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

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Projects Overview

MATERIAL SCIENCE, PROSTETIC MATERIALS
Carbon-based prosthetic materials, interaction with cell membranes; AFM cell/tissue mechanobiology.

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

NANOBIOTECHNOLOGY
DNA-Nanoarrays, DNA-based immunoassay

NANOMEDICINE
Electrochemical microfabricated devices for real-time, cheap and fast protein analysis
Exosome sorting and characterization

Model membrane systems
DNA-barcoded Nanoarrays

Detection in cell lysate

M. Ganau, A. Bosco et al., Nanomedicine and Nanotechnology 2015

RBC 2018, Zreče, Slovenia
ECD-Her2 detection in serum

Limit of sensitivity 100 pM

Lower than the cutoff value of 15 ng/ml commonly used in clinics for Her2 positive breast cancer assessment.
Multiplexing Assay

E. Ambrosetti, E. Tagliabue, A. DeMarco, P. Parisse and L. Casalis, ACS Omega, 2017
Strategies for personalized medicine

Cancer patients
Primary tumor cell collection, culture and single cell sorting

Multiparametric single-cell analysis
i) Single cell mechanics/phenotypic analysis

ii) Nanodevices for single-cell molecular profiling

iii) Microfluidic single-cell sorting

Fluorescence/IR/Raman readout

Personalized treatment

Intratumor cell subpopulation and biomarker variation

Phenotype/biomarker expression variation of different tumor clones upon anti-cancer treatment

Prediction of tumor evolution
Cell Biomechanics
How do cells perceive a mechanical stimulus and translate it into a biochemical response

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
*δ₀* ... indentation of the sample
*E* ... elastic modulus
*μ* ... Poisson’s ratio

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RBC 2018, Zreče, Slovenia
Calcific aortic valve disease
Calcific Aortic Valve Disease (CAVD)

- H = High
- MH = Medium High
- ML = Medium Low
- L = Low
Aortic Valve Interstitial Cells (VICs) on Polyacrylamide gels

**Diagram:**
- Surface activation
- Polyacrylamide polymerization
- Polyacrylamide surface activation
- Collagen I coating
- VICs seeding

**Graph:**
- Substrate Stiffness [kPa]
- Percentage of calibrator stiffness
- Categories: H, MH, ML, L
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: low levels of α-SMA and co-localization;
purple arrow: no α-SMA
Rearrangement of the Cytoskeleton and cell stiffness

red: phalloidin; blue: DAPI; green: α-SMA;
High cytoskeleton tensioning determines high levels of YAP nuclear localization

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
CVD growth 2D CNTs substrates

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

- T ≈ 650–750 °C
- Growth Catalyst: Fe nanoparticles from Fe film (2-5 nm) on SiO₂, annealed at 650–670 °C in H₂ (partial pressure 3∙10⁻¹ mbar)
- Precursor: C₂H₂ (up to a partial pressure of 10⁻⁴–20 mbar)

*In collaboration with Andrea Goldoni, Elettra Carbon Lab
CVD growth 2D CNTs substrates*

*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

CNTs

SiO₂

Si
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

4% PFA fixation after 12-72 hrs

Phalloidin
DAPI

MFib
Fib
SMc

50%
28%
22%

10%
46%
44%

SMc
Fib
MFib
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.

Set samples =5
Number force curve =60
* → p < 0.05
*** → p < 0.001
t-CNTs influences Focal Adhesions

Number of FA (vinculin)

Preliminary results: further studies needed

Set samples = 3
Number images = 25

** → p < 0.001
*** → p < 0.001
CNTs-membrane interaction

**GLASS**

![AFM image of CNTs on glass](image1)

**CNT**

![AFM image of CNTs without glass](image2)
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.
NanoInnovation Lab Members

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Fundings

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EXOTHERA

Centro Cardiologico Monzino