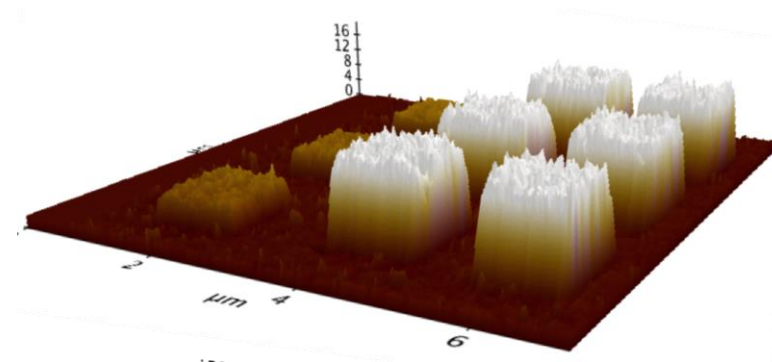
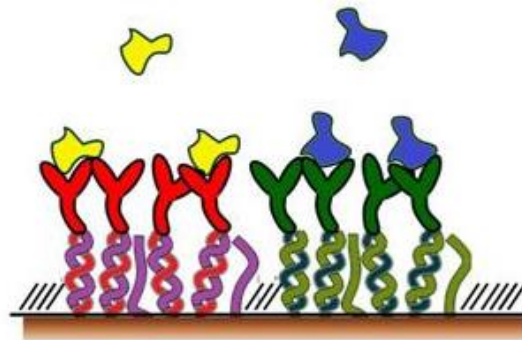
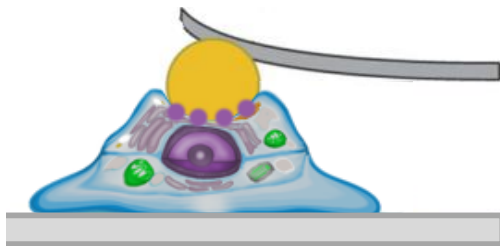


Cell Biomechanics as a marker of disease development: the case of calcific aortic valve disease

Loredana Casalis





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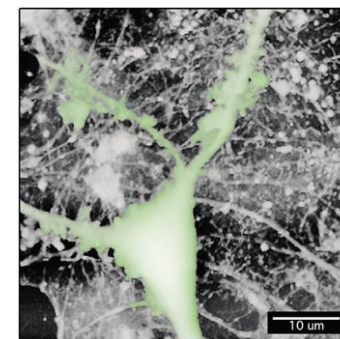


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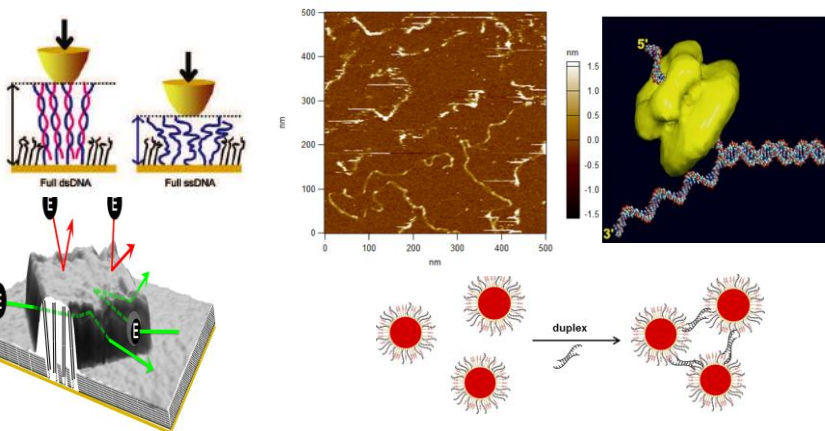
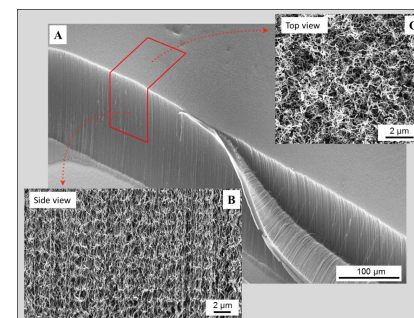
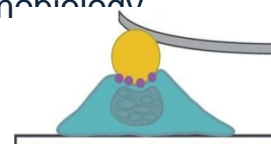
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NanoInnovation Lab Projects Overview



MATERIAL SCIENCE, PROSTETIC MATERIALS

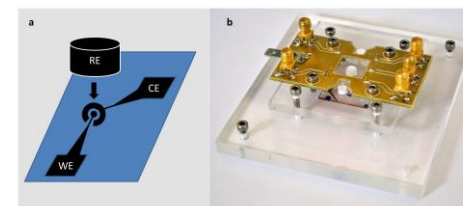
Carbon-based prosthetic materials, interaction with cell membranes; AFM cell/tissue mechanobiology



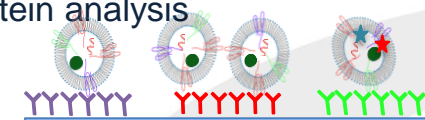
Enzymatic reactions on DNA nanobrushes and on DNA coated Au Nanoparticles.
Single molecule DNA-Helicase interactions

NANO-BIOPHYSICS

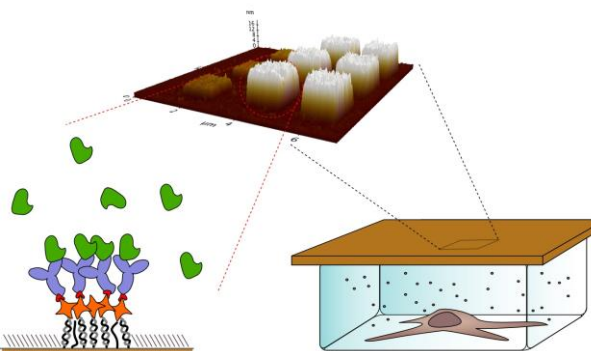
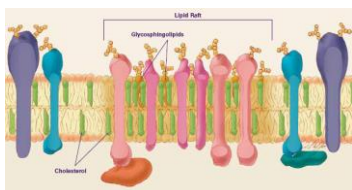
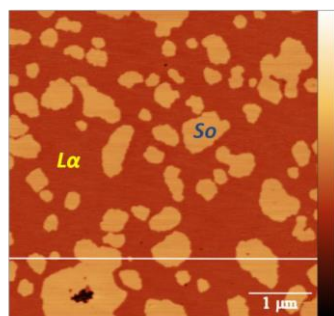
NANOBIOTECHNOLOGY NANOMEDICINE



Electrochemical microfabricated devices for real-time, cheap and fast protein analysis



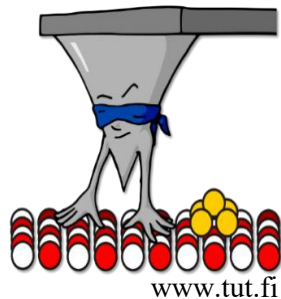
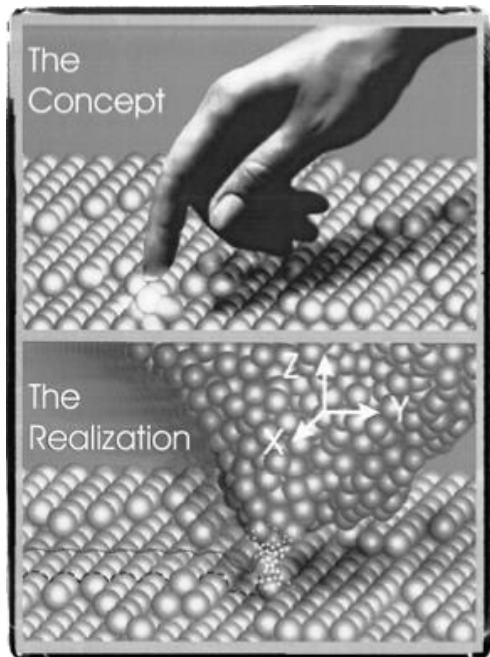
Exosome sorting and characterization



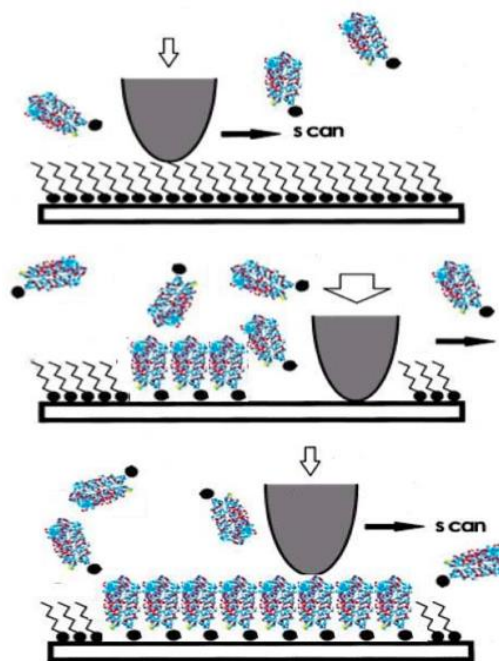
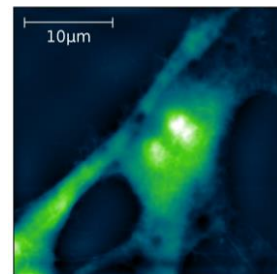
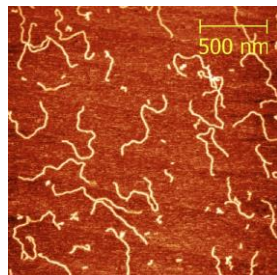
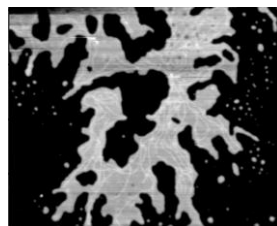
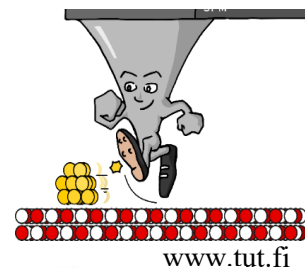
DNA-Nanoarrays, DNA-based immunoassay

An Atomic Force Microscopy Lab

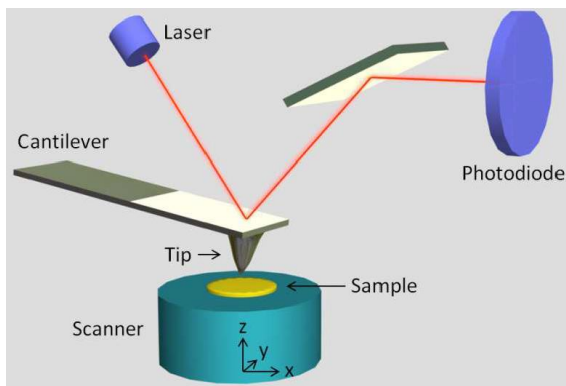
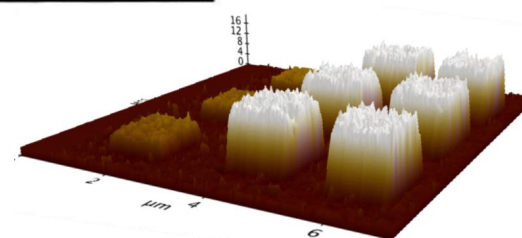
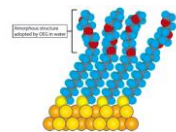
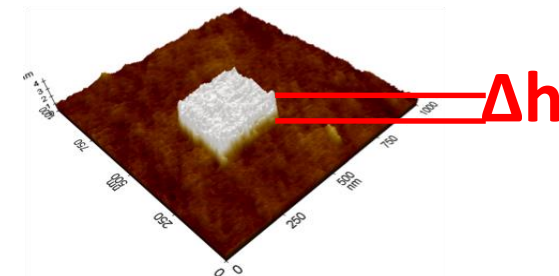
Imaging



Nanografting

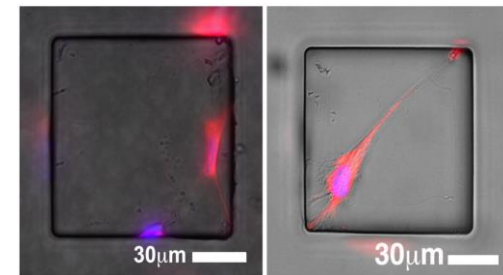
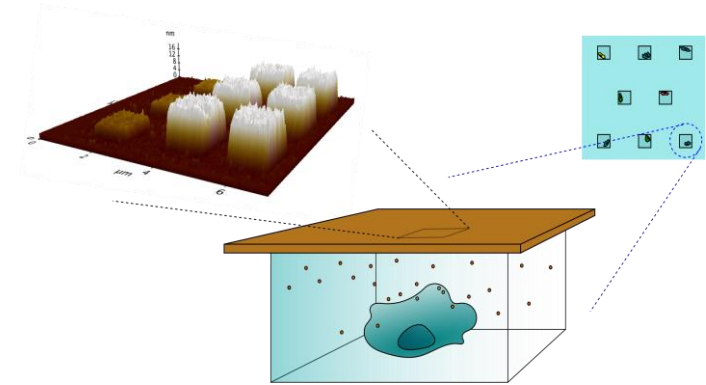
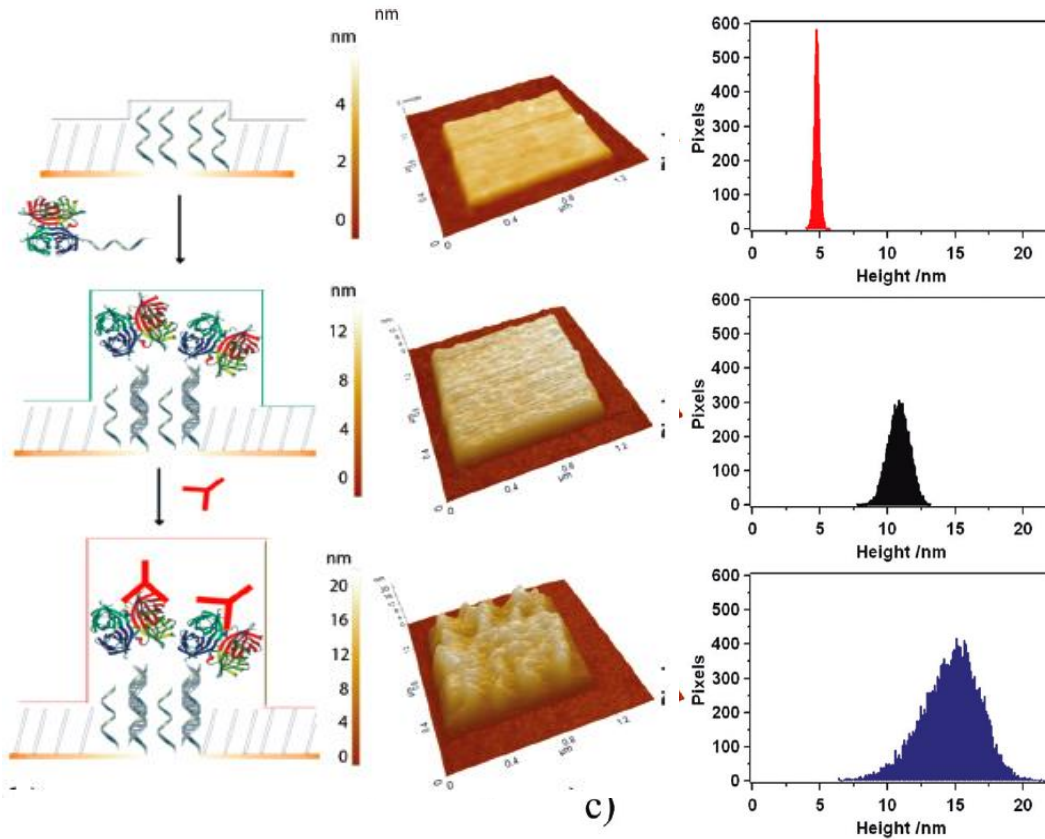
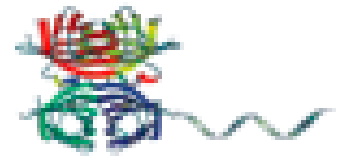


ssDNA into a C11-OEG3 SAM





DNA-barcoded Nanoarrays

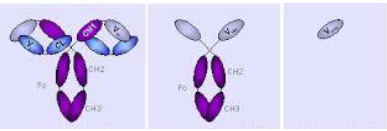


Detection in cell lysate

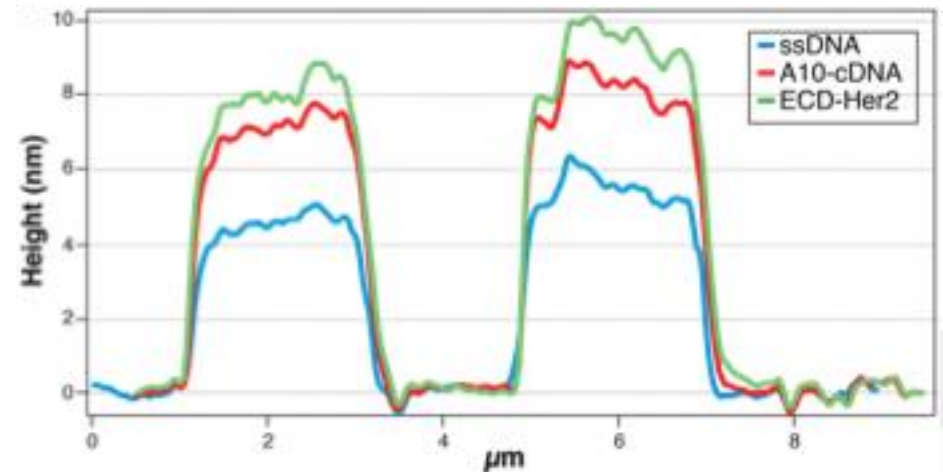
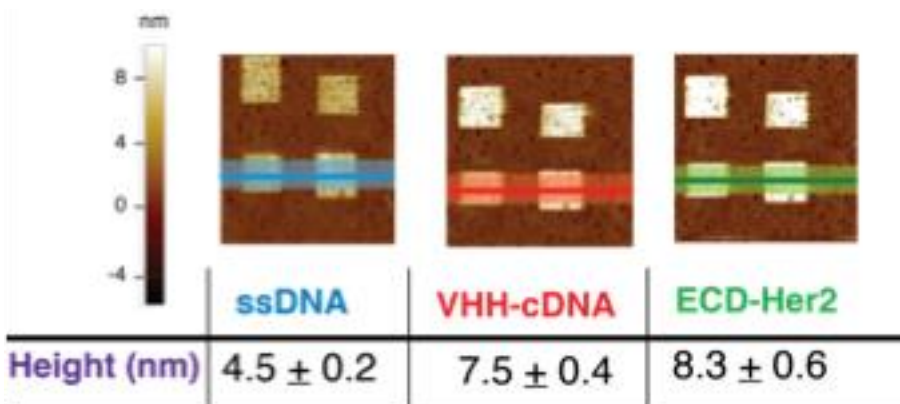
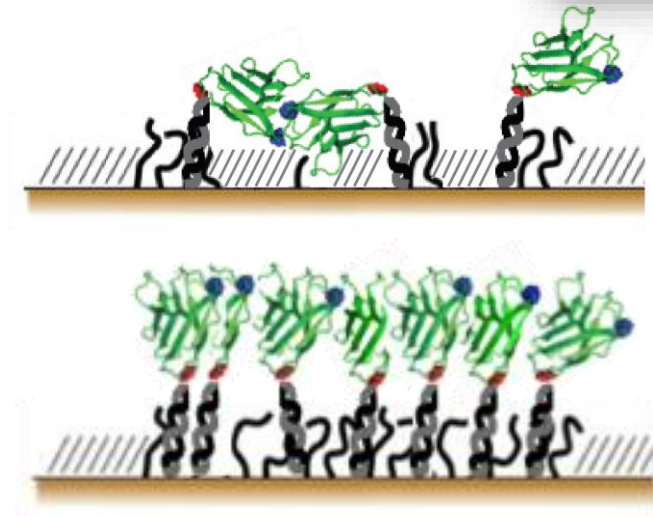
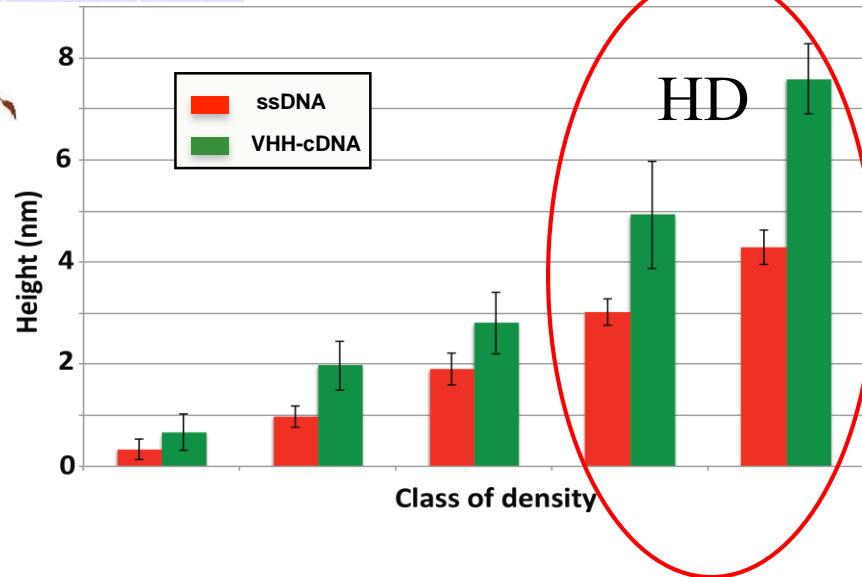


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ECD-Her2 detection in serum



Nanobody

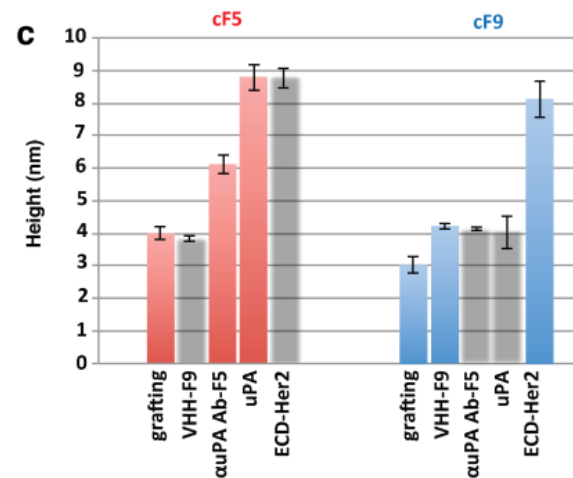
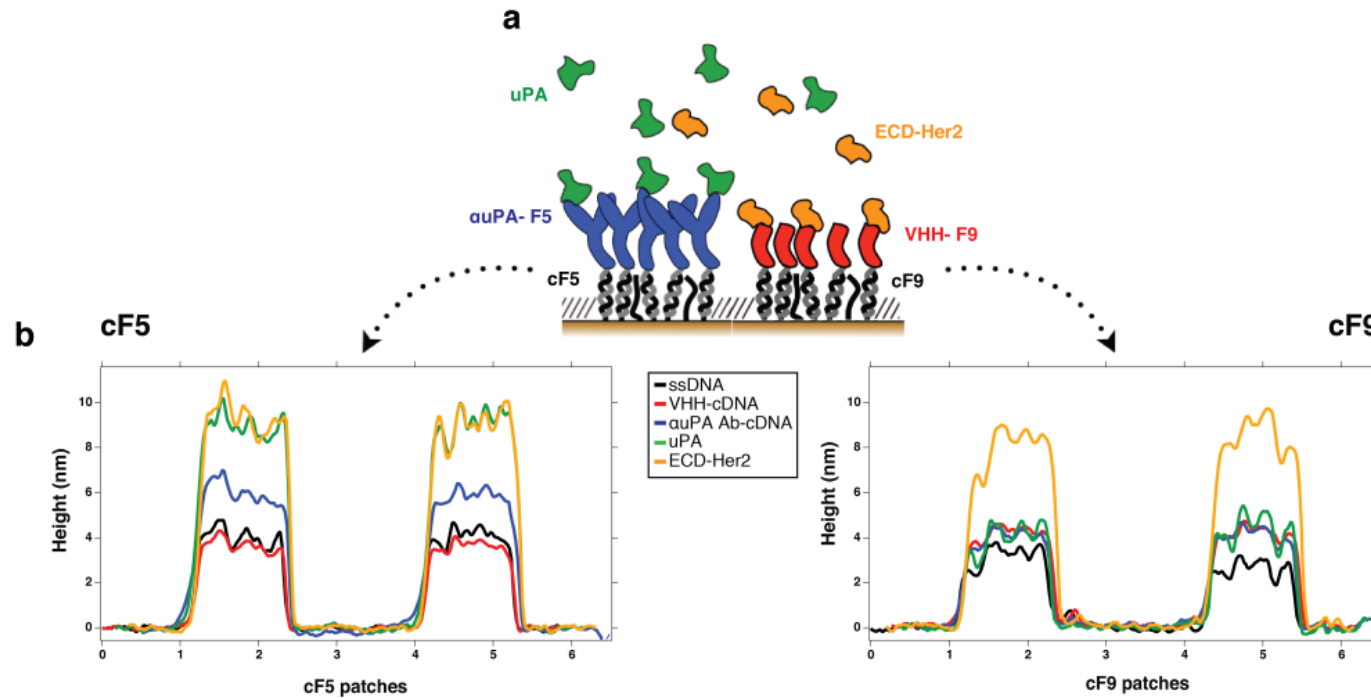


Limit of sensitivity **100 pM**

Lower than the cutoff value of 15 ng/ml commonly used in clinic for Her2 positive breast cancer



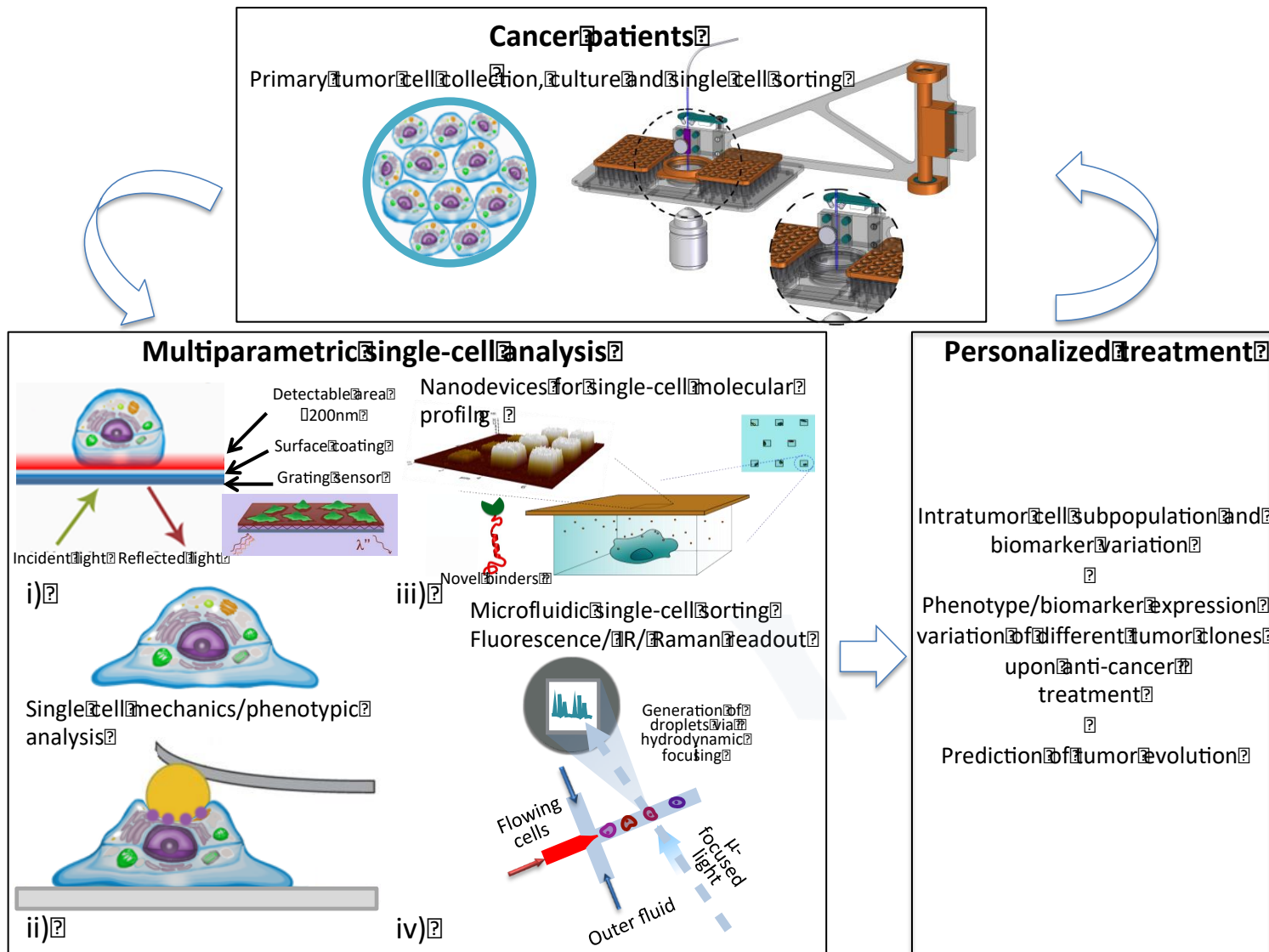
Multiplexing Assay



	Height
ssDNA	4.0 ± 0.2
VHH-F9	3.9 ± 0.1
auPA Ab-F5	6.1 ± 0.3
uPA	8.8 ± 0.4
ECD-Her2	8.8 ± 0.3

	Height (nm)
ssDNA	3.1 ± 0.3
VHH-F9	4.2 ± 0.1
auPA Ab-F5	4.1 ± 0.1
uPA	4.0 ± 0.5
ECD-Her2	8.2 ± 0.6

Strategies for personalized medicine





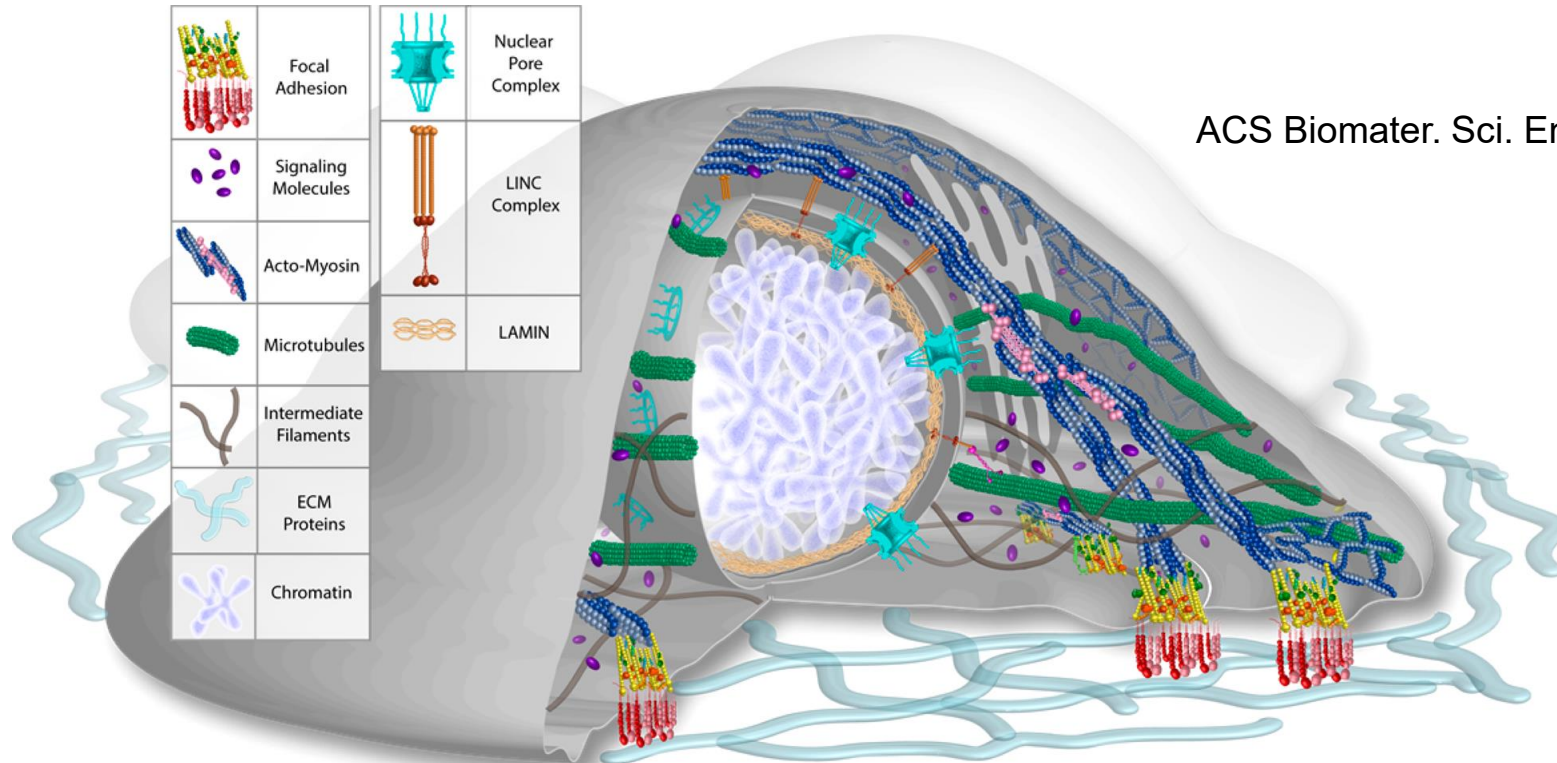
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Cell Biomechanics



Mechanobiology

How do cells perceive a mechanical stimulus and translate it into a biochemical response



ACS Biomater. Sci. Eng. 2017, 3, 2712–2726

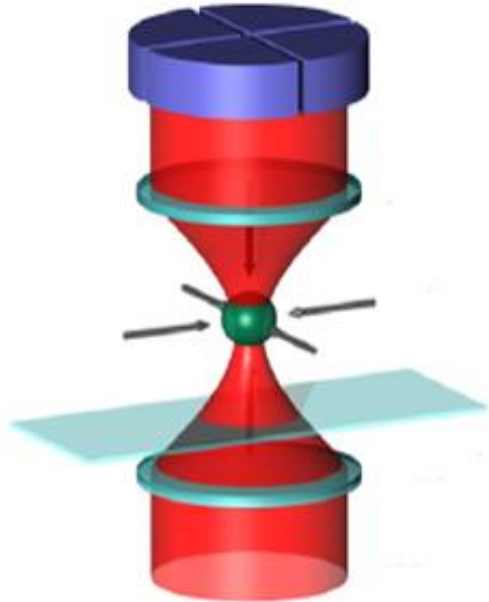
Cells respond to extracellular matrix (**ECM**) cues generating and transducing mechanical forces into biochemical signals and genomic pathways which affect cell properties.

Such forces define tissue architecture and drive specific cell differentiation programs. In adults perturbation of ECM (stiffness, mutations) cause pathologies in different organs, including ageing and malignant progression.

Signalling induced by ECM stiffness regulate the onco-factor YAP (Yes-associated protein) promoting its translocation from the cytoplasm into the nucleus to promote cell division/apoptosis and controlling the formation of Focal Adhesion (FA) to stabilize the anchor of the actin cytoskeleton to the cell

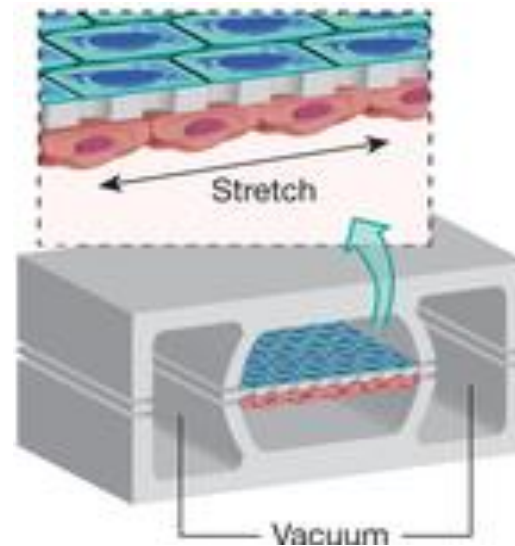


How to study cell mechanics

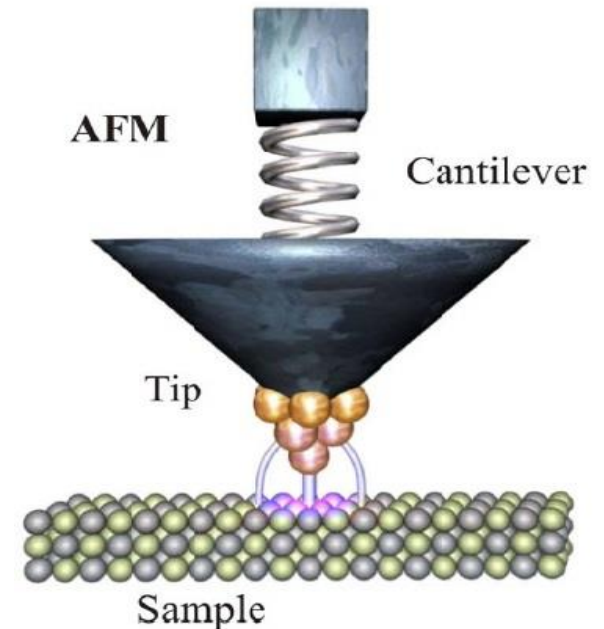


OPTICAL TWEEZER

- Two lasers in order to trap a bead
- The bead displacement converted to force by the software
- Force applied from 0.1 to 100 pN



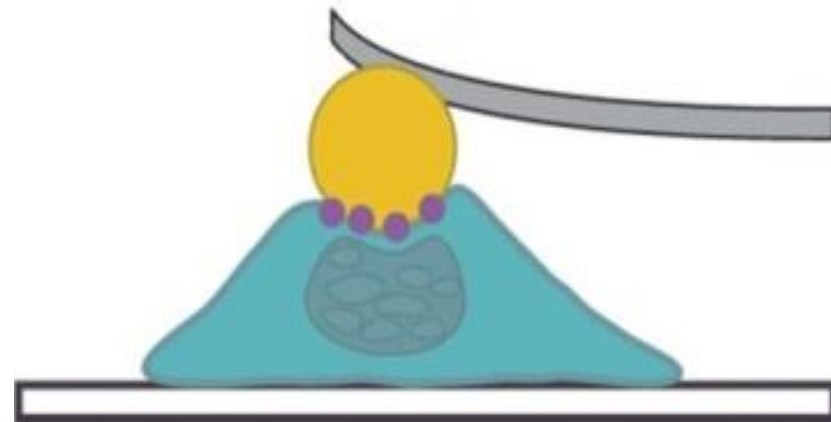
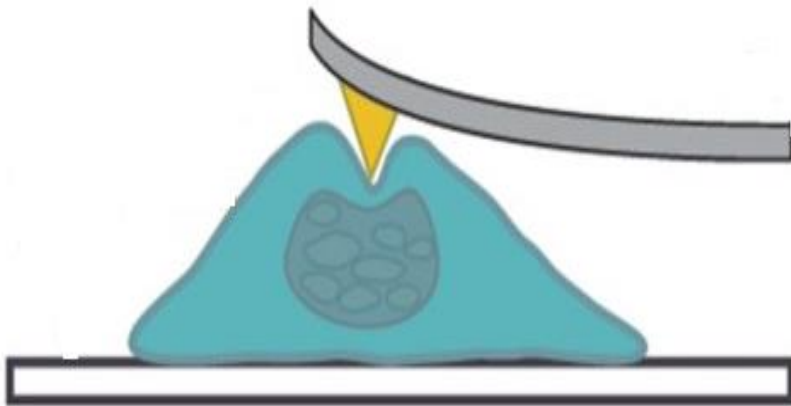
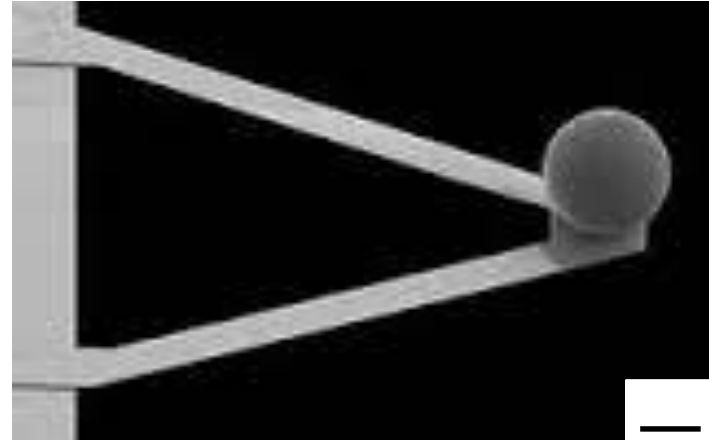
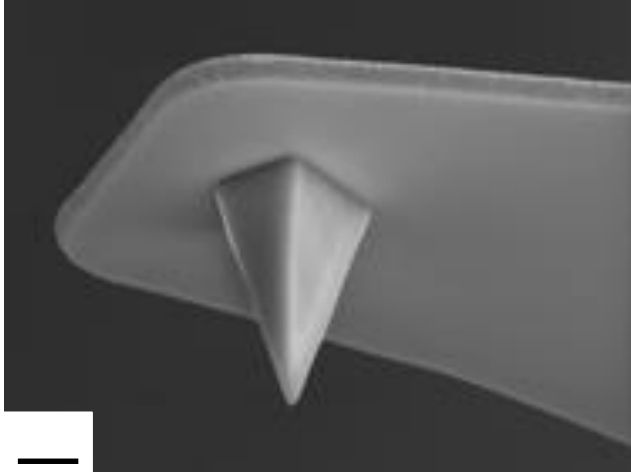
STRETCHING IN MICROFLUIDIC CHANNELS



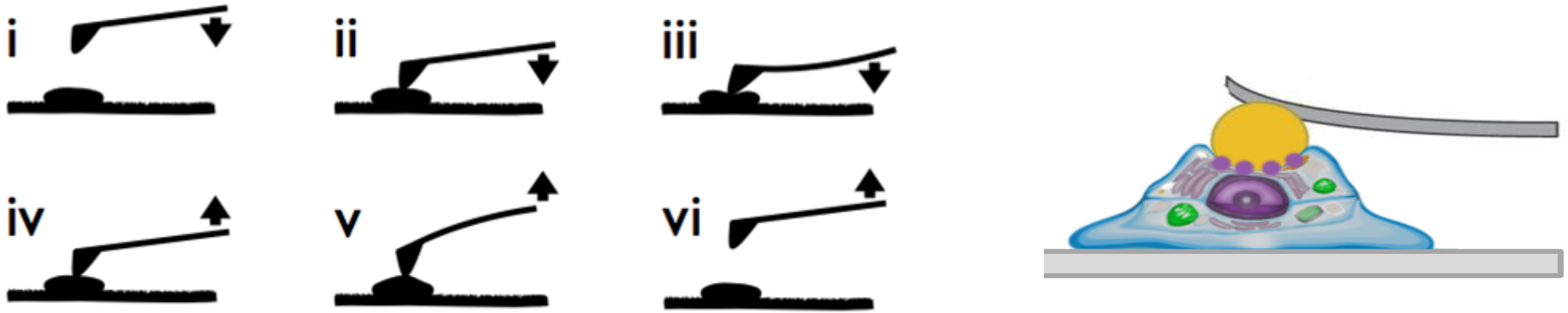
ATOMIC FORCE MICROSCOPY

- Tip mounted on a flexible cantilever
- Tip/sample interaction monitored by a laser
- Force applied from 10 pN to 100 nN

AFM Force-Spectroscopy

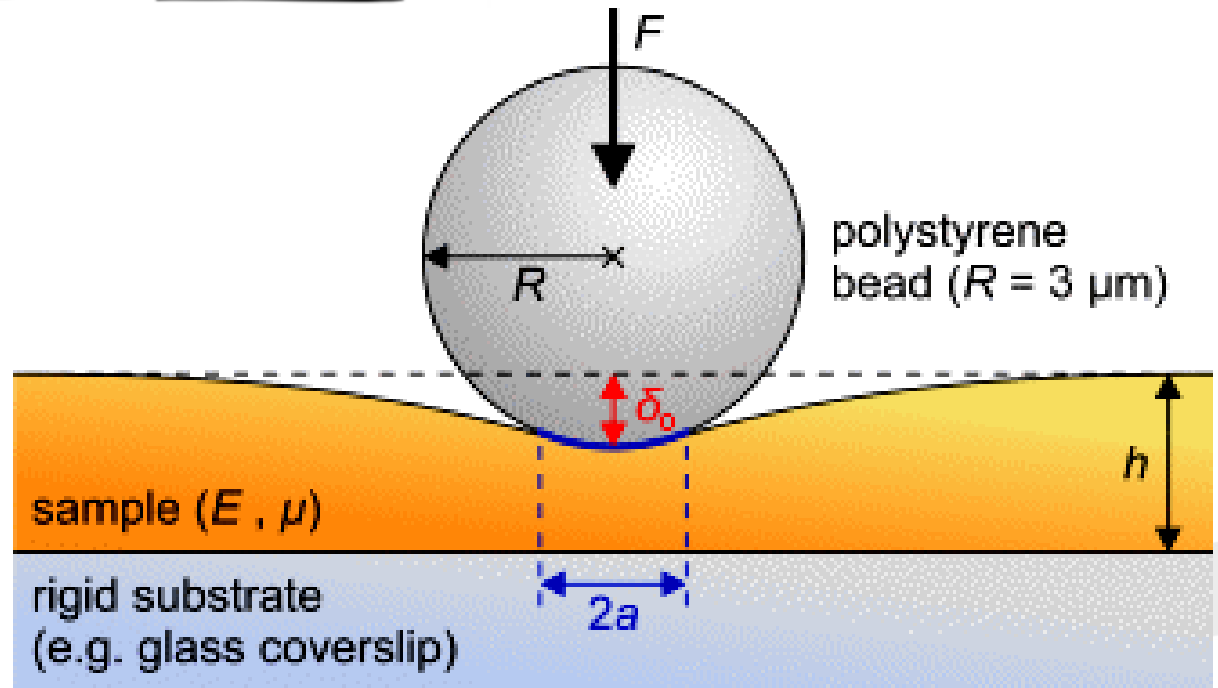


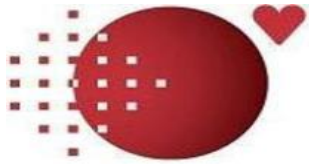
AFM Force-Spectroscopy



$$F = \frac{4}{3} \frac{E}{1-\mu^2} \sqrt{R \delta_0^3}$$

- F ... applied force
- R ... radius of the probe
- δ_0 ... indentation of the sample
- E ... elastic modulus
- μ ... POISSON'S ratio

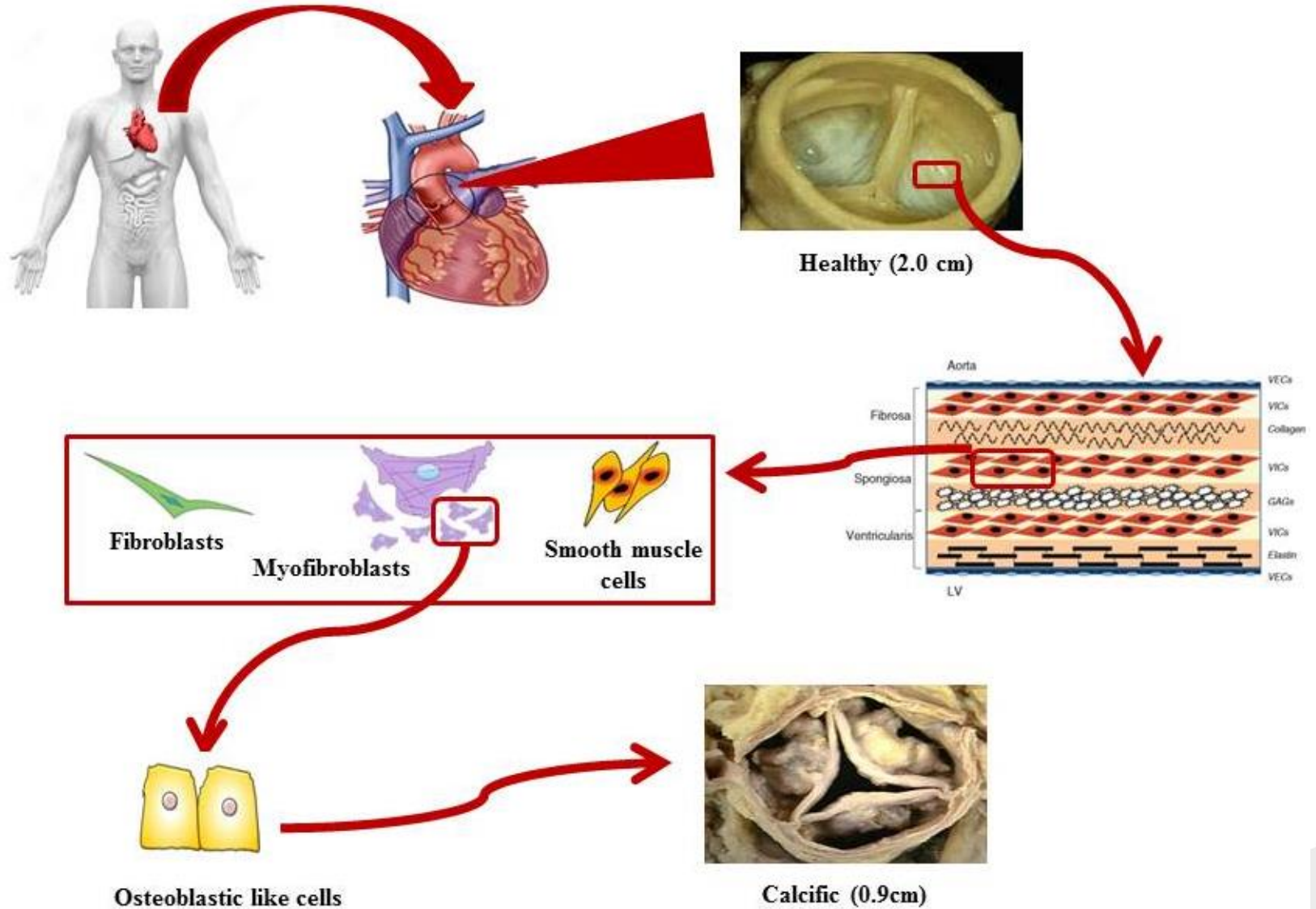




Calcific aortic valve disease



Calcific Aortic Valve Disease (CAVD)

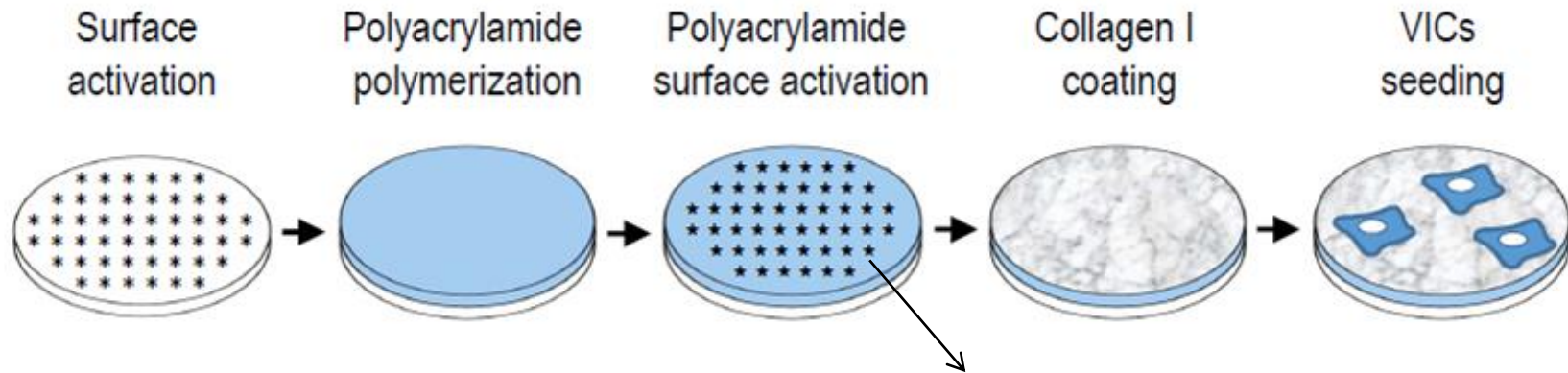




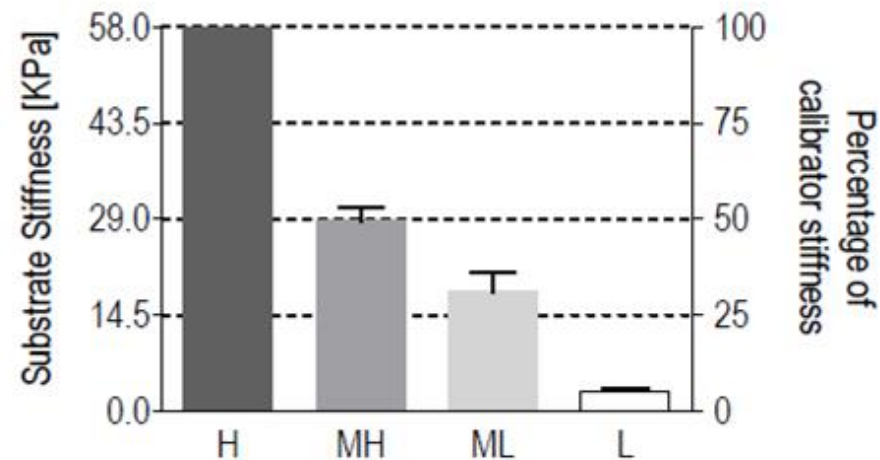
Aortic Valve Interstitial Cells (VICs) on Polyacrylamide gels



a



b



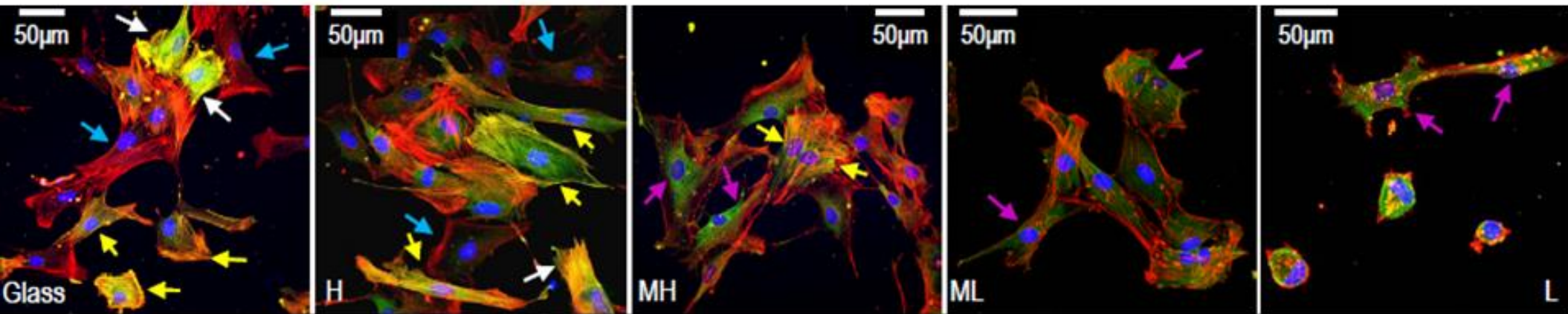


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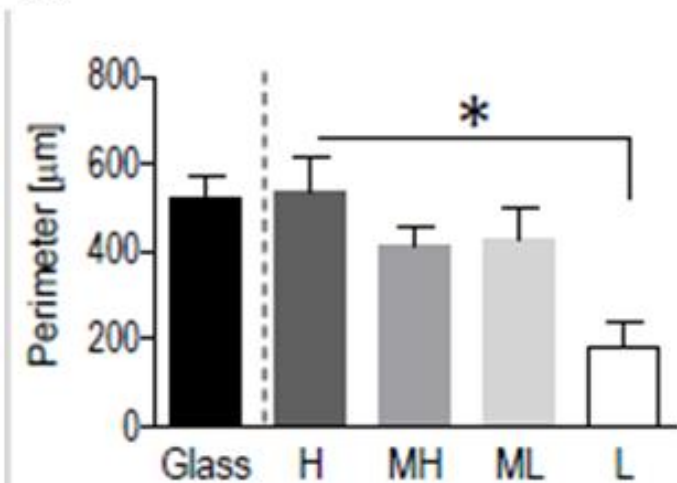
Cellular rigidity is determined by the rearrangement of the cytoskeleton

red: phalloidin; blue: DAPI; green: α -SMA;
white arrow: high levels of α -SMA and co-localization; yellow arrow: intermediate levels of α -SMA and co-localization; blue arrow: low levels of α -SMA and co-localization; purple arrow: no α -SMA

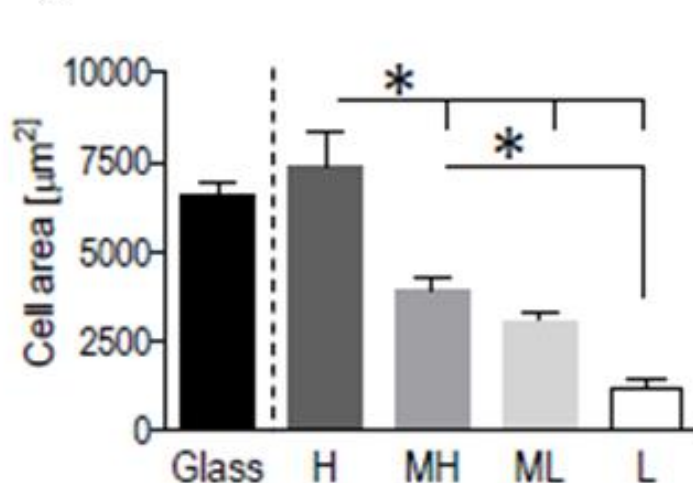
a



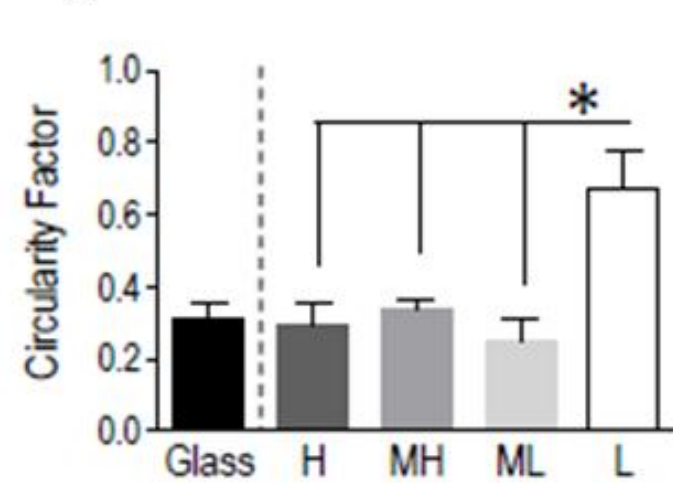
b



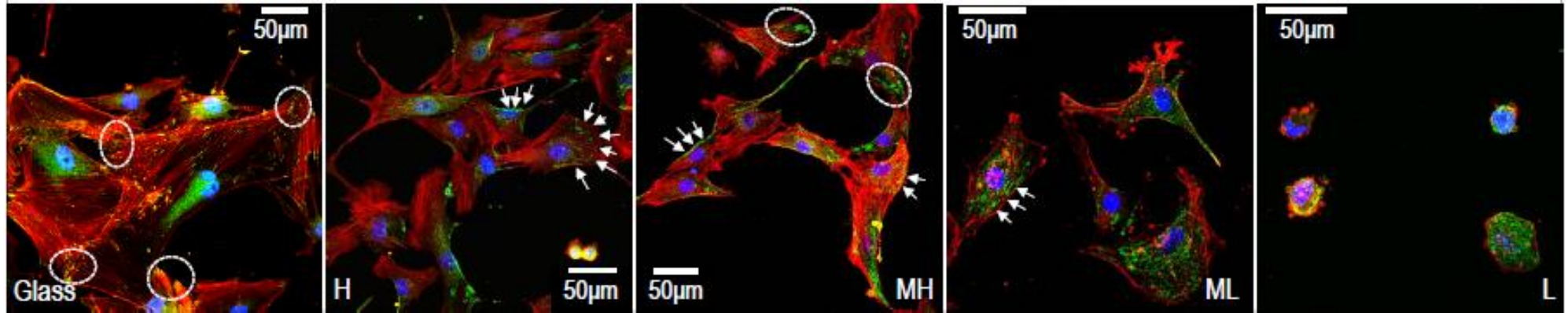
c



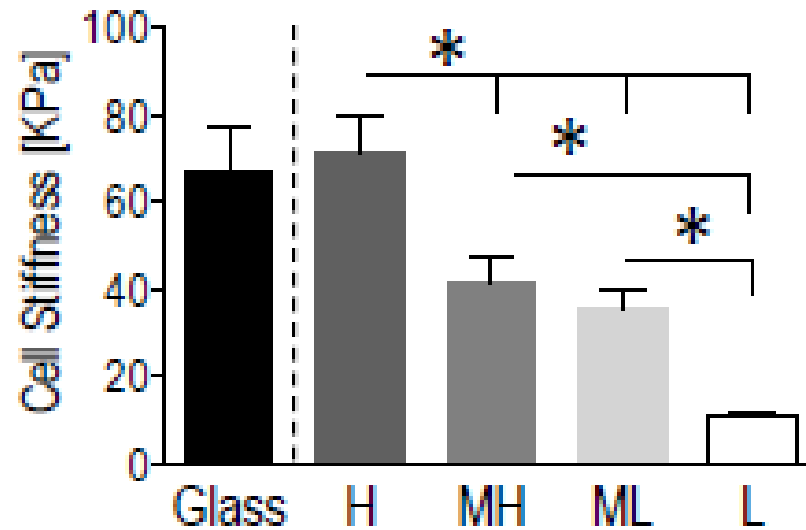
d



Rearrangement of the Cytoskeleton and cell stiffness

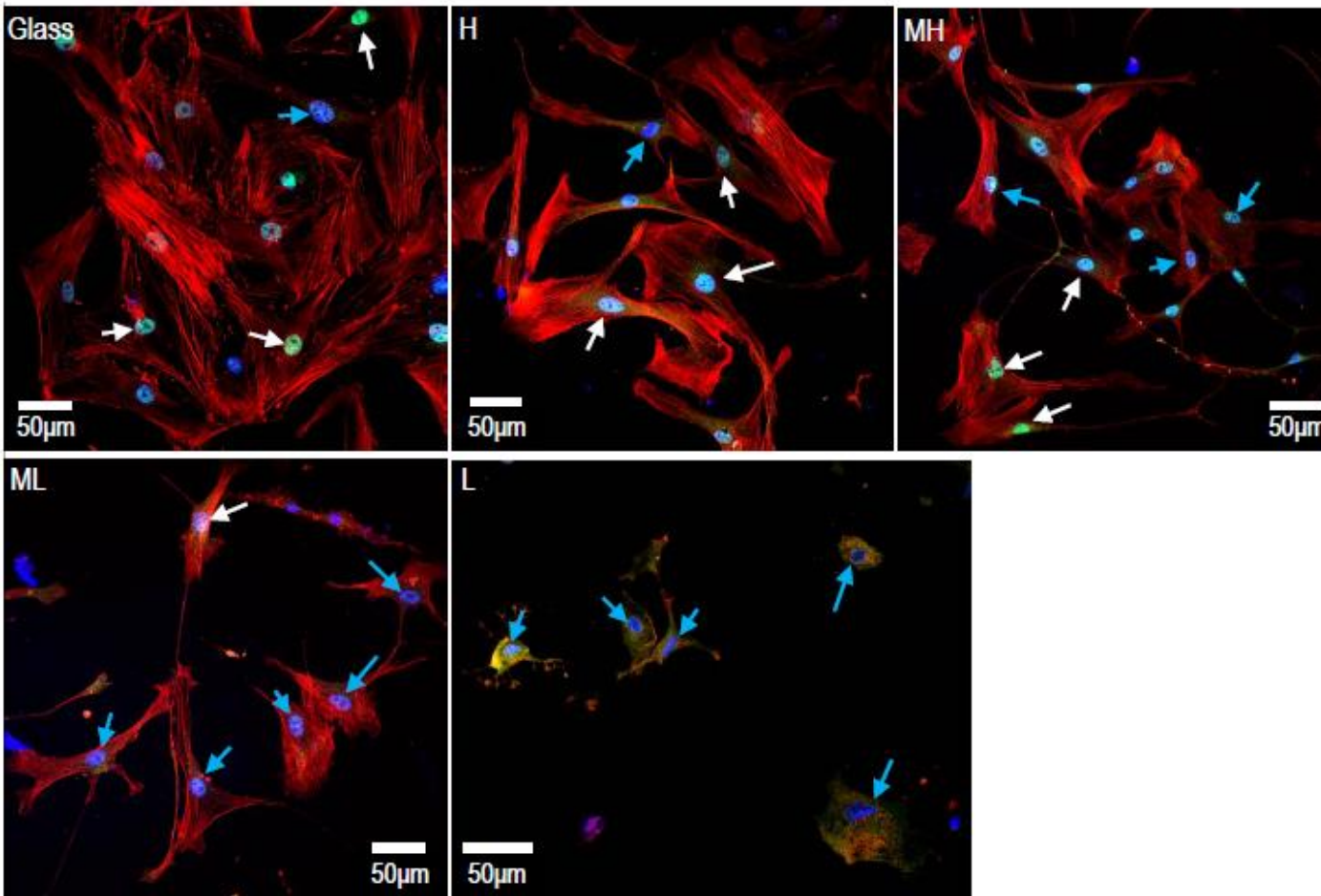


red: phalloidin; blue: DAPI; green: α -SMA;

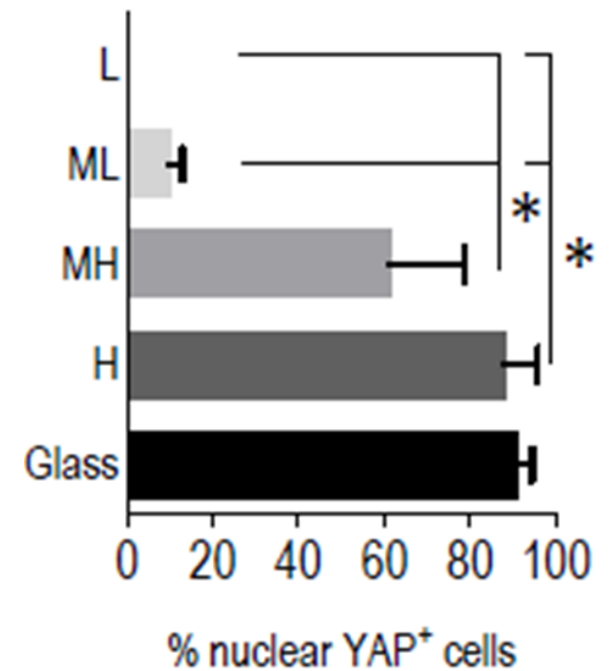


High cytoskeleton tensioning determines high levels of YAP nuclear localization

a

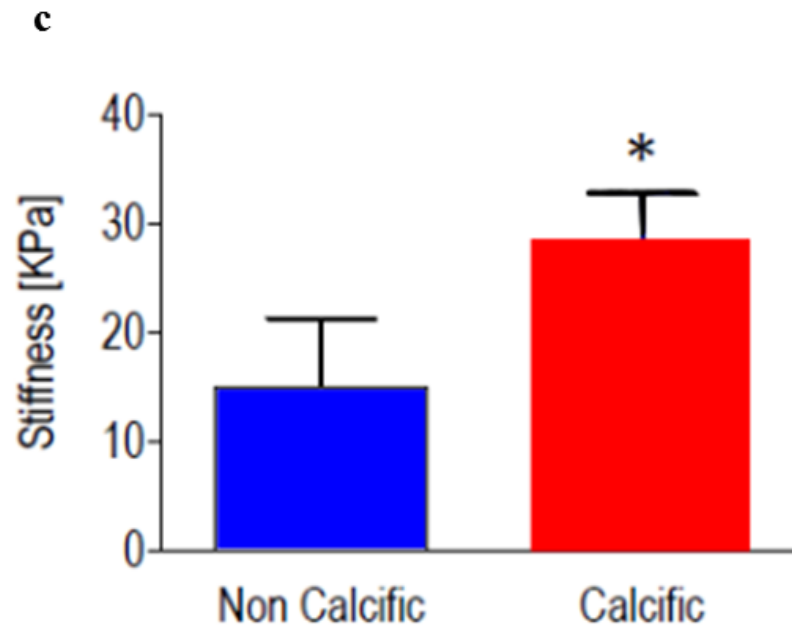
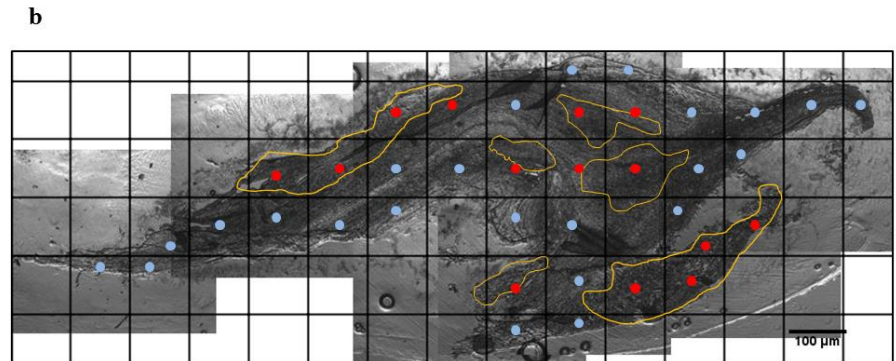
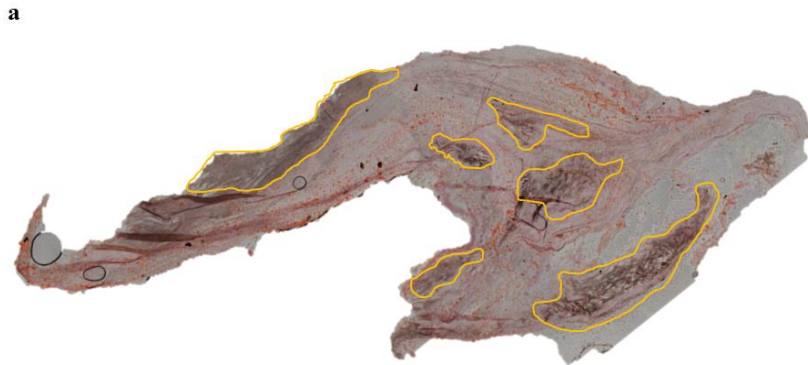


b



red: phalloidin; blue: DAPI; green:
YAP

Ex-vivo tissue: stiffness of human aortic valve leaflet



ECM morphology contribution to Calcific Aortic Valve Disease: the carbon nanotubes matrix



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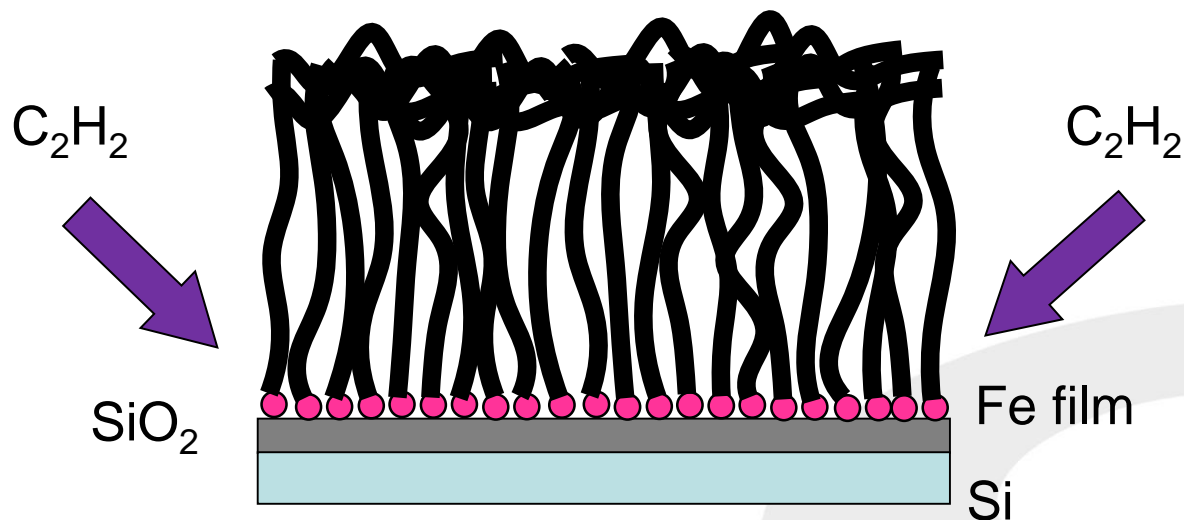
CVD growth 2D CNTs substrates



Thermal decomposition of a gaseous precursor on catalytic nanoparticles in a high-vacuum reaction chamber



nanoparticles from Fe film (2-5 nm) on SiO_2 , annealed at 650–670 °C (at a pressure of 10–20 mbar)

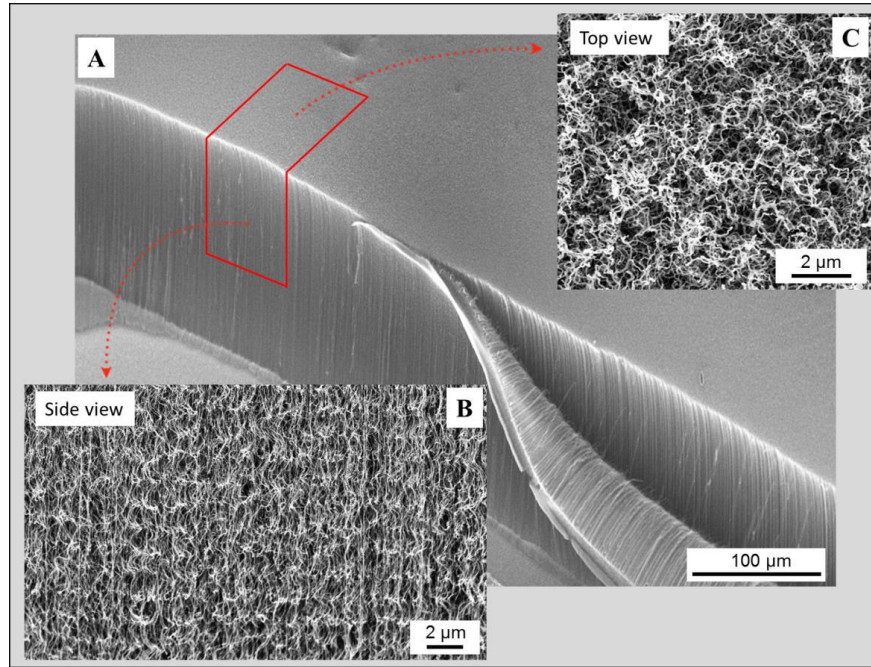


*In collaboration with Andrea Goldoni, Elettra Carbon Lab

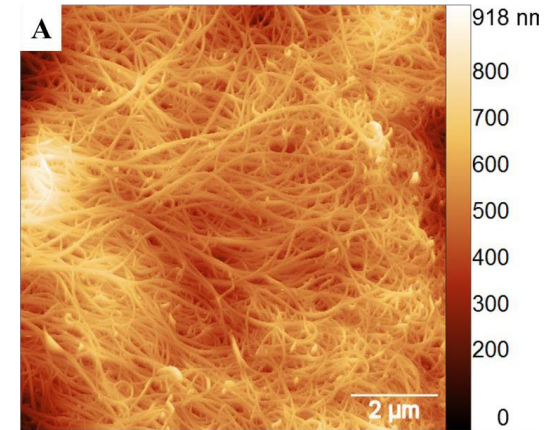


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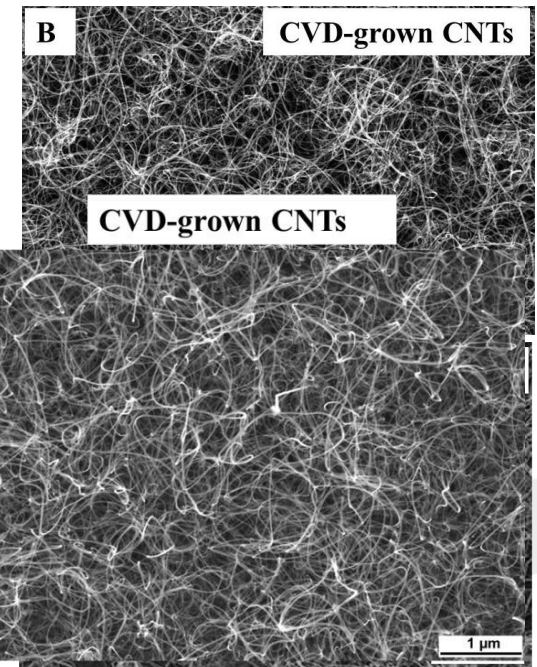
CVD growth 2D CNTs substrates*



AFM CNT

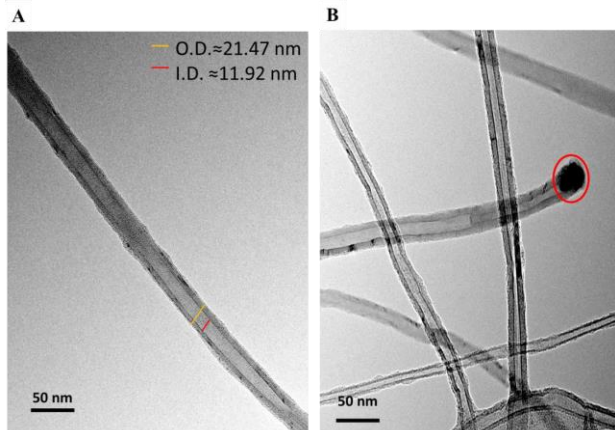


SEM CNT



Drop-casted CNTs

CVD-grown CNTs

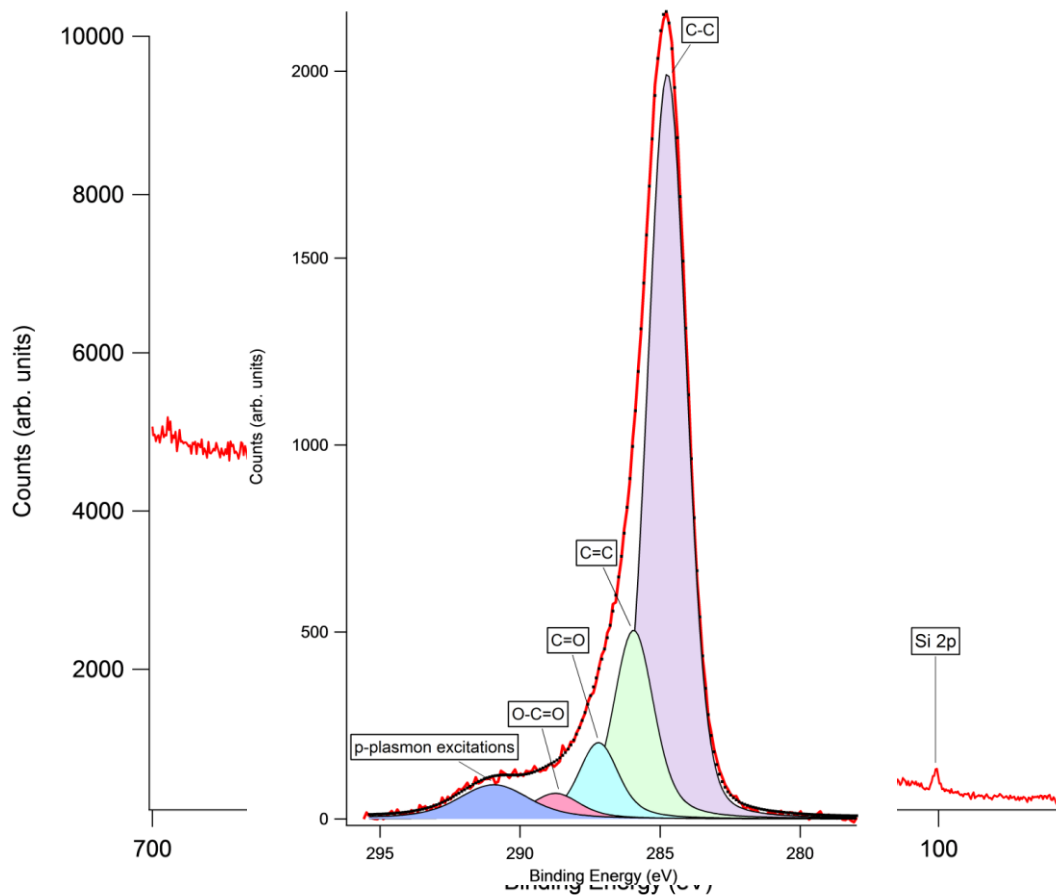
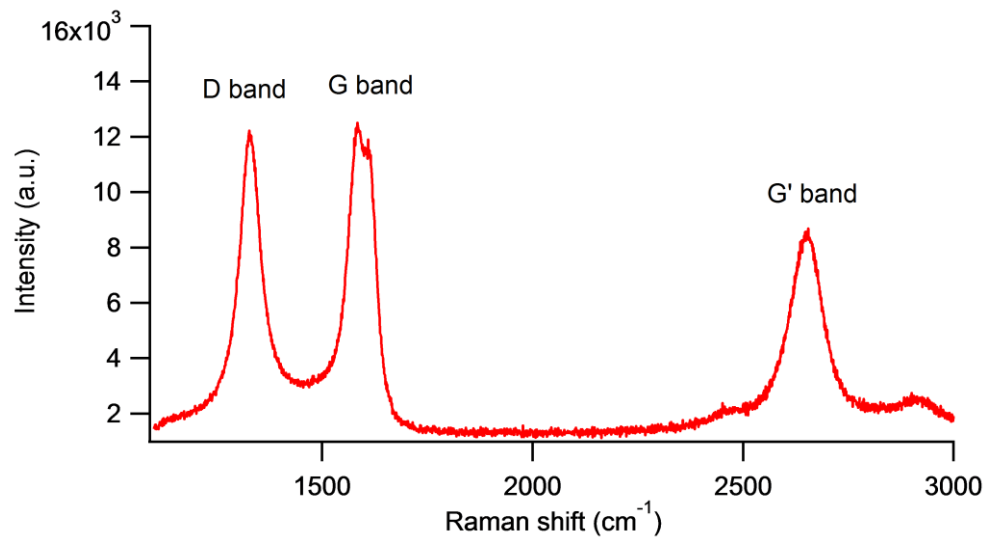


*In collaboration with Denis Scaini, SISSA, Trieste

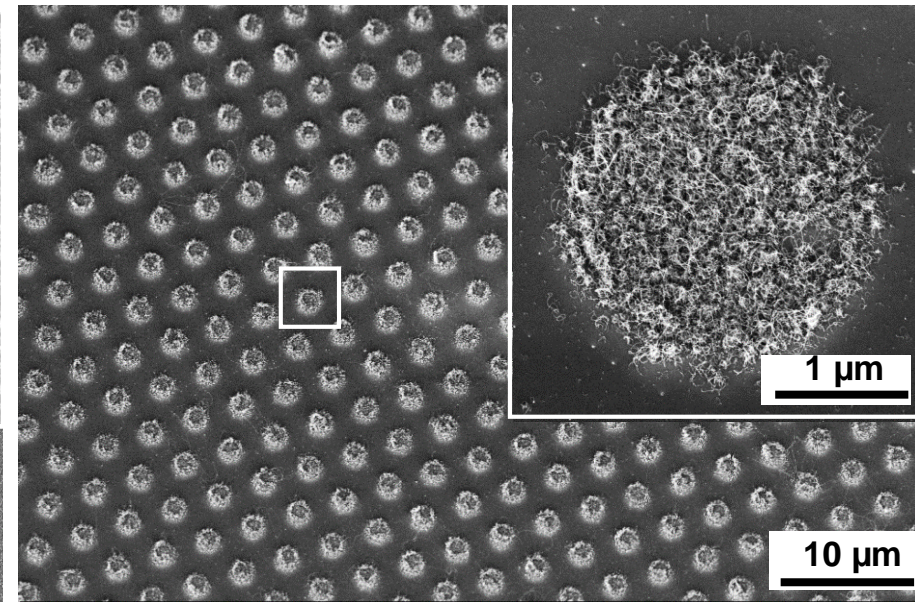
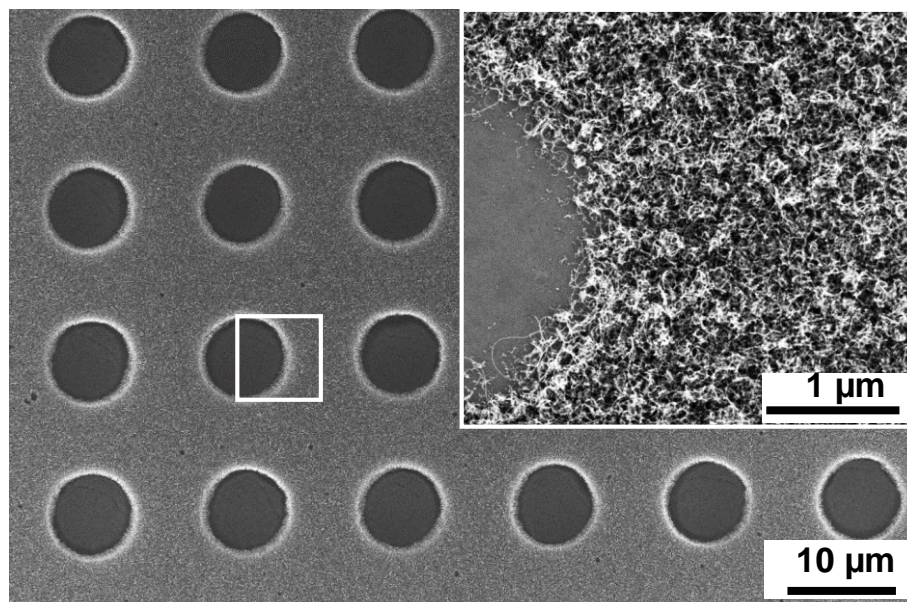
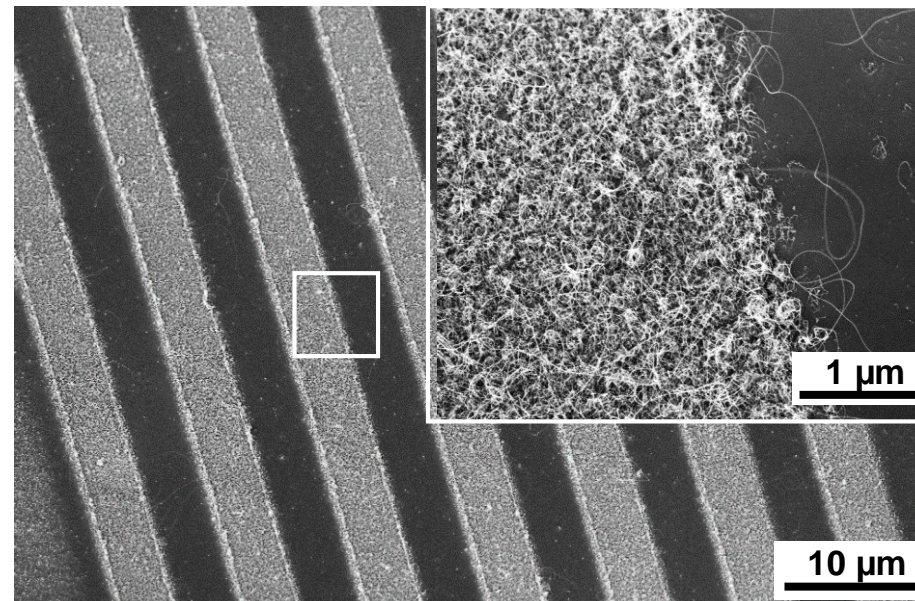
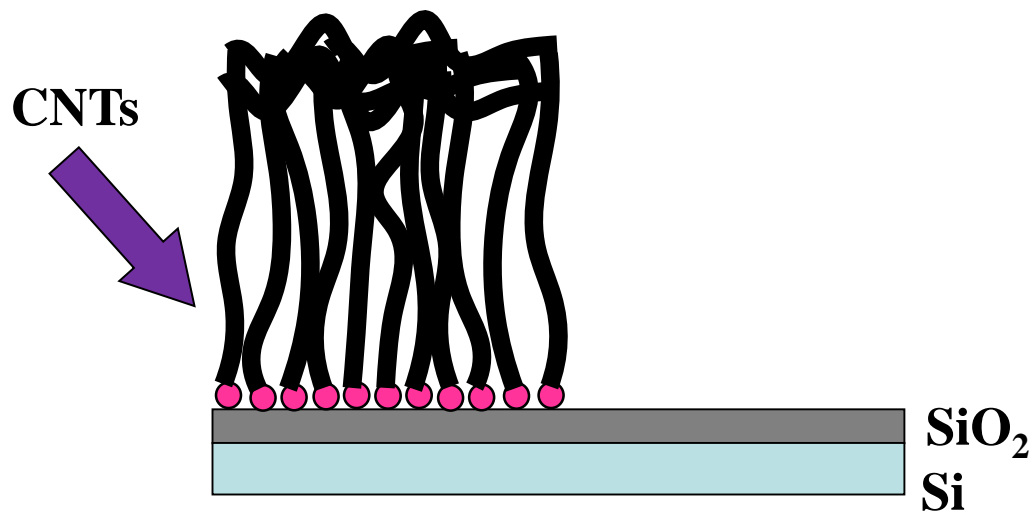


Raman and XPS characterization

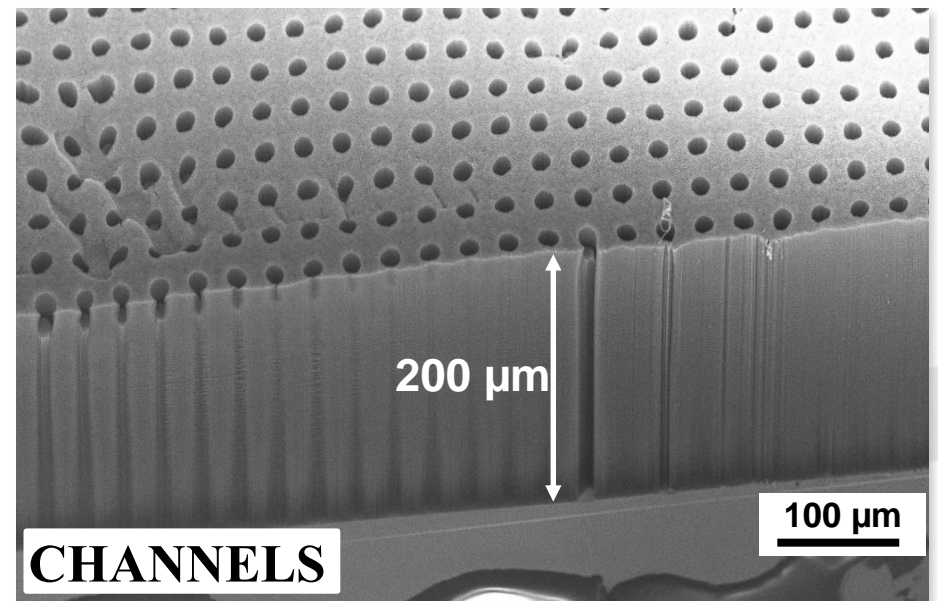
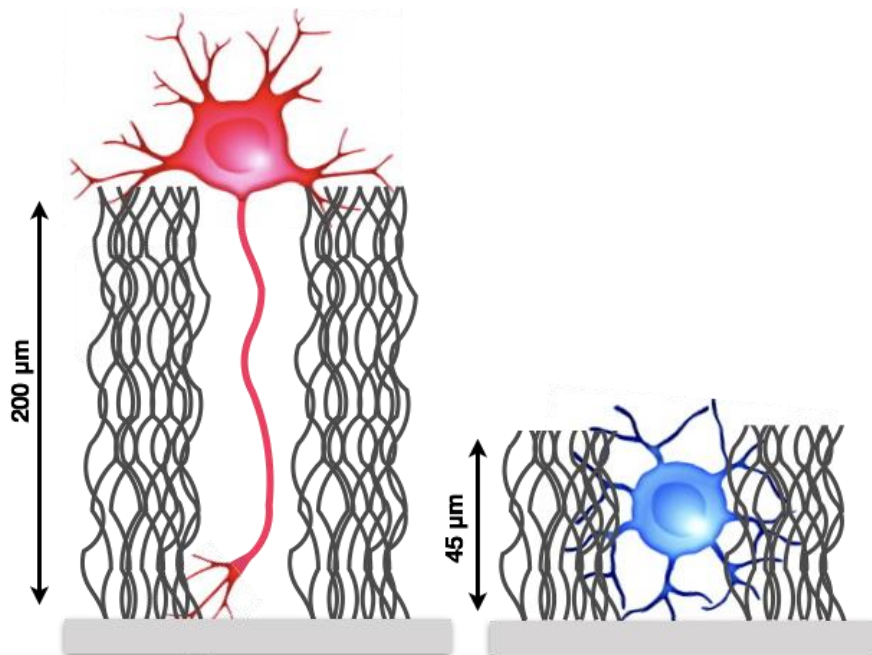
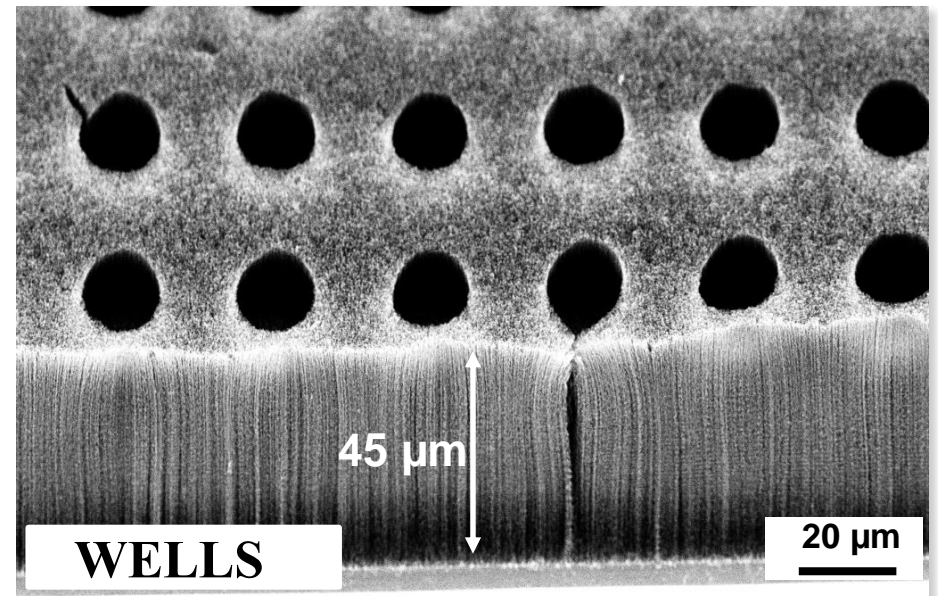
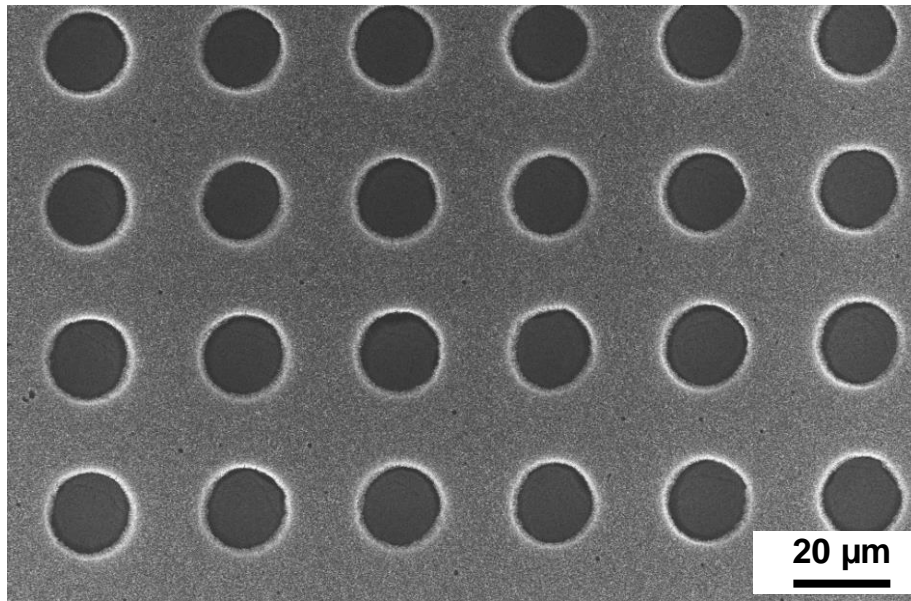
Confocal Raman Microscopy
excitation line at 632.8 nm (He/Ne
laser)



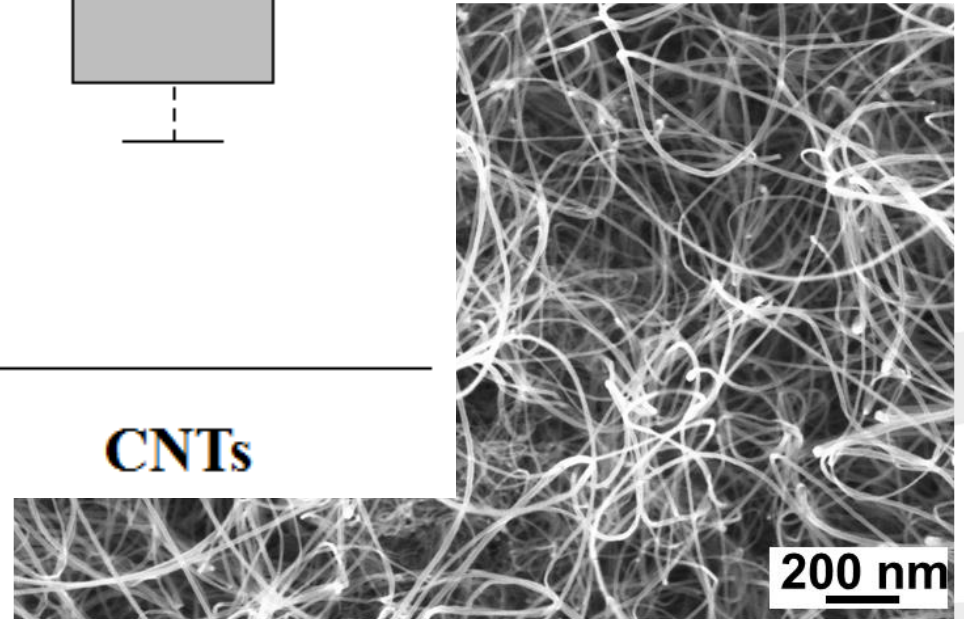
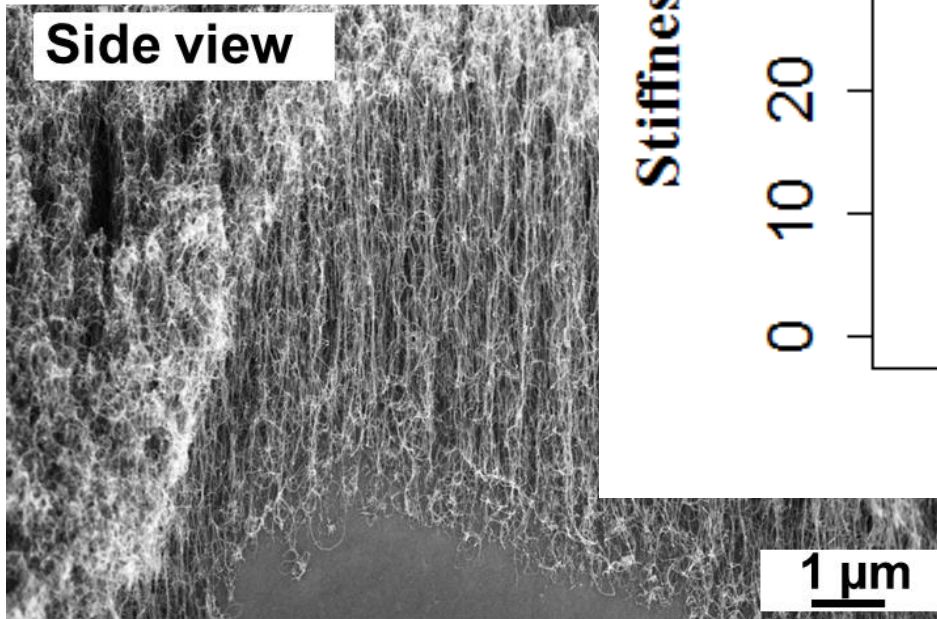
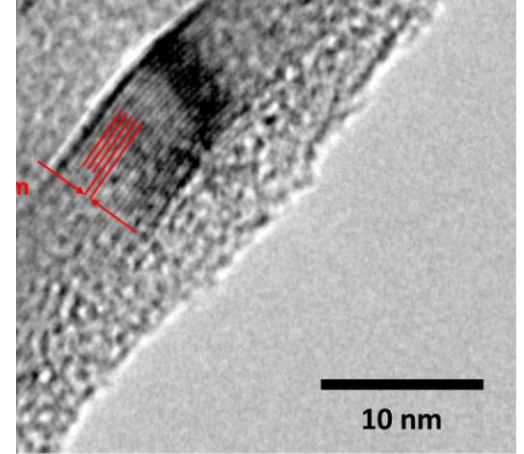
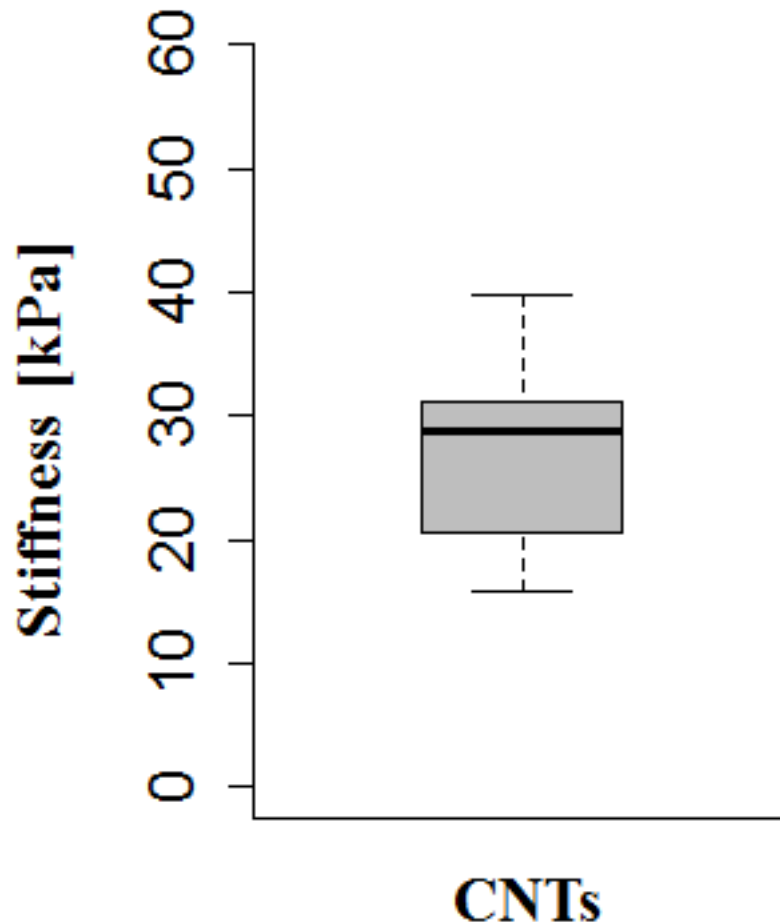
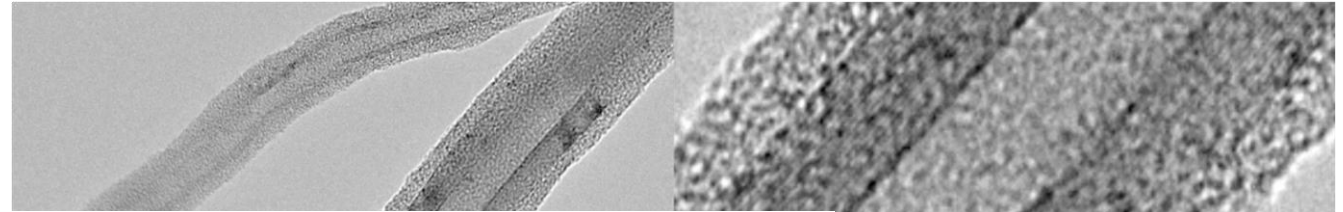
Patterned CVD grown CNTs



Patterned CVD grown CNTs

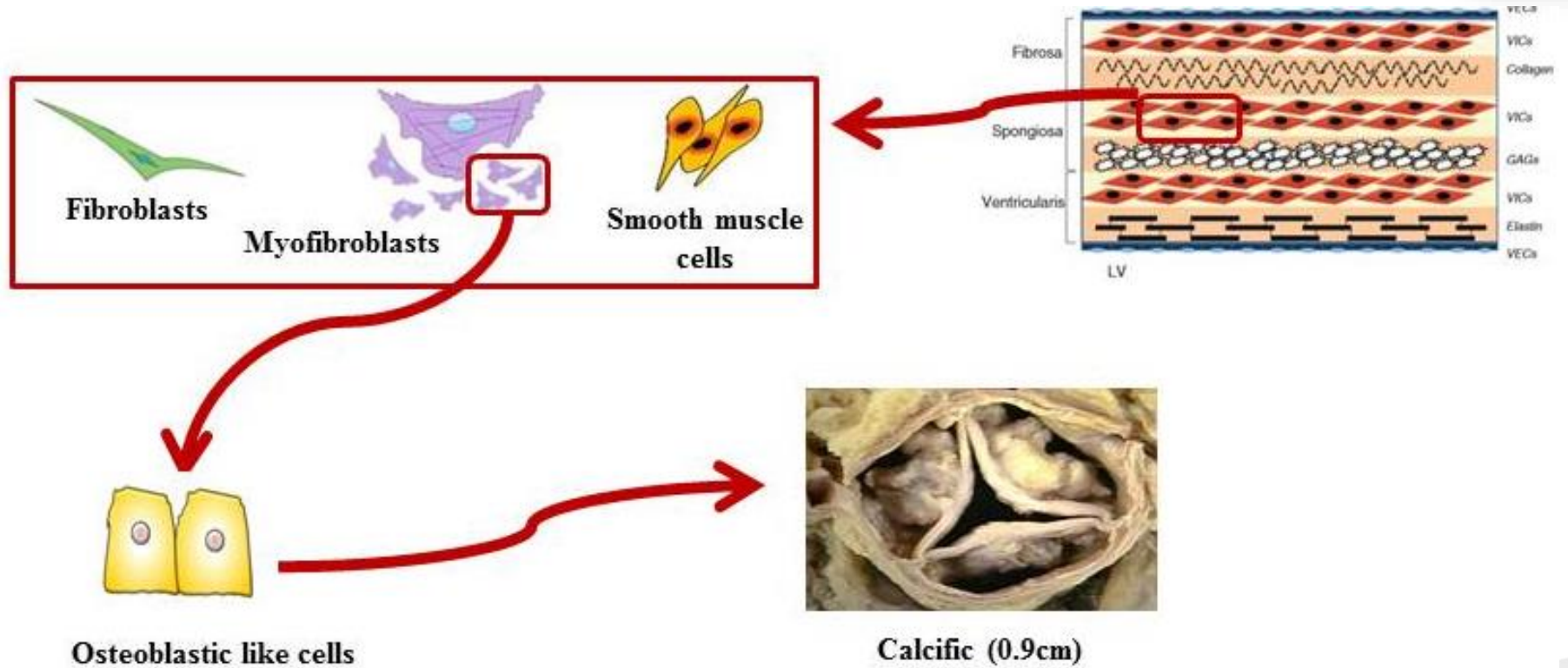


CVD assisted growth of CNTs on transparent substrates



t-CNTs influences cell morphology

aortic valve interstitial cells

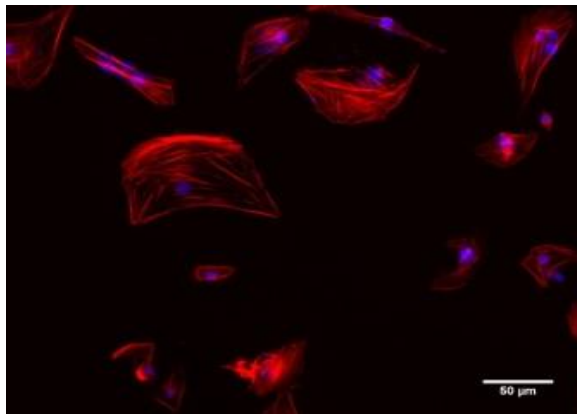


Stiffness of the **healthy valve** leaflet: 20-30 kPa; Myofibroblast about 5-10 %

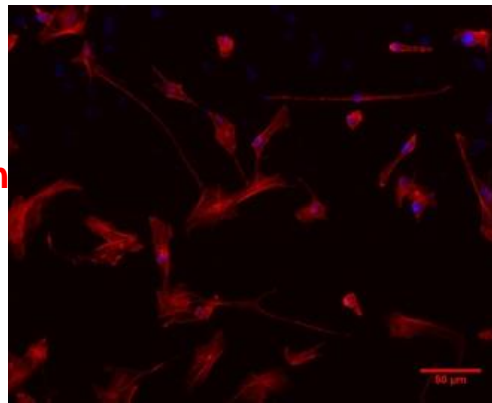
t-CNTs influences cell morphology

aortic valve interstitial cells

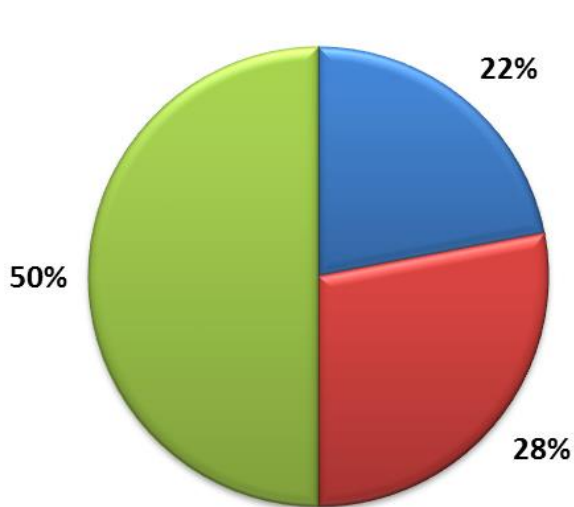
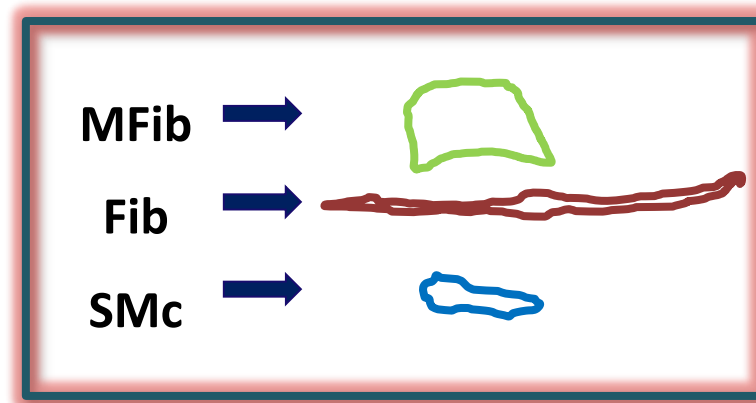
Number of cells per type on glass Number of cells per type on t-CNT



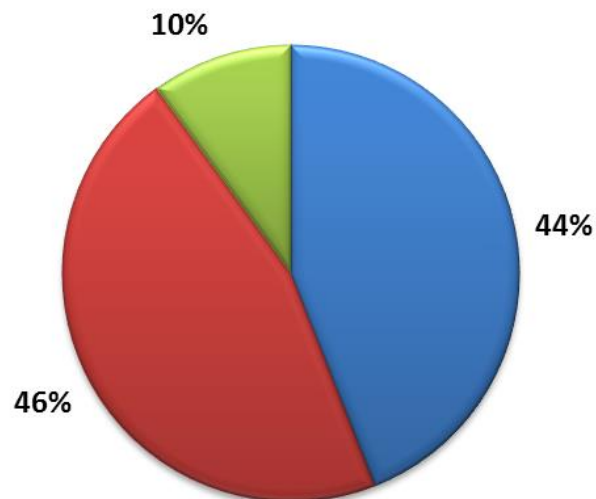
Phalloidin
DAPI



4 % PFA fixation after 12-72 hrs

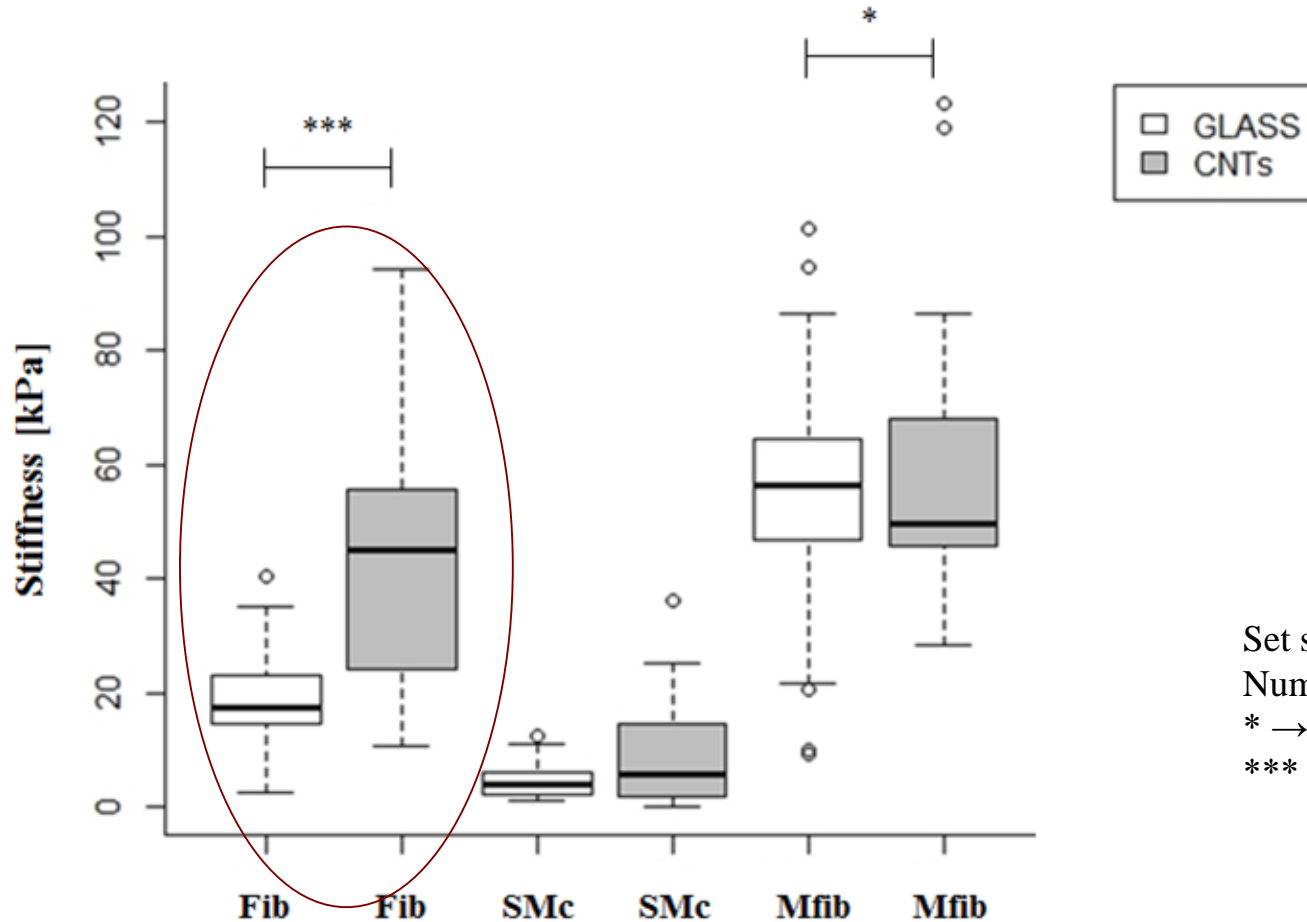


■ SMc
■ Fib
■ MFib



■ SMc
■ Fib
■ MFib

t-CNTs influences VICs stiffness



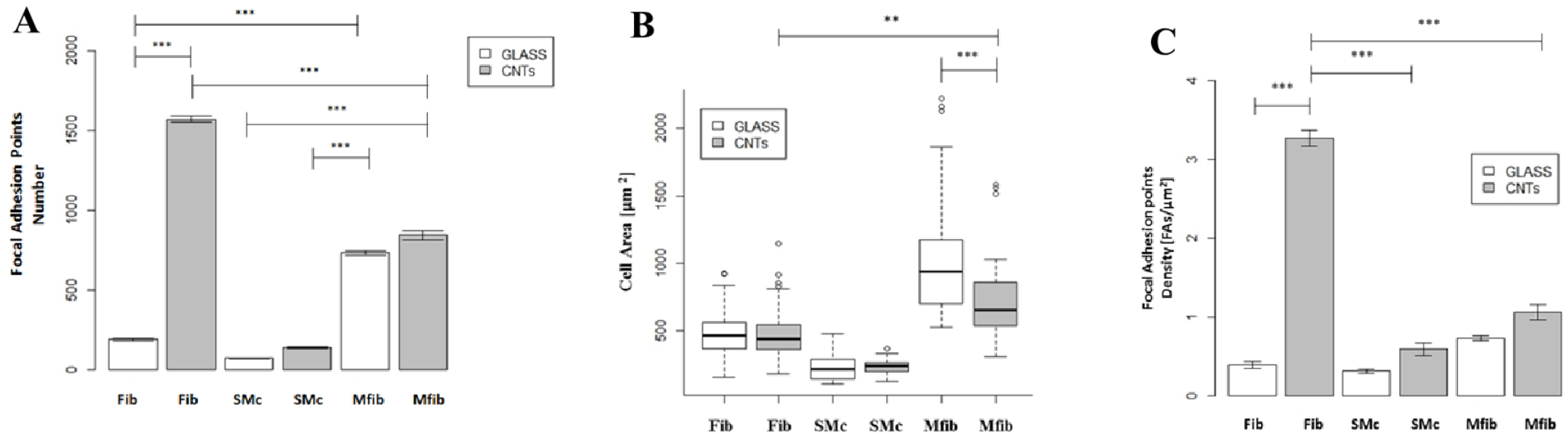
Set samples =5
 Number force curve =60
 * → $p < 0.05$
 *** → $p < 0.001$

Possible explanation : cells characterized by a small body area (i.e. cells of elongated shape) feel the nanometric stiffness of the CNTs (structural contribution) more than cells having a large body surface that, instead, feel the micro- or macroscopic stiffness of the CNTs carpet .

Nanotubes perturb more effectively VIC stiffness when the contact area between cells and the underneath CNT mat is small.

t-CNTs influences Focal Adhesions

Number of FA (vinculin)



Preliminary results: further studies needed

Set samples = 3

Number images = 25

** $\rightarrow p < 0.001$

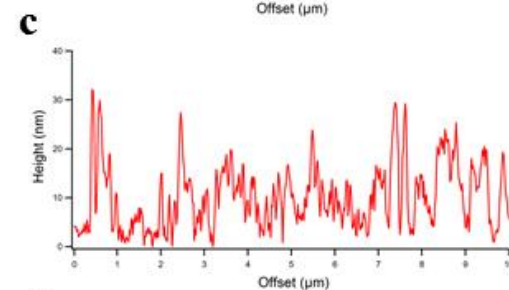
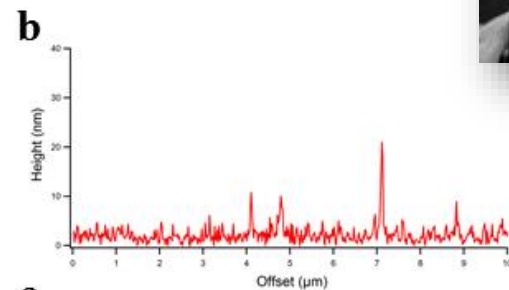
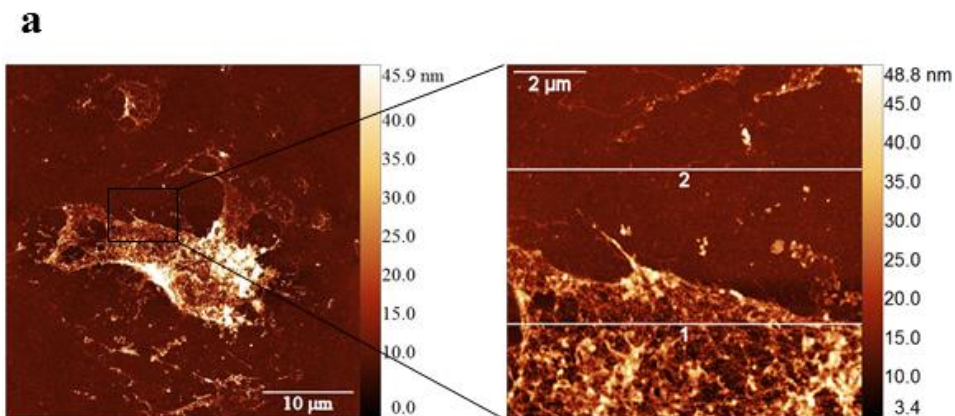
*** $\rightarrow p < 0.001$



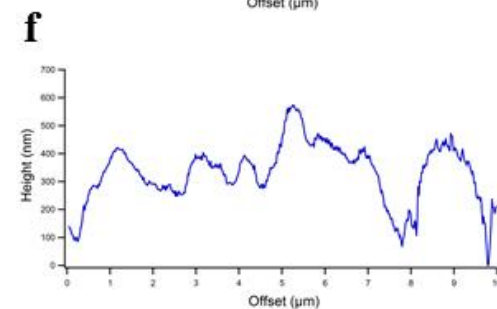
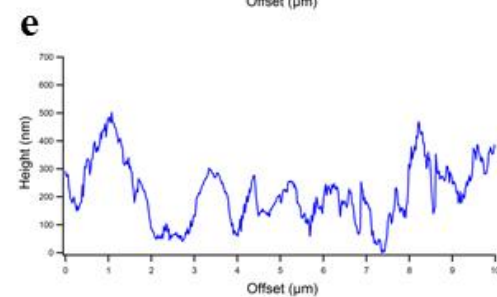
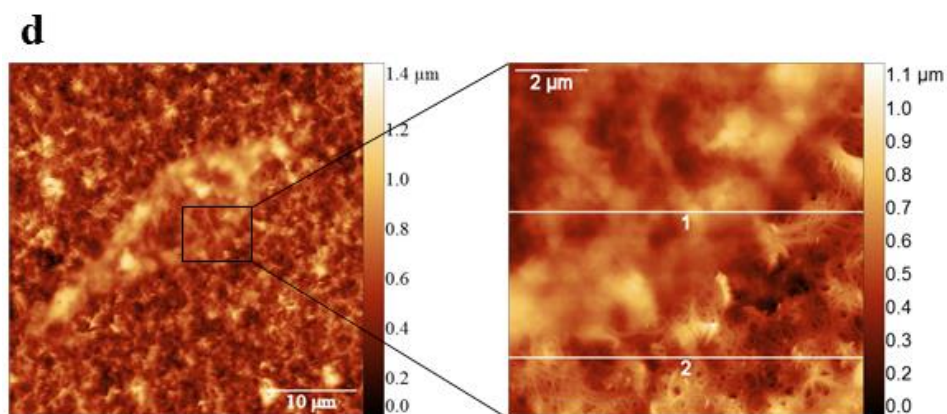
CNTs-membrane interaction



GLASS



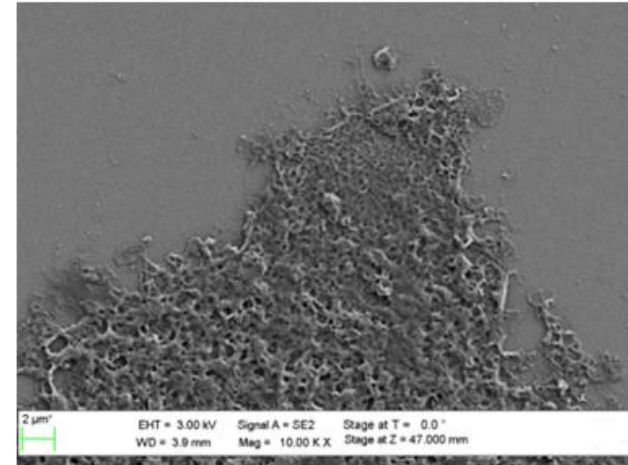
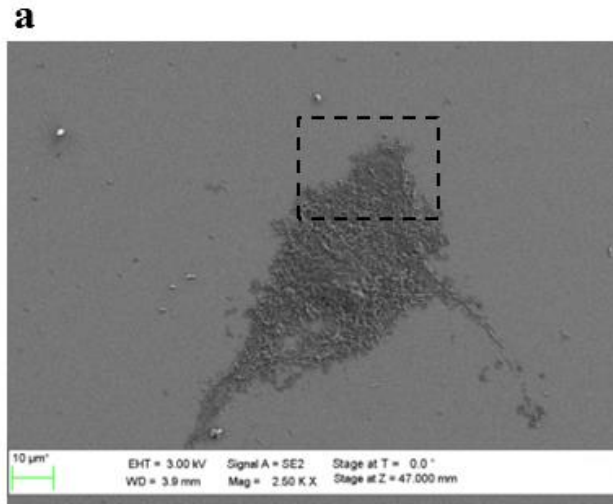
CNT



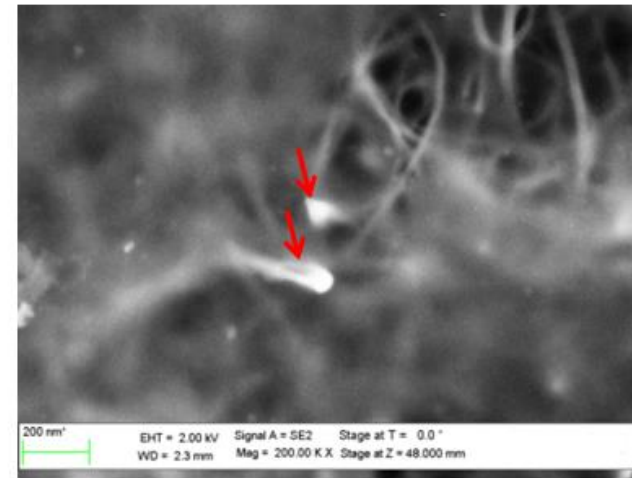
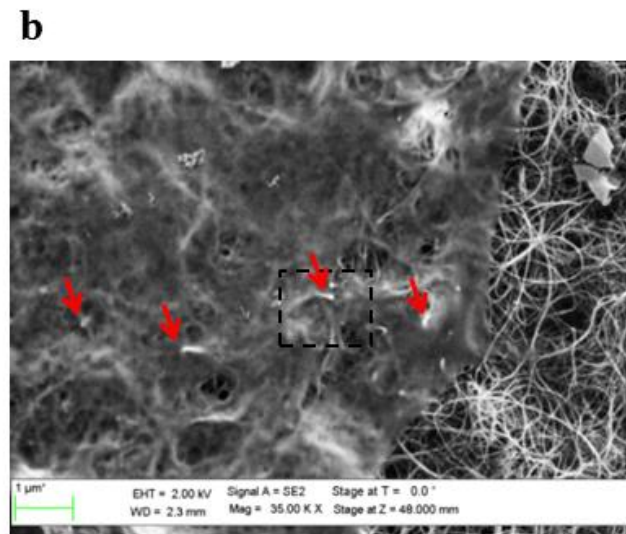


CNTs-membrane interaction

GLASS



CNT



Conclusions

1. VICs' Mechanical properties are dependent on substrates stiffness and induce different cytoskeleton rearrangements.
2. YAP / TAZ activity is involved in the variation of cell mechanical properties
3. CNTs have a positive effect on the VICs differentiation, promoting the formation of a low number of myofibroblast with respect to fibroblast, and therefore conditions for a healthy valves.

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Fundings



**Regione Autonoma
Friuli Venezia Giulia**



Ministero della Salute

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