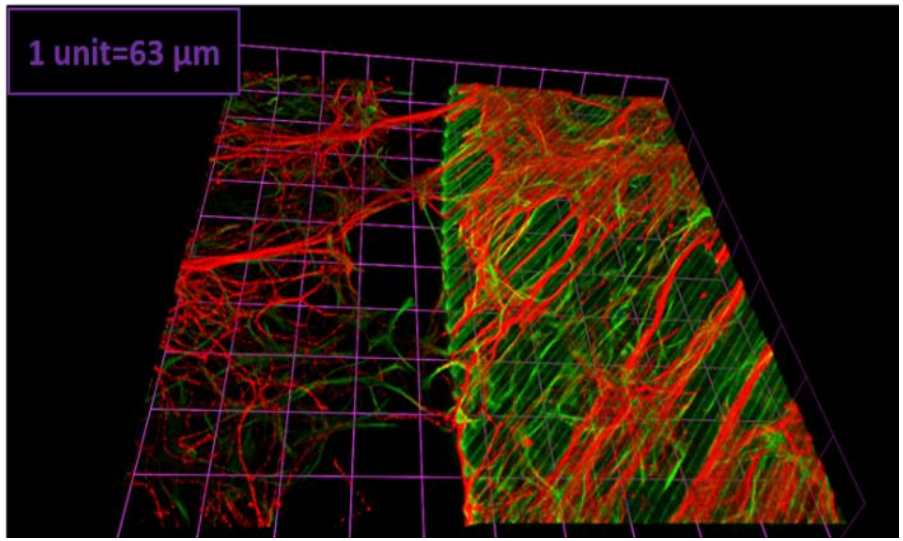


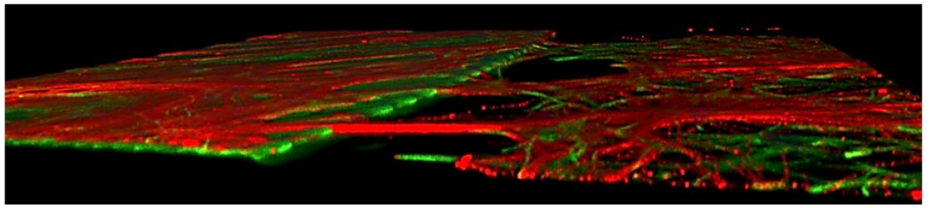
Cell alignement on patterned substrates

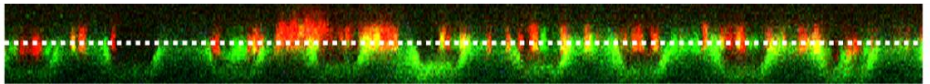












DRG neurons are grown on suspended graphene periodic substrates microfabricated at IOM

*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

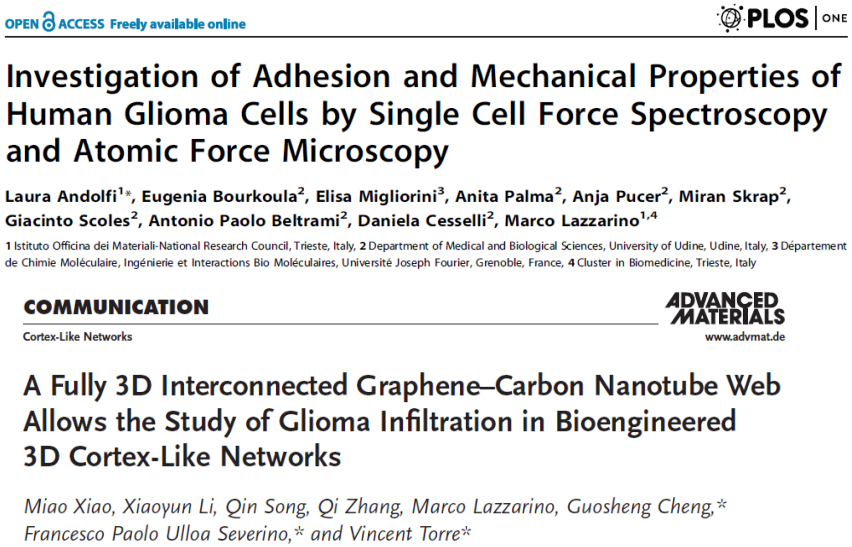
Control fibroblasts do not align

The role of forces in biology

Many important diseases involve forces.

E.g.

- Malaria
- Osteoporosis
- Heart failure
- Cancer
- Pain – (mechanotransduction)
- ….



OPEN ACCESS Freely available online

PLOS ONE

Investigation of Adhesion and Mechanical Properties of Human Glioma Cells by Single Cell Force Spectroscopy and Atomic Force Microscopy

Laura Andolfi[1]*, Eugenia Bourkoula[2], Elisa Migliorini[3], Anita Palma[2], Anja Pucer[2], Miran Skrap[2], Giacinto Scoles[2], Antonio Paolo Beltrami[2], Daniela Cesselli[2], Marco Lazzarino[1,4]

1 Istituto Officina dei Materiali-National Research Council, Trieste, Italy, 2 Department of Medical and Biological Sciences, University of Udine, Udine, Italy, 3 Département de Chimie Moléculaire, Ingénierie et Interactions Bio Moléculaires, Université Joseph Fourier, Grenoble, France, 4 Cluster in Biomedicine, Trieste, Italy

COMMUNICATION

Cortex-Like Networks

ADVANCED MATERIALS

www.advmat.de

A Fully 3D Interconnected Graphene–Carbon Nanotube Web Allows the Study of Glioma Infiltration in Bioengineered 3D Cortex-Like Networks

Miao Xiao, Xiaoyun Li, Qin Song, Qi Zhang, Marco Lazzarino, Guosheng Cheng, Francesco Paolo Ulloa Severino,* and Vincent Torre**

eLIFE elifesciences.org

RESEARCH ARTICLE

Acetylated tubulin is essential for touch sensation in mice

Shane J Morley[1,2*†], Yanmei Qi[3†], Loredana Iovino[1,2], Laura Andolfi[4], Da Guo[3], Nereo Kalebic[1,5], Laura Castaldi[1], Christian Tischer[6], Carla Portulano[1], Giulia Bolasco[1], Kalyanee Shirlekar[1], Claudia M Fusco[1], Antonino Asaro[1], Federica Fermani[1], Mayya Sundukova[1], Ulf Matti[6], Luc Reymond[7], Adele De Ninno[8], Luca Businaro[8], Kai Johnsson[7], Marco Lazzarino[4], Jonas Ries[6], Yannick Schwab[6], Jing Hu[3], Paul A Heppenstall[1,2*]

nature COMMUNICATIONS

ARTICLE

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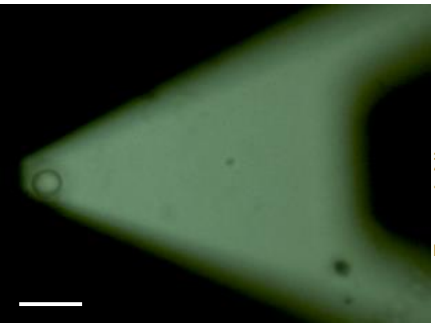
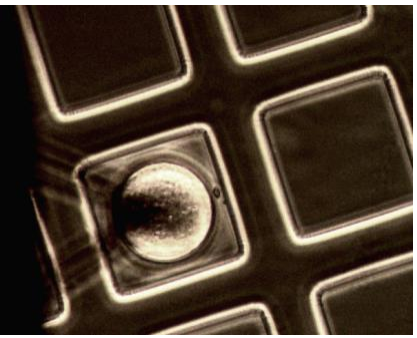
DOI: 10.1038/ncomms9512 OPEN

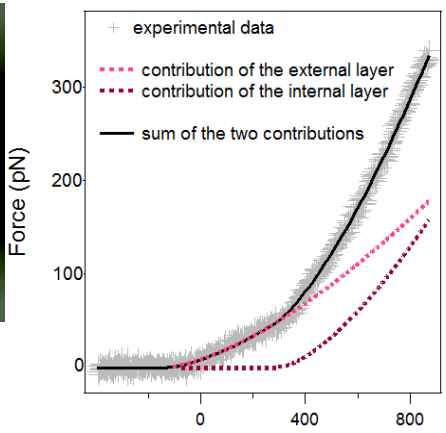
Membrane stiffening by STOML3 facilitates mechanosensation in sensory neurons

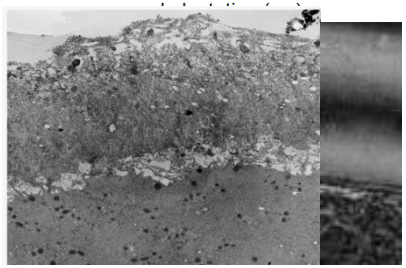
Yanmei Qi[1], Laura Andolfi[2], Flavia Frattini[1], Florian Mayer[1], Marco Lazzarino[2] & Jing Hu[1]

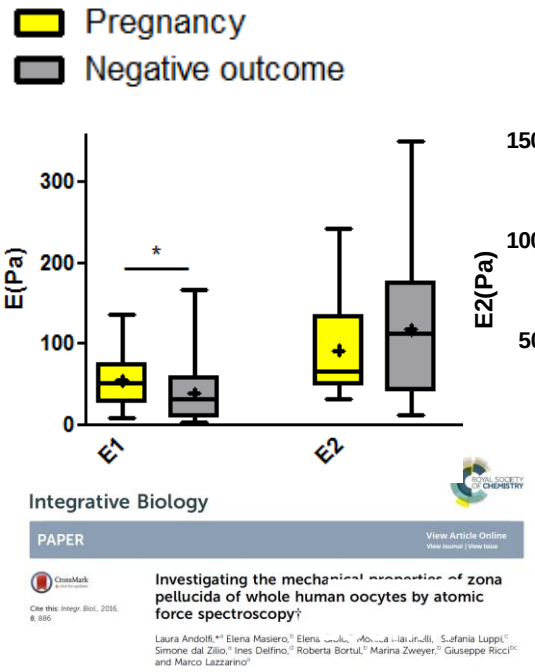
Oocyte mechanical sorting for IVF

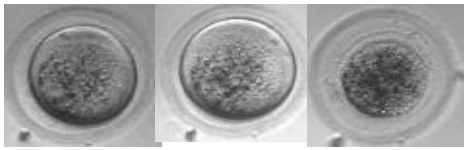


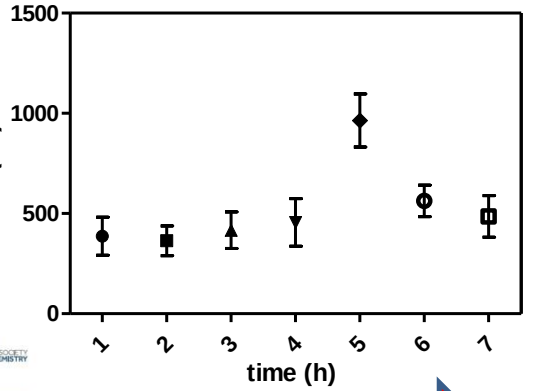












Oocite ageing

ZP is a multilayered structure wich undergoes morpholoigical and mechanical changes during maturation.

We followed ZP mechanical evolution as a score to monitor maturationa dn select the competertnt oocite for IVF

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

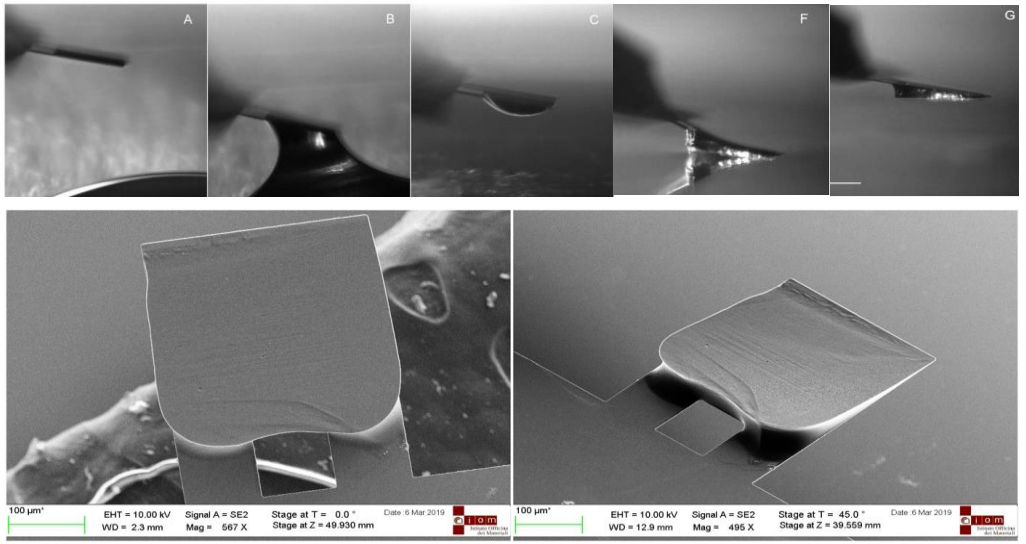
Oocuite ZP Young modulus can be used to predict oocite degradation 1 to 2 hours **before** degradation becomes visible at the microscope.

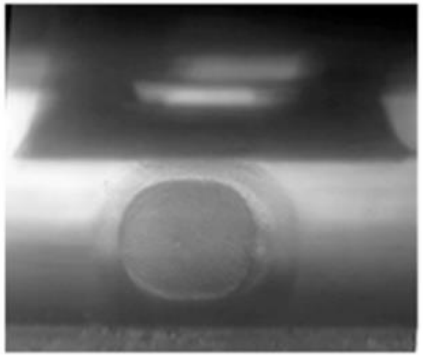
Oocyte mechanical sorting for IVF

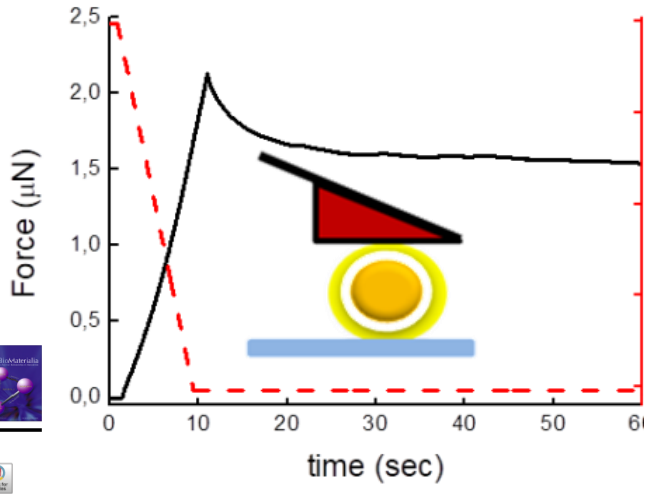
AFM indentation provides information limited to the ZP

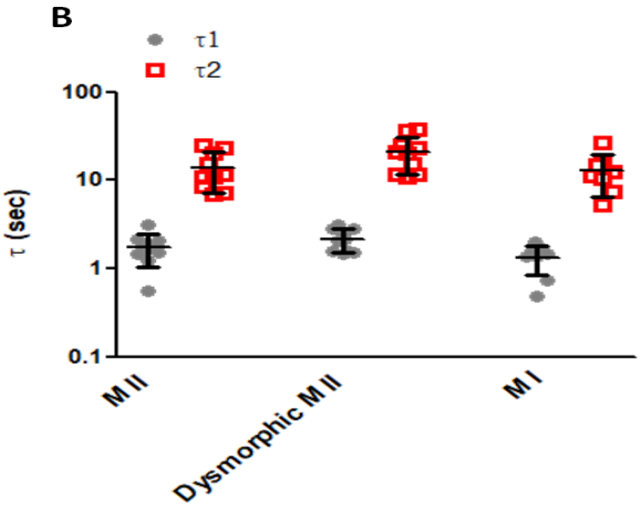
To investigate the mechanical behavior of the whole cells we fabricated flat and large cantilevers for

- *stress-relaxation*
- *creep measurements*









Acta Biomaterialia 94 (2019) 505–513

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Planar AFM macro-probes to study the biomechanical properties of large cells and 3D cell spheroids

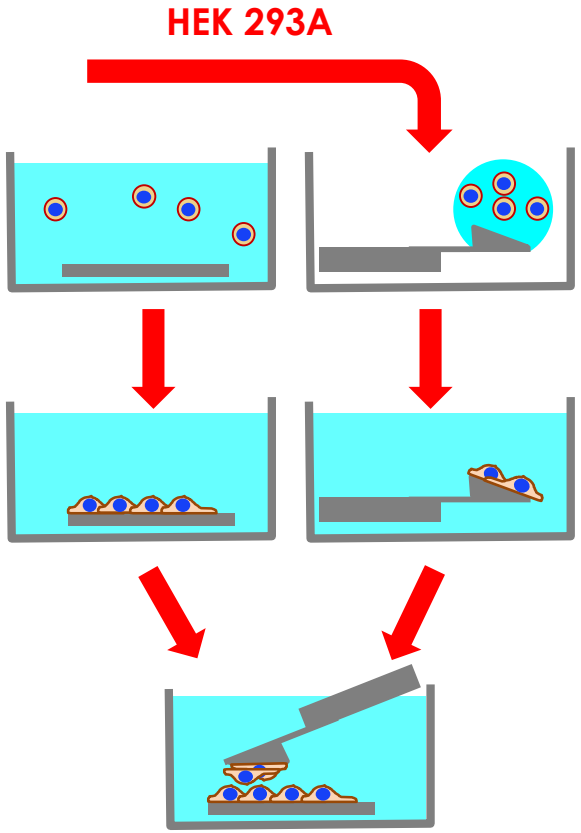
Laura Andolfi [a,*], Silvio L.M. Greco [a], Domenico Tierno [a,b], Roberto Chignola [c], Monica Martinelli [d], Elena Giolo [d], Stefania Luppi [d], Ines Delfino [e], Michele Zanetti [a,b], Alice Battistella [a,b], Giovanna Baldini [f], Giuseppe Ricci [d,f,*], Marco Lazzarino [a]

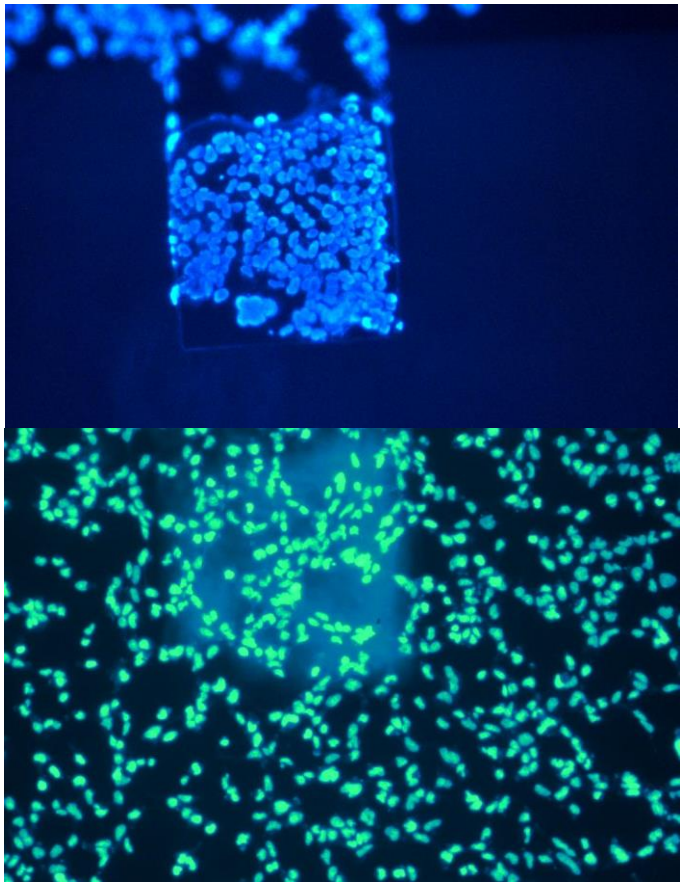
Many-cell/tissue force spectroscopy

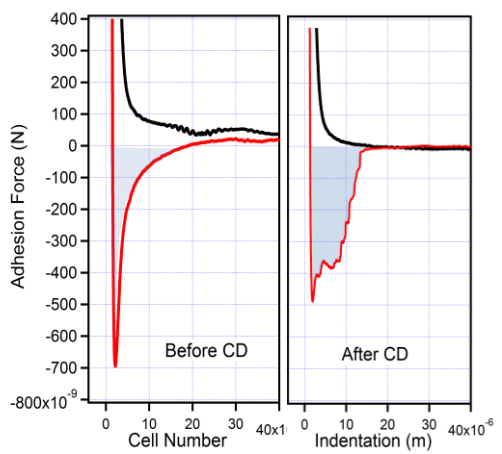
Single cell force spectroscopy suffer from severe drawback in term of Statistics

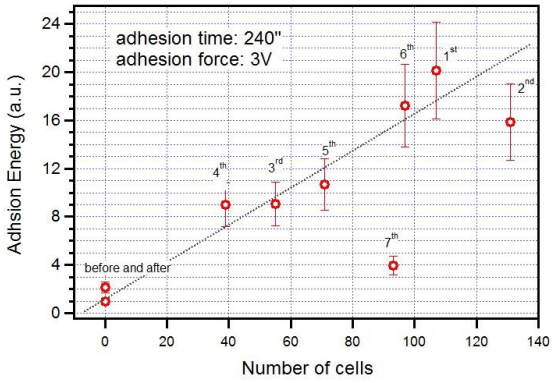
Proper cell adhesion and growth on the cantilever.

Our large and flat cantilever can address also these issues.

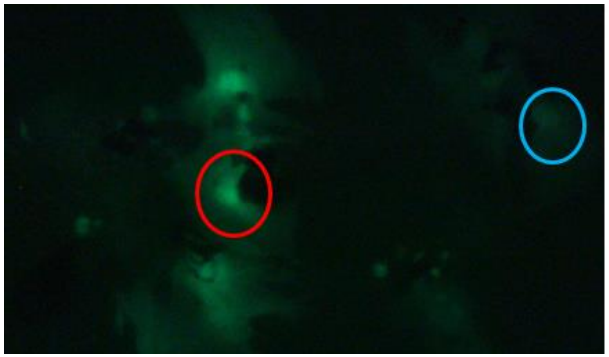


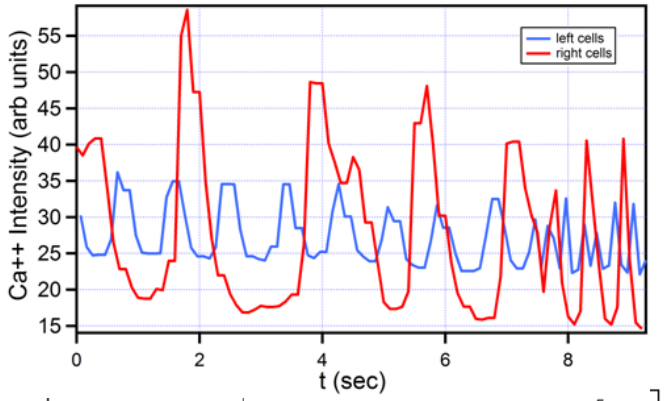


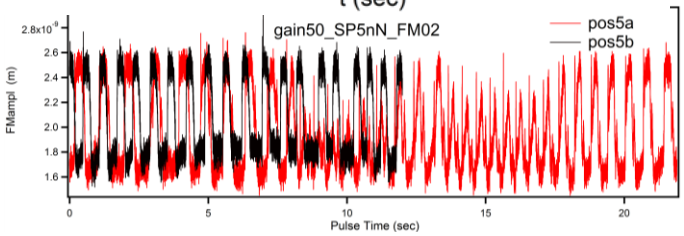




Ongoing & future projects: 1. Ca++ imaging

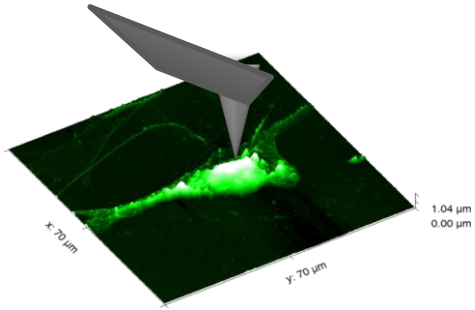


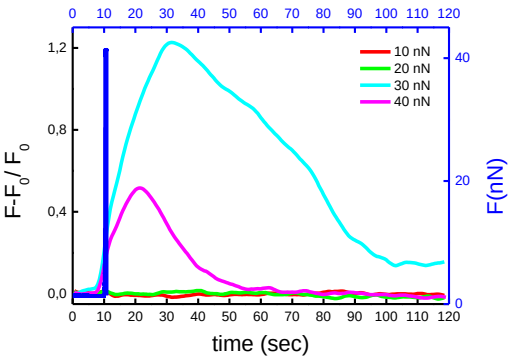


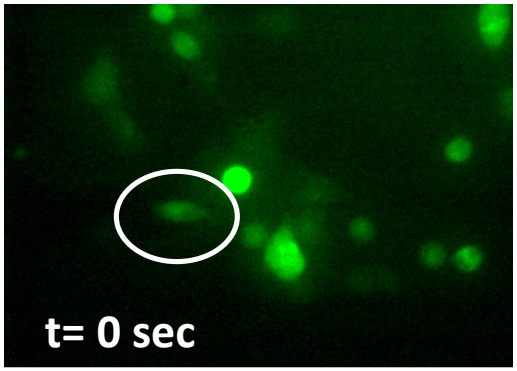


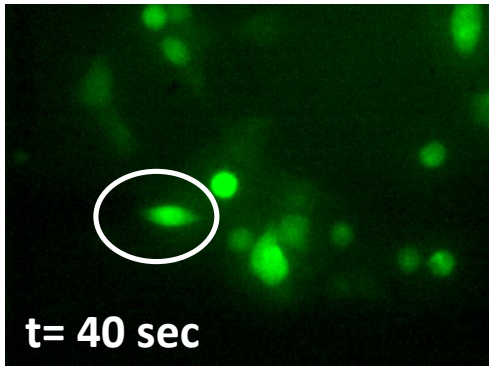
Beating recorded by AFM while recording Ca++ imaging pulse

Calcium wave is spontaneous









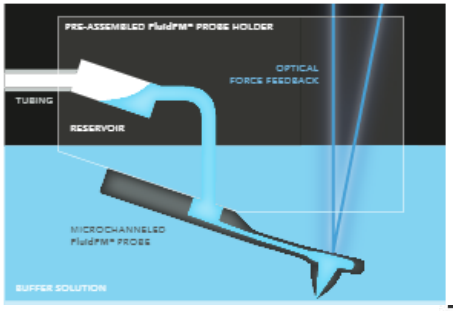
Mechanotransduction:

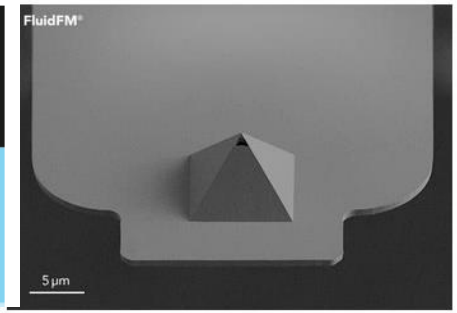
AFM for controlled mechanical stimulation
AND
Calcium Imaging to monitor meccanosensitive channels

Calcium wave is stimulated

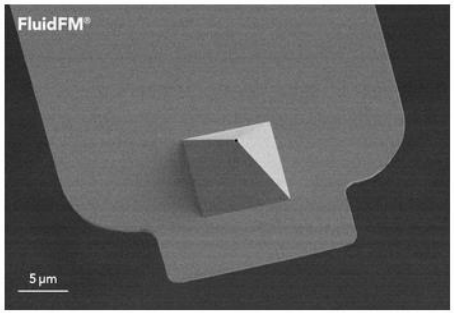
The stimulation can be localized or wide depending on the tip

Ongoing & future projects: 2. FluidFM

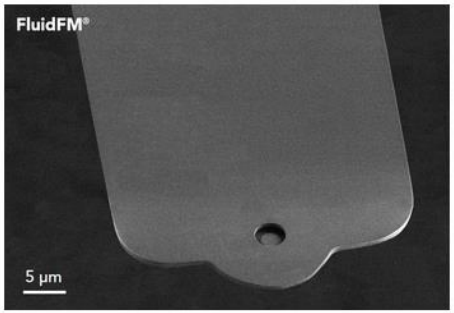




FluidFM nanosyringe



FluidFM nanopipette



FluidFM micropipette

Single cell adhesion

Spotting

Colloidal spectro-scopy

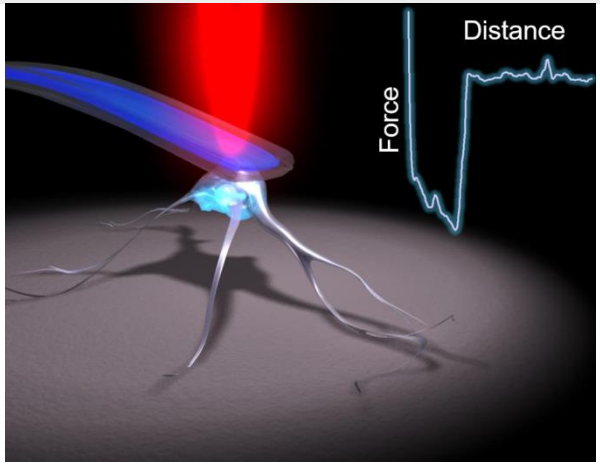
Single bacteria adhesion

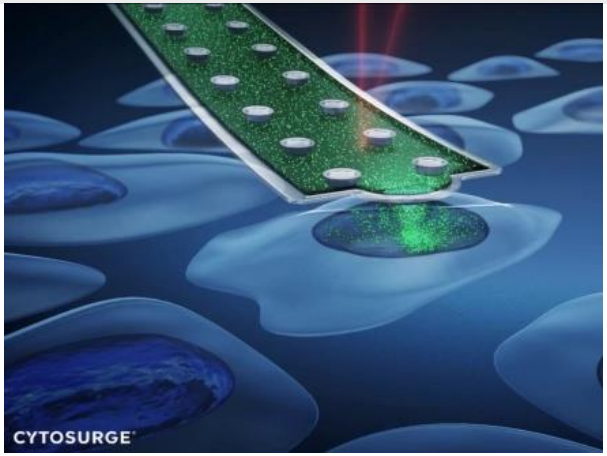
Nano-printing

Nano-injection

Nano-extraction

Cell isolation





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