Surface treatment of biomaterials for bone tissue engineering

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UNIVERSITÉ LAVAL

LABORATOIRE LIS D'INGÉNIERIE DE SURFACE

Durrieu's research activities

- Surface functionalization of biomaterials to control cell fate
  - Design of innovative active principles
  - Synthesis of dendritic and metallodendritic structures
  - Nano/micropatterning of surfaces
- Synergistic effect between biochemical cues and biomaterials mechanical properties to control cell fate
- Cell culture
  - Mesenchymal stem cells, osteoblasts, endothelial cells,...
  - Production and purification of human cell-derived microvesicles
  - Control of stem cell adhesion, proliferation & differentiation (mineralization and tube-like formation)
  - Characterization of cell culture (immunofluorescence, chromogenic assay, western-blot, RT-qPCR, q-PCR)

Laroche's research activities

- Plasma surface modification/functionalization/coatings
  - Improving the biocompatibility of biomaterials
  - Drug delivery systems
  - Antibacterial coatings
  - Nano/micro patterning of surfaces to control cell fate
  - Anti fogging materials

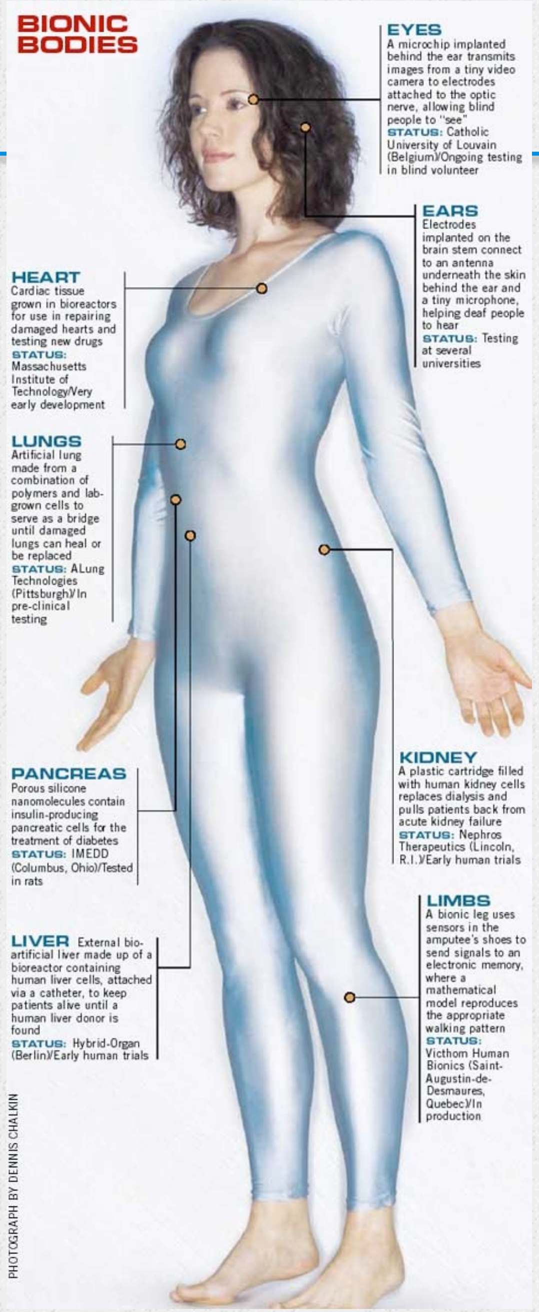
- Synergistic effect between biochemical cues and biomaterials mechanical properties to control cell fate

What is tissue engineering?

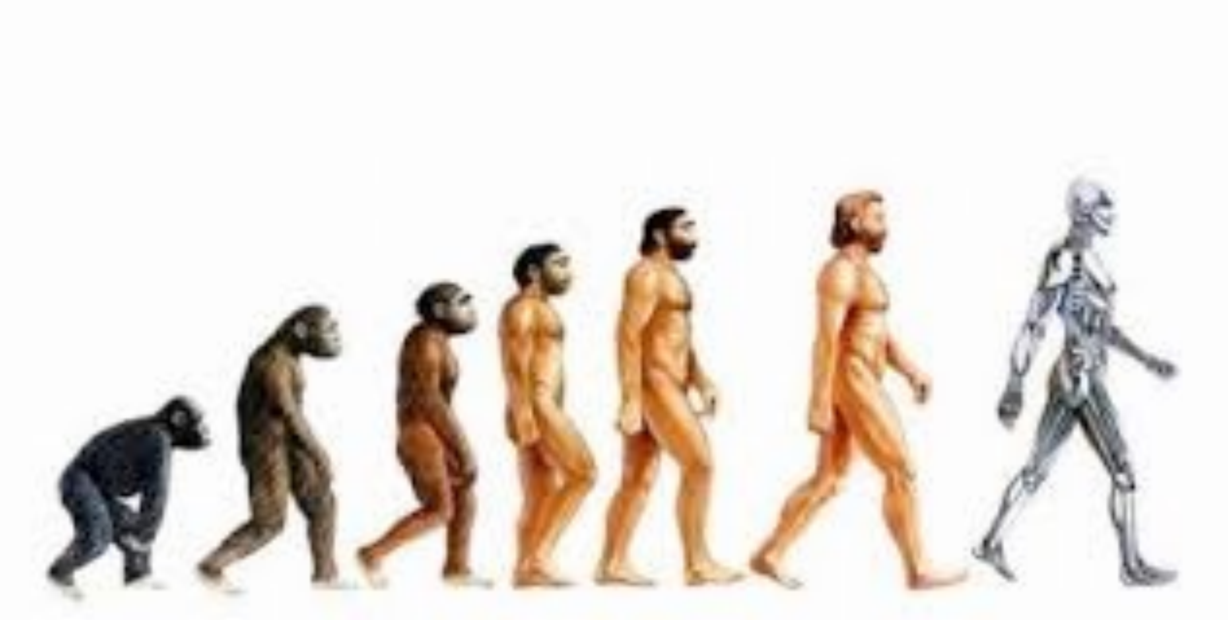
What is tissue engineering?

Tissue engineering is an extraordinarily simple concept that everybody can understand. It's simply accelerating the pace at which the body heals itself to a clinically relevant timescale.

Business Week magazine

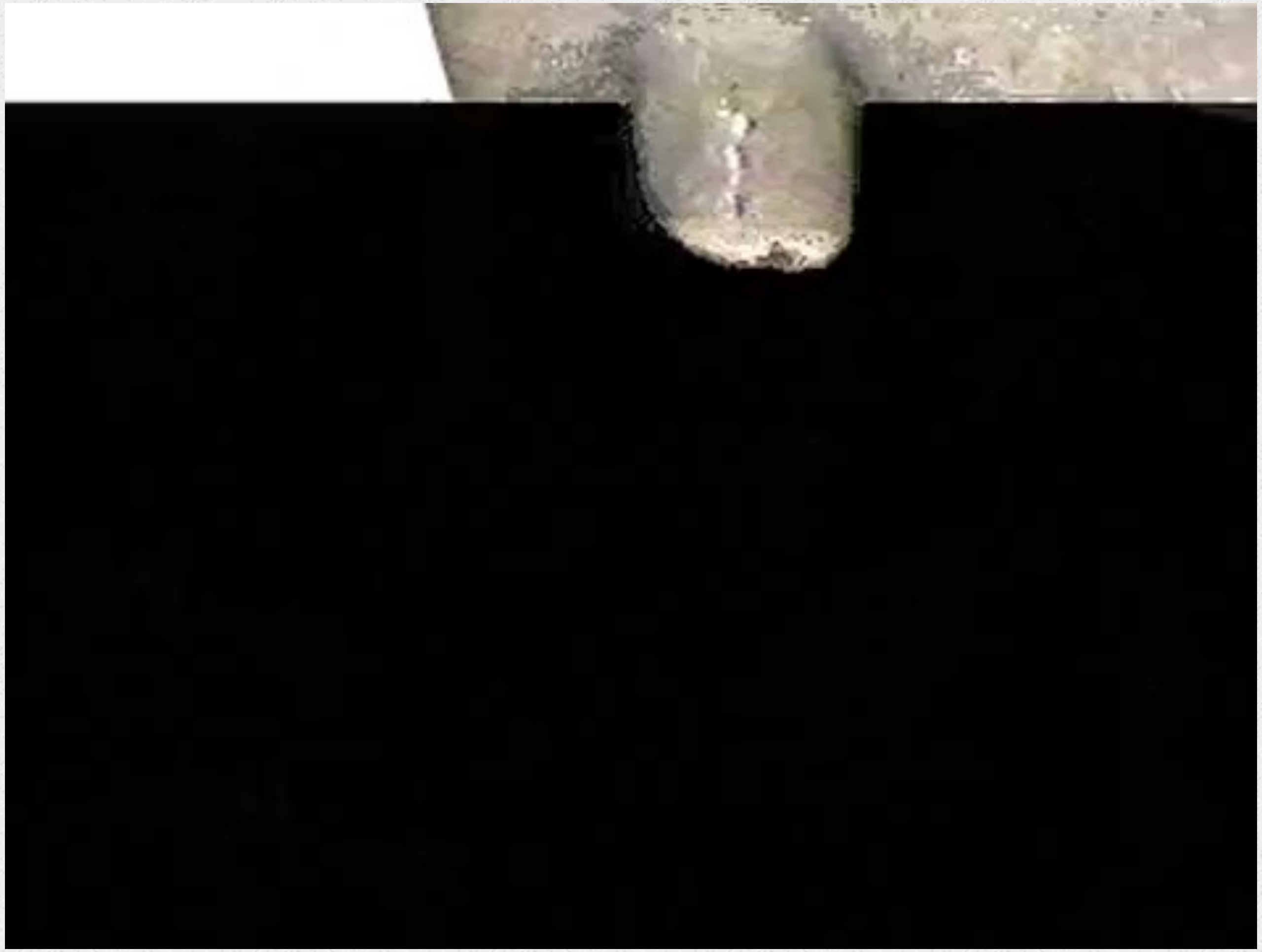


The limits of tissue engineering



So that at the end of the treatment, you are the same (or perhaps better) as you were!

DELIVER CURES INSTEAD OF TREATING SYMPTOMS: IF A NEWT CAN DO IT WHY CAN'T WE?

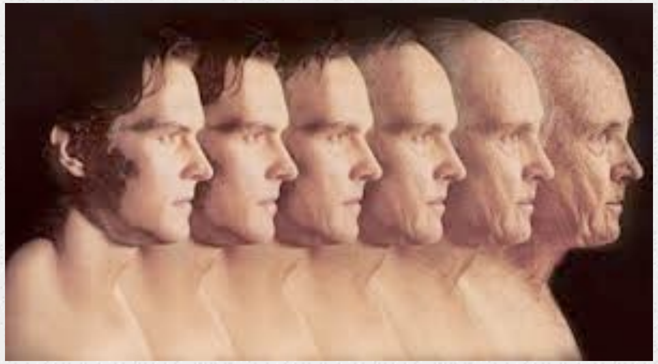


Why can't humans regenerate?

Actually, we can regenerate !!

A mammalian fetus, if it loses a limb during the first trimester of pregnancy, will re-grow that limb

Before the age of about six months, if children lose their fingertip in an accident, they'll re-grow their fingertip

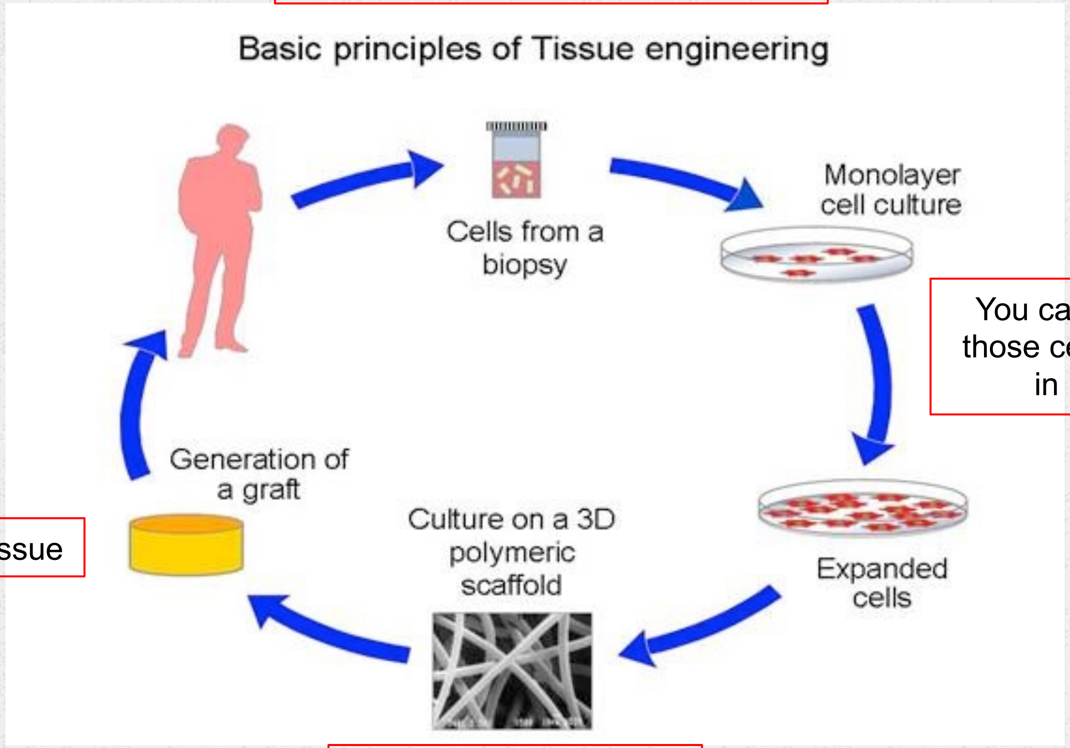


Your bone regenerates every 10 years. Your skin regenerates every 2 weeks. **So your body is constantly regenerating!!**

How can we do that?
We need to learn to speak the body's language

WHAT CAN WE DO TODAY BY USING SMART BIOMATERIALS?

you can take a very small piece of tissue from that organ or from biologic fluids

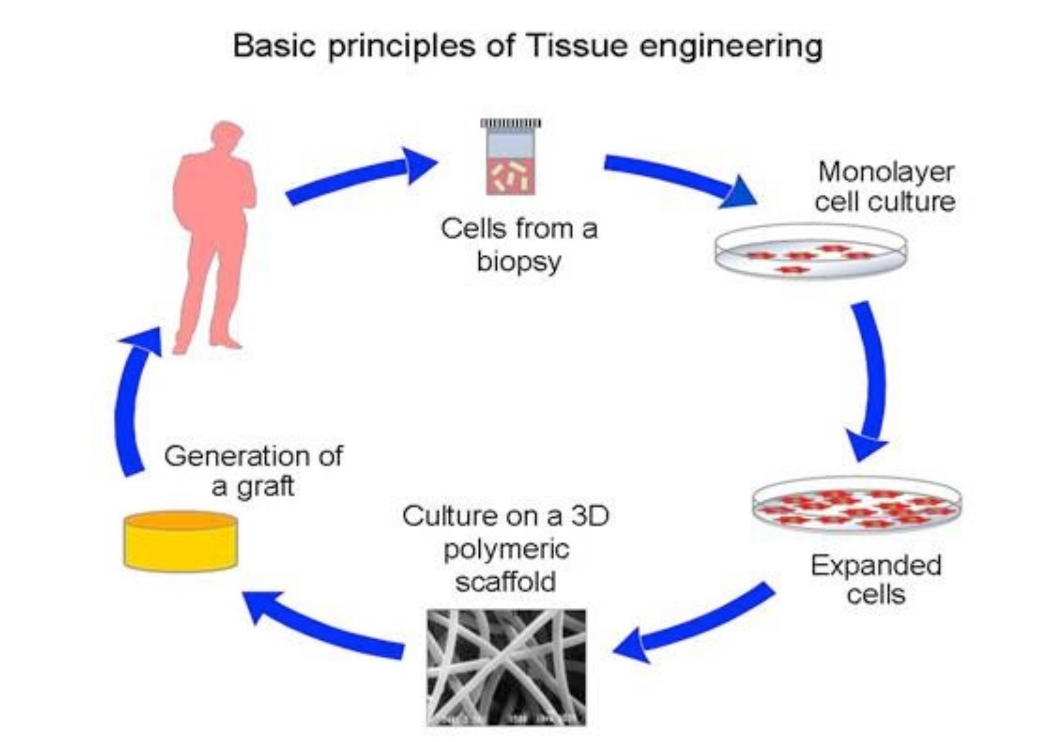


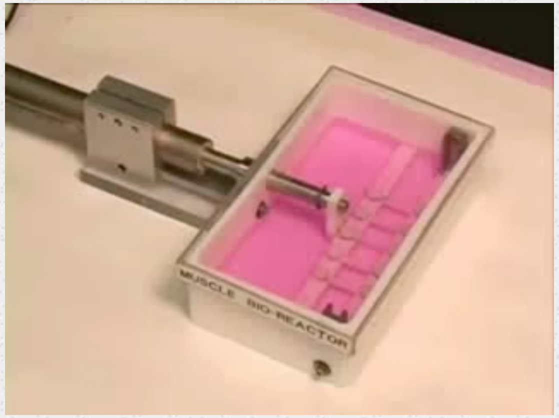
You can grow and expand those cells outside the body in large quantities

Engineered tissue

We can seed these cells on a smart scaffold material to regenerate tissues

WHAT CAN WE DO TODAY BY USING SMART BIOMATERIALS?





Researchers exercice this muscle

TISSUE ENGINEERING: STATE OF THE ART ?

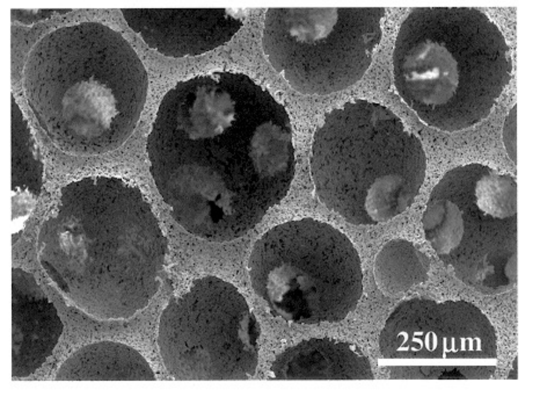
For most types of tissues, research in this field is almost still experimental on animals.

BUT inability of cells to become self-organized into tissues or organs

⬇

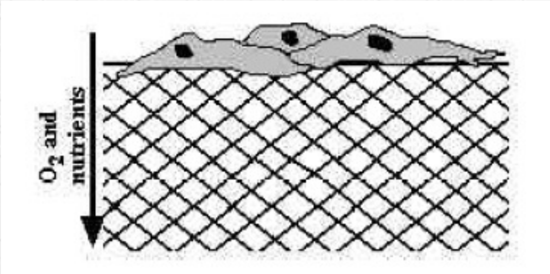
Cells need signals and external guides ("scaffolds") to form 3D functional tissues or organs.

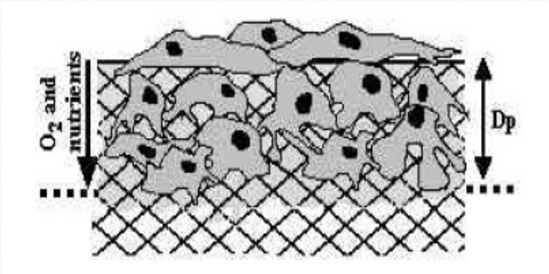
The currently used method is the
***in vitro* growth of cells**
onto a bioactive scaffold structure
that has a specific structure and geometry.



TISSUE ENGINEERING: TODAY'S LIMITS

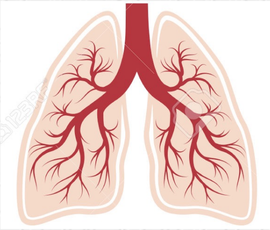
Today, the biggest obstacle to the growth of complex tissues is the difficulty **to vascularize them**. As long as this aim is not reached, the dimension of cultivated tissues will be limited by the maximum distance **of nutrients, gases and waste diffusion.**

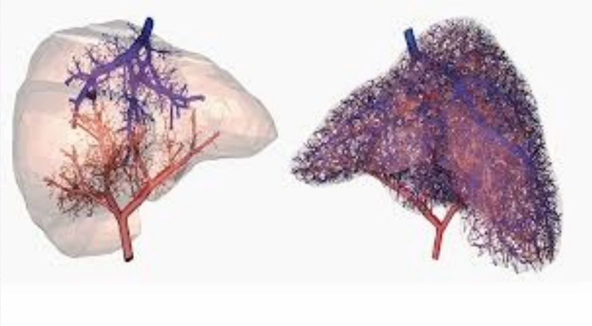




<u>Today, the maximum size of regenerated tissues is around 3 mm^3.</u>

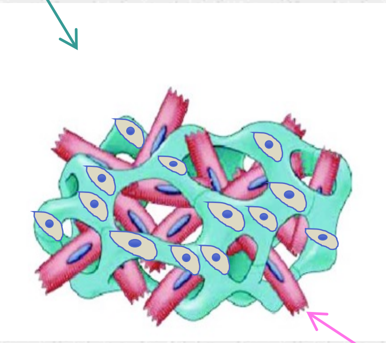
Therefore, it is of the utmost importance to promote **tissue vascularization**





Our objective : Smart materials synthesis for bone tissue engineering

Favour bone formation (mesenchymal stem cell differentiation)



Engineer microchannels for vascularization

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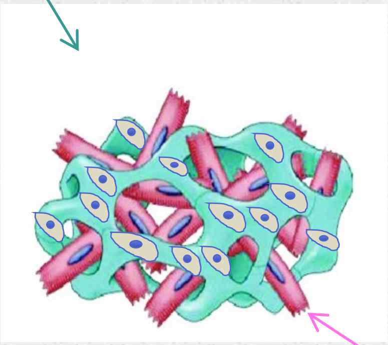
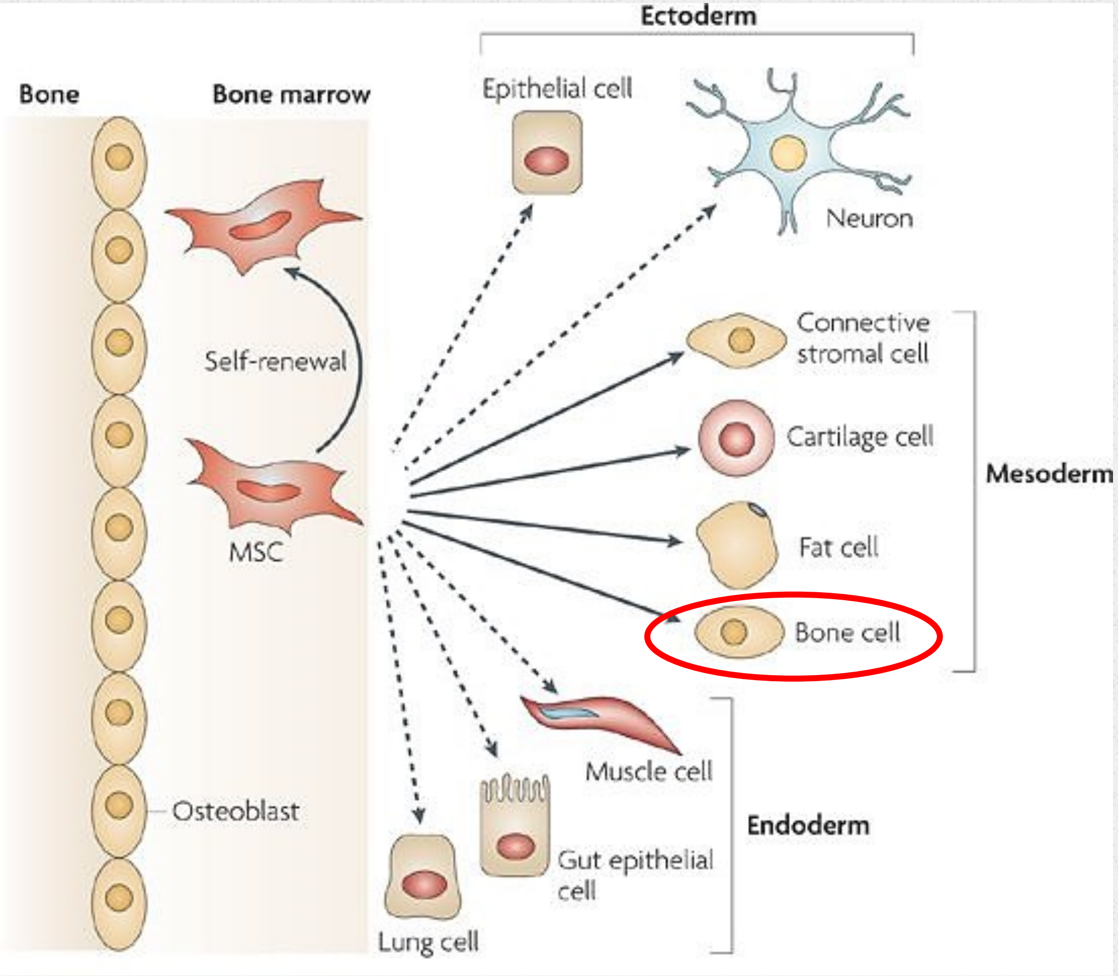


Engineer microchannels for vascularization

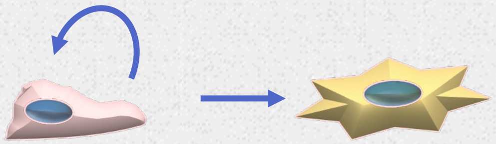
FAVOUR BONE FORMATION (MESENCHYMAL STEM CELL DIFFERENTIATION)



Uccelli et al. ***Nature Reviews Immunology***, 2008

Mesenchymal Stem Cells (MSC) & Bone tissue engineering

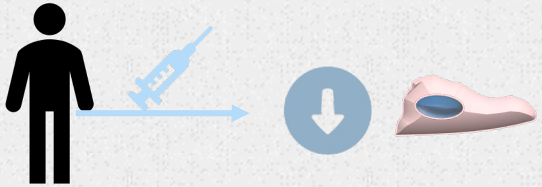
☺Self-renewal



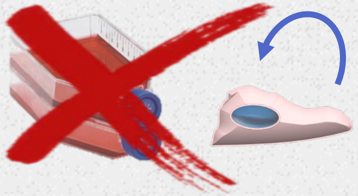
☺Differentiation into osteoblasts

☺High proliferation rate

☹Low availability



☹challenging to exclusively differentiate MSC into a single desired cell-type

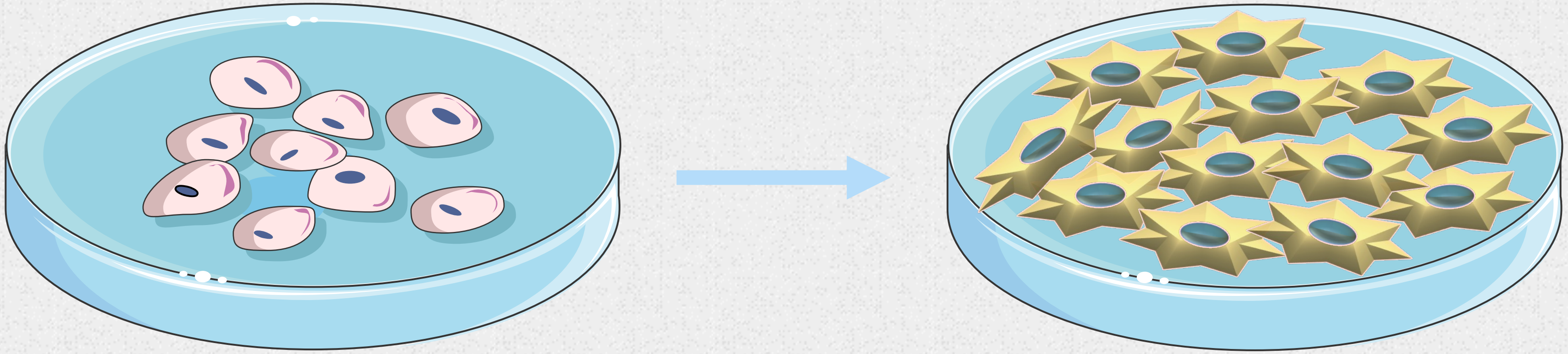


Ullah et al. ***Biosci Rep*** *2015*

SMART *IN VITRO* SYSTEMS

Seed MSC on *smart* material

Large number of osteoblasts



For applications in:

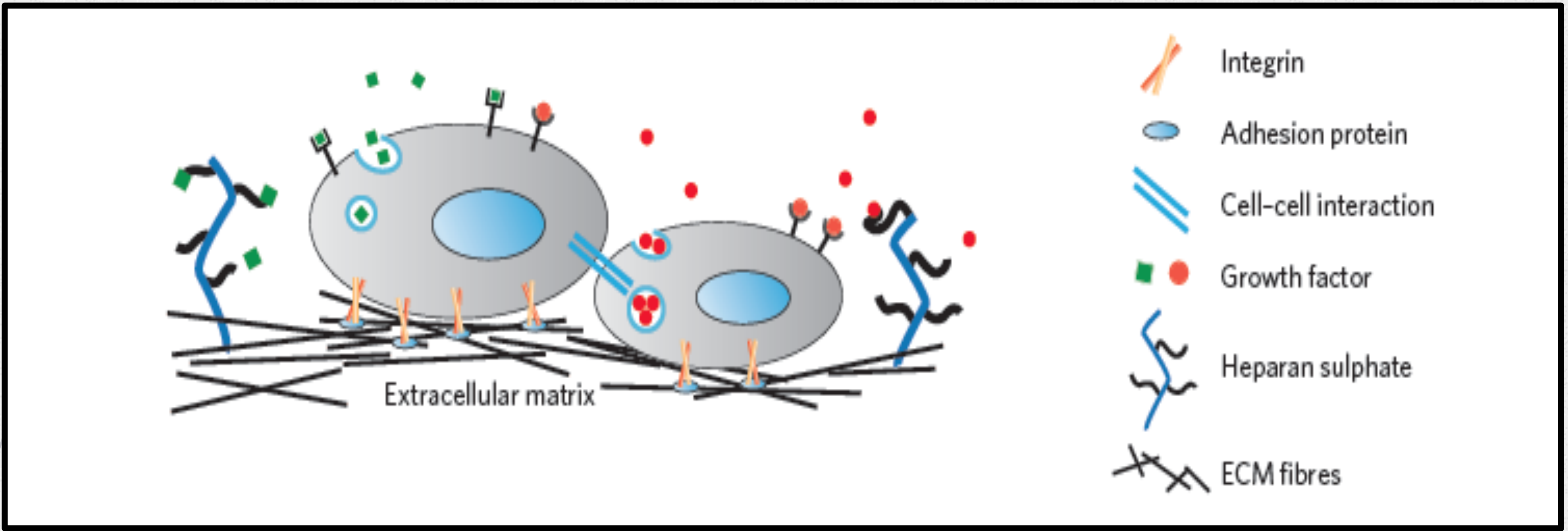
- Drug testing
- Bone disease models
- Bone tissue engineering

Longer future…

Treatment of bone defects

Cell therapy applications

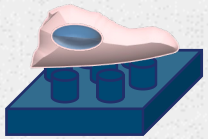
HOW TO MIMIC THE EXTRACELLULAR MATRIX (ECM)?

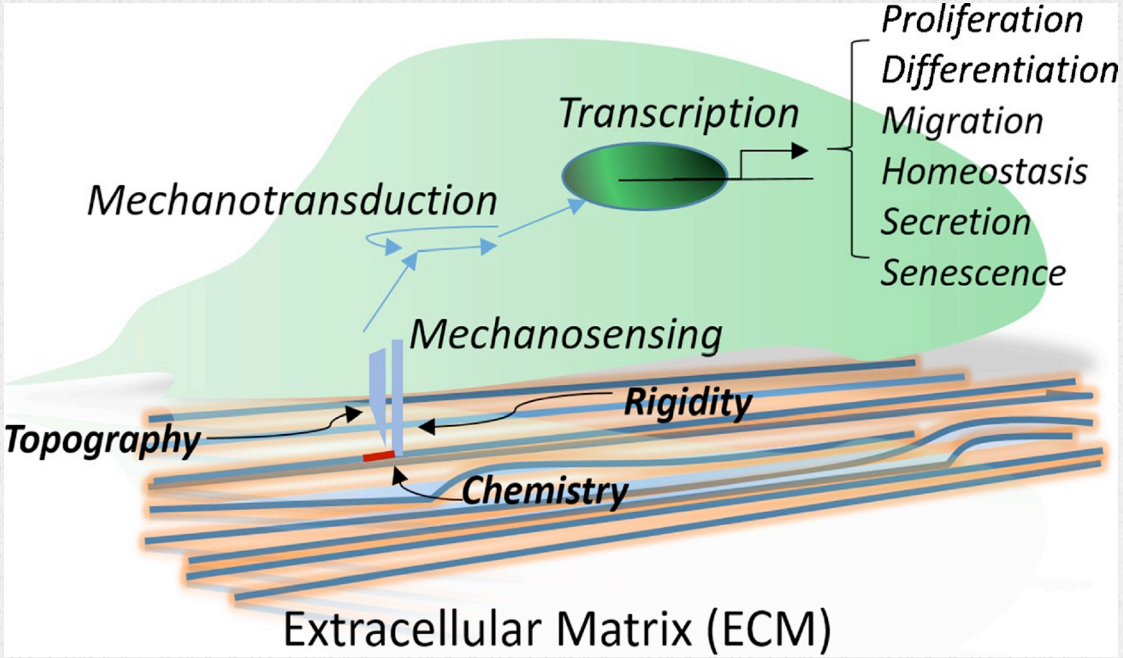


<u>ECM is characterized by biophysical, mechanical and biochemical properties</u>

State of Art

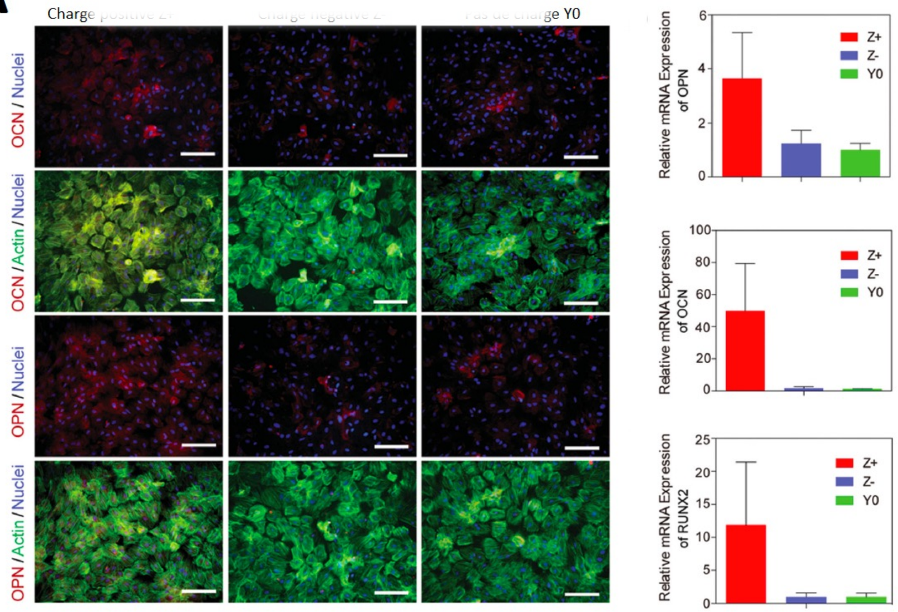
Stem cell micro, nanoenvironments (**biochemical, topographical & mechanical features**) impact on cell fate.





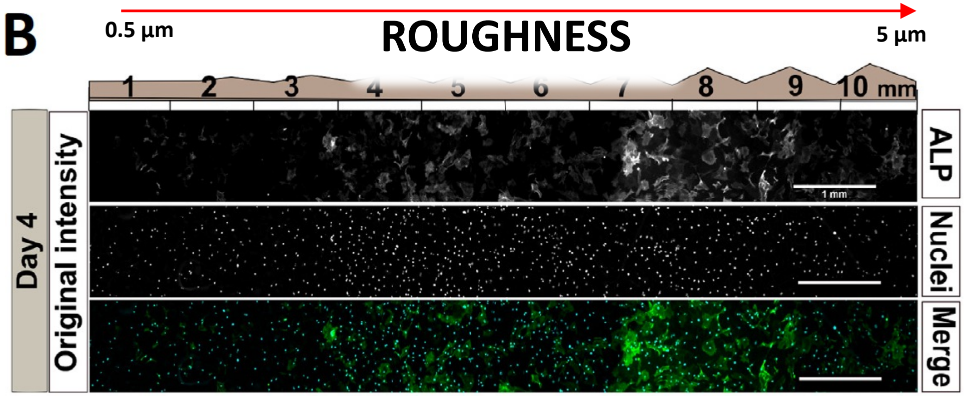
Kshitiz et al. ***Curr Stem Cell Rep***, 2016

A **SURFACE CHARGES**



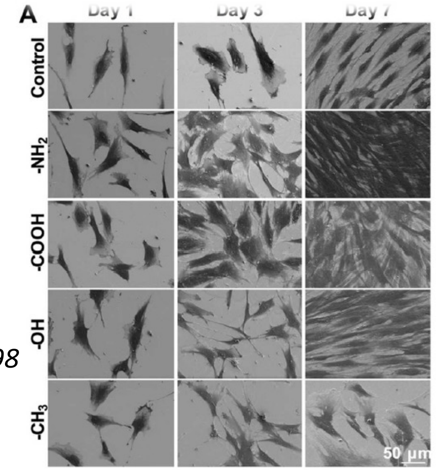
Li et al, Adv. Healthcare Materials, 2015, 4, 998

B



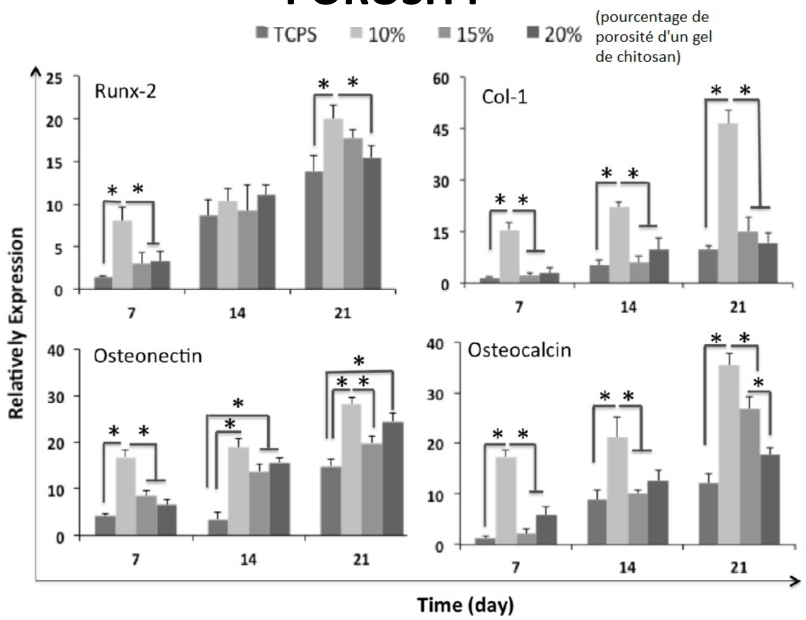
Faia-Torres et al., Biomaterials, 2014, 35, 9023, Adv. Healthcare Materials, 2015, 4, 998

C **SURFACE CHEMISTRY**



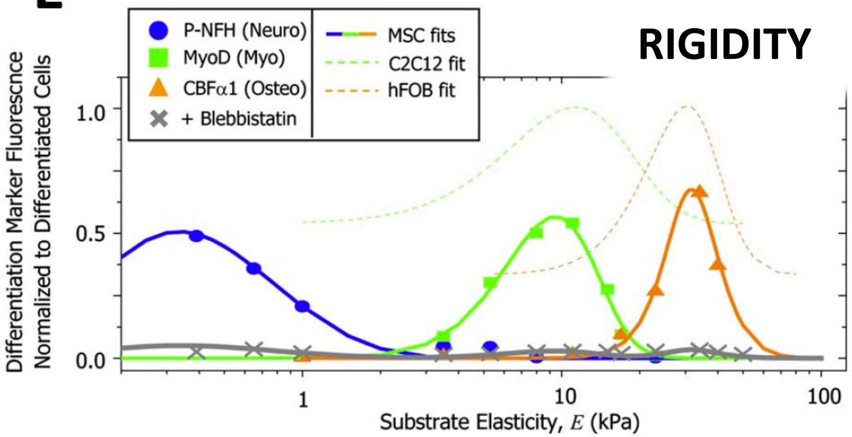
Yu et al., ACS Biomater Sci Eng, 2017, 3, 1119

D **POROSITY**



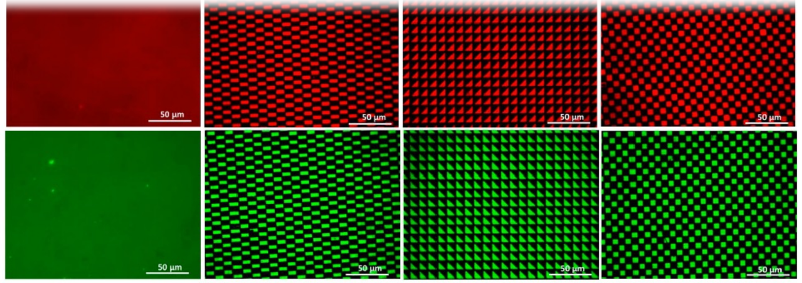
Ardeshirylajimiet al., J Cell Biochem, 2018, 119, 625

E **RIGIDITY**



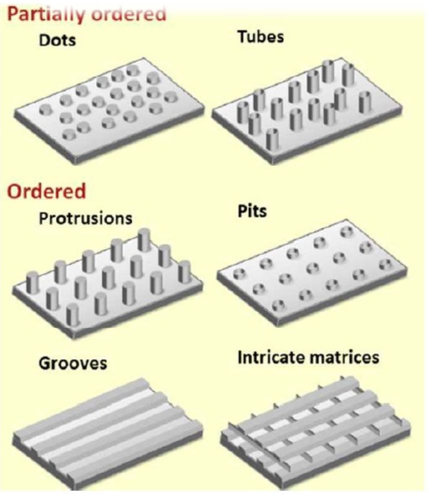
Engler et al., Cell, 2006, 126, 677

F **MICRO, NANOPATTERNING OF BIOMOLECULES**



Bilem et al, ACS Biomater Sci Eng, 2017, 3, 2514 ; J Biomed Mater Res, 2018, 106, 959

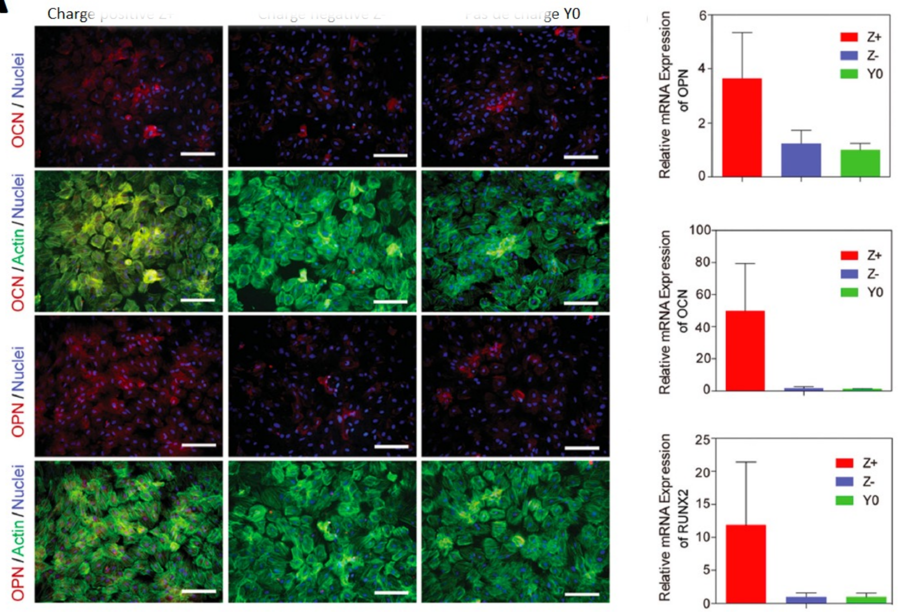
G **MICRO & NANO TOPOGRAPHIES**



Gui et al, , Biomater Sci, 2018, 6, 250

A

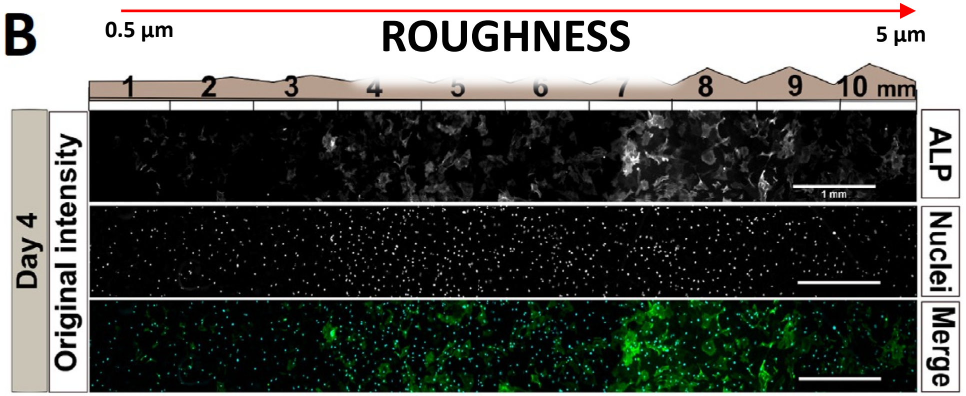
SURFACE CHARGES



Li et al, Adv. Healthcare Materials, 2015, 4, 998

B

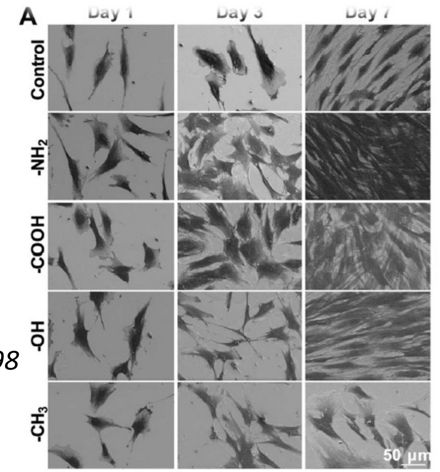
ROUGHNESS



Faia-Torres et al., Biomaterials, 2014, 35, 9023, Adv. Healthcare Materials, 2015, 4, 998

C

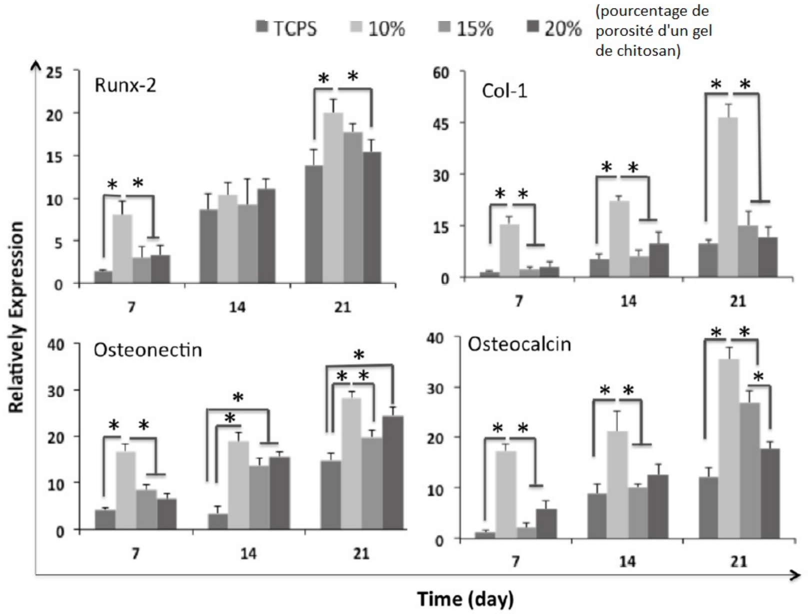
SURFACE CHEMISTRY



Yu et al., ACS Biomater Sci Eng, 2017, 3, 1119

D

POROSITY



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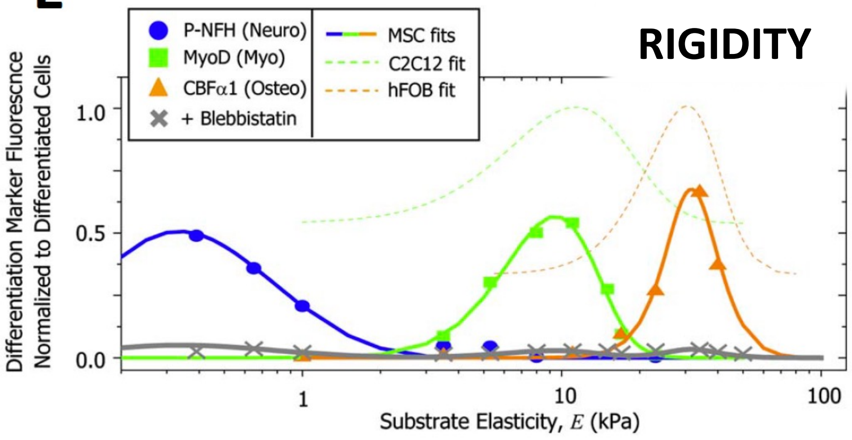
E

RIGIDITY



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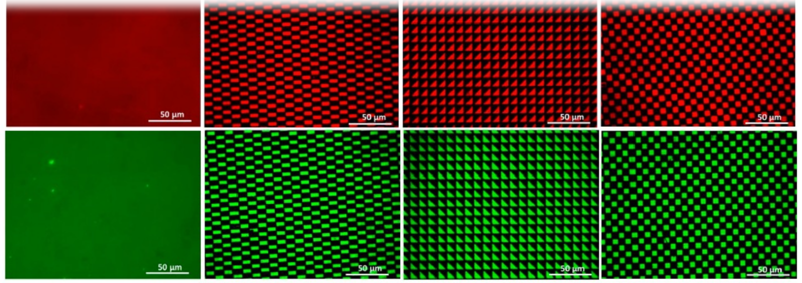

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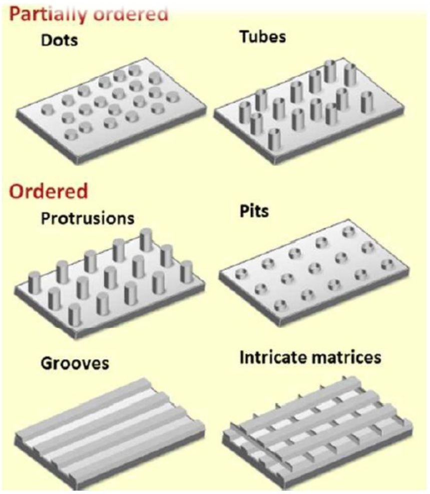


Gui et al, , Biomater Sci, 2018, 6, 250

C - BIOFUNCTIONALIZATION

Adsorption

Covalent binding

Bioactive molecules able to elicit a cell response when immobilized on a surface

Proteins

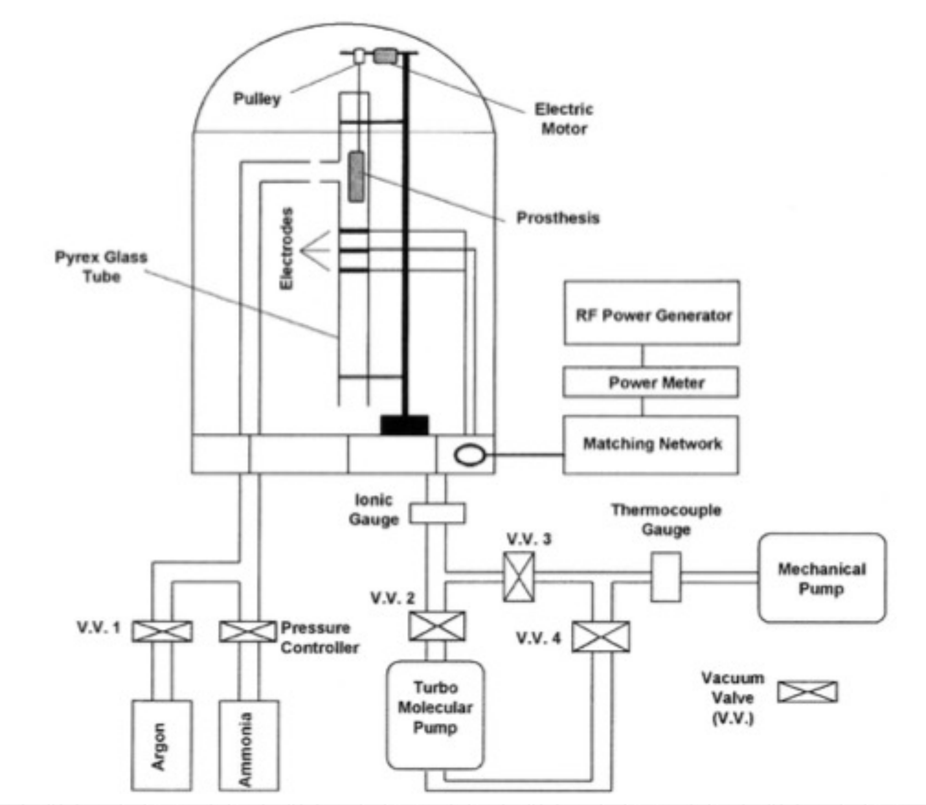
- ☺ Better affinity to receptors
- ☺ Multiple binding sites
- ☹ Poor stability
- ☹ Difficult handling
- ☹ Immunogenicity

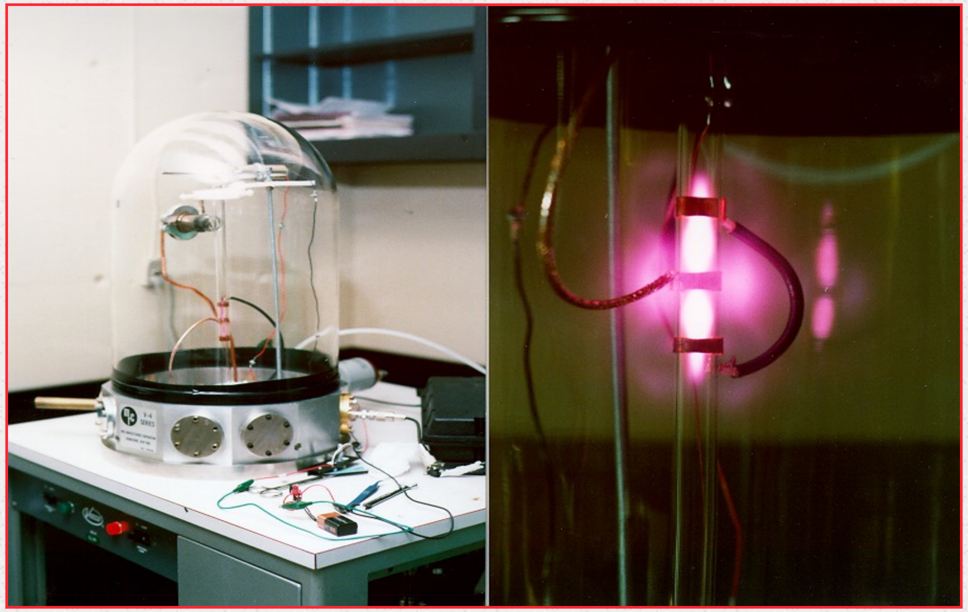
Peptides

- ☺ Less expensive
- ☺ High purity
- ☺ Improved stability
- ☺ Controlled density
- ☹ Lower specificity

Dalby et al. ***Nat Rev Mater*** 2018

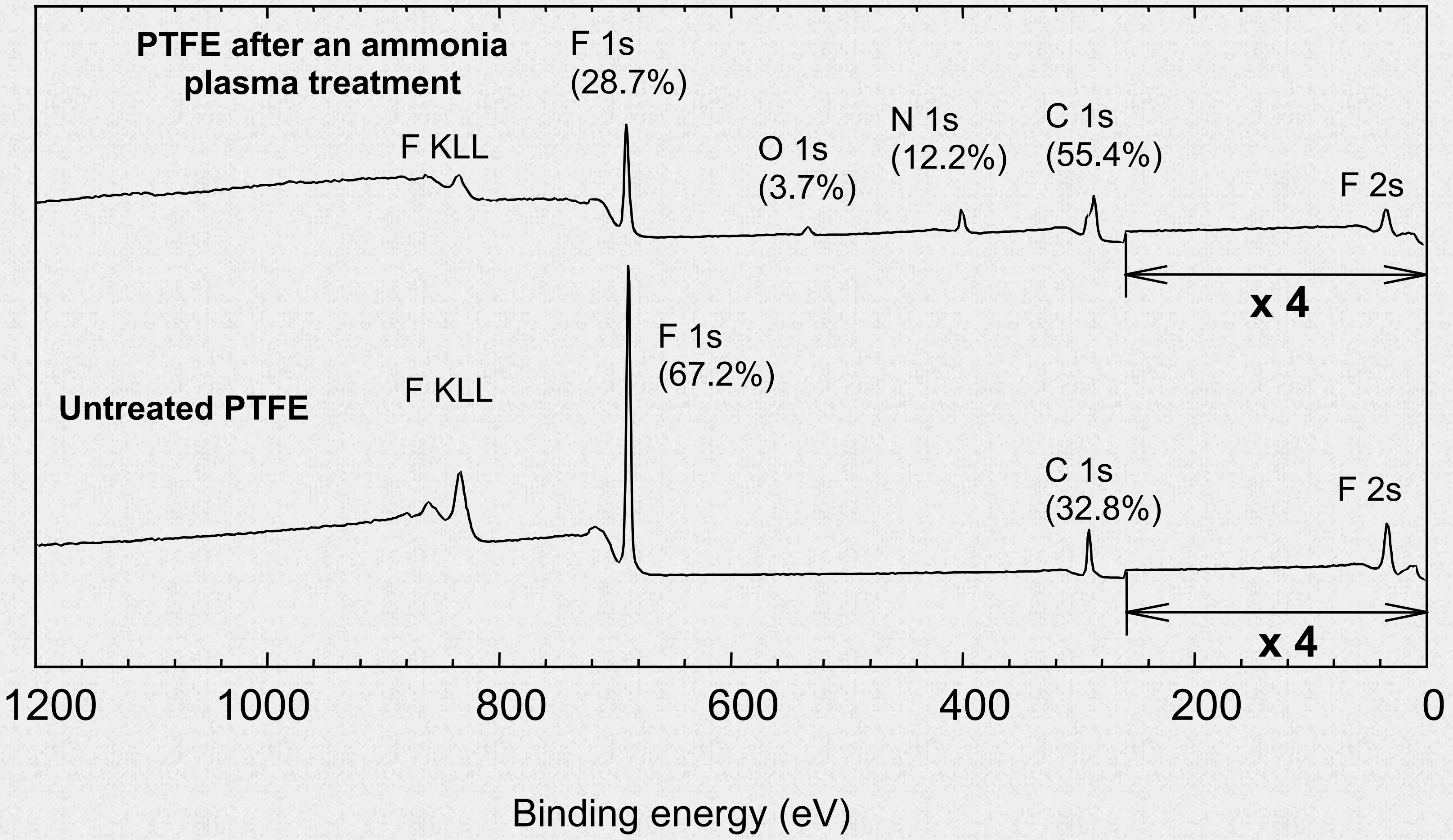
C-Biomaterials surface functionalization





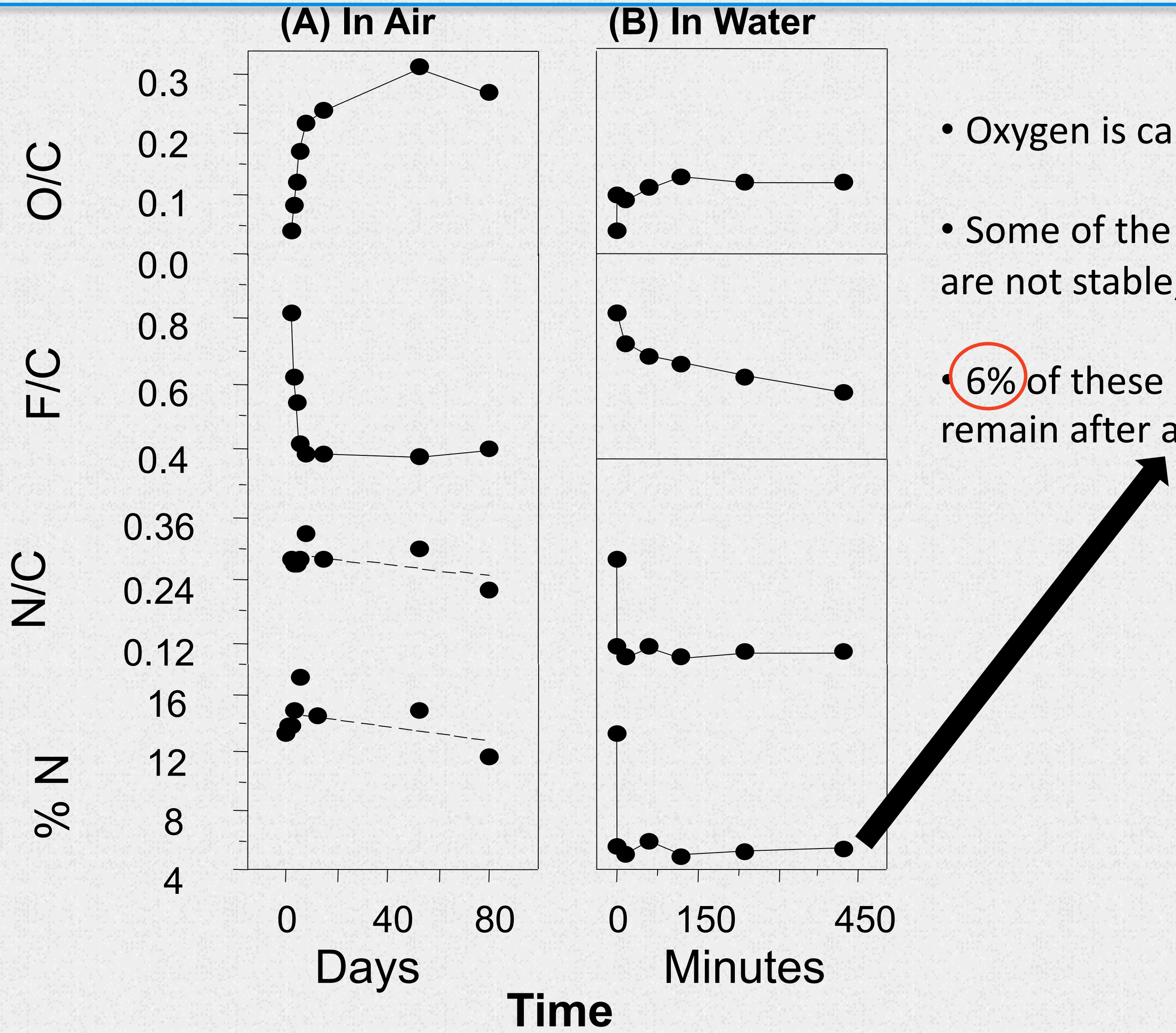
Chevallier et al., J. Phys Chem, 2001, 105, 12490

Biomaterials surface functionalization



BIOMATERIALS SURFACE FUNCTIONALIZATION

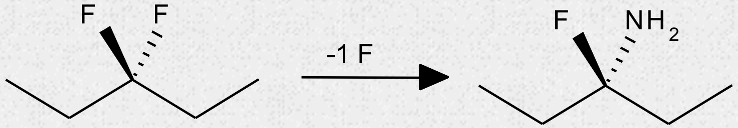
- Aging experiments

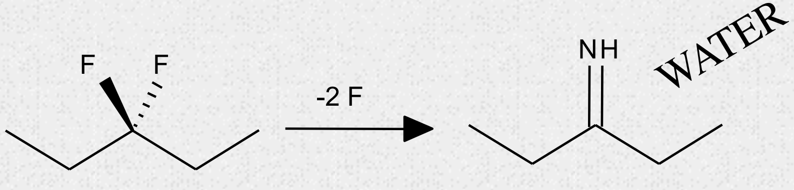


- Oxygen is captured by the surface
- Some of the nitrogen-containing species are not stable, especially in water
- 6% of these nitrogen-containing species remain after aging in water.

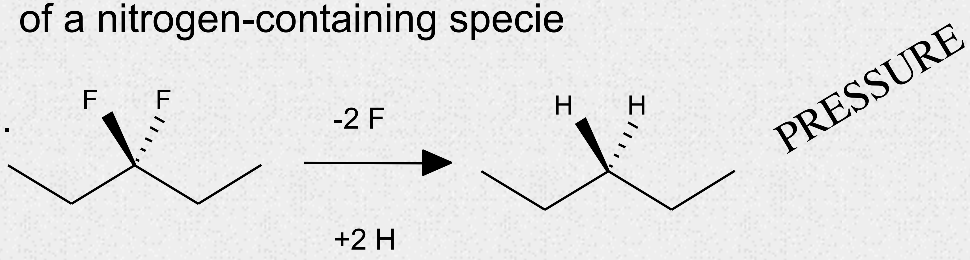
Biomaterials surface functionalization

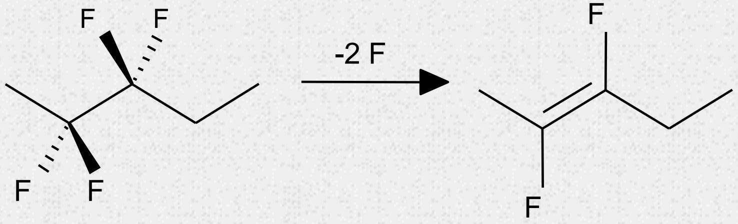
Removal of fluorine, replacement by a nitrogen-containing specie

1. 

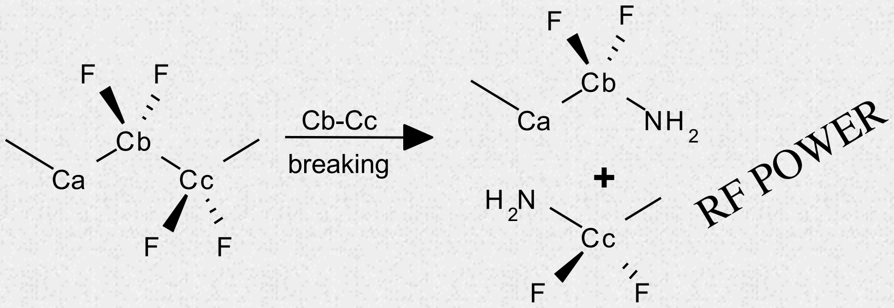
2. 

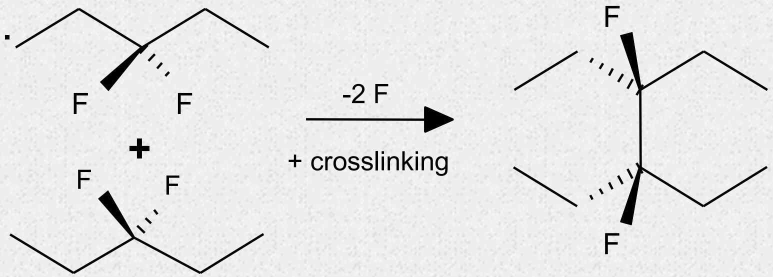
Removal of fluorine without addition of a nitrogen-containing specie

4. 

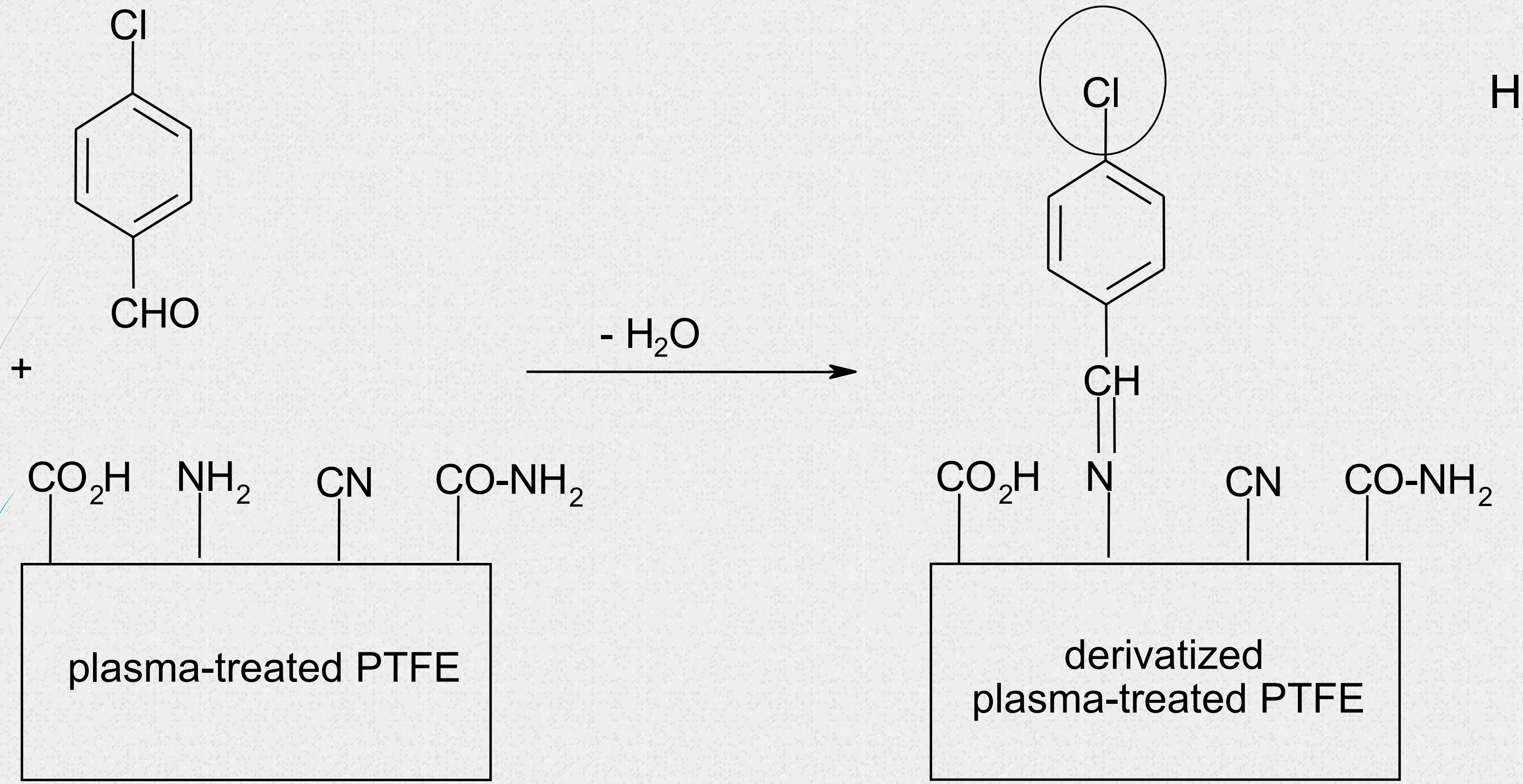
5. 

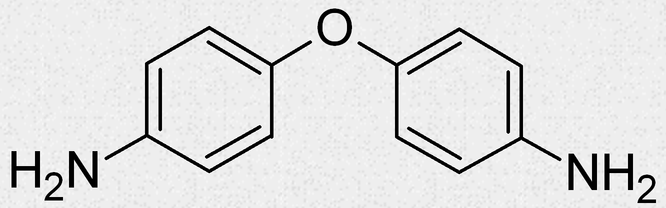
No removal of fluorine but addition of a nitrogen-containing specie

3. 

6. 

Biomaterials surface functionalization

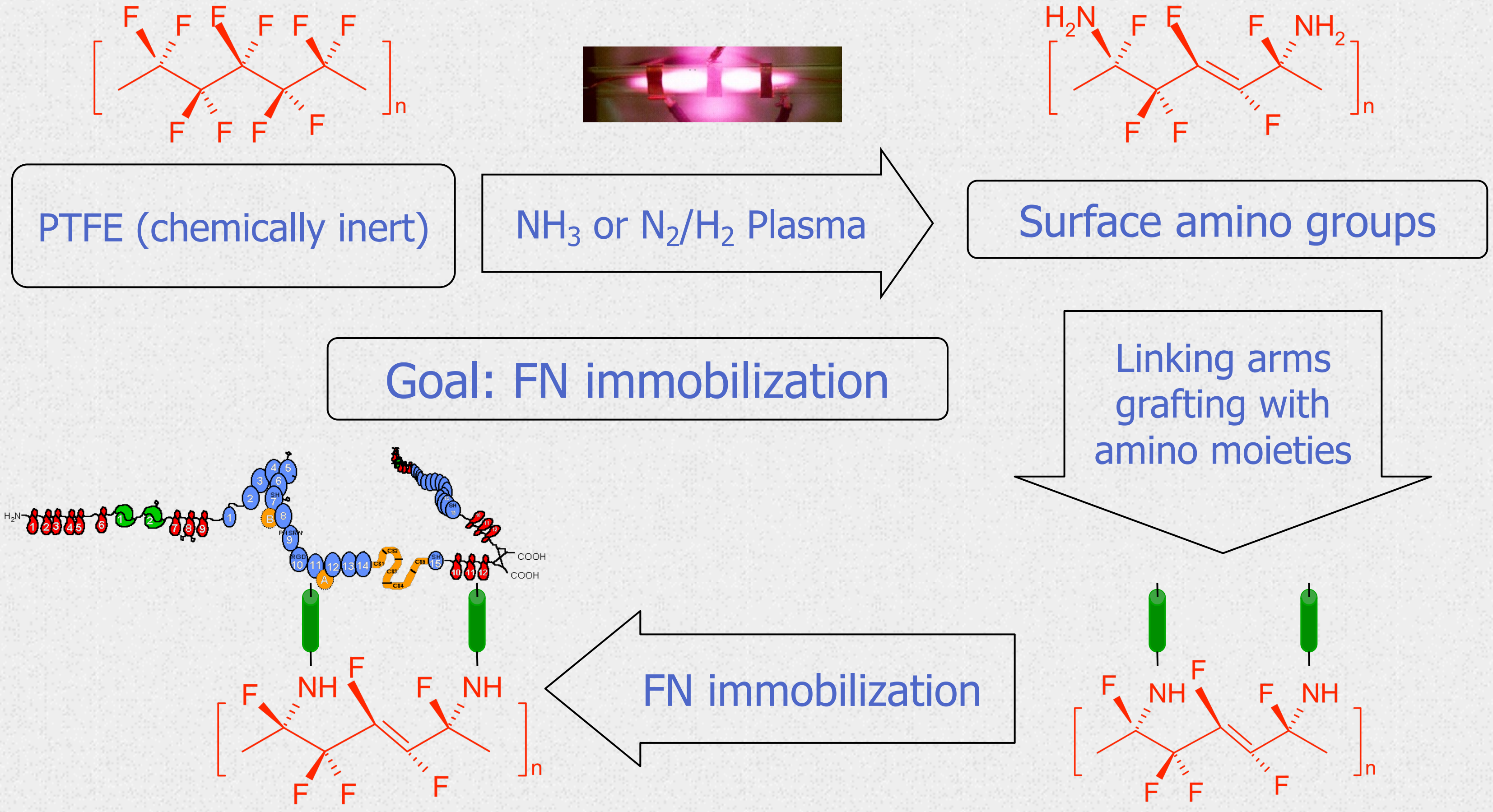




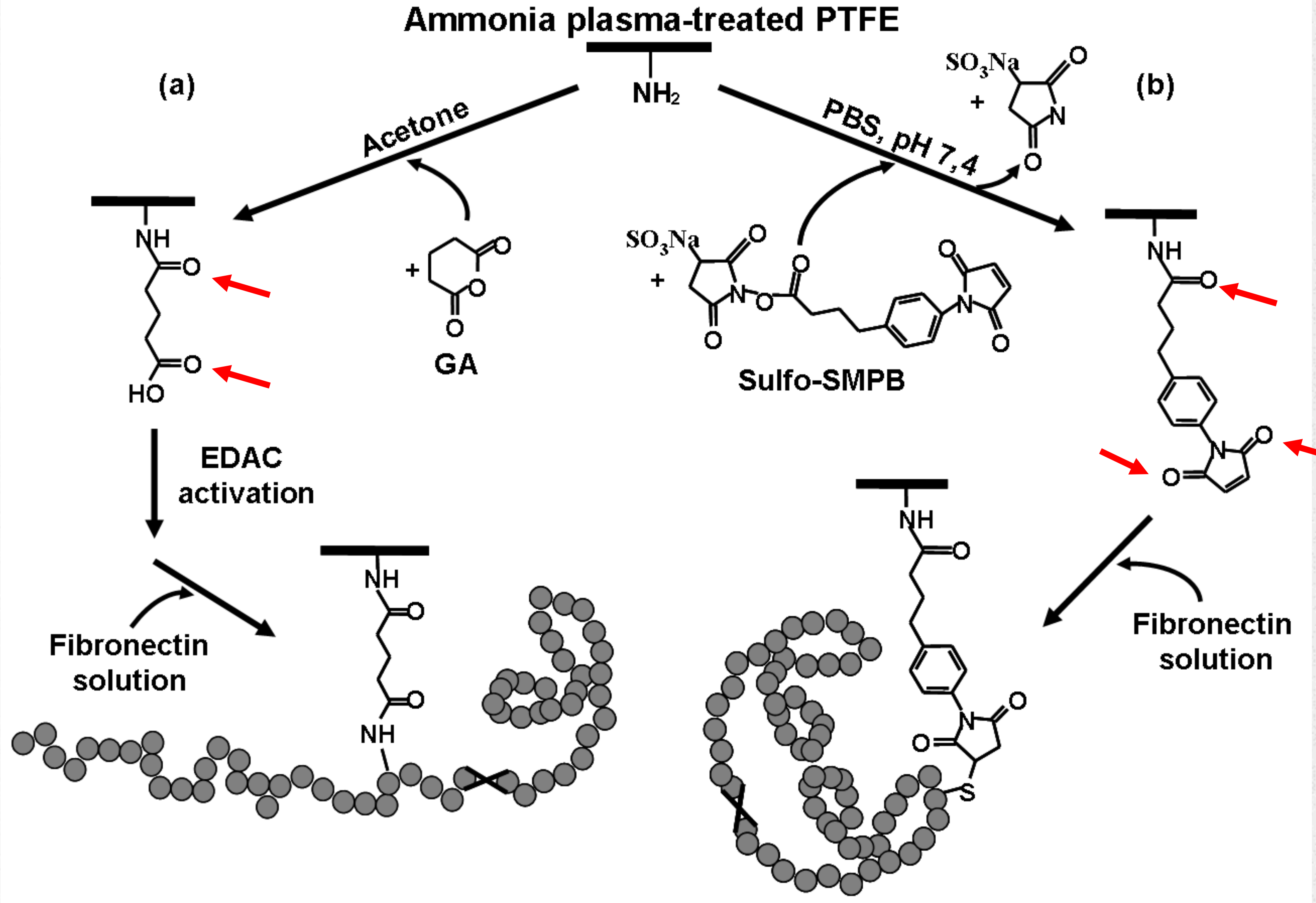
Biomaterials surface functionalization

Plasma treatment Duration	%N_0	F/C	N/C	%NH_2 / Ntotal	%NH_2 / surface *
250s	**14.3**	**0.499**	**0.269**	**42**	**6.0**
100s	**11.9**	**0.626**	**0.229**	**42**	**5.0**
50s	**11.6**	**0.865**	**0.249**	**31**	**3.6**

SURFACE CONJUGATION STRATEGY AND BIOACTIVITY



Surface conjugation strategy and bioactivity



Vallières et al., Macromol. Biosci., 2007, 7, 738; Langmuir, 2007, 23, 9745

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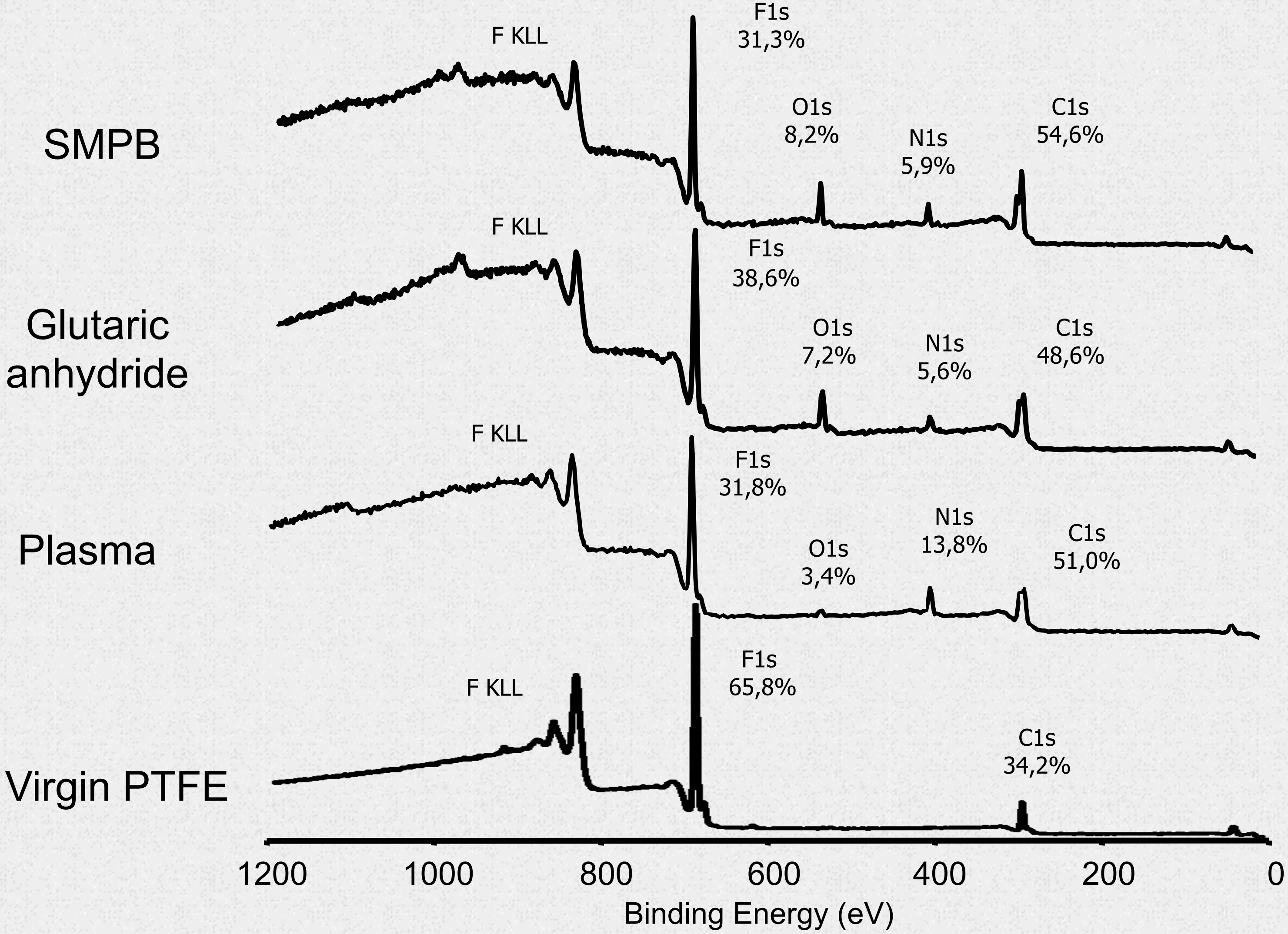

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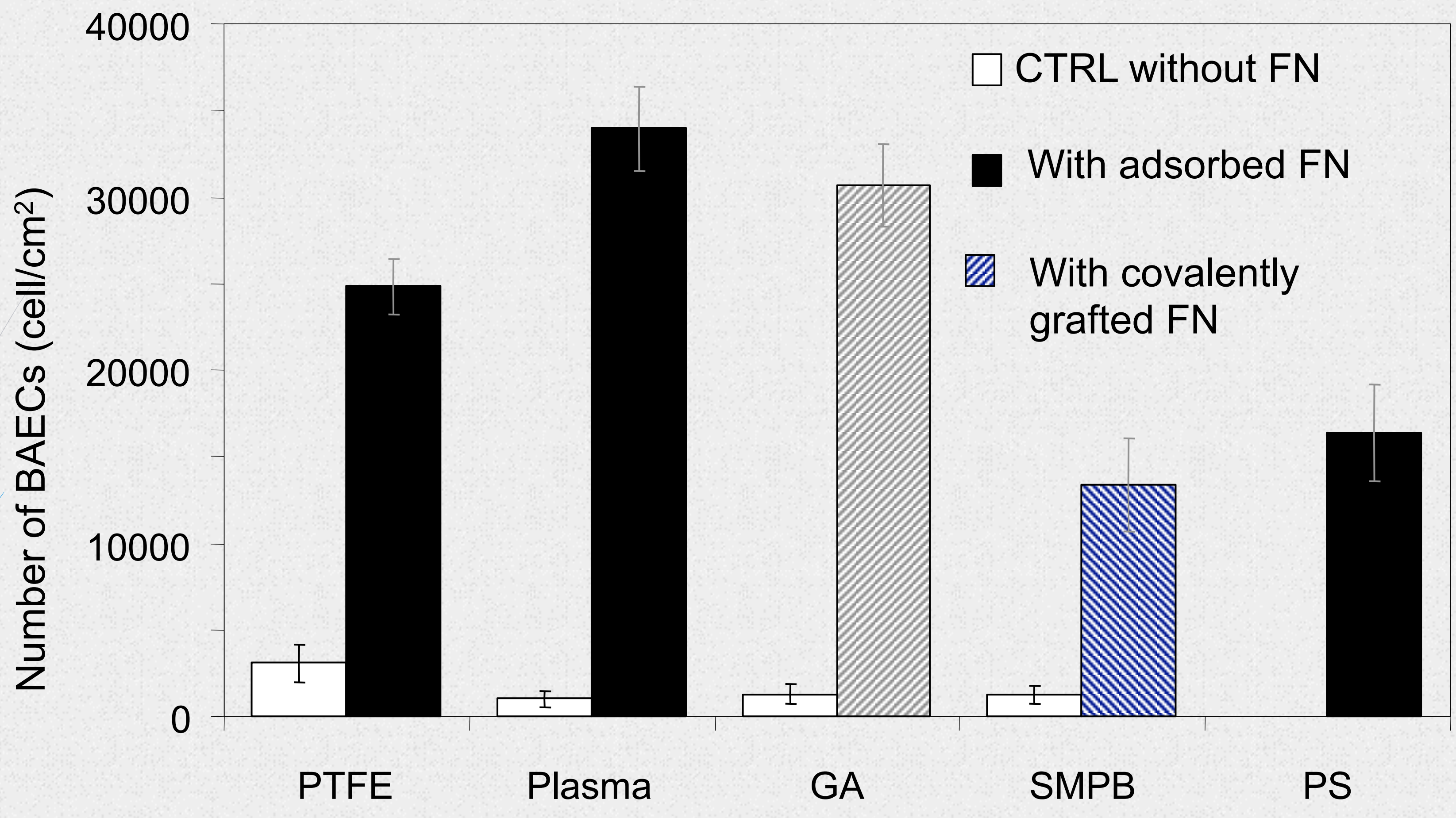
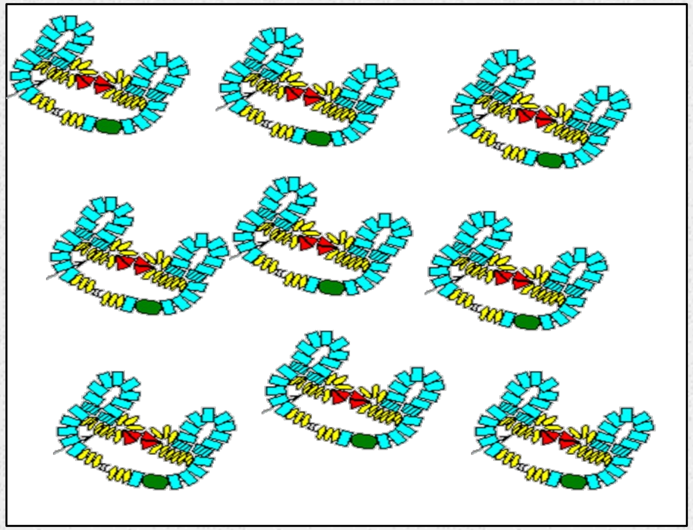


Conjugation strategy influences FN biological activity

Surface conjugation strategy and bioactivity

Is it a surface concentration effect?

GA



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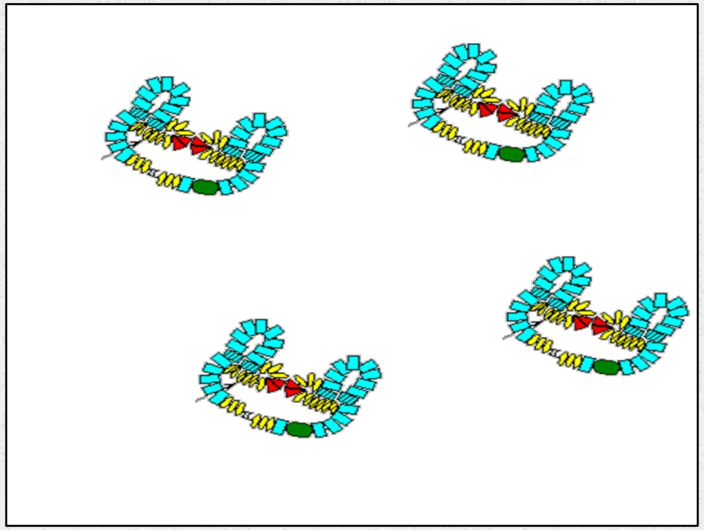
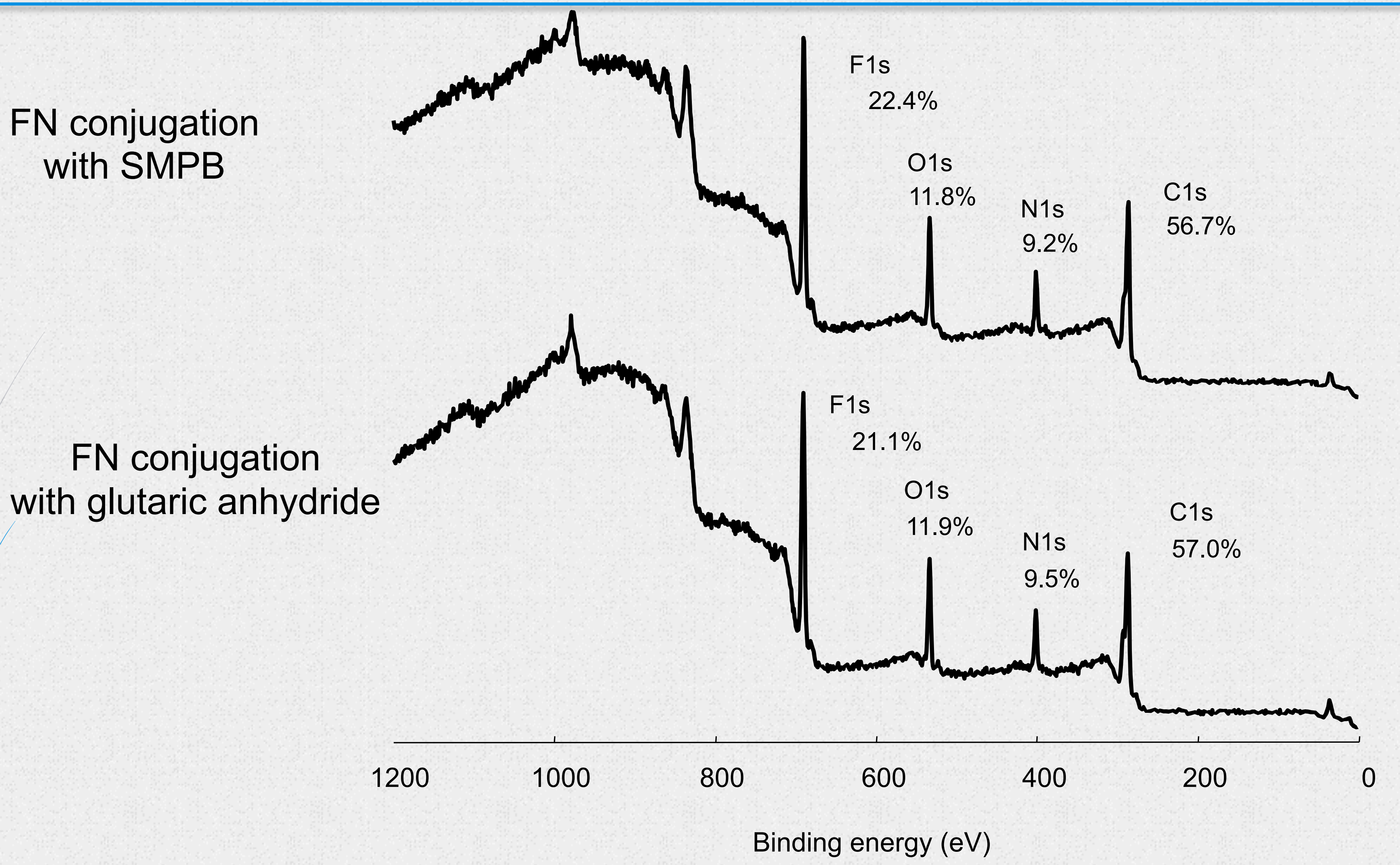
SMPB

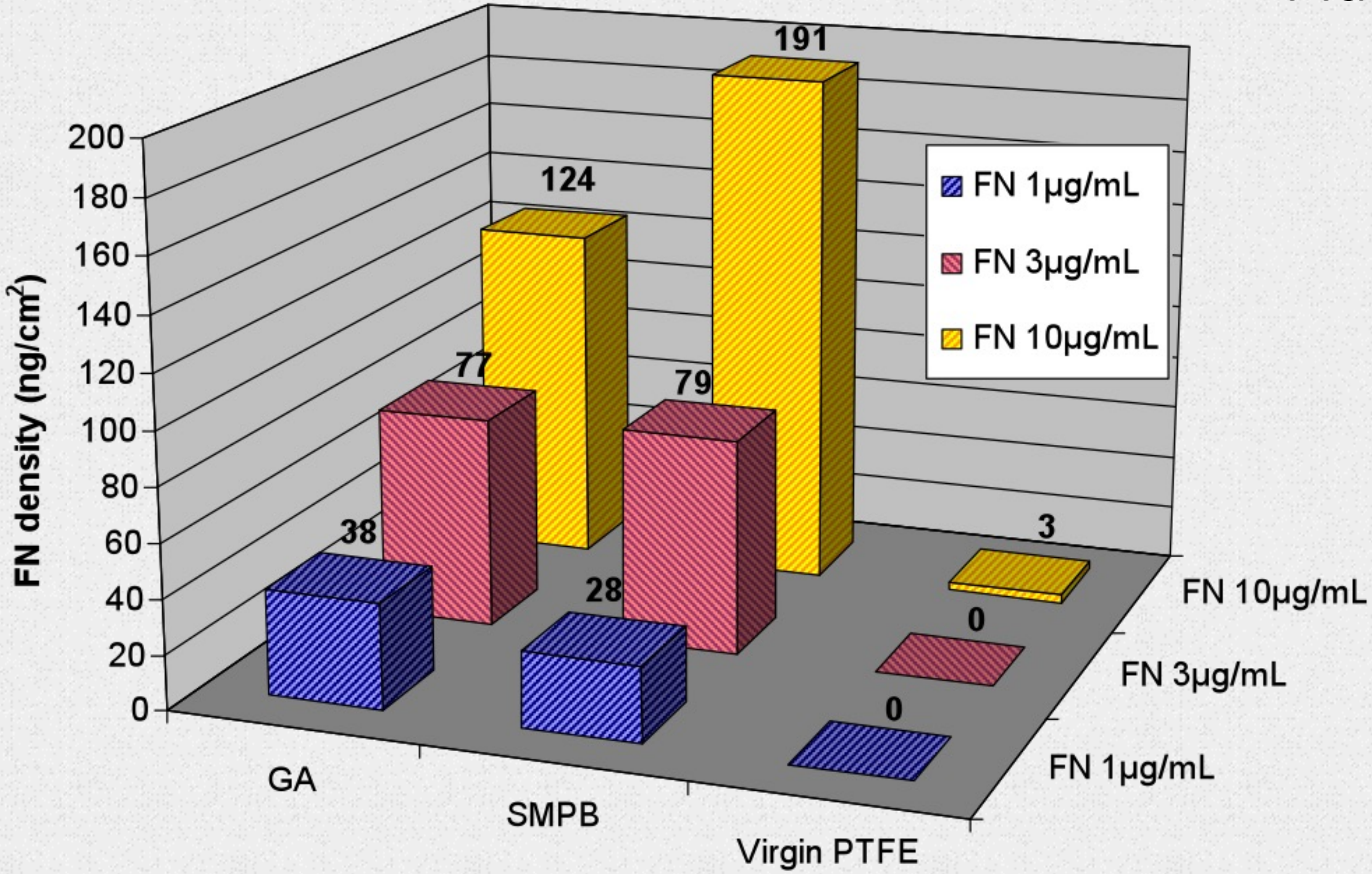


SURFACE CONJUGATION STRATEGY AND BIOACTIVITY



Surface conjugation strategy and bioactivity

^{125}I labeling

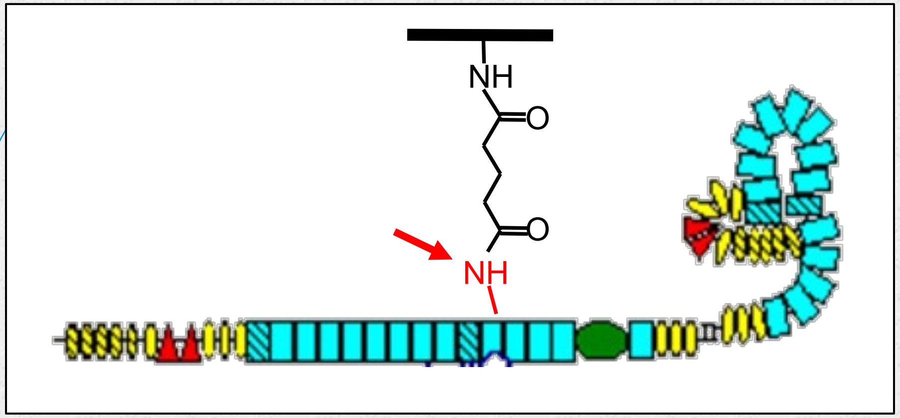


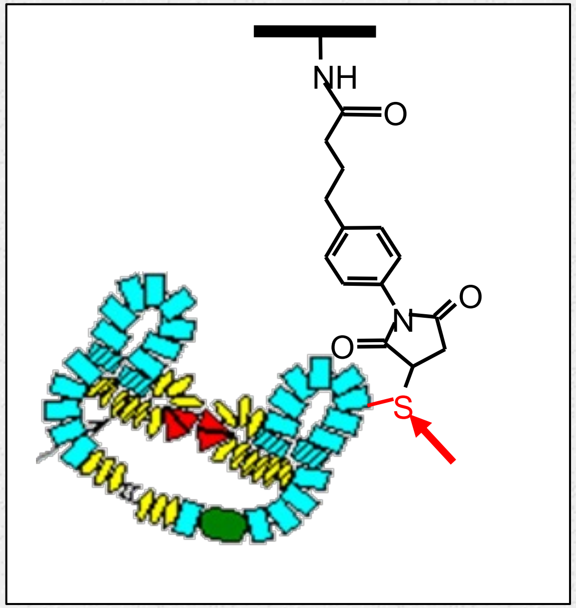
SURFACE CONJUGATION STRATEGY AND BIOACTIVITY

Is it a protein conformation effect?

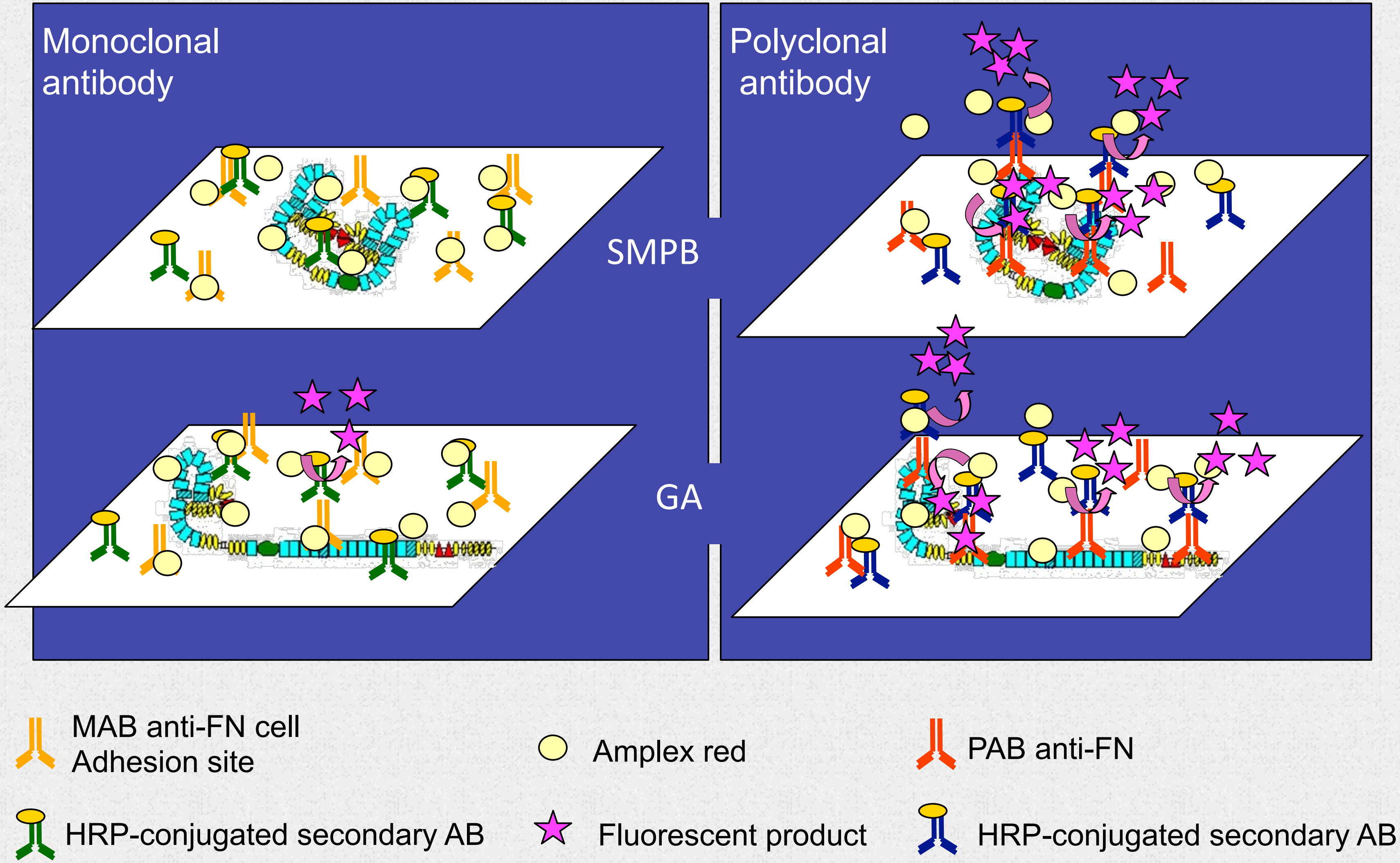
SMPB

Glutaric Anhydride

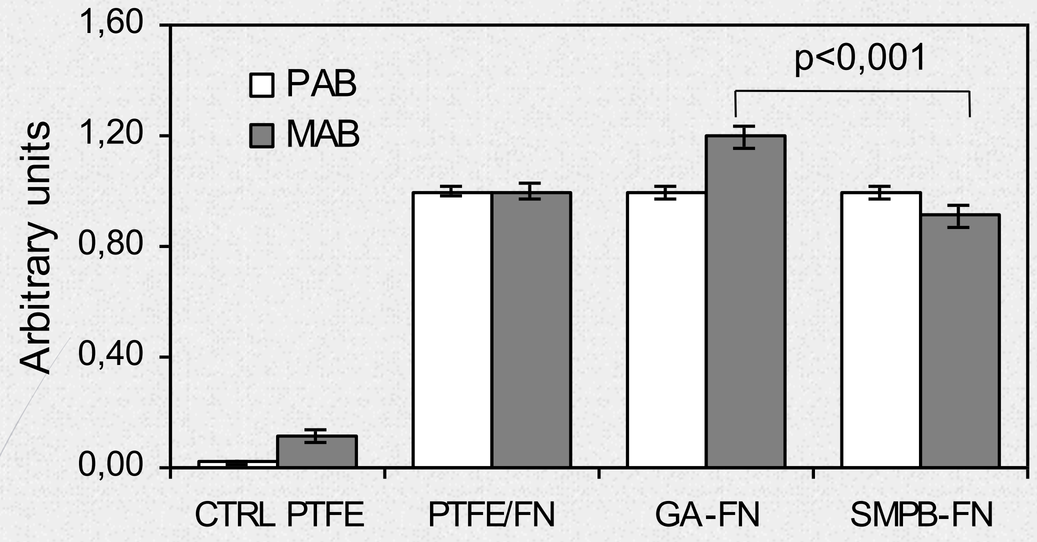


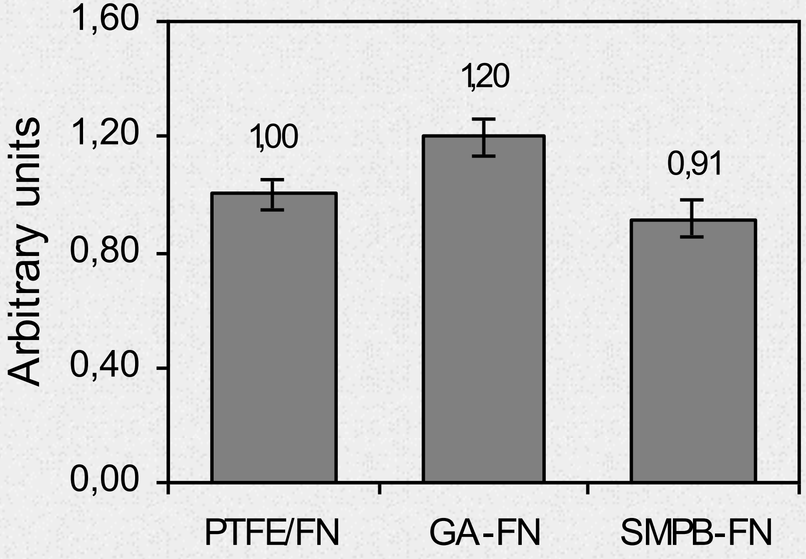


Surface conjugation strategy and bioactivity



SURFACE CONJUGATION STRATEGY AND BIOACTIVITY





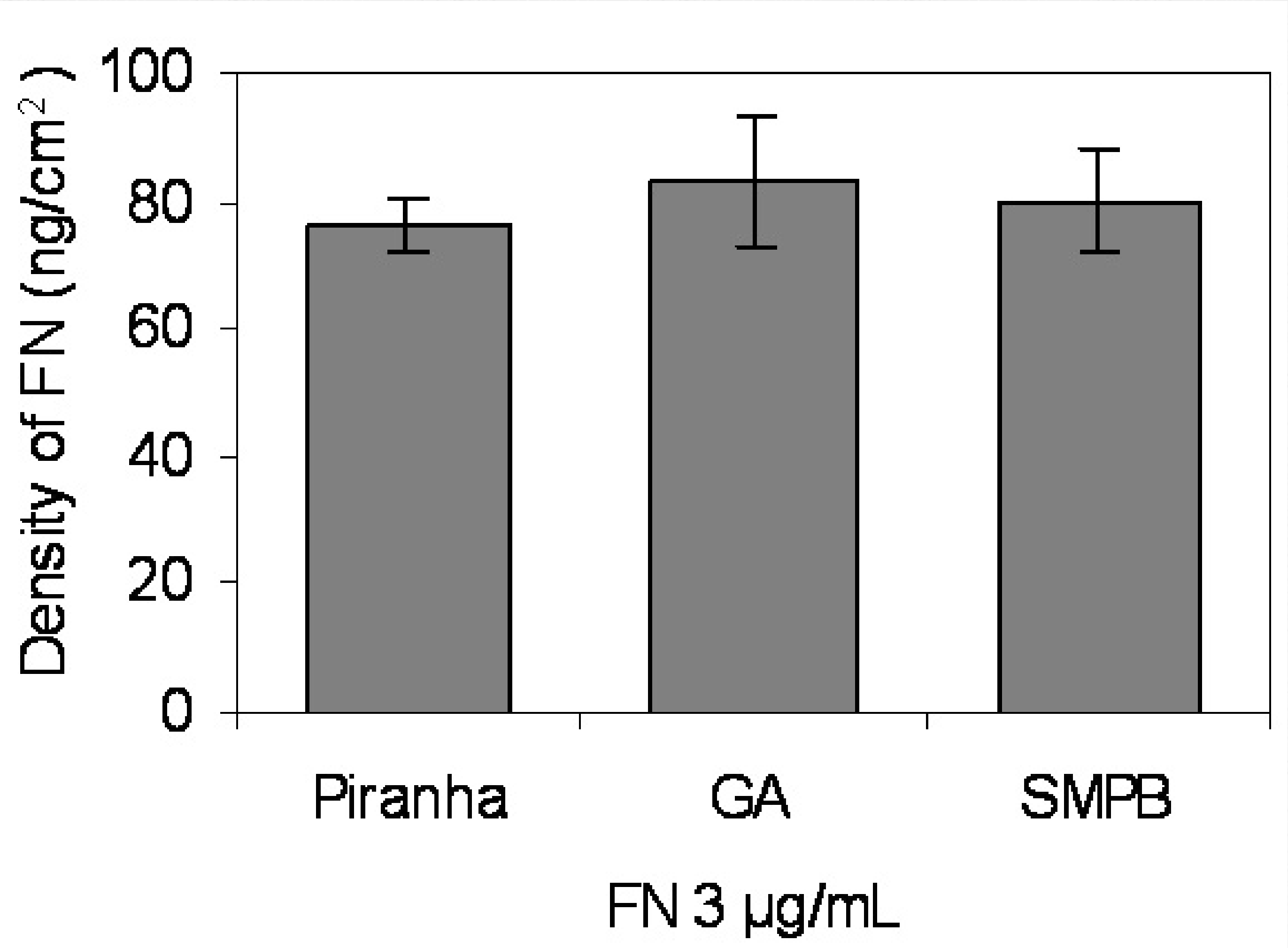
Surface conjugation strategy and bioactivity

- A closer look…

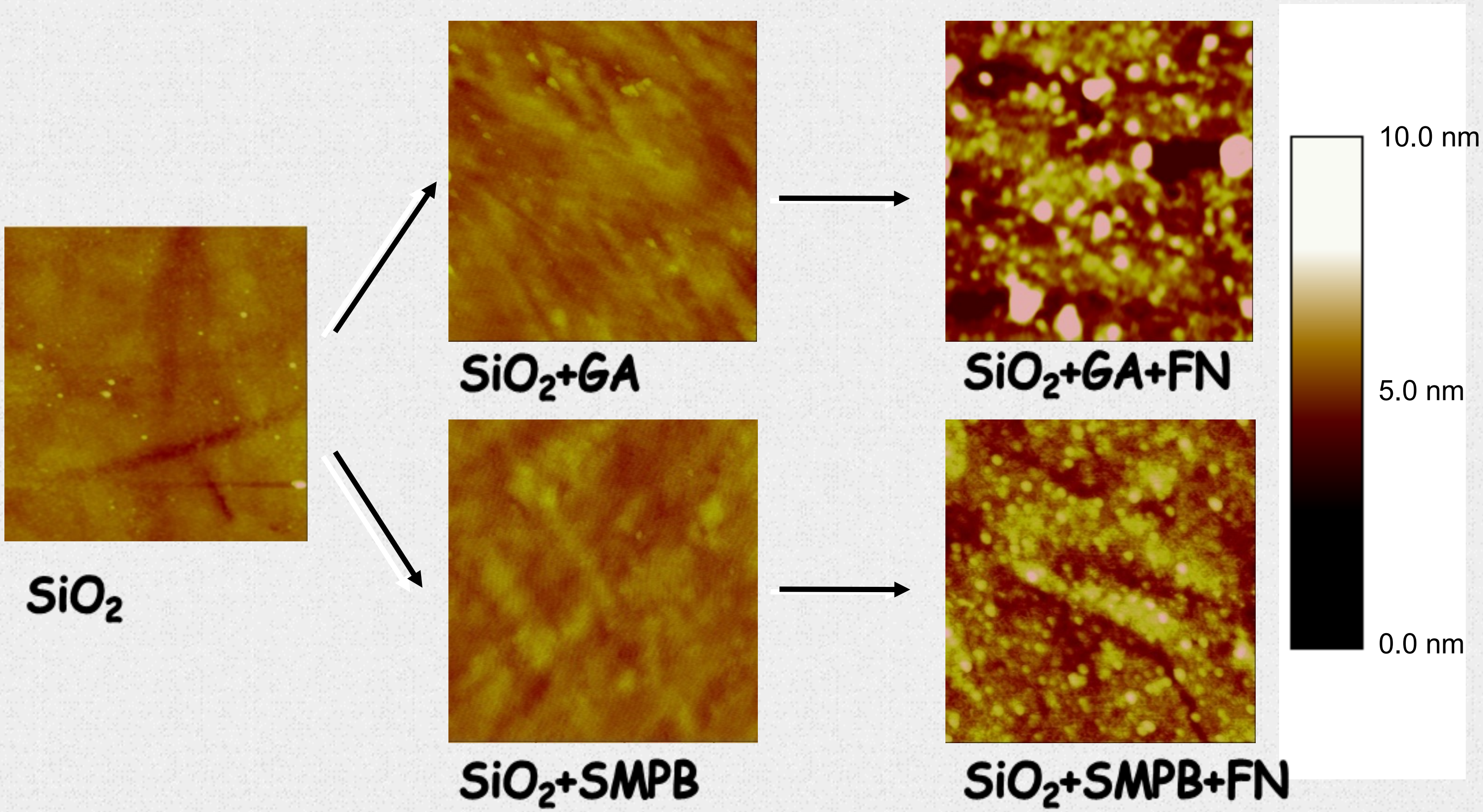
  - Atomic Force Microscopy: nanometer resolution.
  - FN: about 120 nm long x 4-5 nm high when fully elongated (Bergkvist, 2002)
  - PTFE : surface roughness too high (48 nm RMS) to allow FN imaging.
  - A softer surface is required: SiO_2 (RMS=0,29nm)
  - Conjugation of FN on plasma-treated SiO_2 via GA or SMPB.

Surface conjugation strategy and bioactivity

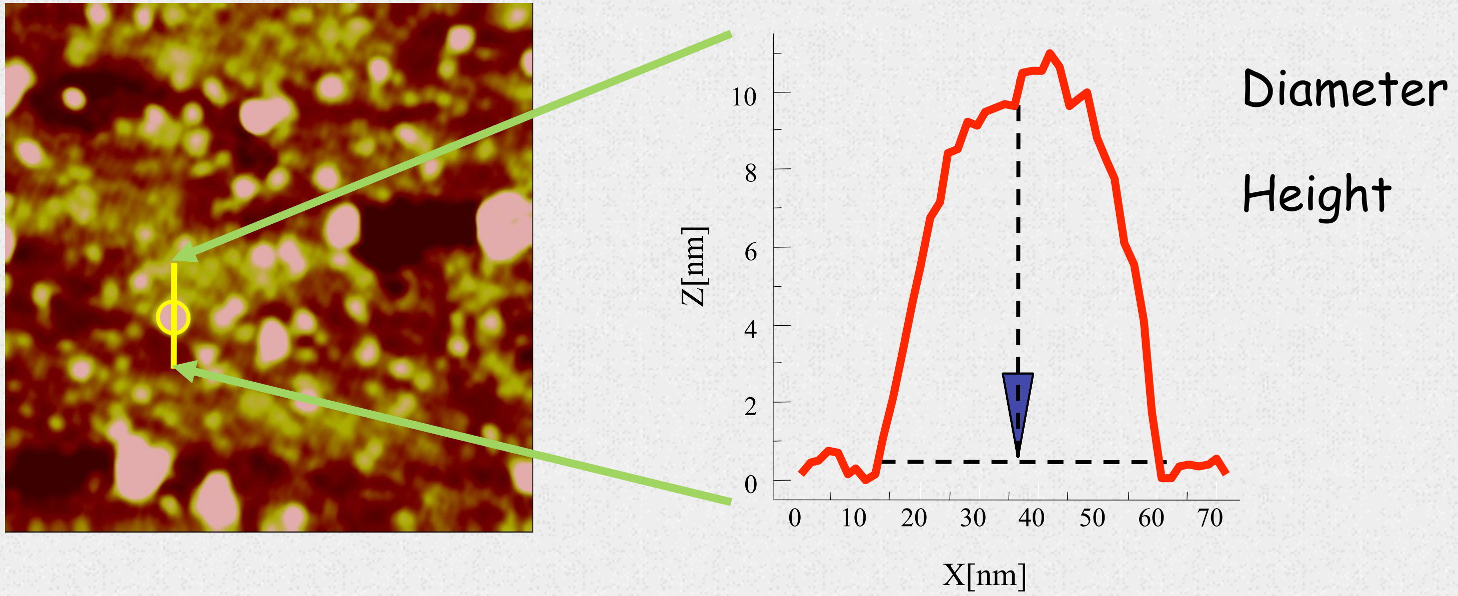
- FN on glass



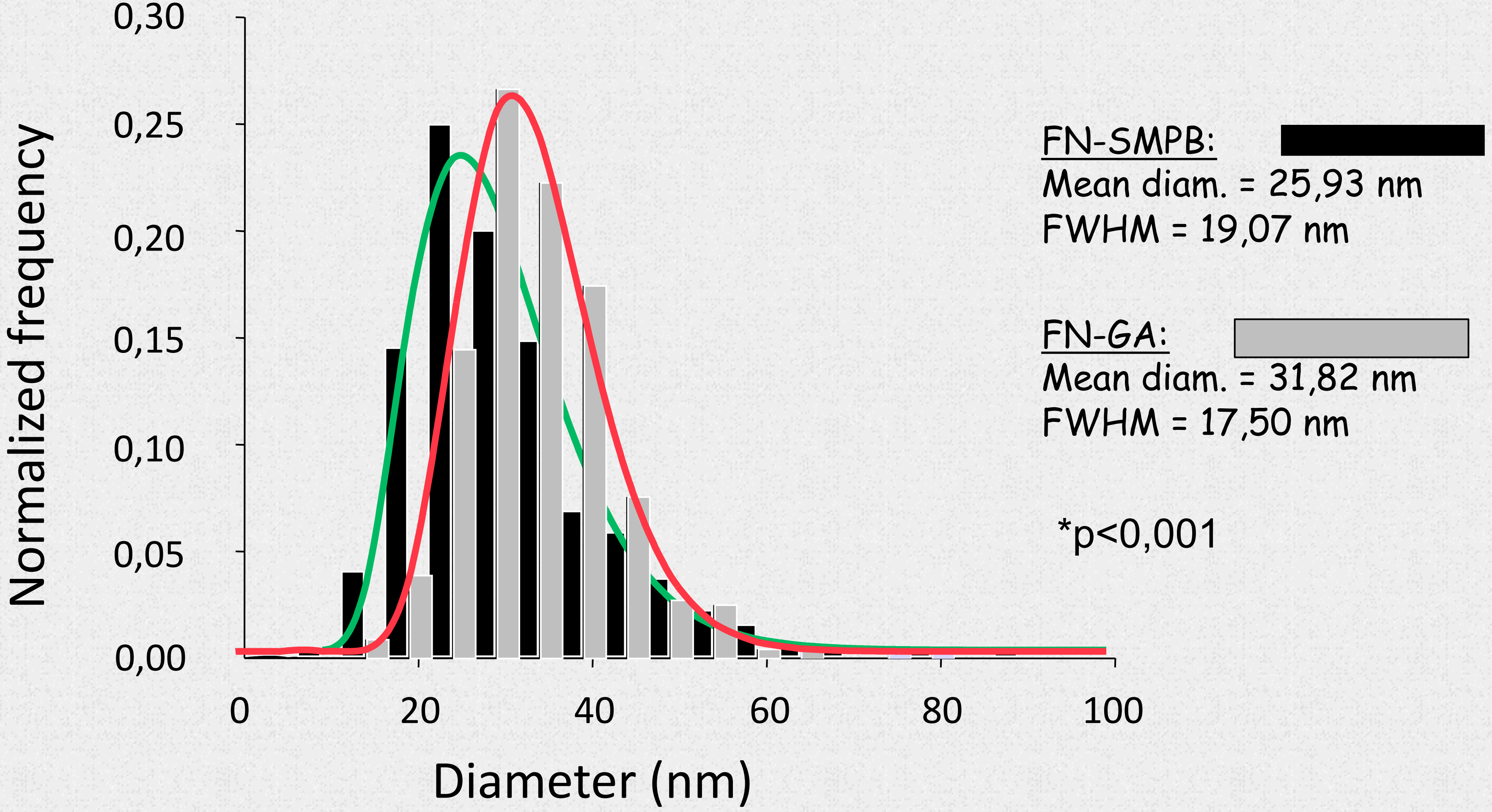
SURFACE CONJUGATION STRATEGY AND BIOACTIVITY



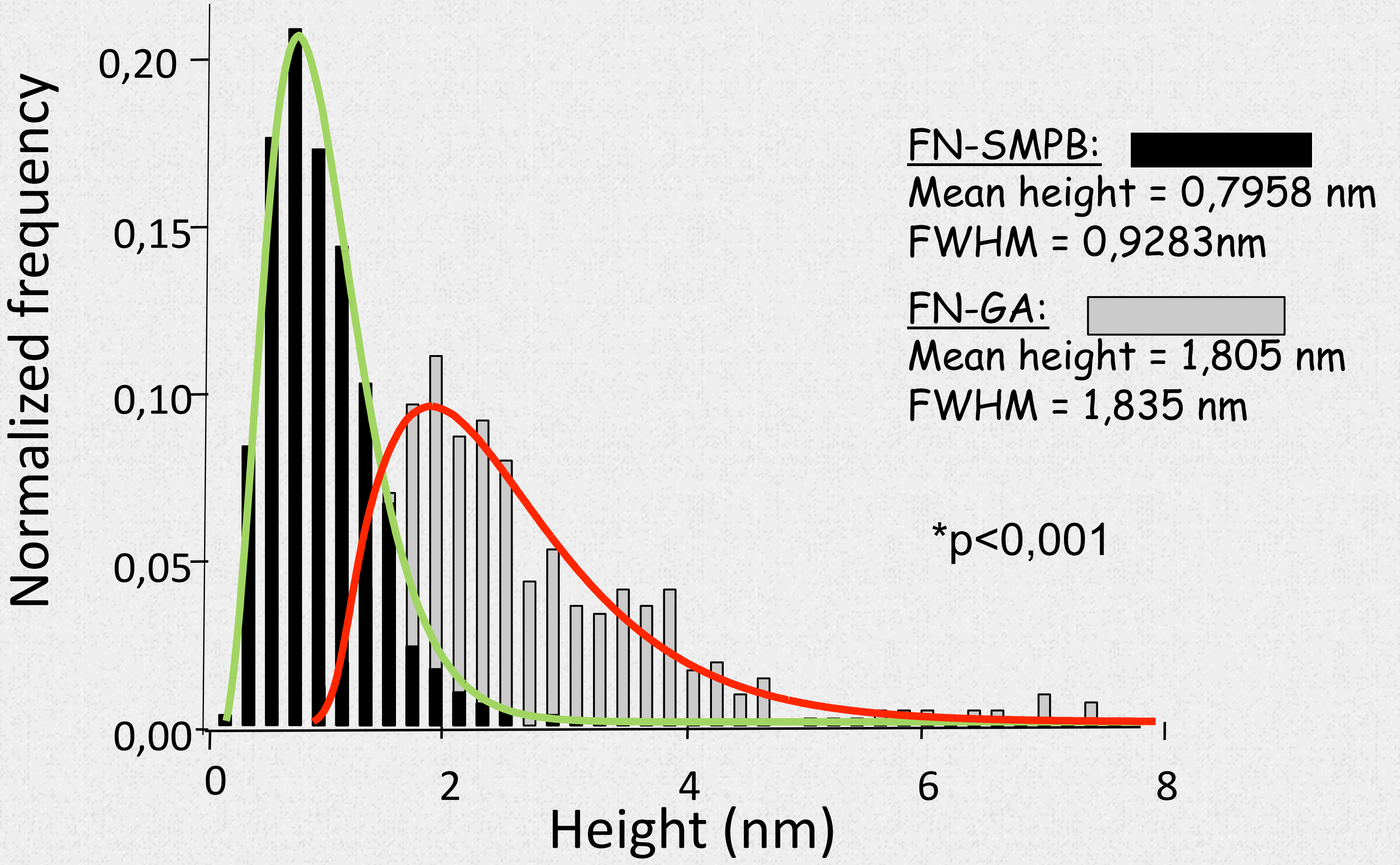
Surface conjugation strategy and bioactivity



Surface conjugation strategy and bioactivity



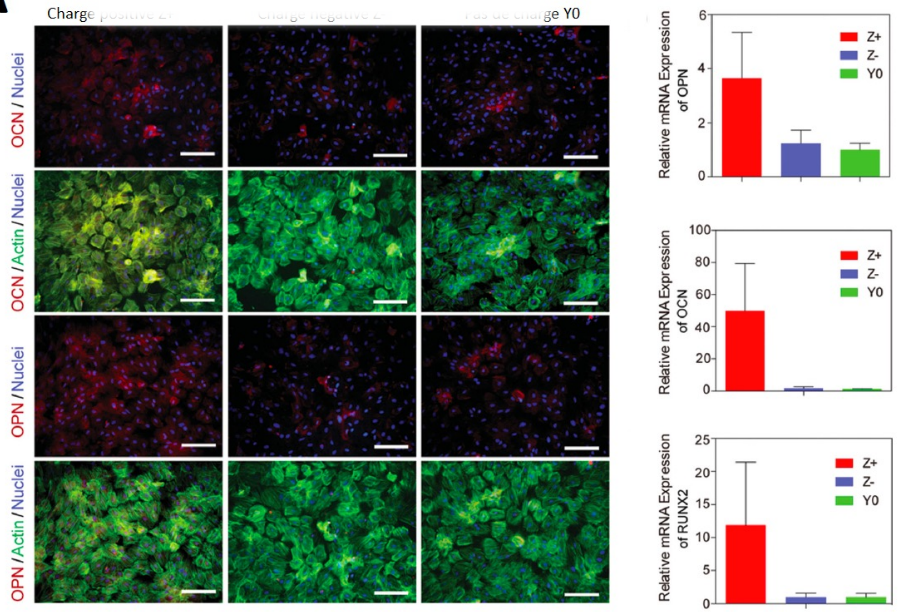
Surface conjugation strategy and bioactivity



Surface conjugation strategy and bioactivity

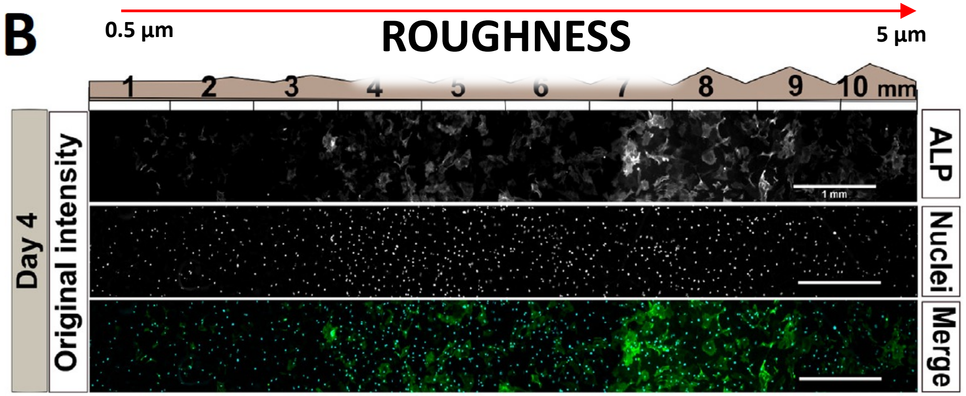
- Conclusion
  - The surface conjugation strategy drive the conformation and organization of fibronectin.
  - This conformation/organization determines the surface bioactivity in terms of celle binding site availability and cell adhesion.

A SURFACE CHARGES



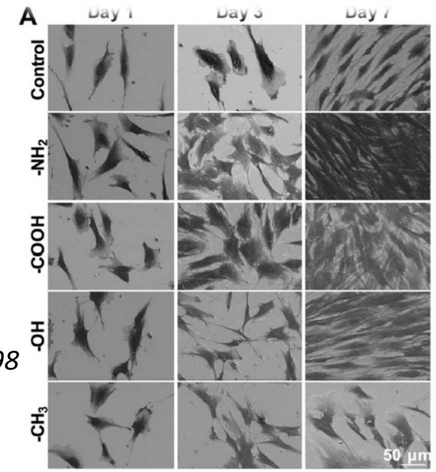
Li et al, Adv. Healthcare Materials, 2015, 4, 998

B ROUGHNESS



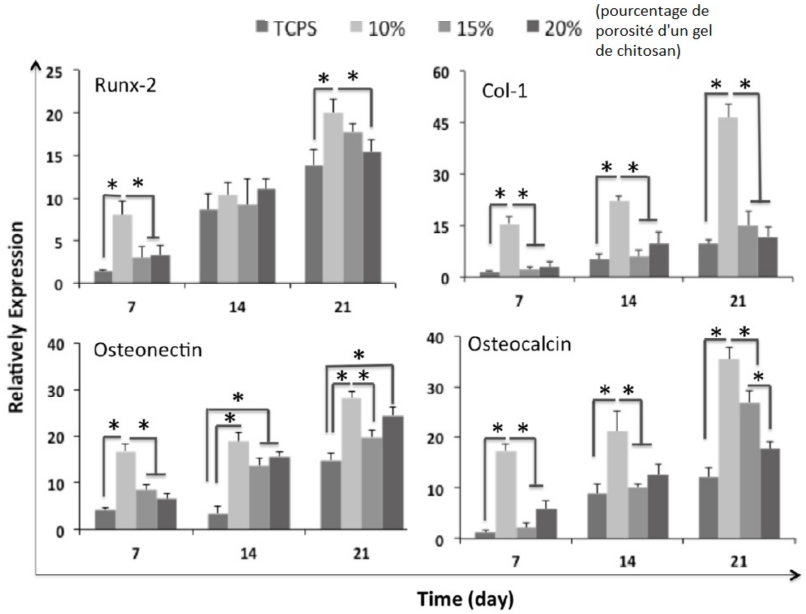
Faia-Torres et al., Biomaterials, 2014, 35, 9023, Adv. Healthcare Materials, 2015, 4, 998

C SURFACE CHEMISTRY



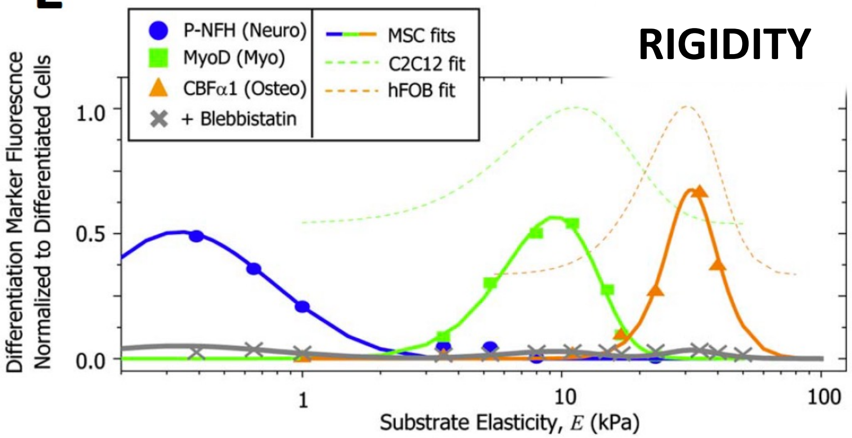
Yu et al., ACS Biomater Sci Eng, 2017, 3, 1119

D POROSITY



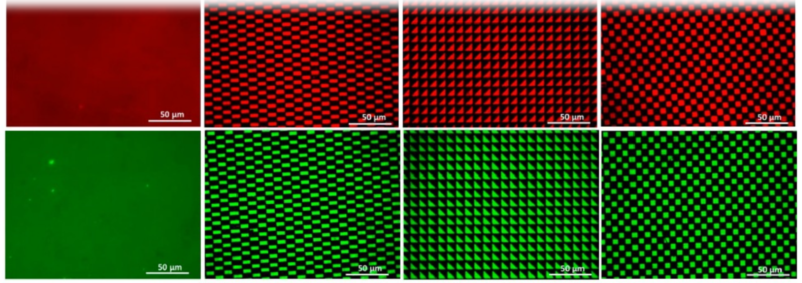
Ardeshirylajimiet al., J Cell Biochem, 2018, 119, 625

E RIGIDITY



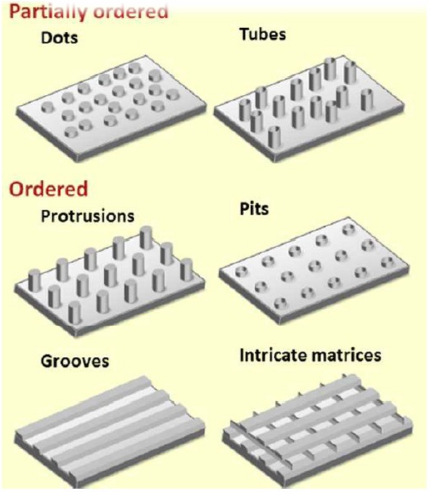
Engler et al., Cell, 2006, 126, 677

F MICRO, NANOPATTERNING OF BIOMOLECULES



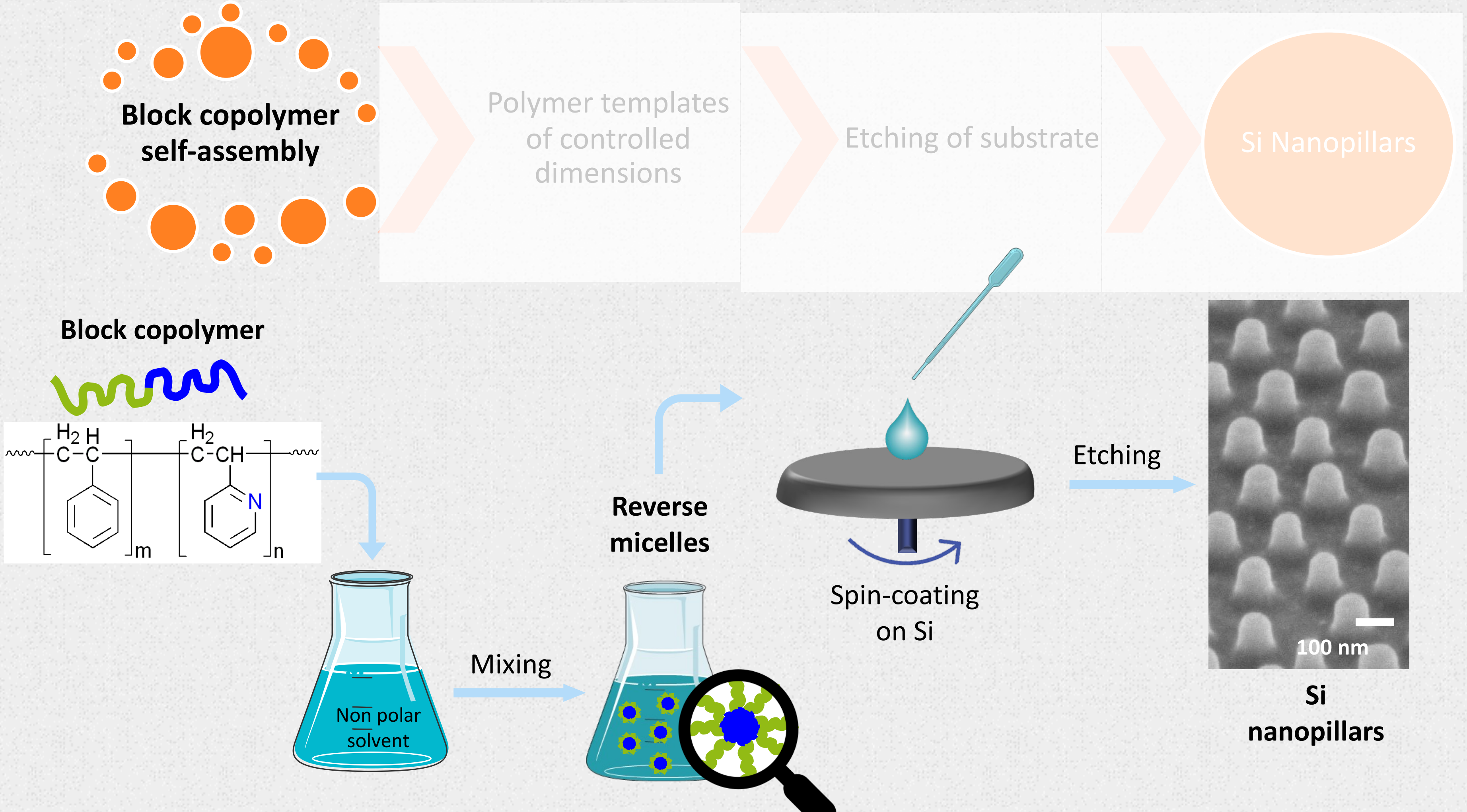
Bilem et al, ACS Biomater Sci Eng, 2017, 3, 2514 ; J Biomed Mater Res, 2018, 106, 959

G MICRO & NANO TOPOGRAPHIES



Gui et al, , Biomater Sci, 2018, 6, 250

G-Nanopillar arrays



Krishnamoorthy et al. *Adv Funct Mater* 2011

G - SILICON NANOPILLARS

Colloidal lithography

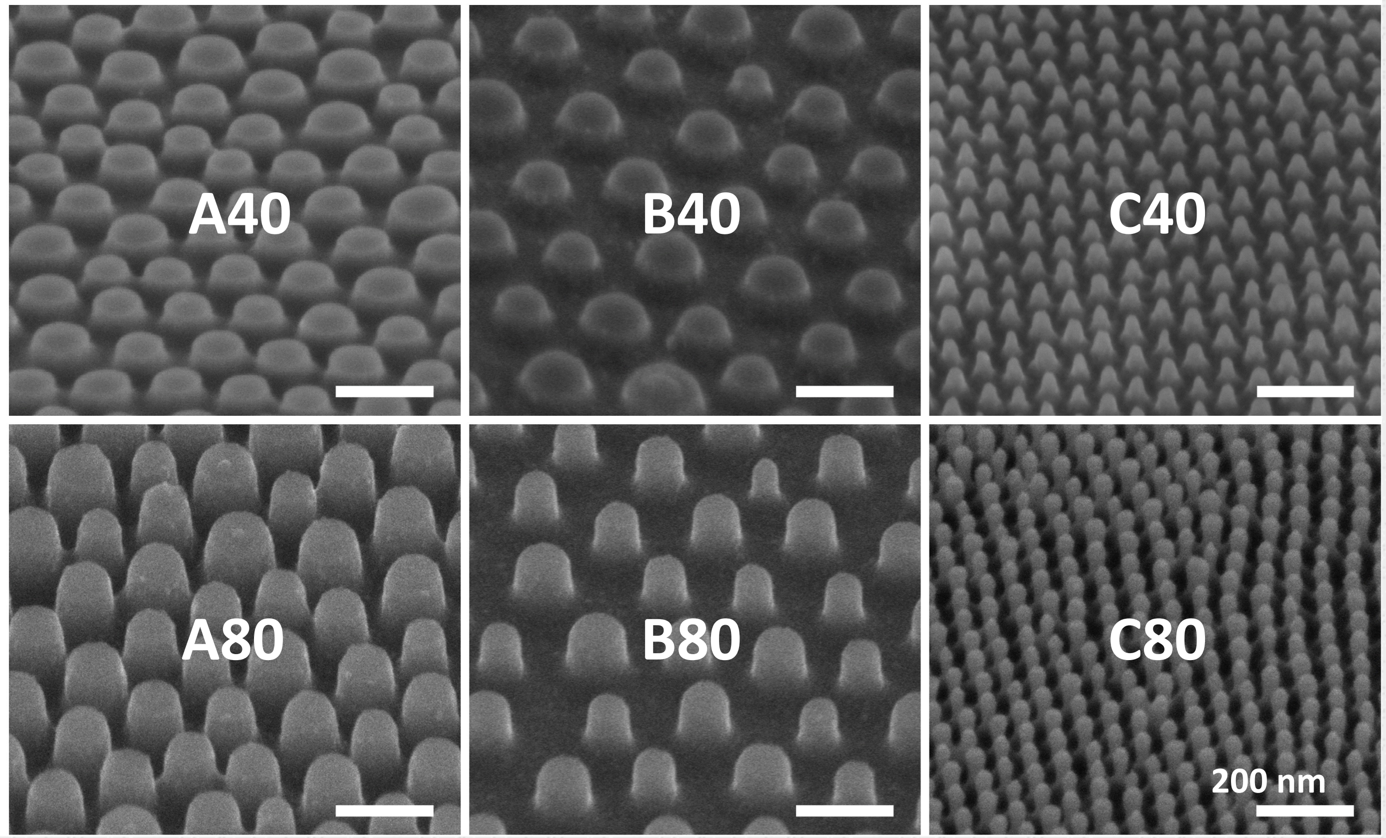
Diameter 100 nm
Pitch 140 nm

Diameter 100 nm
Pitch 200 nm

Diameter 50 nm
Pitch 70 nm

Height
40 nm

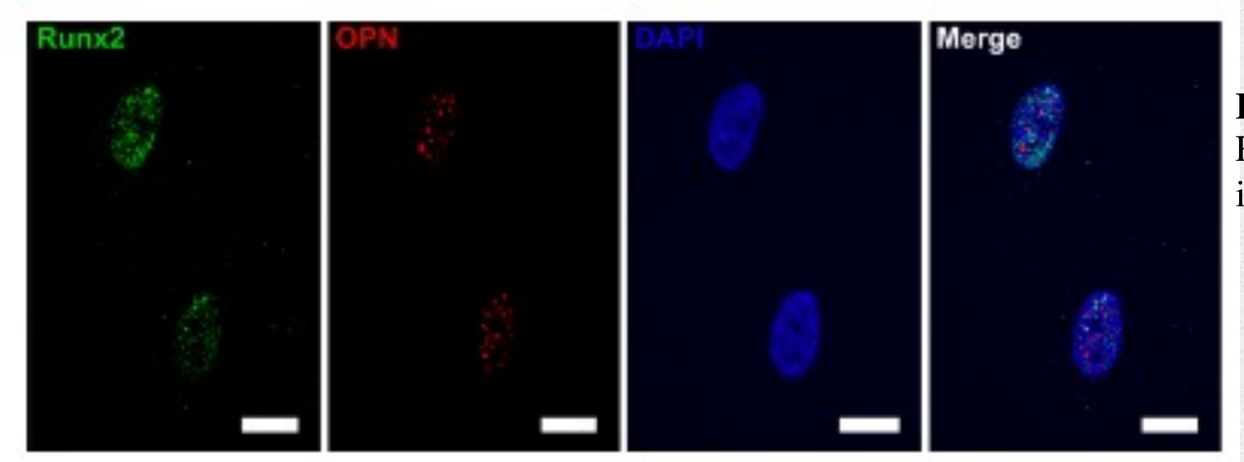
Height
80 nm



SEM micrographs of nanopillar samples.

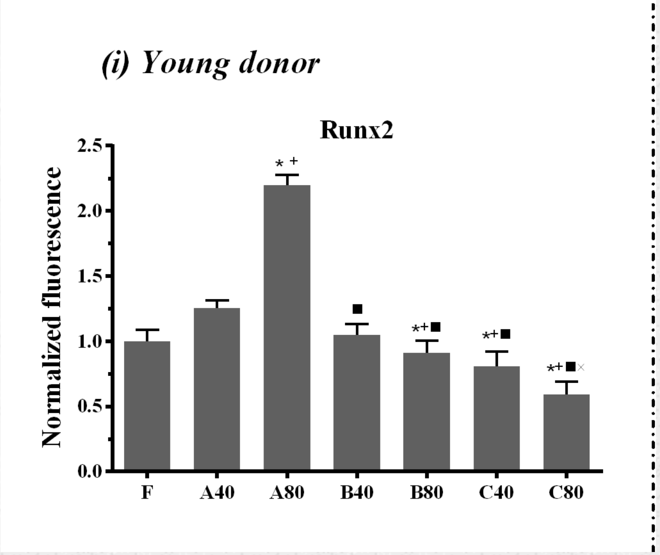
ACS Appl. Mater. Interfaces *2019, 11, 8858–8866 ;*
Appl Sci , 2021, 11, 11209

G – IMPACT OF SURFACE NANOTOPOGRAPHIE ON STEM CELL DIFFERENTIATION



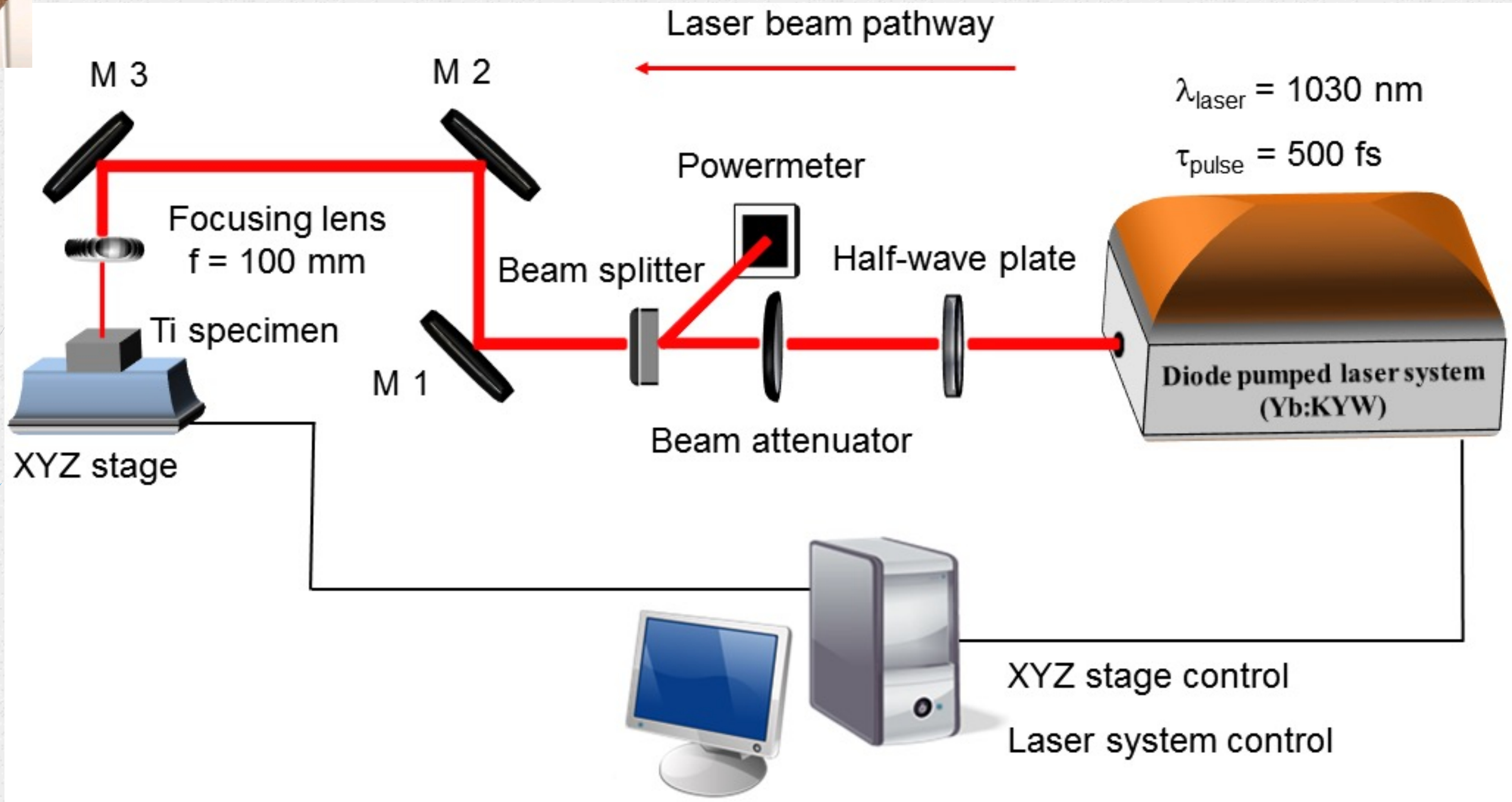
Example of immunofluorescence images obtained for the characterization of Runx2 and OPN expression (nucleus marked with DAPI) for understanding of intracellular distribution of these proteins on flat silicon. (Scale bar 15 µm).

Fluorescence intensity related with the expression of markers for osteoblastic differentiation of hMSCs after 2 weeks of culture on the nanostructured Si samples in basal medium was normalized against flat Si (F) control.
Expression in cells from young donor.



ACS Appl. Mater. Interfaces 2019, 11, 8858–8866 ; *Appl Sci* , 2021, 11, 11209

G- FEMTOSECOND LASER SURFACE TREATMENT SETUP



G - Impact of surface nanotopographie on stem cell differentiation

Polished titanium

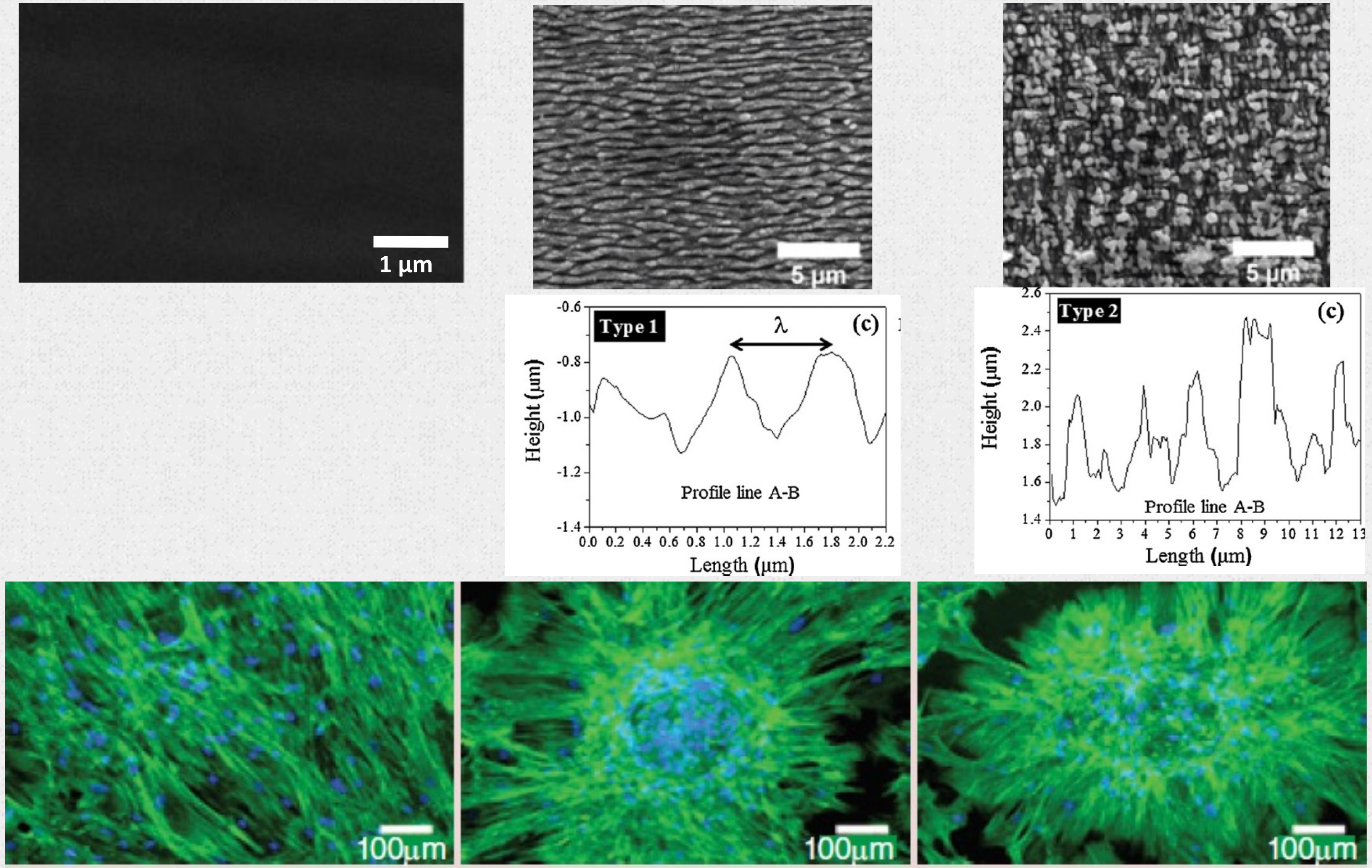
Laser-induced periodic surface structures

Nanopillars on titanium

I

II

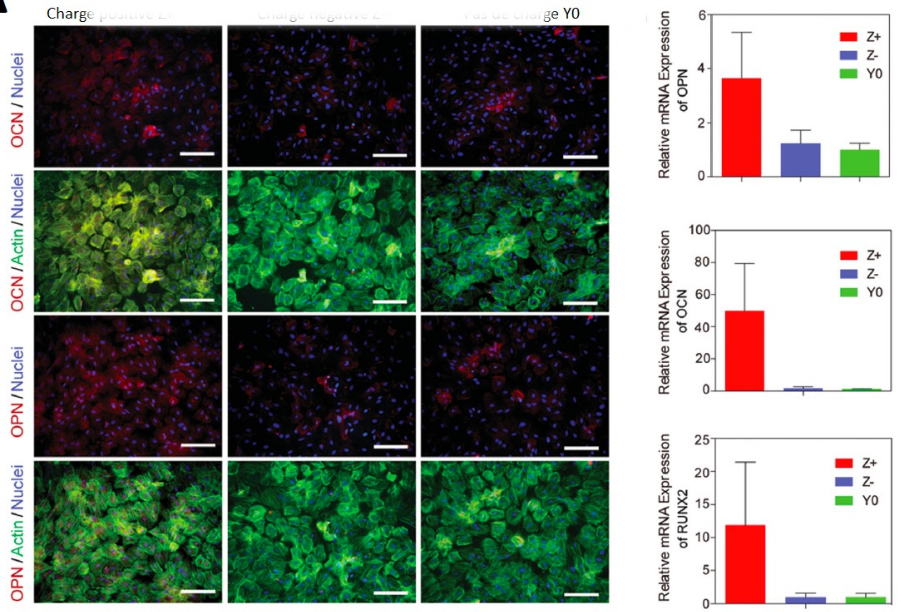
III



(I) SEM micrographs, **(II)** optical profilometer measurements and **(II)** Fluorescence images of cells on polished or nanostructured surfaces (4 weeks after cell seeding, F-actin fibers (green), cell nucleus (blue))

***J Nanomedicine**, 2015, 10(5), 725 ;*
***Appl Surf Sci**, 2013, 265, 688*

A SURFACE CHARGES



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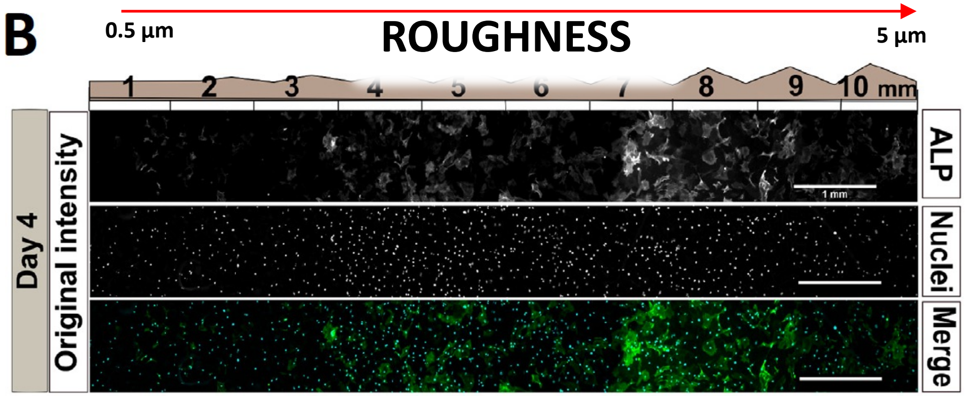

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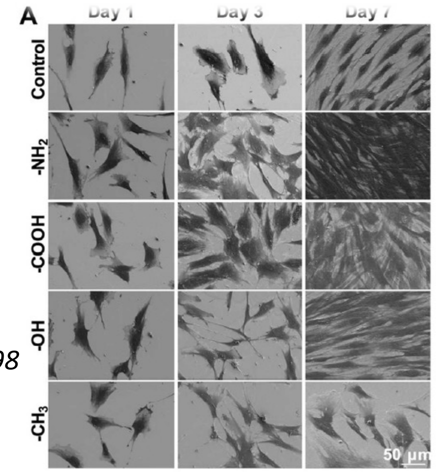

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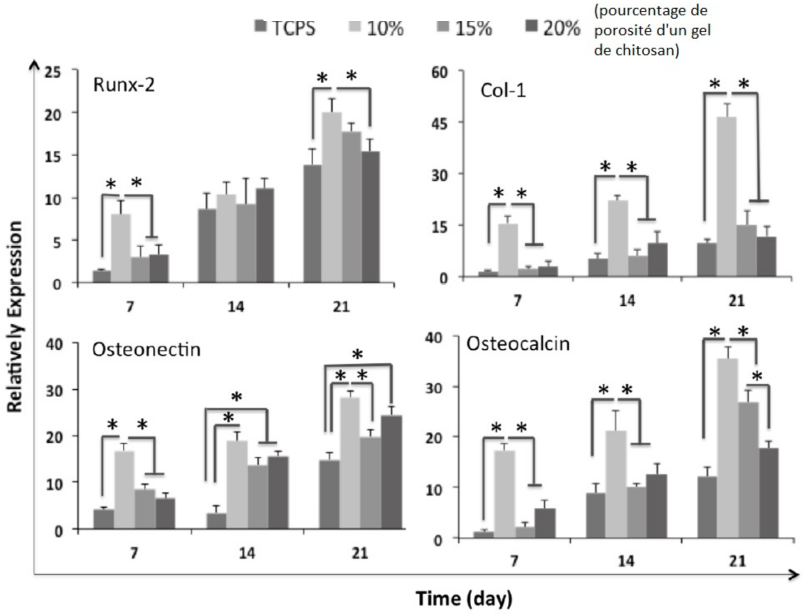

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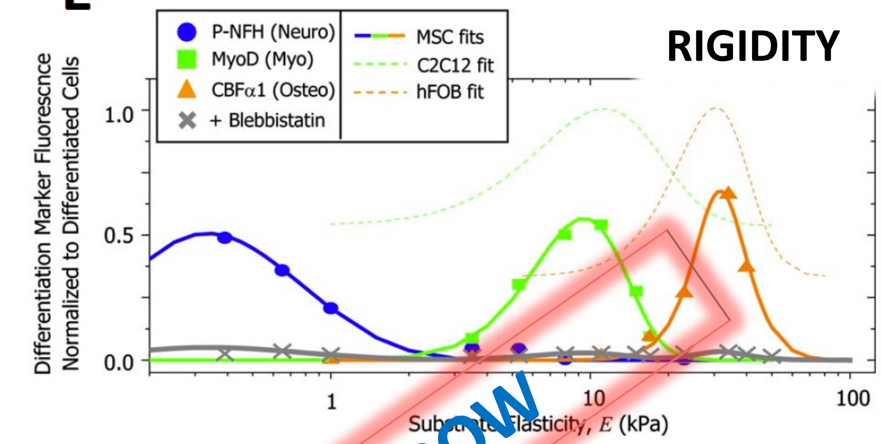

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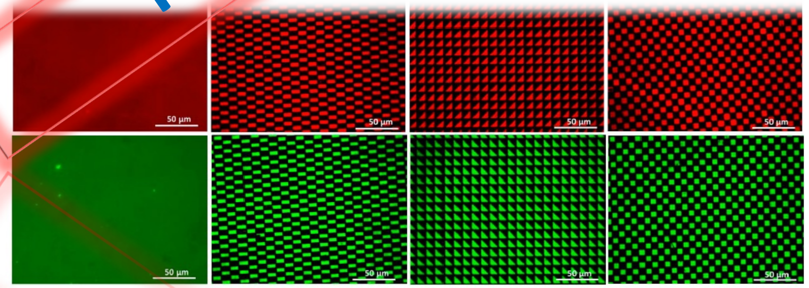

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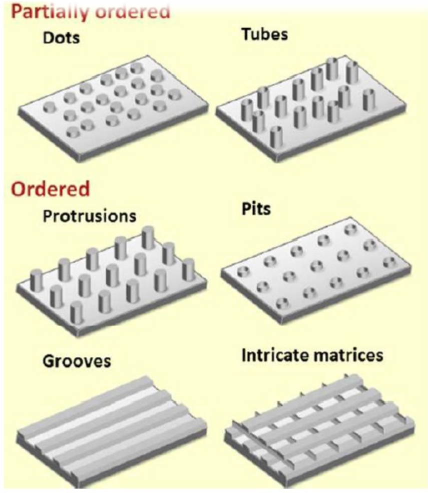

Gui et al, , Biomater Sci, 2018, 6, 250

TOMORROW

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AMA Advanced Materials in Aquitaine

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AMADEus Advanced MAterials by DEsign

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Investissements d'avenir

Région Nouvelle-Aquitaine

CHU de Québec

Université Laval

Fonds de recherche Nature et technologies Québec

Laboratoire LIS D'ingénierie de surface

NSERC CRSNG

université de BORDEAUX

China Scholarship Council

HORIZON 2020 Le programme de recherche et d'innovation de l'Union européenne

European Commission ERASMUS MUNDUS

SPARK BORDEAUX

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cqmf centre québécois sur les matériaux fonctionnels

PRIMA

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