Synergy between biochemical and biophysical cues to promote cell differentiation

Marie-Christine Durrieu

Gaétan Laroche

GDR-Les Houches

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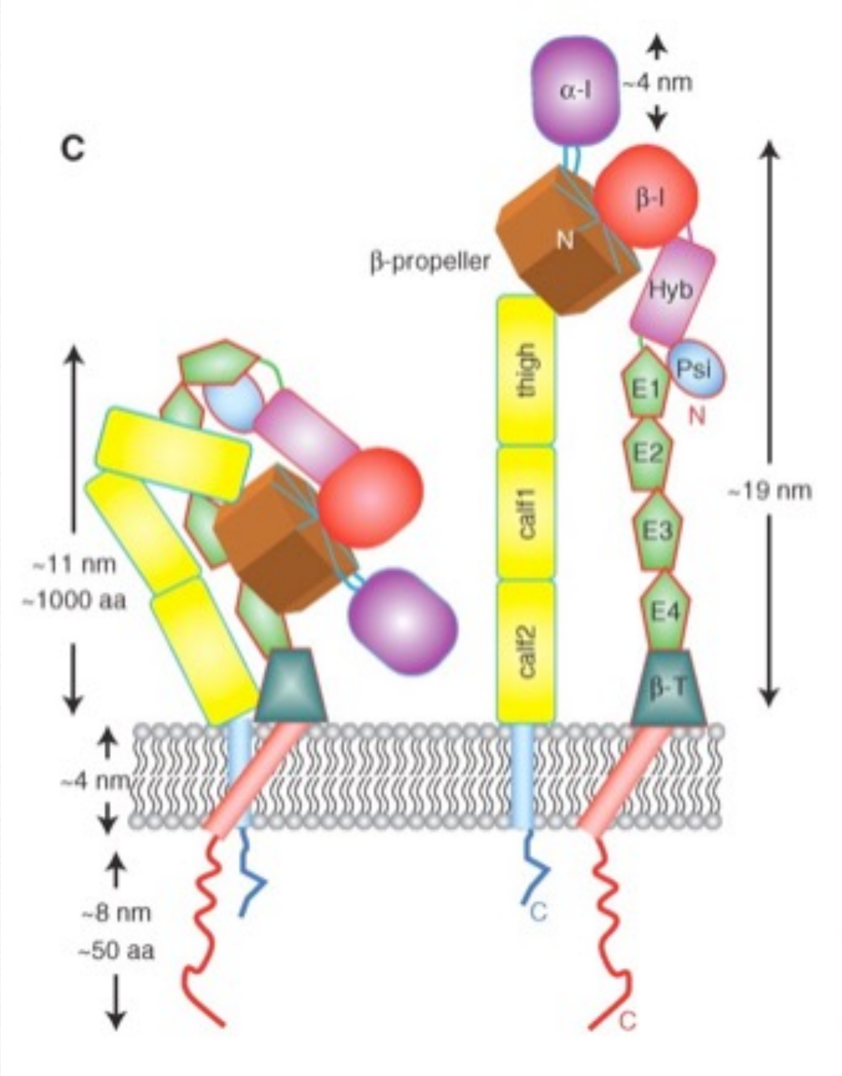
université de BORDEAUX
cbmn Chimie & Biologie des Membranes et des Nano-objets
UNIVERSITÉ LAVAL
LABORATOIRE LIS D'INGÉNIERIE DE SURFACE

WHY MICRO/NANOSTRUCTURING?

- Why micro/nanostructuring



ID Campbell and MJ Humphries, Cold Spring Harb Perspect Biol 2011; **3**: a004994

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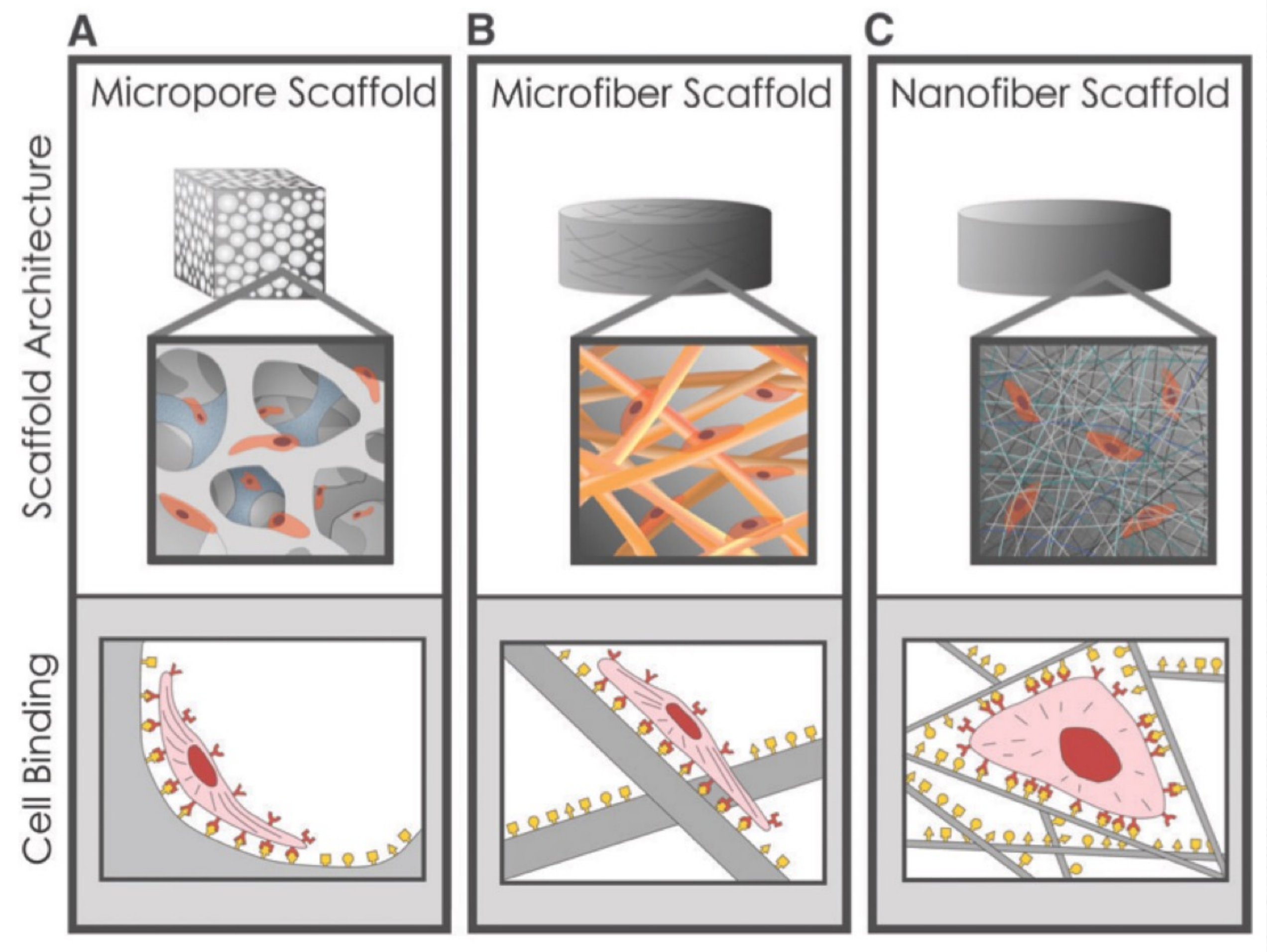


MM Stevens and JH George, Science, 2005; **310**: 1135-1138.

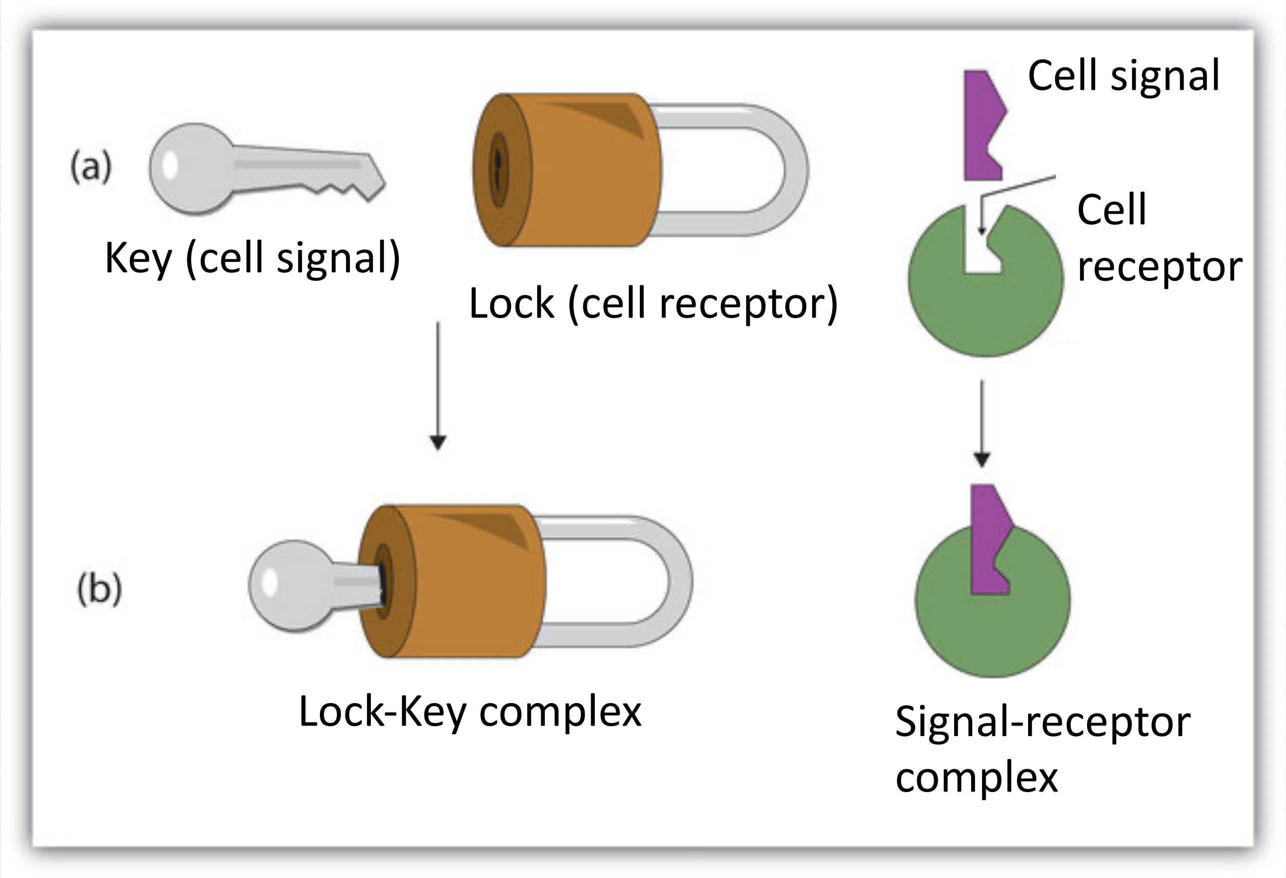
Why micro/nanostructuring?

- The interaction between integrins (or more generally cell receptors) and the extracellular matrix (specific amino acid sequences) proceeds through self-assembly mechanisms.

- While the interaction with cell receptors occurs at the nano scale through self-assembly mechanisms, the extracellular matrix self-organizes at the micro scale.

Why micro/nanostructuring?

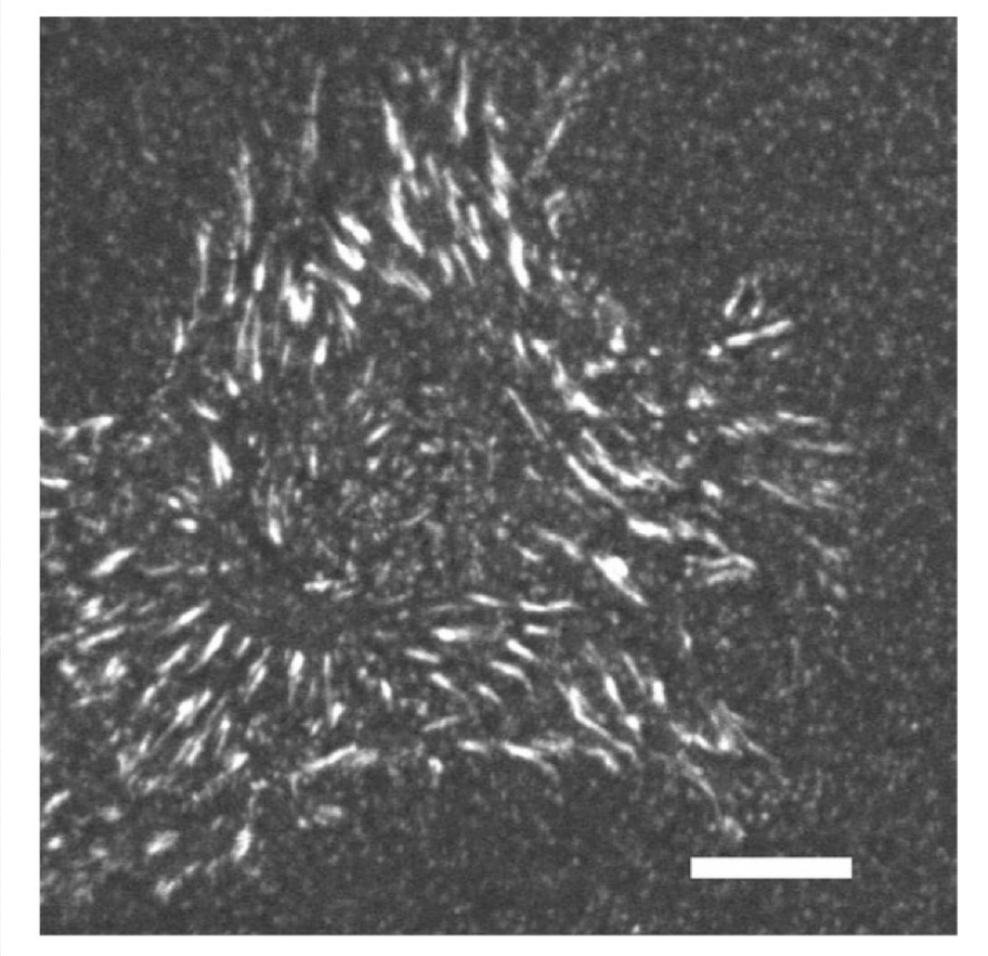
Nanoscale organization



Adapted from Liubov Poshyvailo, PhD Thesis, National University of Kyiv-Mohyla Academy, 2015

WHY MICRO/NANOSTRUCTURING?

Macroscale organization

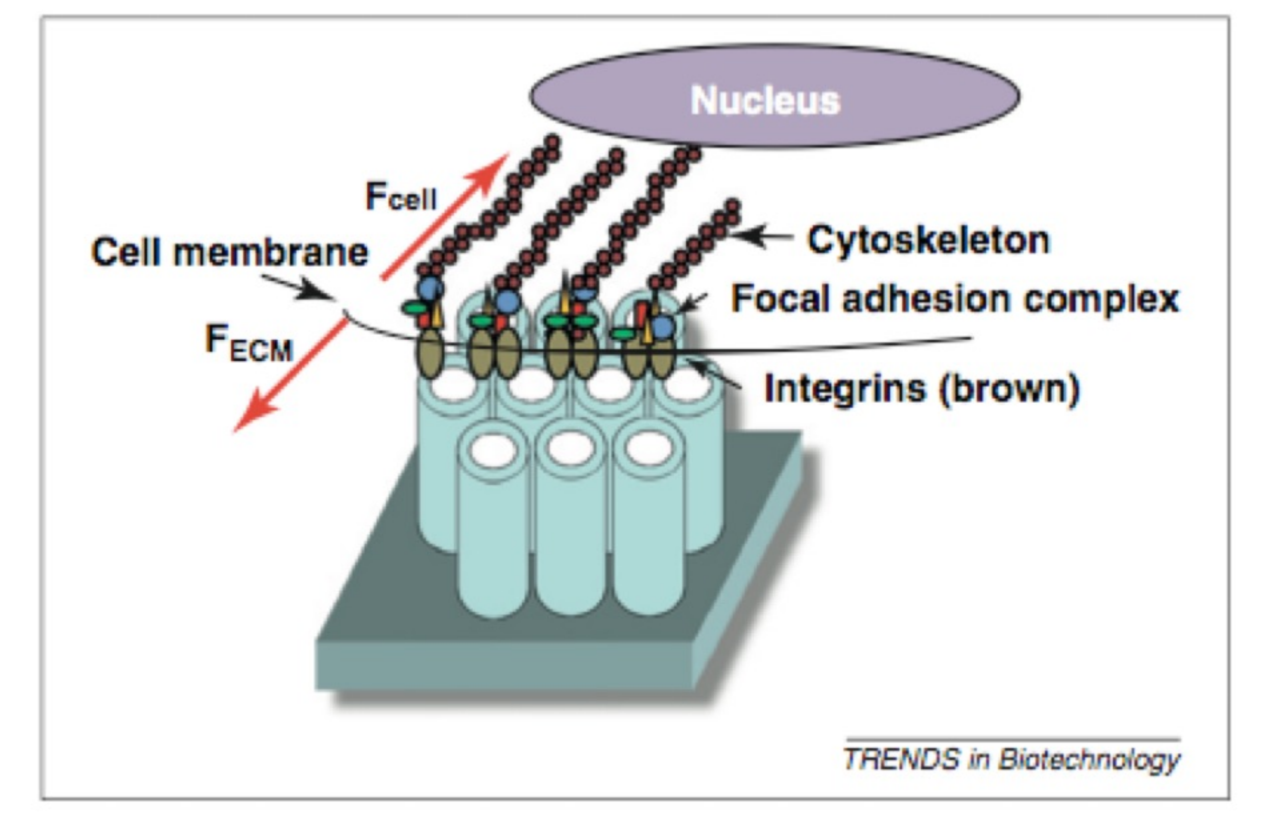


Typical fluorescence image of rhodamine-conjugated fibronectin fibrils reorganized by endothelial cells on poly(propene alt-maleic anhydride) copolymer after 50 min of cell culture.
Scale bar: 10 μm.

T Pompe, C Mitdank, and C Werner, J. Phys.: Condens. Matter, 2004; **16**: S2421-S2426

WHY MICRO/NANOSTRUCTURING?

- To promote the interaction between the material and the cell receptors (integrins).



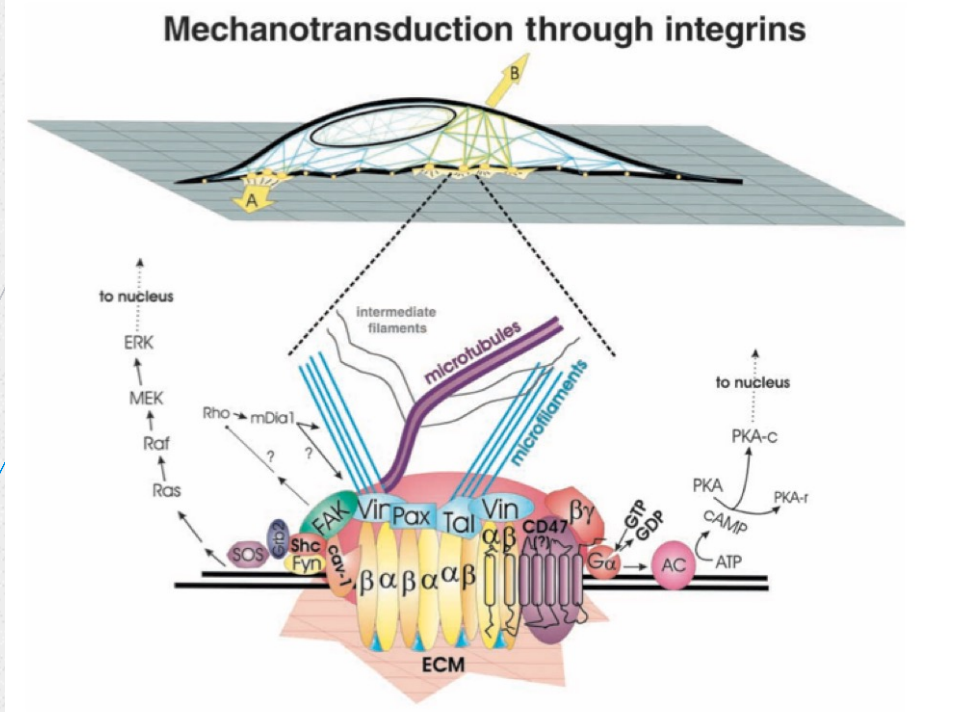
The focal adhesion complex experiences stress from a cell generated contractile force pulling against the extracellular matrix (this role is played by the nanostructured surface).

This stress is transformed trough the cytoskeleton to the nucleus in the form of signalling molecules.

KS Brammer, CJ Frandsen, and S Jin, Trends in Biotechnology, 2012; **30**: 315-322

WHY MICRO/NANOSTRUCTURING

- To mimic the extracellular matrix self-organization upon interaction with cells.

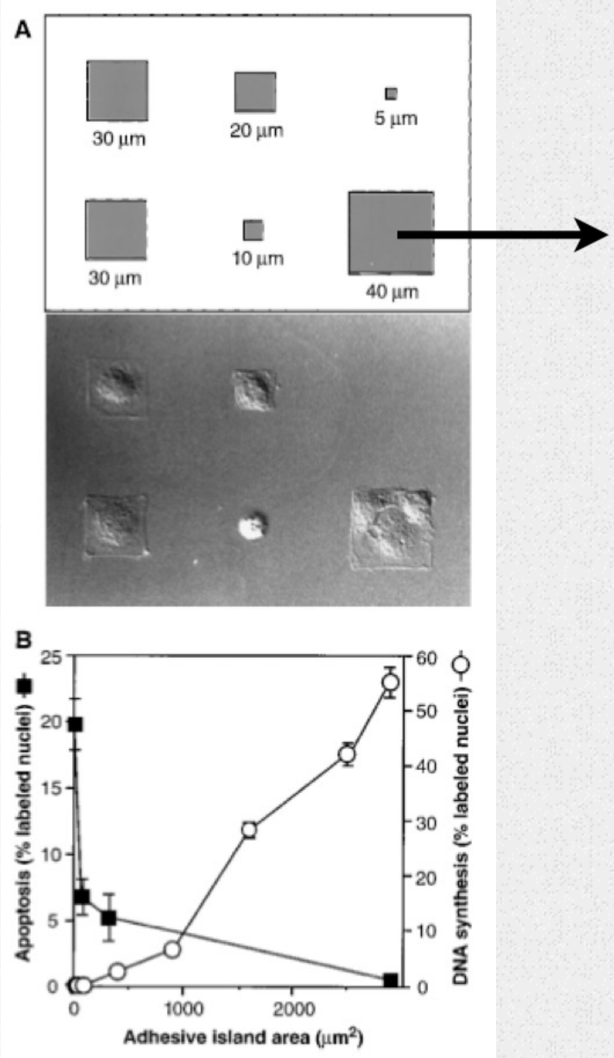


Forces concentrated within the focal adhesion can stimulate clustering of integrins and stimulate recruitment of focal adhesion proteins that form microfilaments.

DE Ingber, J. Cell Sci., 2003; **116**: 1397-1408

Effect of micro/nanostructuring

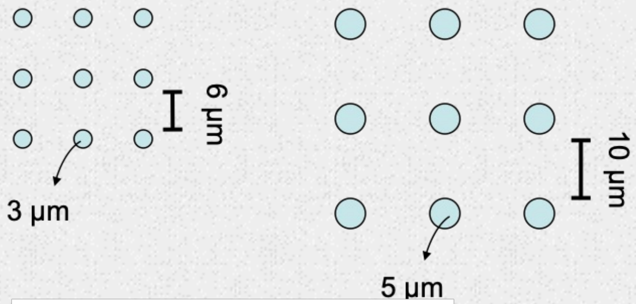
- Increased cell spreading on a homogeneous, high-density coating of FN leads to cell survival and growth.

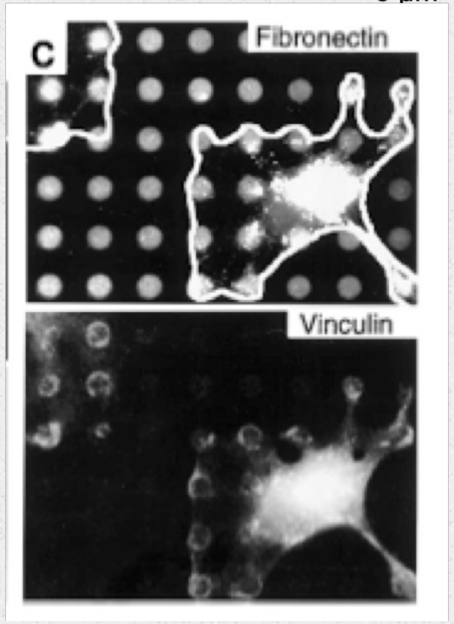


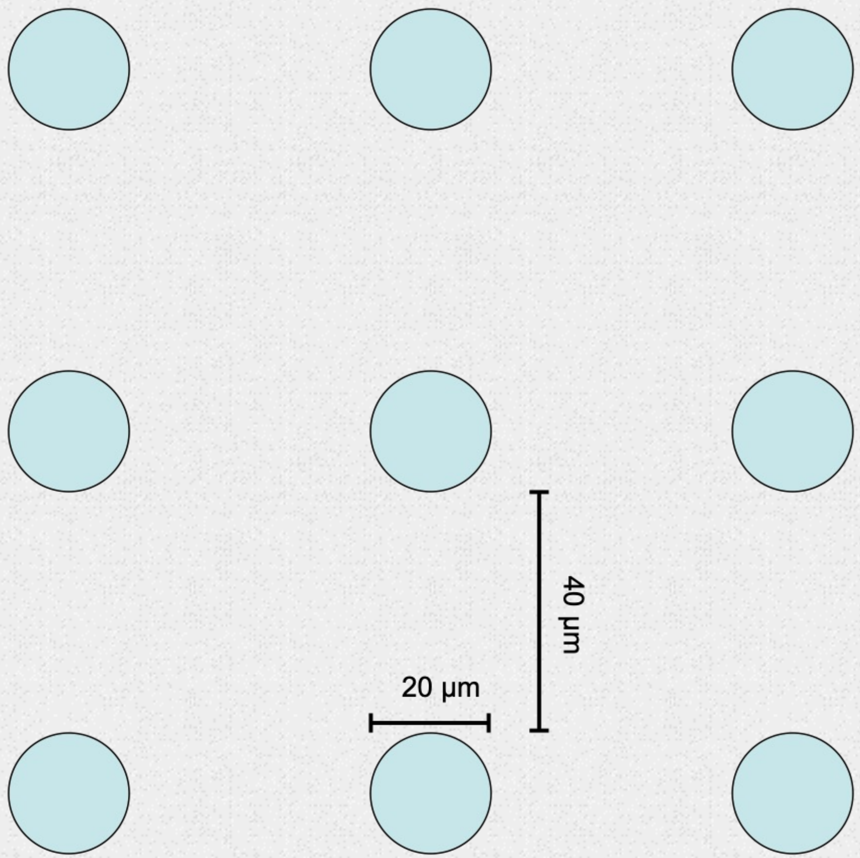
Fibronectin-coated islands

CS Chen, M Mrksich, S Huang, GM Whitesides, and DE Ingber, Science, 1997; **276**: 1425-1428.

Effect of micro/nanostructuring



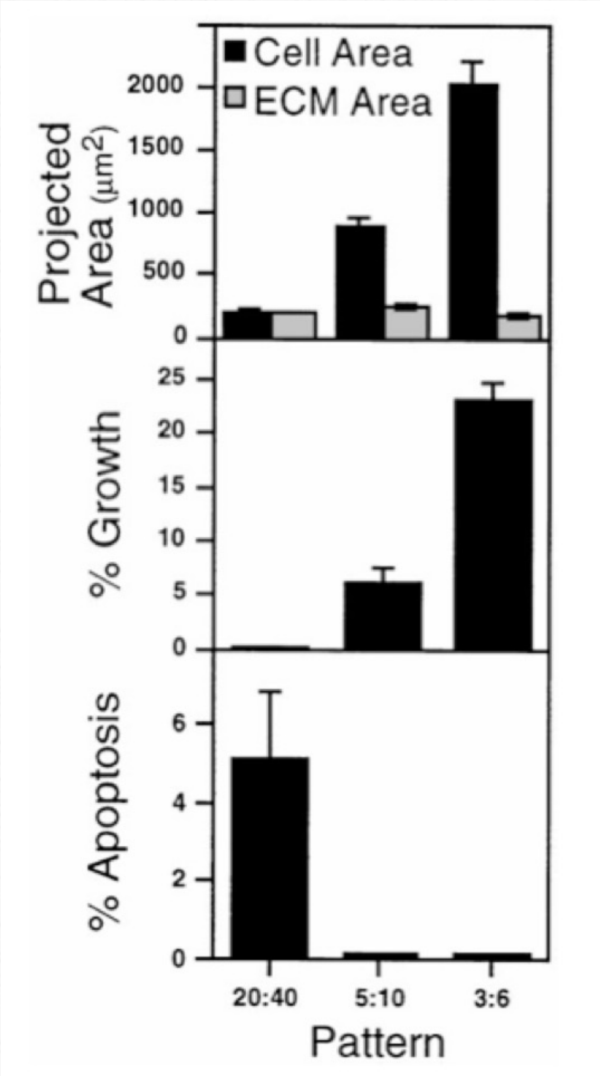


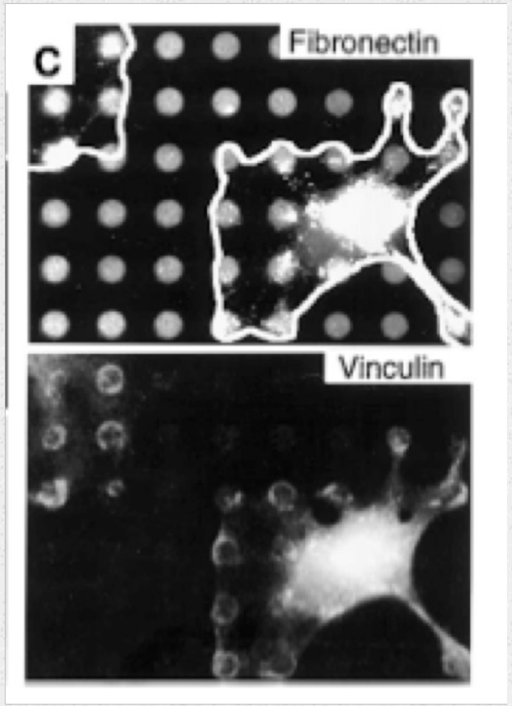


CS Chen, M Mrksich, S Huang, GM Whitesides, and DE Ingber, Science, 1997; **276**: 1425-1428.
RS Kane, S Takayama, E Ostuni, DE Ingber, and GM Whitesides, Biomaterials, 1999; **20**: 2363-2376.

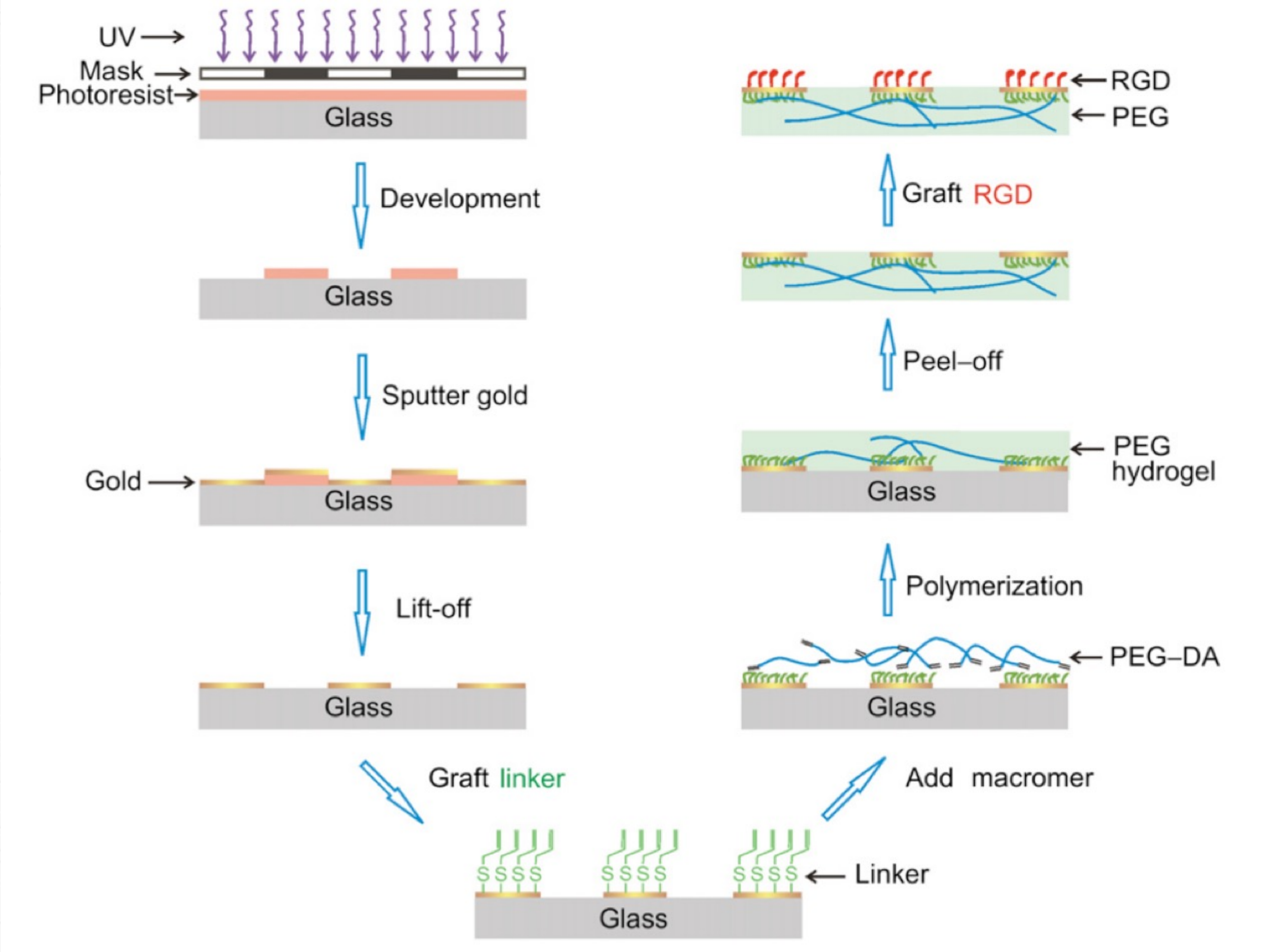
Effect of micro/nanostructuring

- The extent of spreading (the projected surface area of the cell) and not the area of the adhesive contact controlled cell life and death.





Effect of micro/nanostructuring



R Peng, X Yao, and J Ding, Biomaterials, 2011; **32**: 8048-8057.

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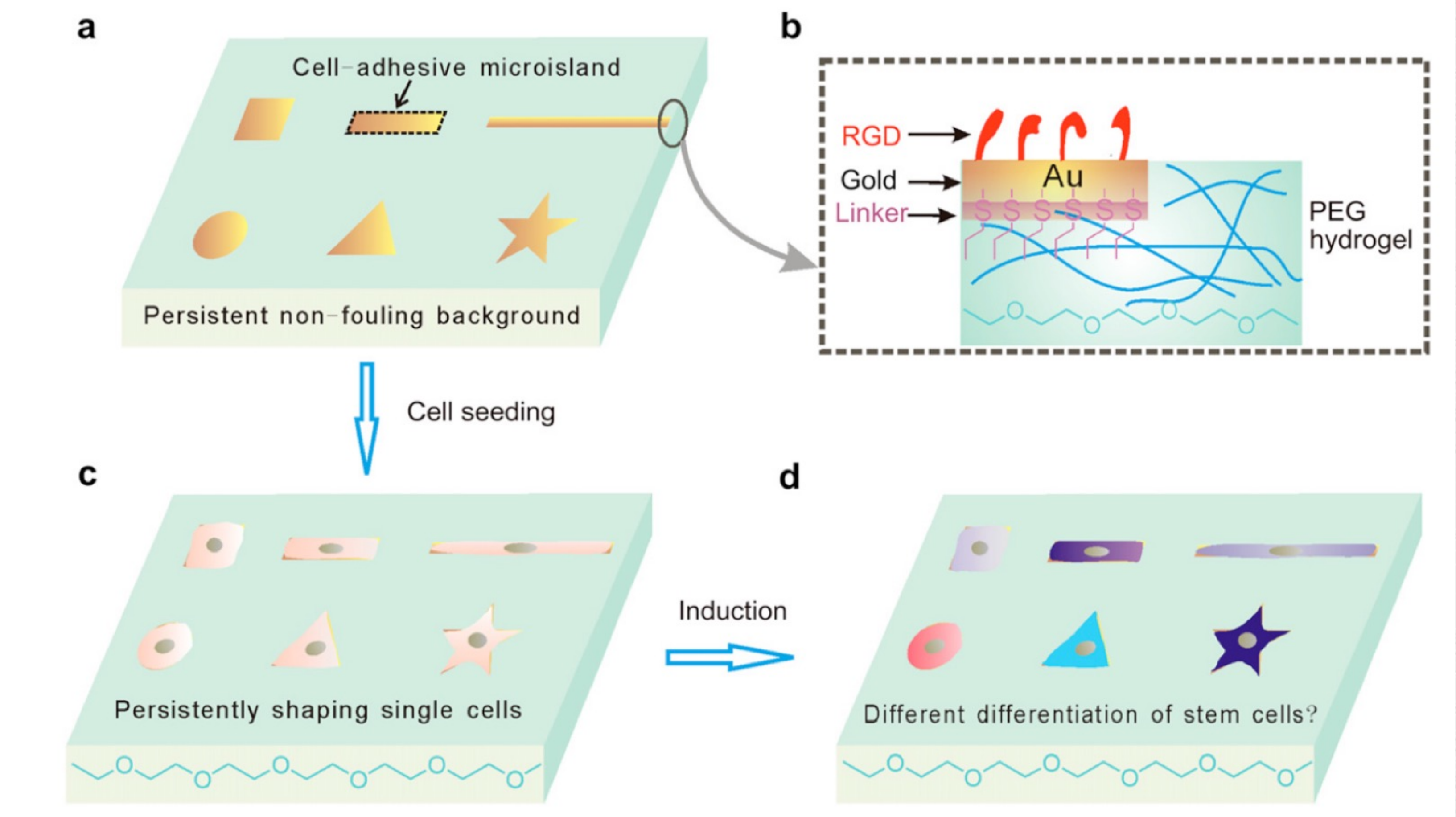

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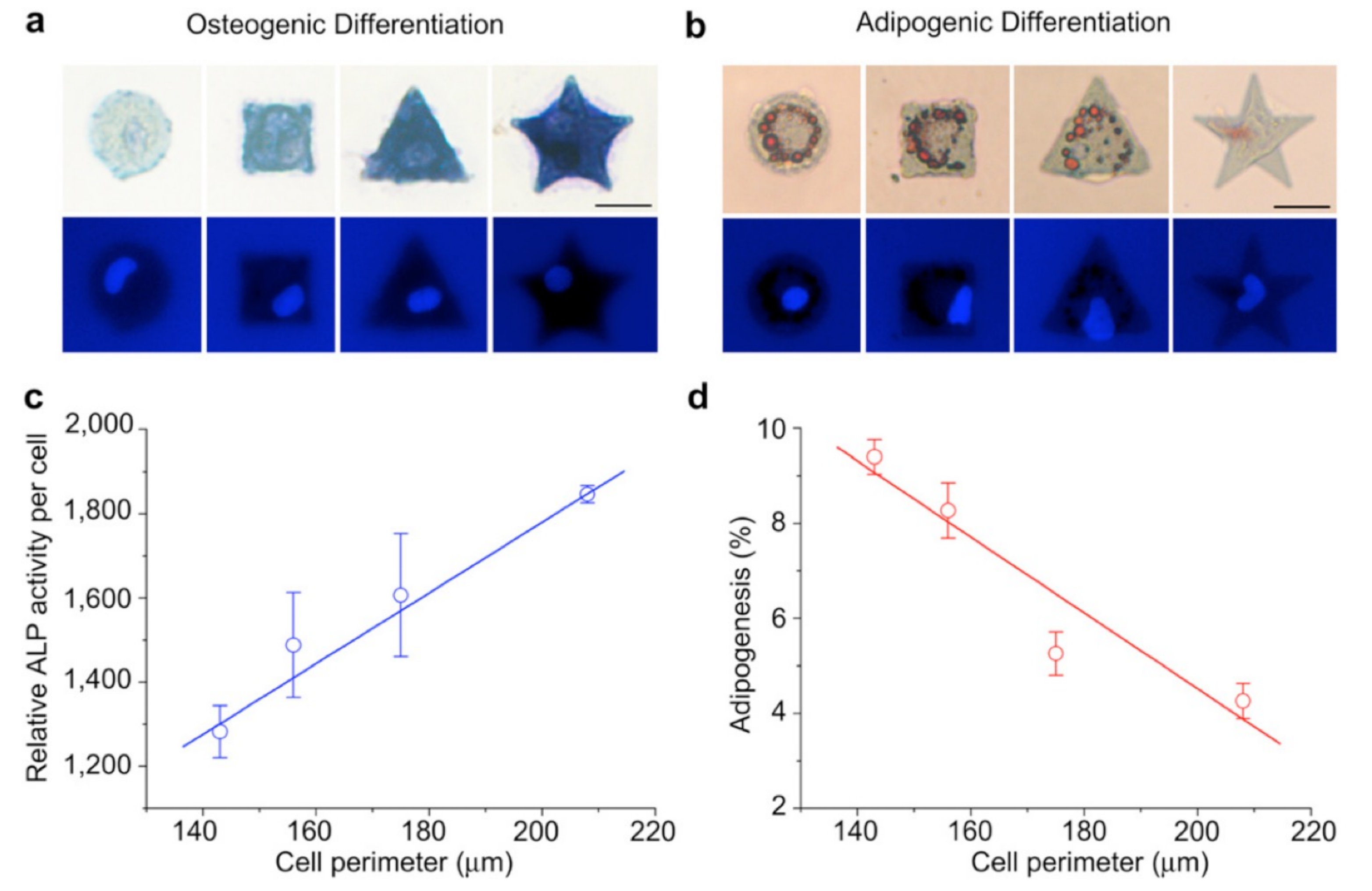

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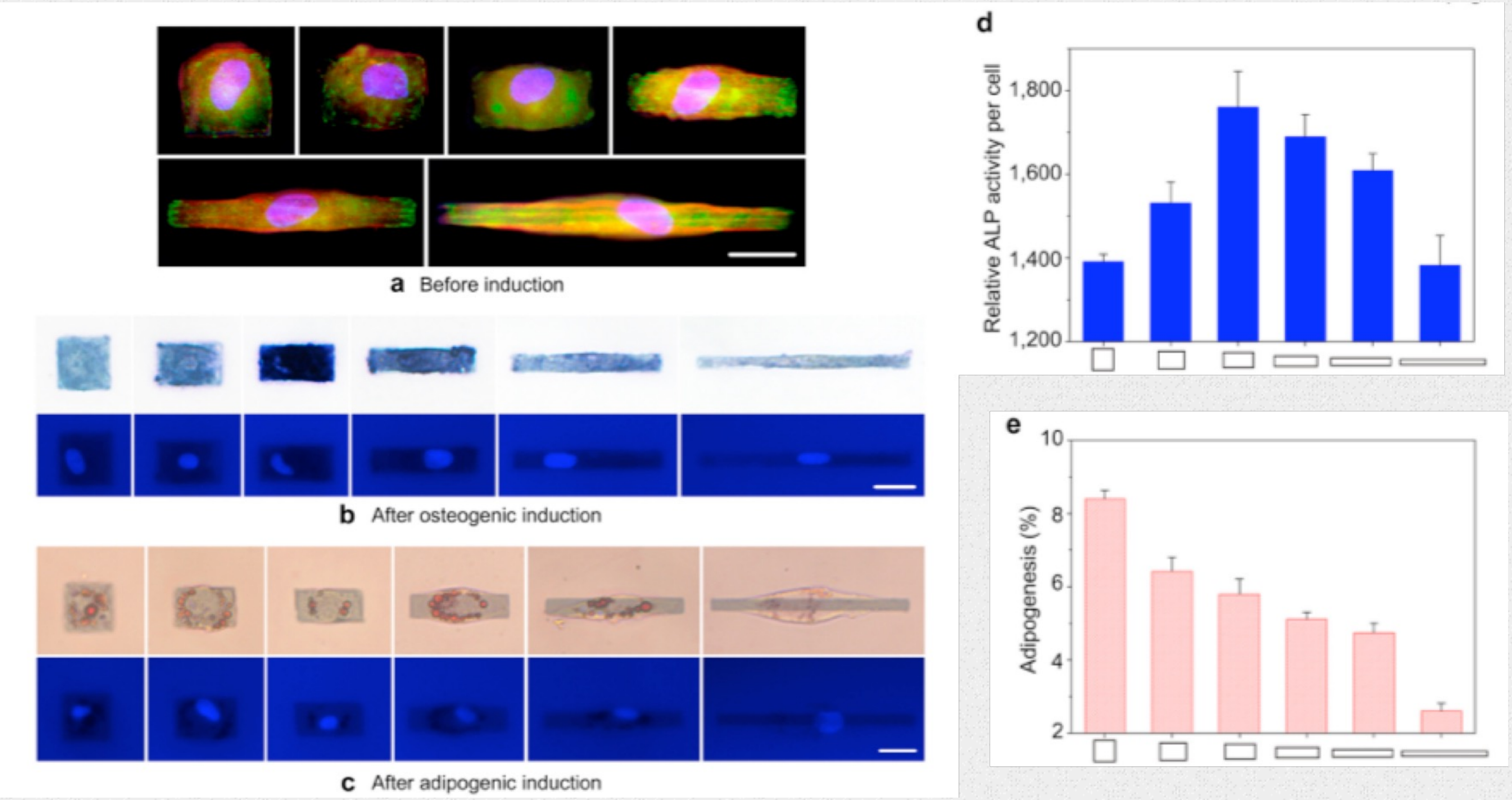

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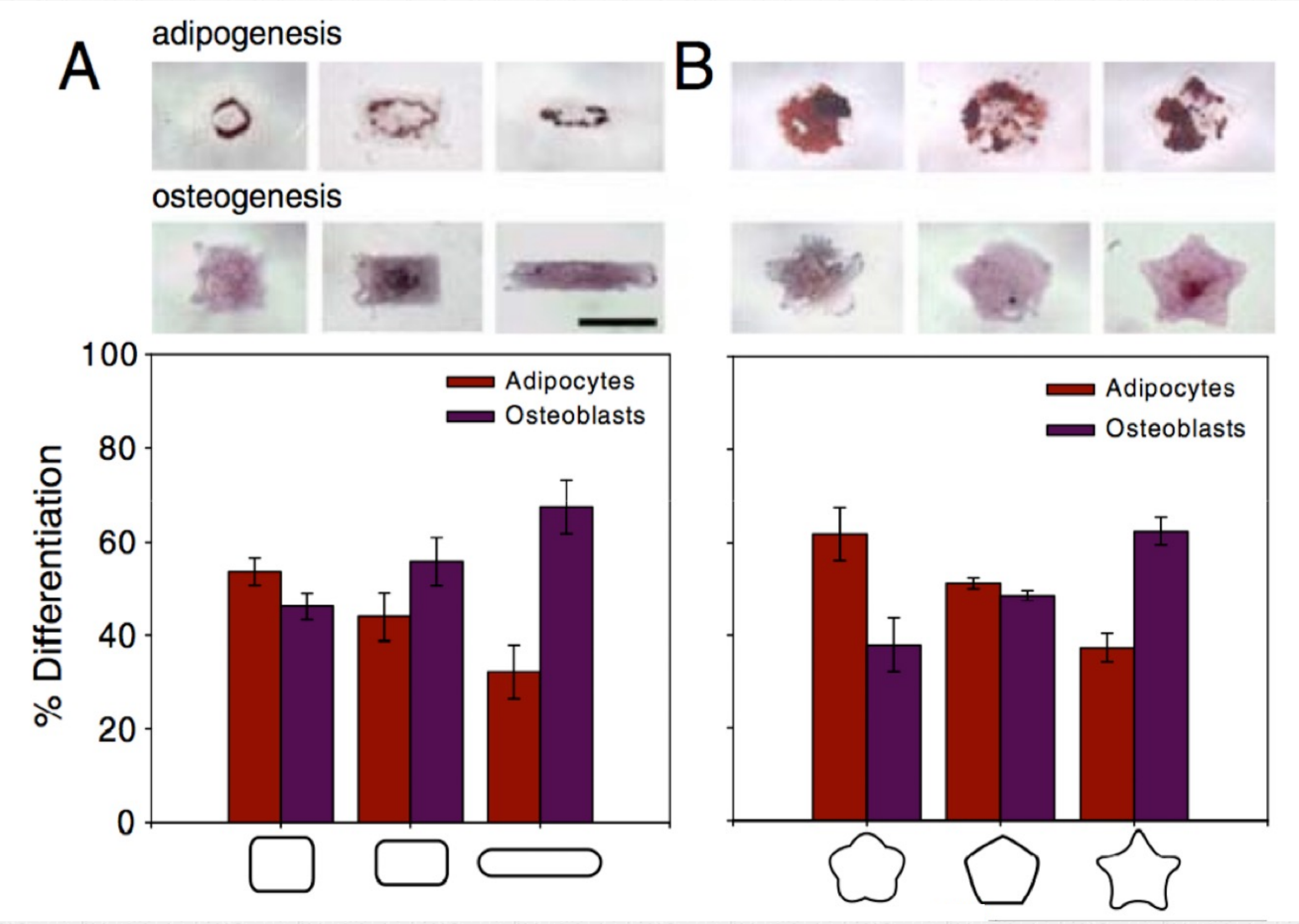

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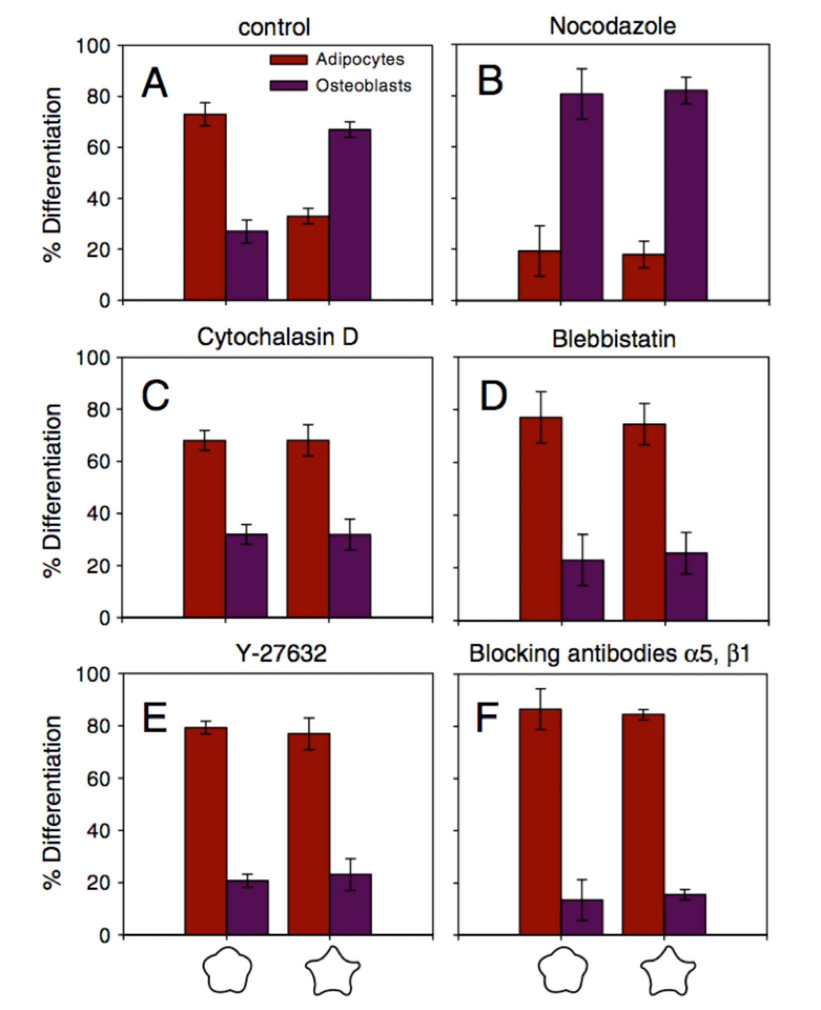
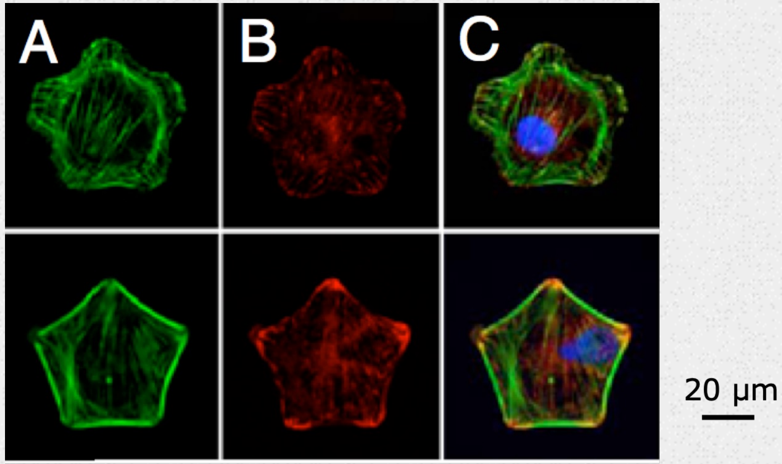
Effect of micro/nanostructuring

Nocodazole: Increases cell contractility

Cytochalasin: Inhibits F-actin polymerization





F-actin

Vinculin

Combined image with nuclei in blue

R Peng, X Yao, and J Ding, Biomaterials, 2011; **32**: 8048-8057.

Over 15 different ECM aspects influence stem cell fate!

Biochemical cues

Ligand bioactivity

(Proteins & growth factors)

Ligand density & gradient

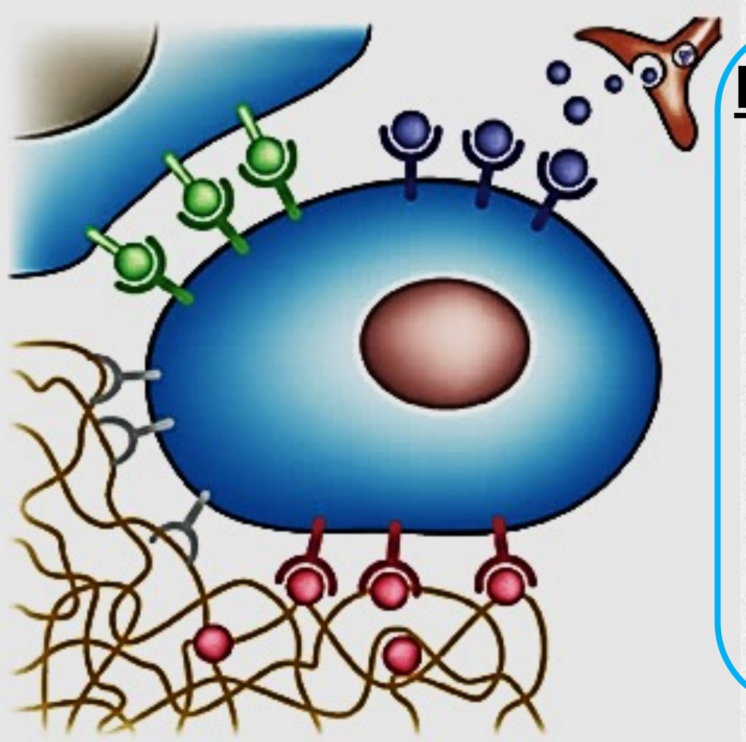
Ligand presentation

(soluble & matrix-bound)

✓ **Ligand interplay**

✓ **Spatial arrangement**

(micro/nano-patterning)



Biophysical cues

Mechanical stimuli

2D/3D structure

Viscoelasticity

Topography

Stiffness

Pore size

Porosity

Cell cues

Shape, dimension, anisotropy, polarity, cell-cell interactions

S. Kobel, M. Lutolf, Biotechniques (2010): **ix-xxii**.

Effect of microstructuring

Objective I

Investigating the effect of ligand interplay (*Parameter 1*) on hMSCs differentiation

Objective II

Investigating the effect of spatially distributed ligands (*Parameter 2*) on hMSCs differentiation

Objective III

Investigating the combinatorial effect of *parameters 1 + 2* on hMSCs differentiation

Ligands

RGD peptide: Cell adhesion

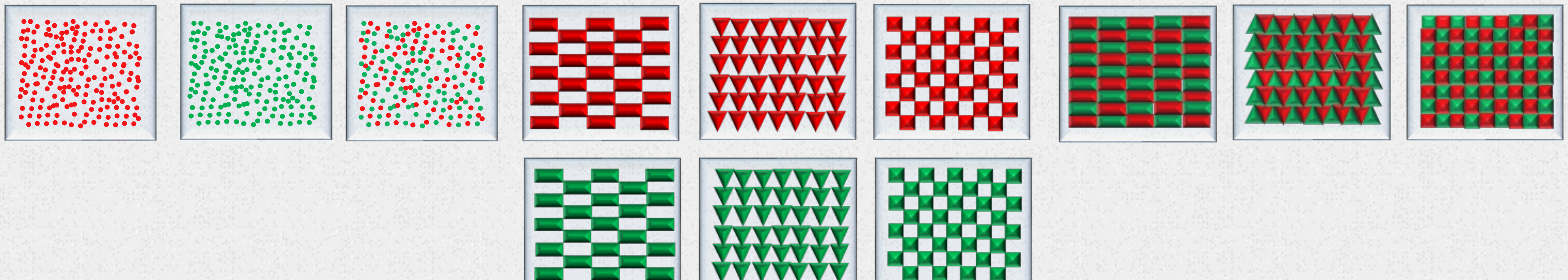
BMP-2 peptide: Osteogenic differentiation

- **Material conditions:**
- RGD-modified surfaces
- BMP-2-modified surfaces
- RGD/BMP-2-modified surfaces

- **Material conditions:**
- RGD micropatterned surfaces
- BMP-2 micropatterned surfaces

- **Material conditions:**
- RGD/BMP-2 micropatterned surfaces

Homogeneous ligand distribution | **Single ligand micropatterning** | **Dual ligand micropatterning**



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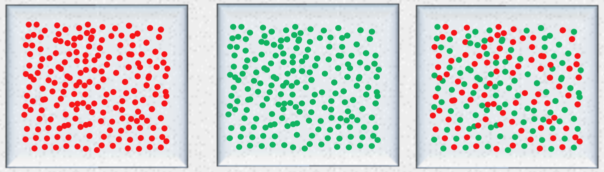

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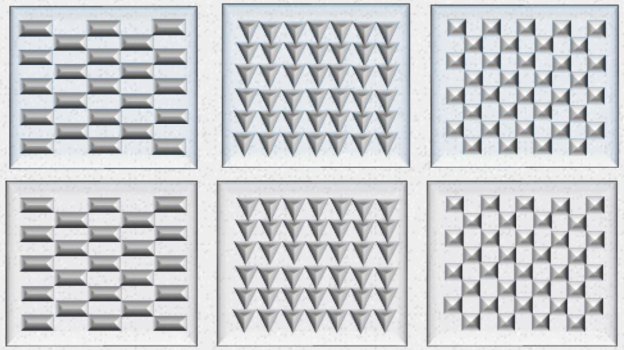
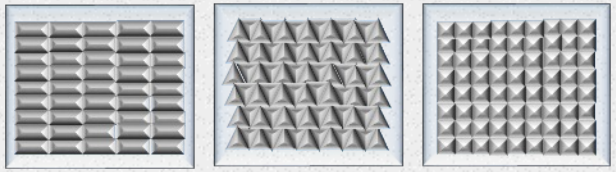
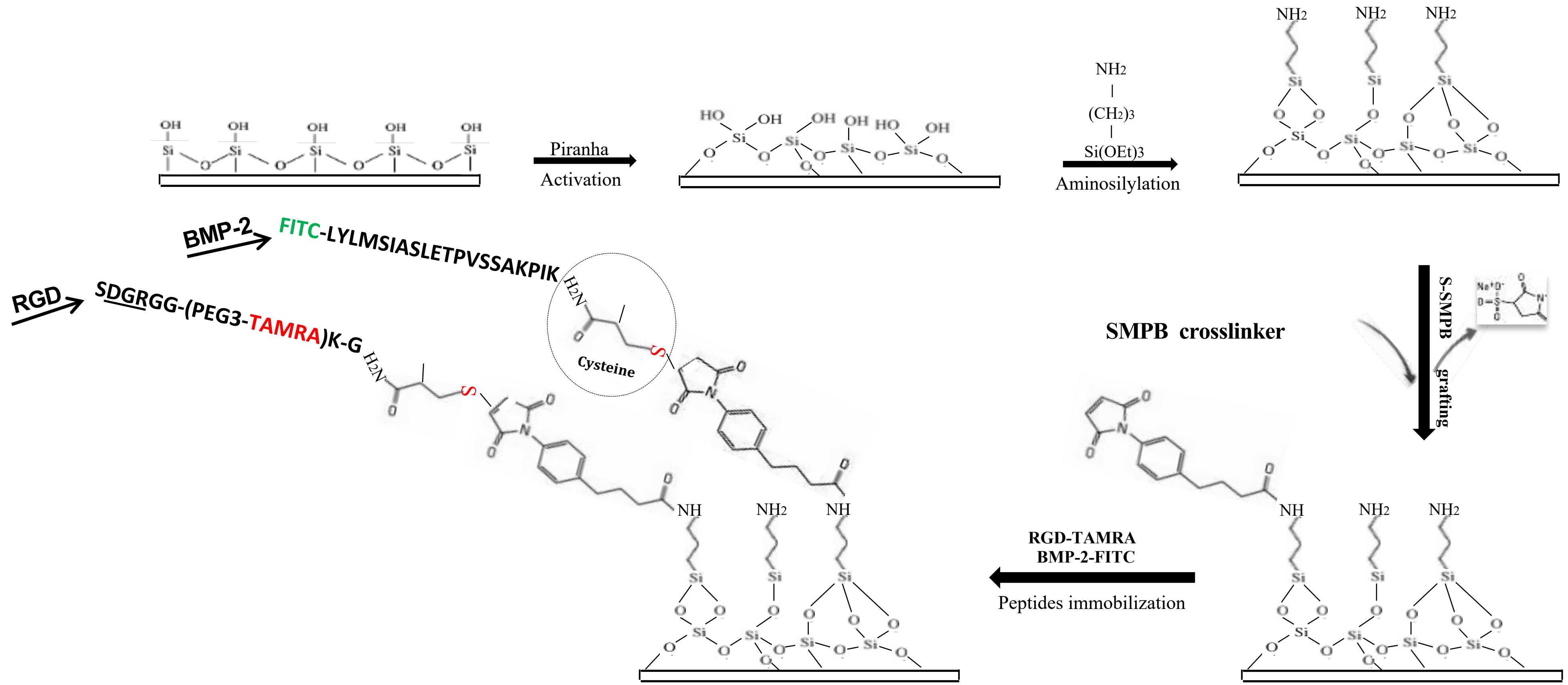


Dual ligand micropatterning



Our strategy for the grafting of bioactive ligands



How to characterize the bioactive surfaces

Methodology: Surface characterization

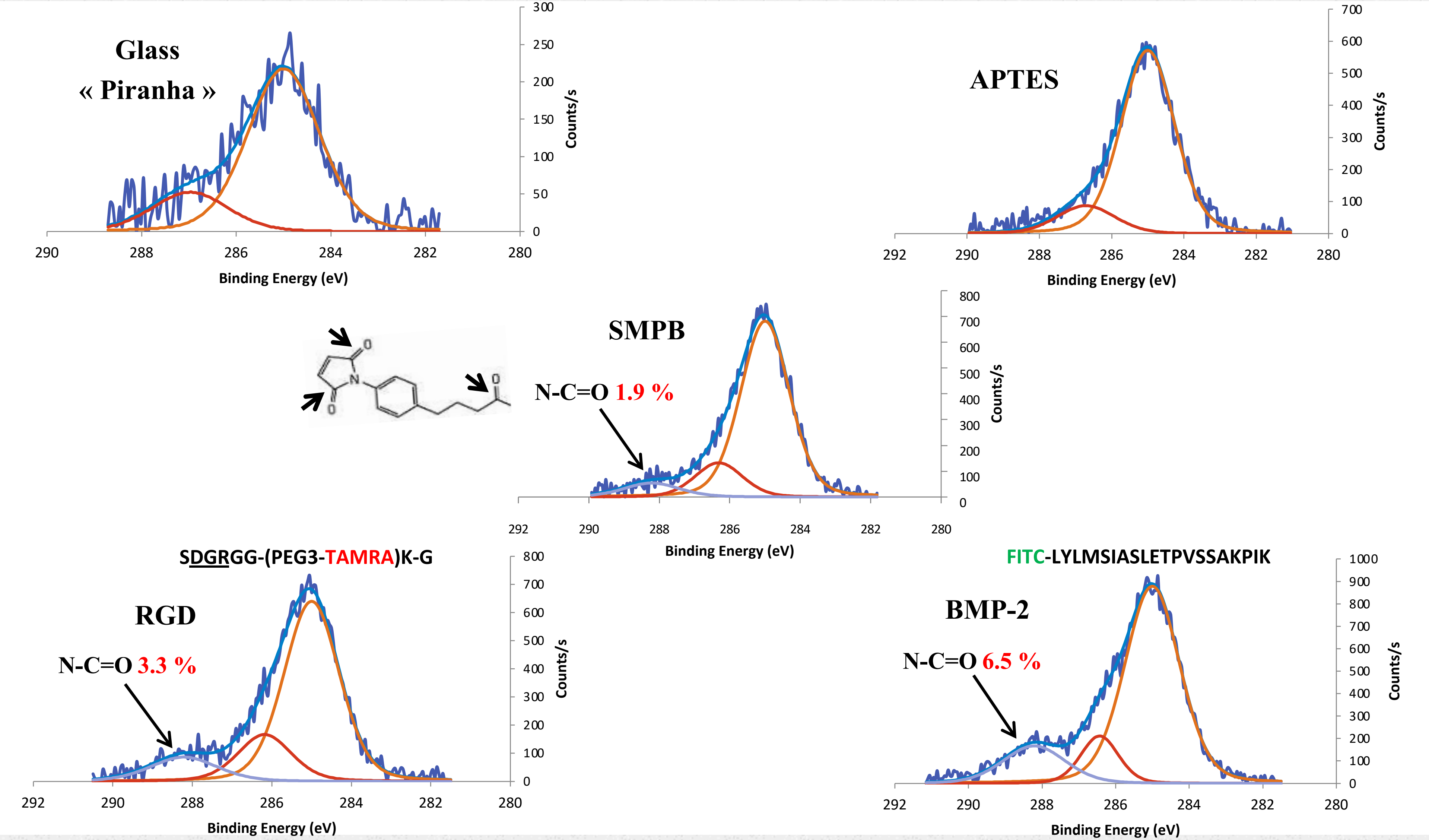
- ➢ **X-Ray photoelectron spectroscopy (XPS)**
  - Surface chemical composition
- ➢ **Fluorescence microscopy**
  - Surface peptide density
  - Peptide imaging
- ➢ **Atomic Force Microscopy (AFM)**
  - Surface topography

How to characterize the bioactive surfaces

Results: XPS survey analyses after each step of peptide grafting

Glass substrate elements (% Si, % O)

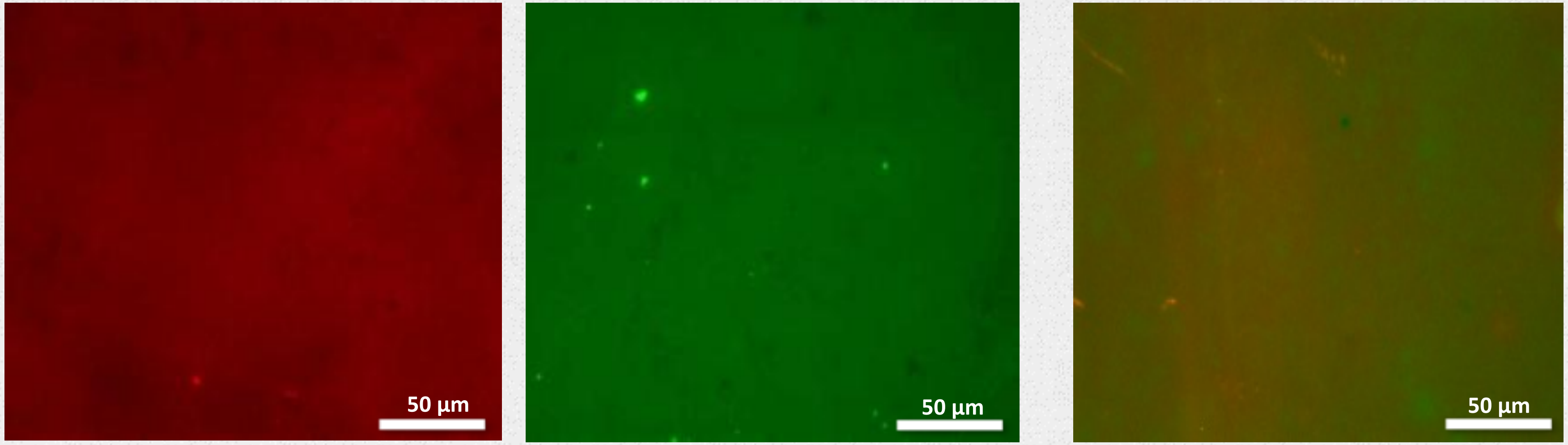
Survey	Glass «Piranha»	APTES	SMPB	RGD	BMP-2
% C	10.0 ± 3.5	19 ± 2	25 ± 1.0	25 ± 2	39 ± 2
% Si	23.7 ± 0.3	22 ± 1	21 ± 1	20.4 ± 0.5	15.2 ± 0.5
% O	65 ± 2	54 ± 1	49 ± 1	50 ± 2	38.2 ± 1.5
% N	–	**1.4 ± 0.4**	**2.2 ± 0.6**	**2.9 ± 0.5**	**5.9 ± 0.3**


Glass
« Piranha »
APTES
SMPB
N-C=O 1.9 %
SDGRGG-(PEG3-TAMRA)K-G
RGD
N-C=O 3.3 %
FITC-LYLMSIASLETPVSSAKPIK
BMP-2
N-C=O 6.5 %
Counts/s
Binding Energy (eV)

How to characterize the bioactive surfaces

Results: **Fluorescence microscopy** (Ligand imaging)

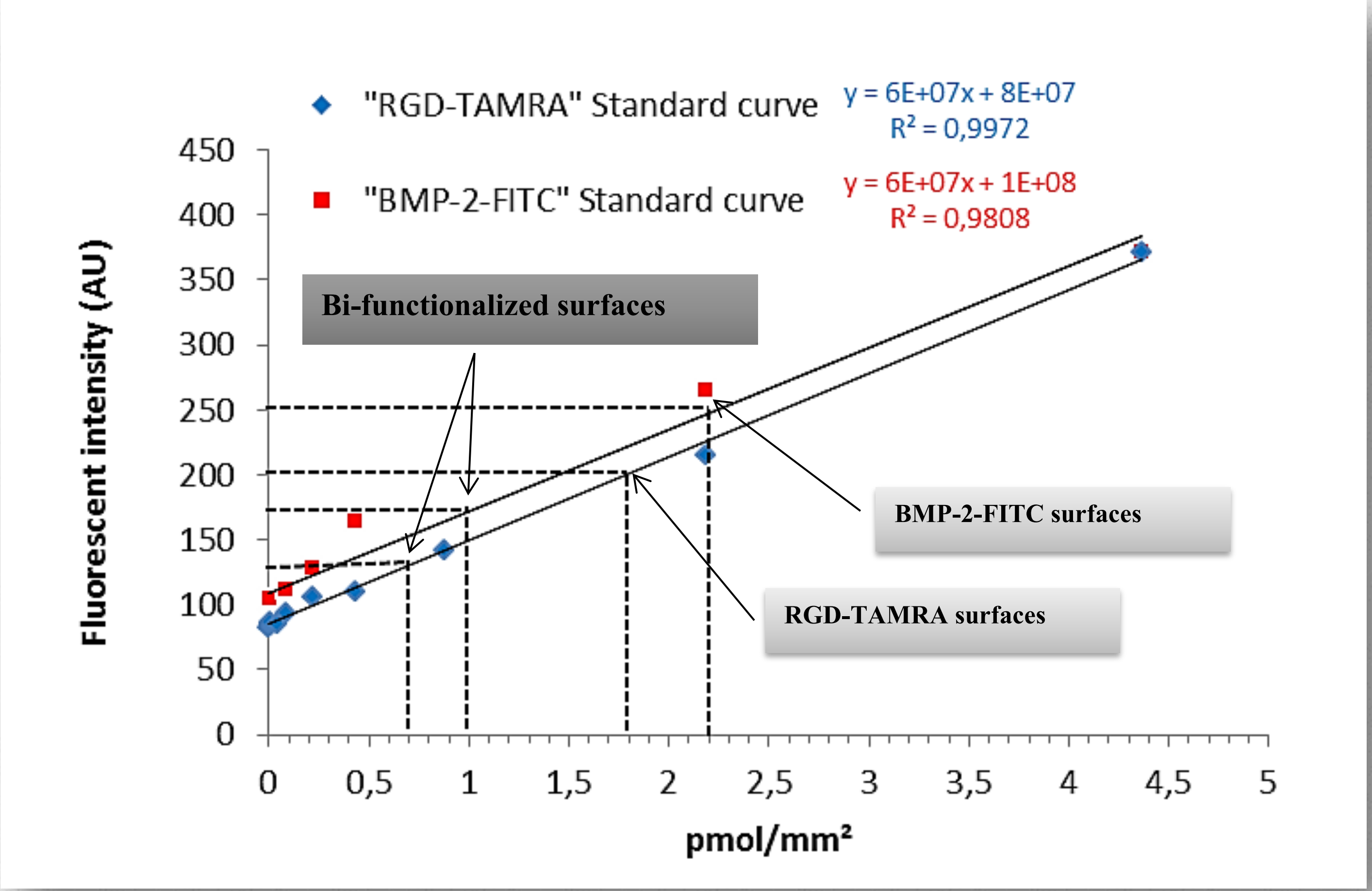
RGD-TAMRA *BMP-2-FITC* *RGD-TAMRA+BMP-2-FITC*



Fluorescent images of RGD-TAMRA and/or BMP-2-FITC peptides randomly grafted on glass surfaces

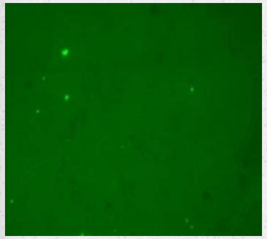
How to characterize the bioactive surfaces

Results: **Fluorescence microscopy** (Surface peptide density)

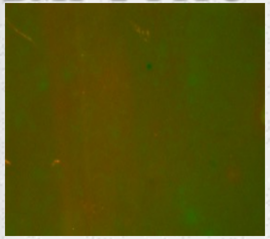


RGD-TAMRA **1.8 ± 0.2 pmol/mm²**

BMP-2-FITC **2.2 ± 0.3 pmol/mm²**

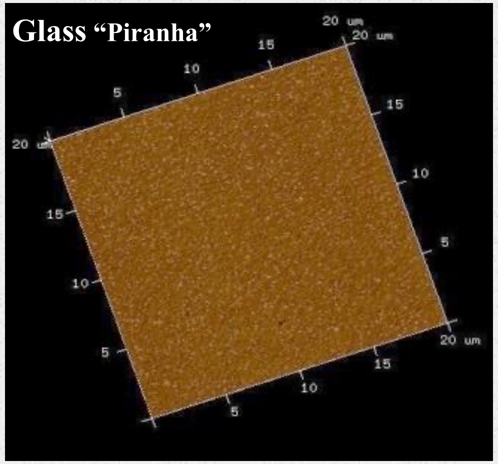


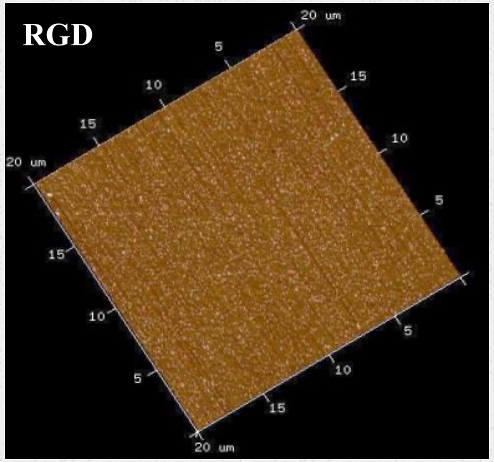
RGD-TAMRA **0.7 ± 0.1 pmol/mm²**
+
BMP-2-FITC **1 ± 0.1 pmol/mm²**

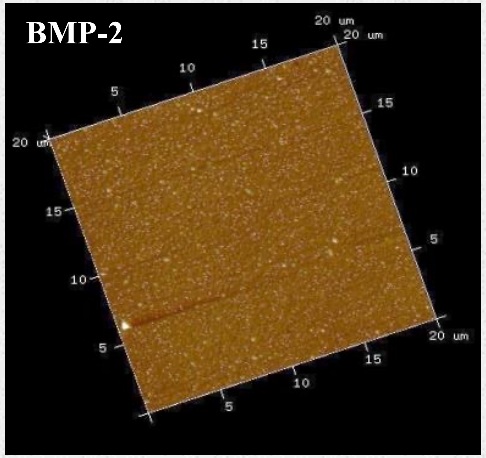


How to characterize the bioactive surfaces

Results: Atomic Force Microscopy (AFM) (tapping mode)







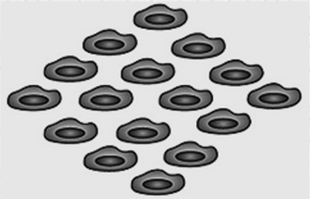
Tapping mode AFM images on bare glass and peptide modified surfaces. *Height scale 60nm.*

	Glass "Piranha"	APTES	SMPB	RGD	BMP-2
R_{rms} *(nm)*	**1.1 ± 0.1**	1.4 ± 0.2	1.3 ± 0.3	**2.2 ± 0.2**	**2.5 ± 0.4**

Cell culture Experiments

Methodology: Lineage-specific differentiation

Patient's iliac crest aspirate



Human mesenchymal stem cells (hMSCs)

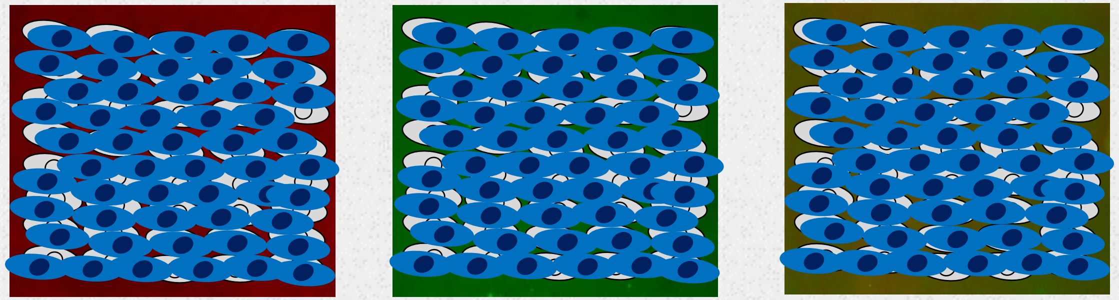
BM: α-MEM + 10 % FBS + 1% ATB

Differentiation markers

Mesenchymal stem cells
Osteoblasts (bone cells)

Immunofluorescence staining
- STRO-1
- Runx-2
- OPN

RGD-TAMRA | *BMP-2-FITC* | *RGD-TAMRA* + *BMP-2*



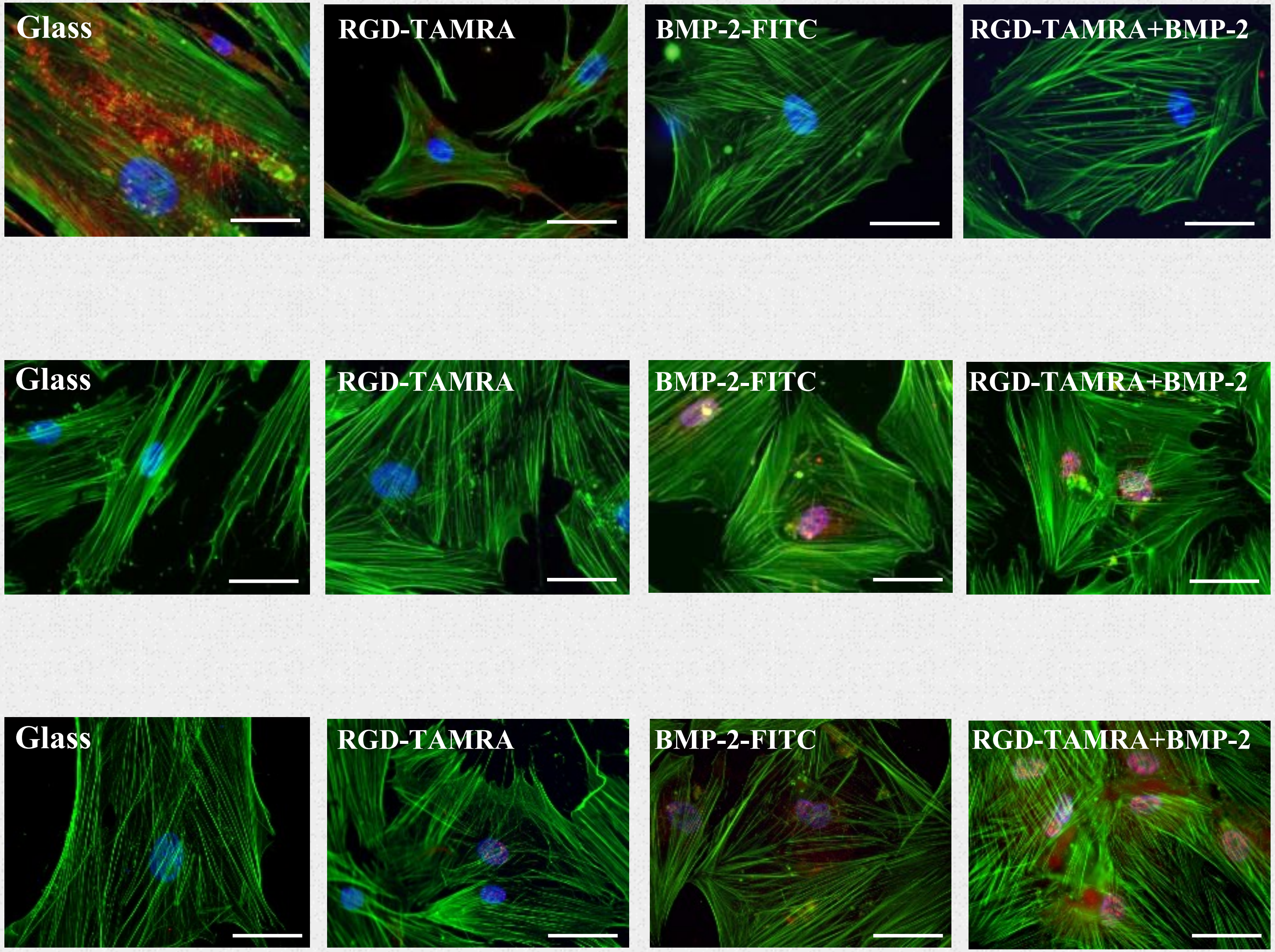
4 weeks of culture without osteogenic media

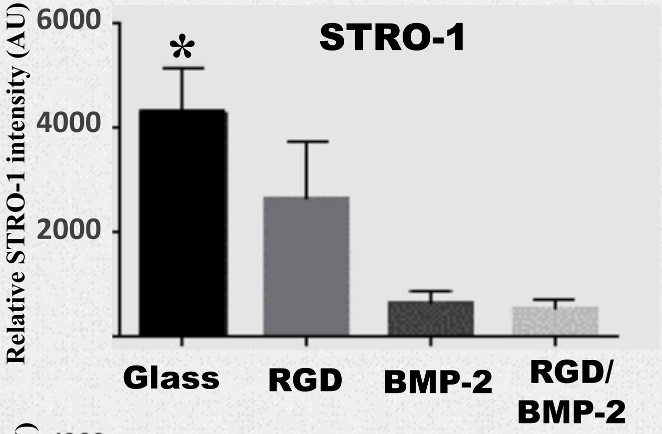
Results: Stem cells & osteogenic markers expression

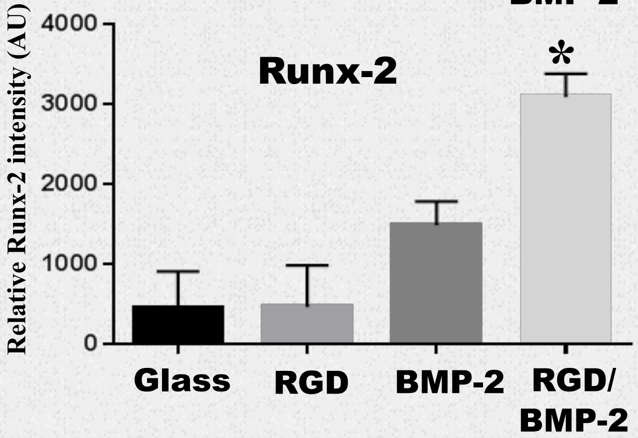
STRO-1

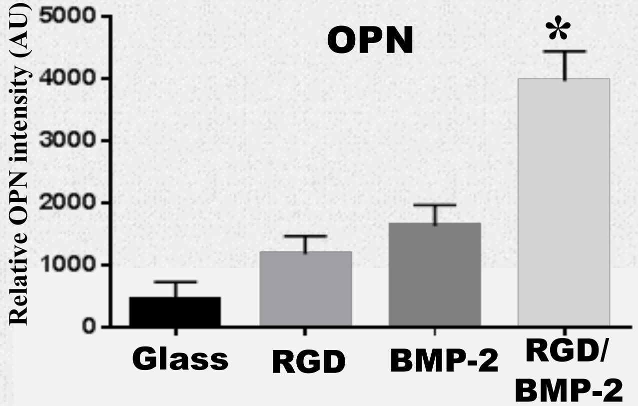
Runx-2
(Early marker)

OPN
(Late marker)





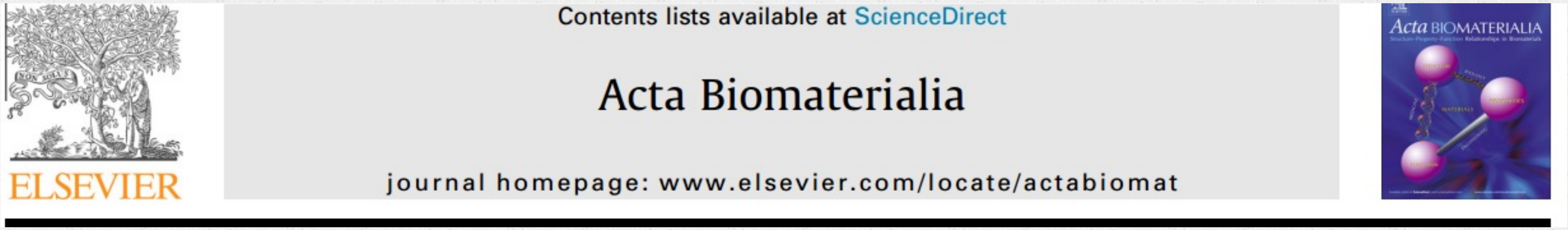




Scale bar: 50 μm^2 *P<0.01*

Conclusion

- ✓ **RGD** and **BMP-2** mimetic peptides **act synergistically to improve** the osteogenic differentiation of hMSCs, **without need of any osteogenic supplements** in the medium.



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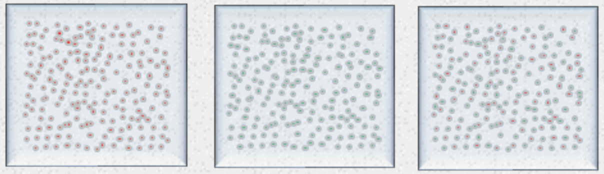
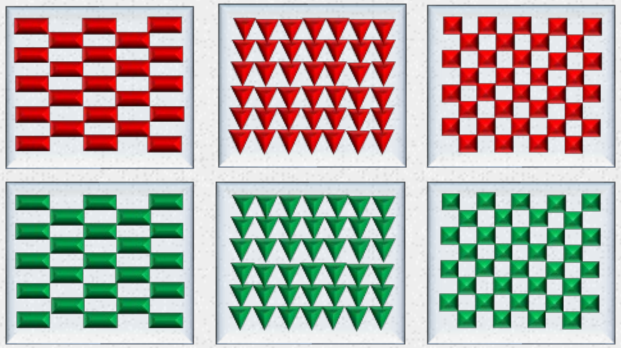
Homogeneous ligand distribution



Single ligand micropatterning



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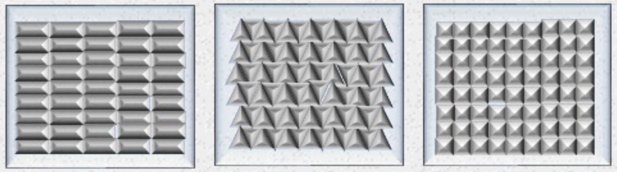
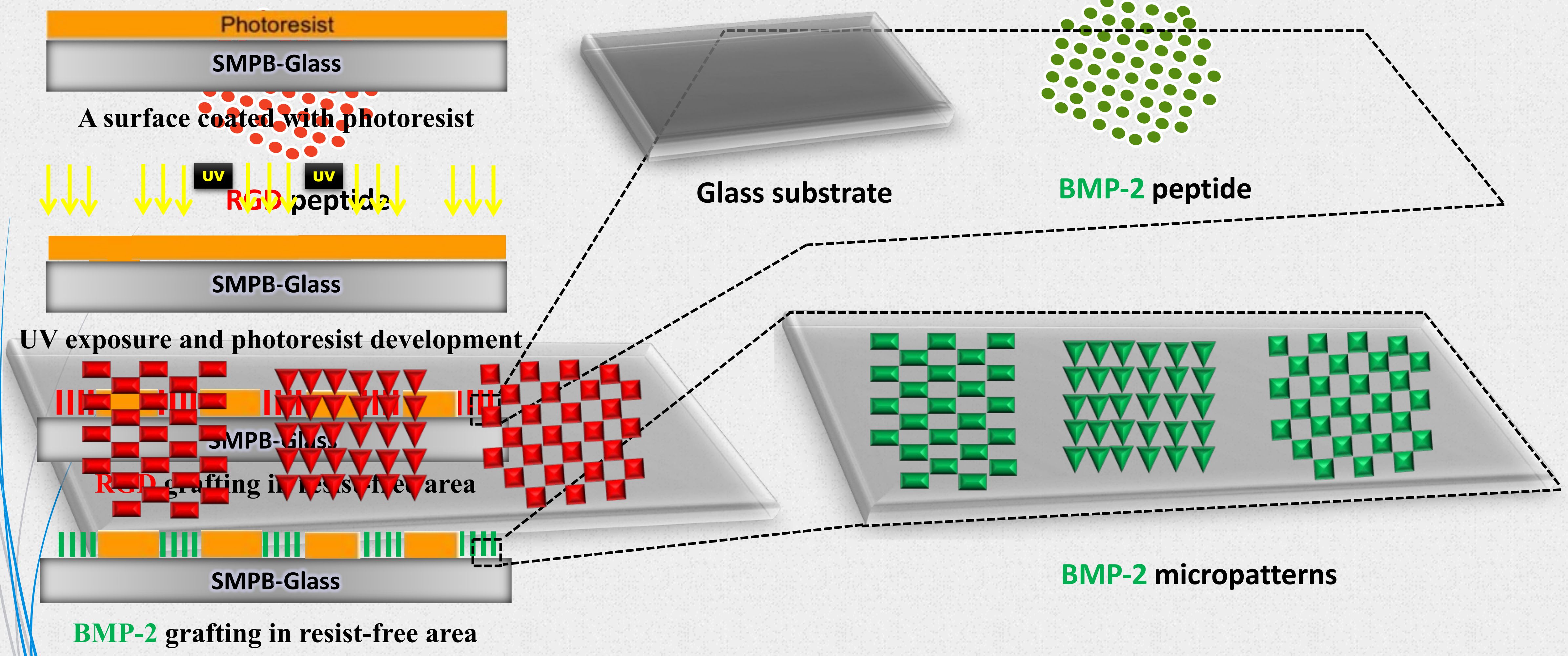


Effect of microstructuring

Methodology: Approach of ligand micropatterning

Photolithography



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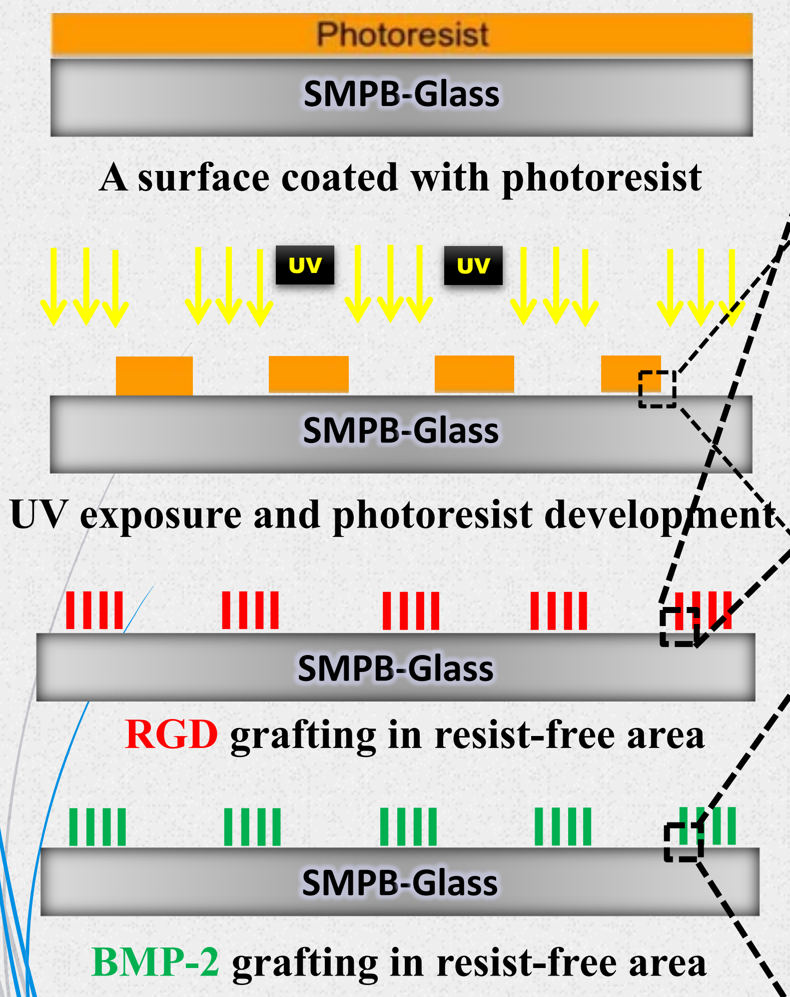

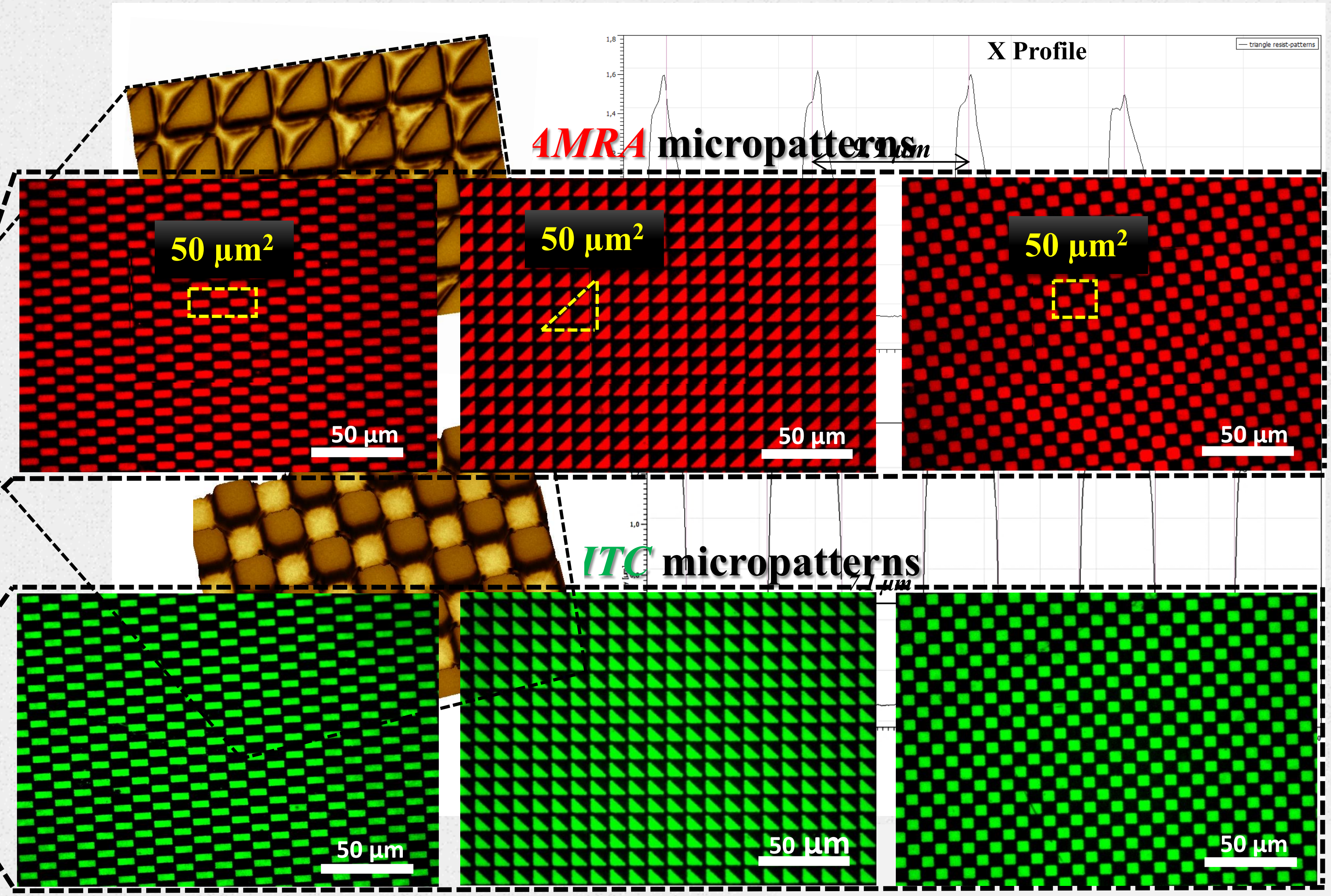

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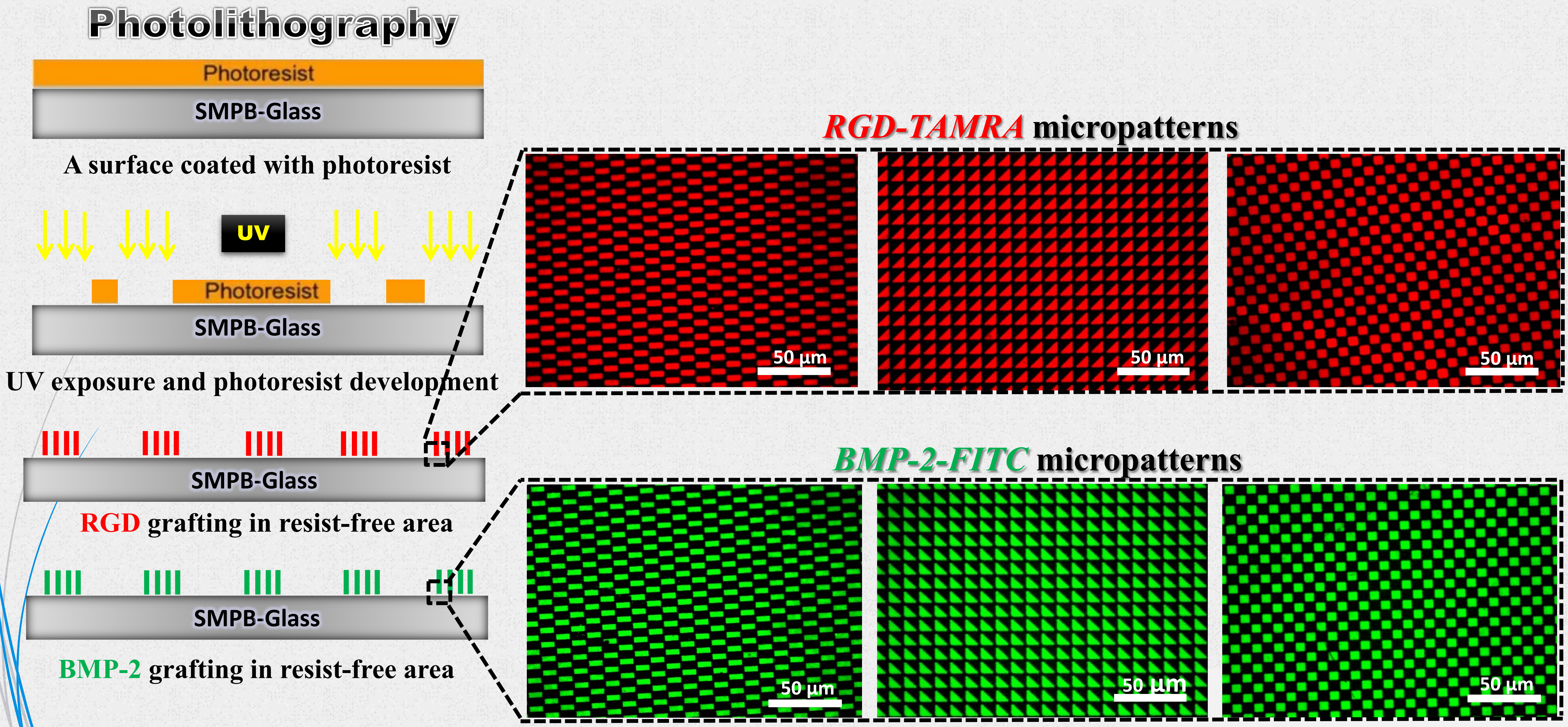

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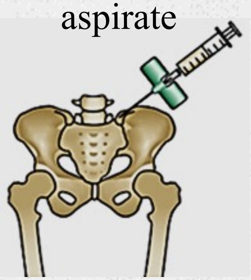
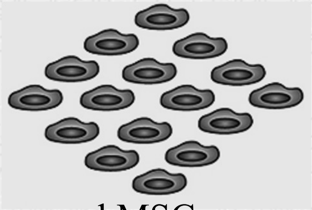
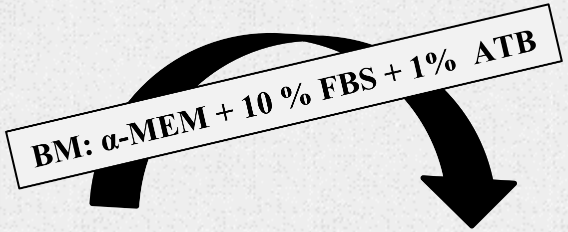
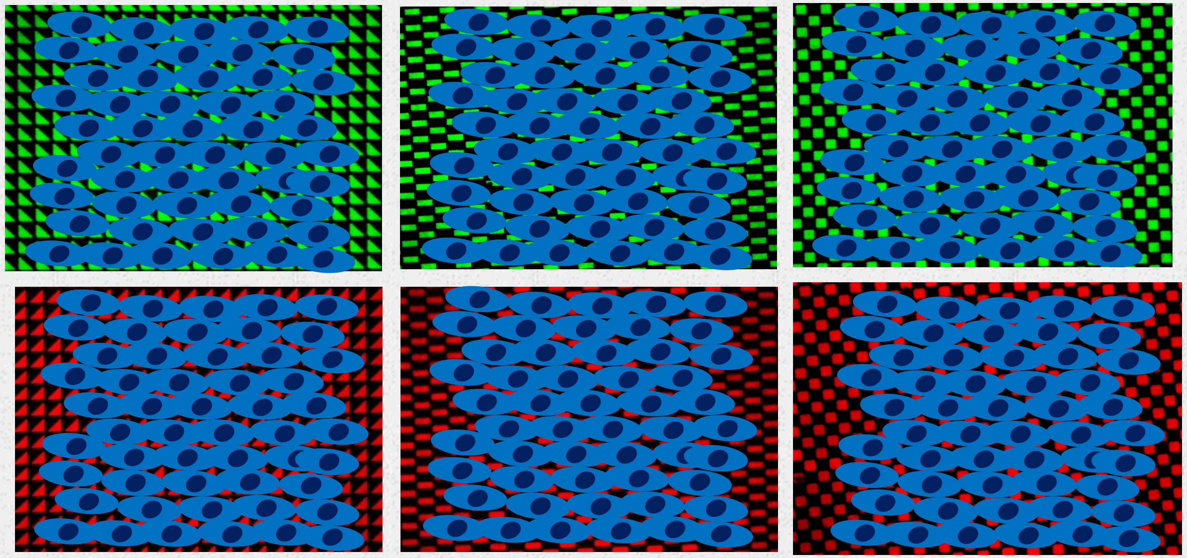




hMSCs



Triangular patterns | Rectangular patterns | Square patterns



Differentiation markers

Osteoblasts (bone cells)

Immunofluorescence staining:
- STRO-1 ⊢
- Runx-2 ✓
- OPN ✓

Colorimetric assay:
- ALP ✓

4 weeks of culture without osteogenic media

Cell culture Experiments

Results: Stem cells & osteogenic markers expression

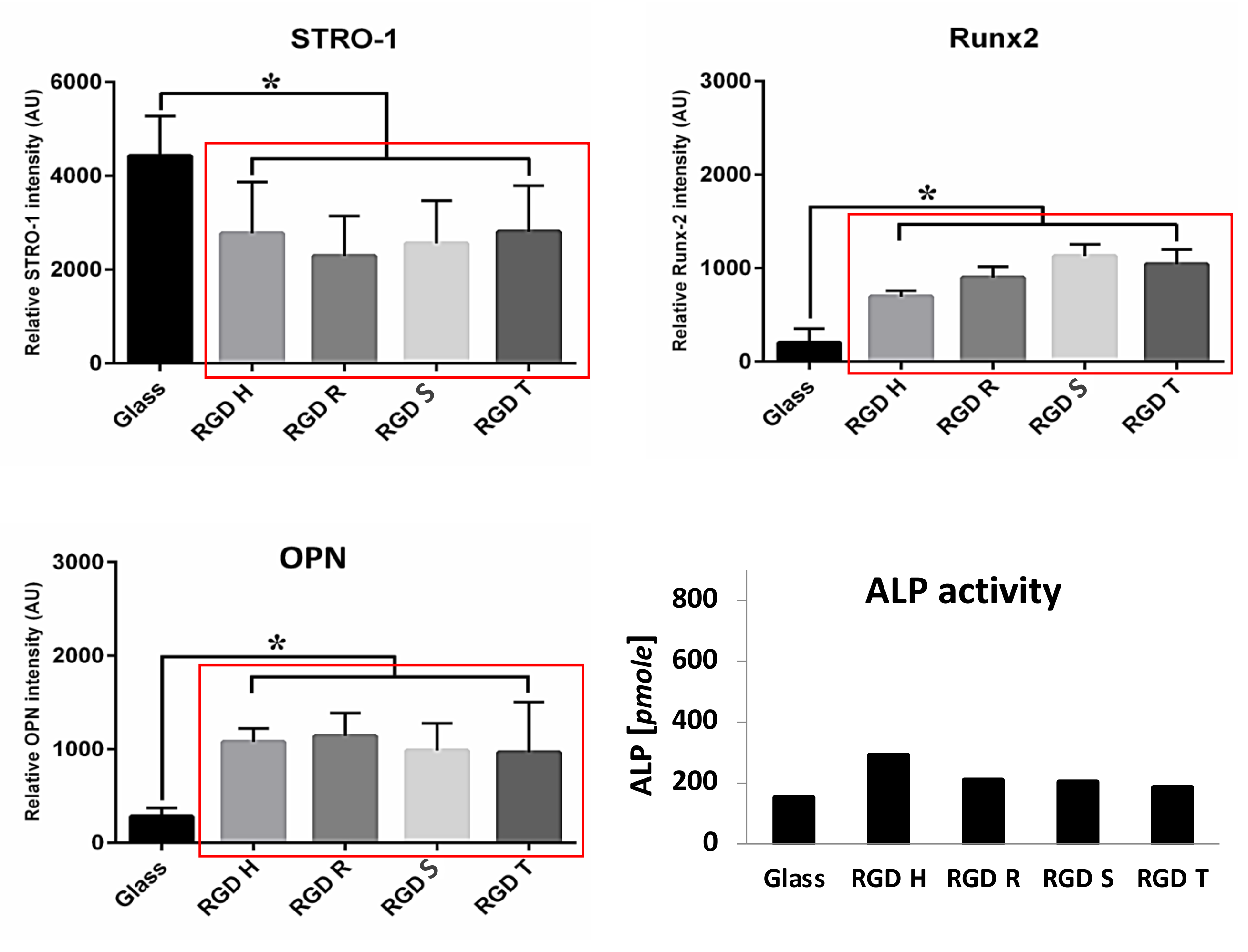
RGD micropatterns / hMSCs differentiation

H: Homogenous grafting

R: rectangular micropatterns

S: square micropatterns

T: triangular micropatterns



Cell culture Experiments

Results: Stem cells & osteogenic markers expression

BMP-2 micropatterns / hMSCs differentiation

H: Homogenous grafting

R: rectangular micropatterns

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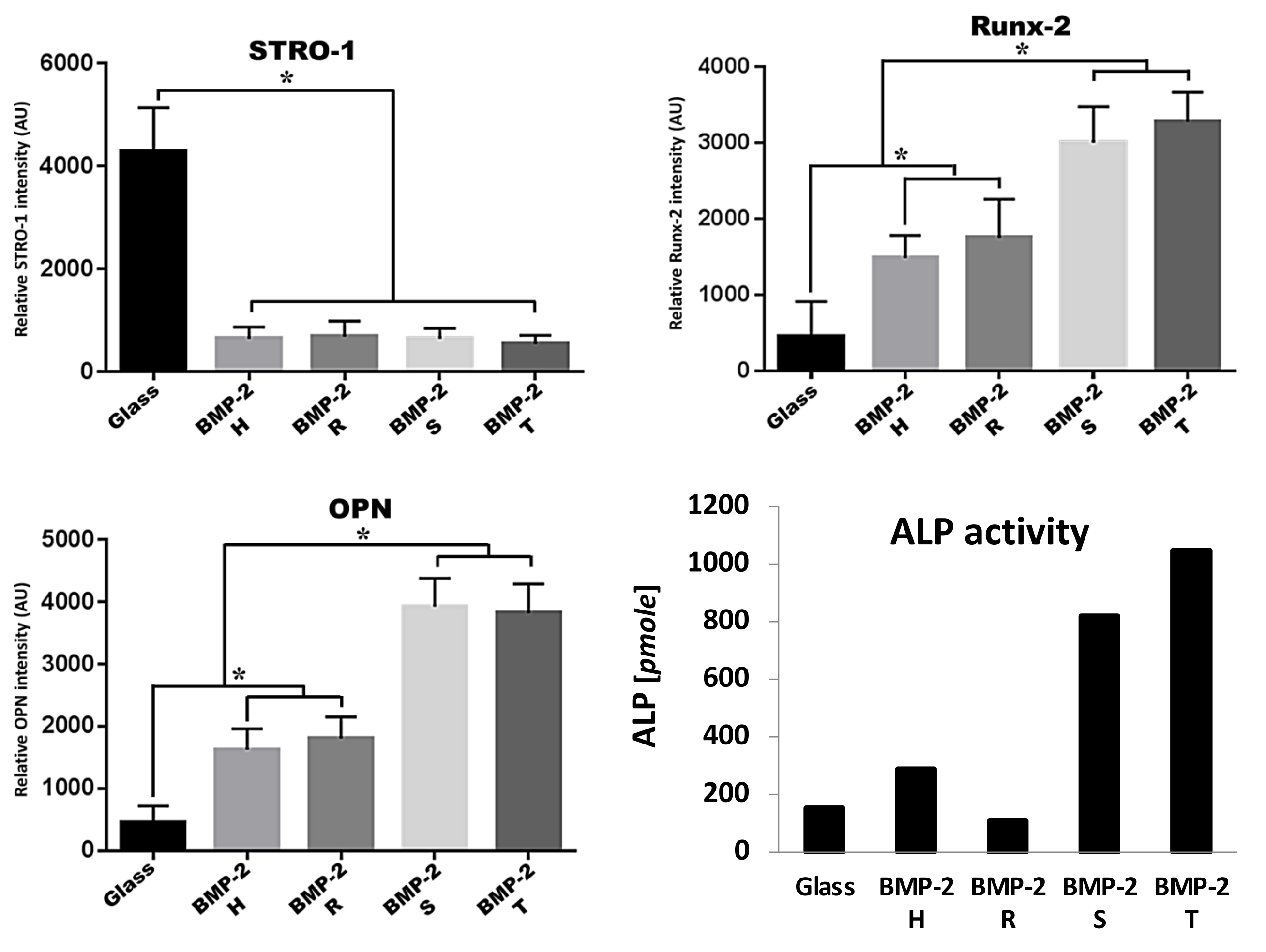
T: triangular micropatterns



Conclusion

- ✓ **RGD** peptide **micropatterning** did **not affect** the expression of both stem cells (STRO-1) and osteogenic markers (Runx-2, Osteopontin, ALP).

- ✓ The osteogenic effect of **BMP-2 peptide micropatterning** was **pattern shape dependent**, with **triangular** and **square micropatterns** as potent effectors of stem cell fate.

Journal of Biomedical Materials Research PART A

Society For Biomaterials

JOURNAL OF BIOMEDICAL MATERIALS RESEARCH A
2018, 106A (4)

Objective I

Investigating the effect of ligand interplay (*Parameter 1*) on hMSCs differentiation

Objective II

Investigating the effect of spatially distributed ligands (*Parameter 2*) on hMSCs differentiation

Objective III

Investigating the combinatorial effect of ***parameter 1 + 2*** on hMSCs differentiation

Ligands

RGD peptide: Cell adhesion

BMP-2 peptide: Osteogenic differentiation

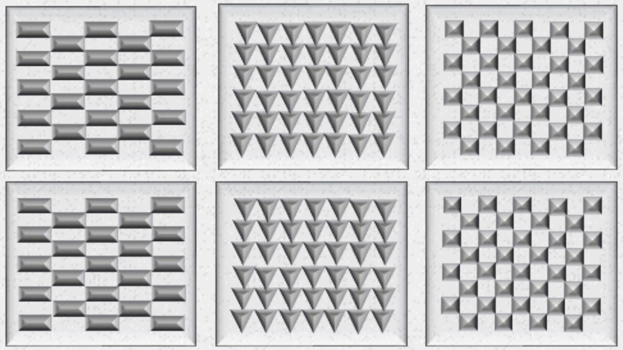
- **Material conditions:**
- RGD-modified surfaces
- BMP-2- modified surfaces
- RGD/BMP-2- modified surfaces

- **Material conditions:**
- RGD micropatterned surfaces
- BMP-2 micropatterned surfaces

- **Material conditions:**
- RGD/BMP-2 micropatterned surfaces

Homogeneous ligand distribution

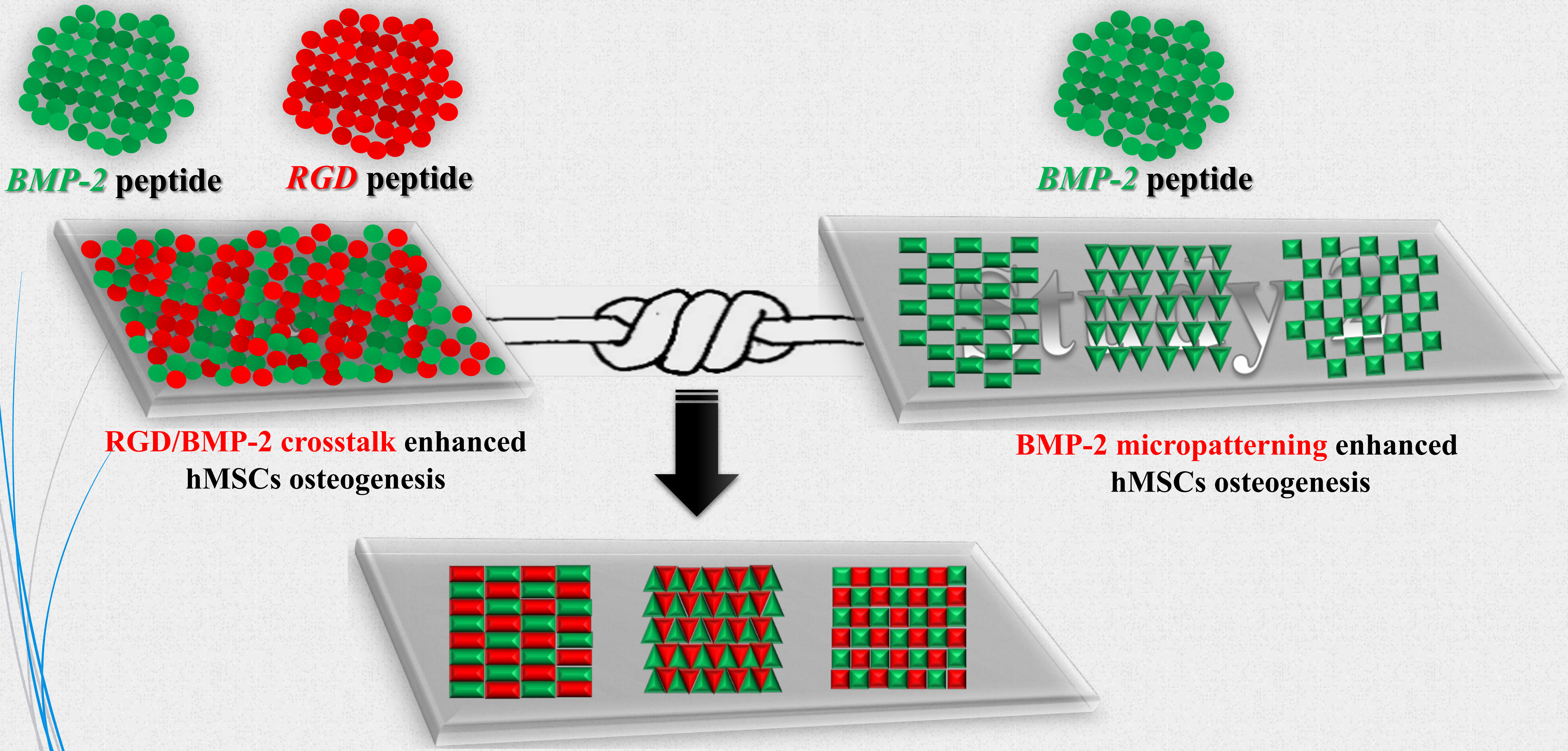
Single ligand micropatterning



Dual ligand micropatterning

Effect of microstructuring

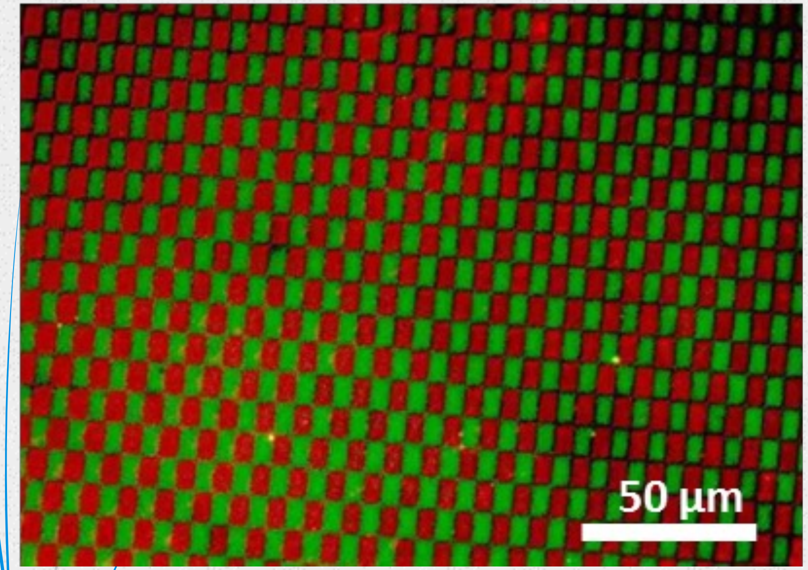
Methodology: **Approach of the dual ligand micropatterning**

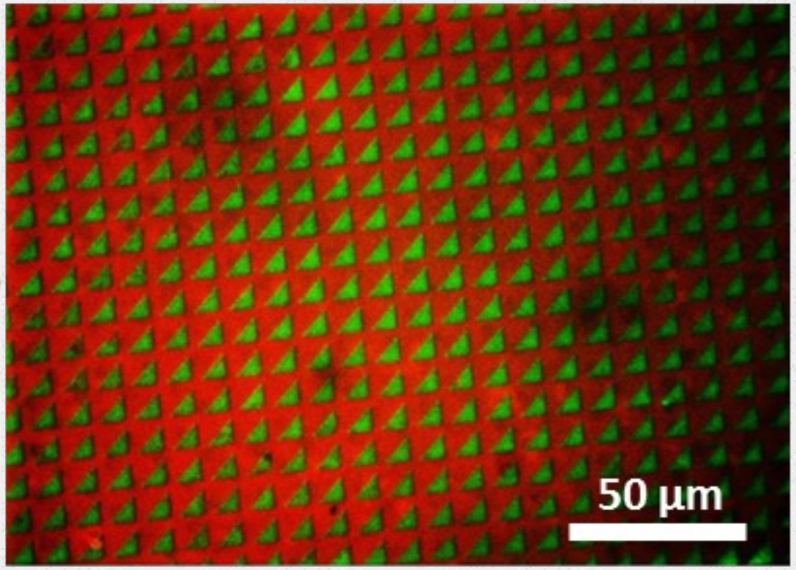


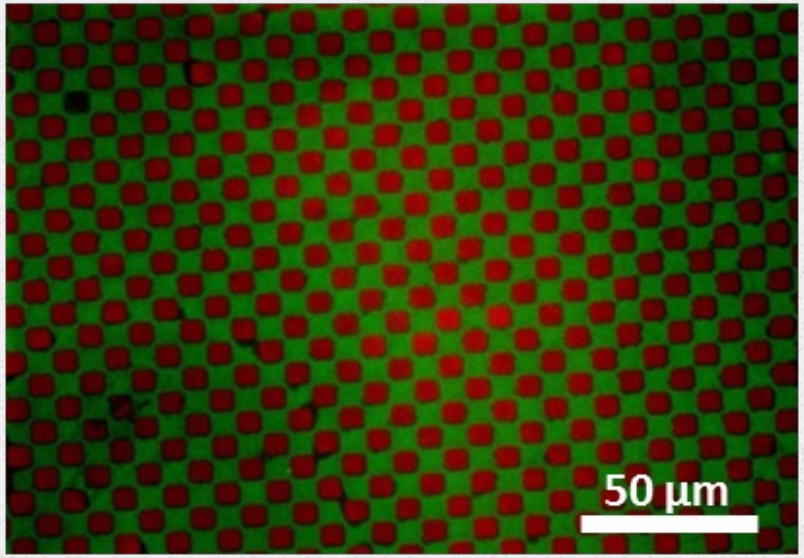
Effect of microstructuring

Results: **Fluorescence microscopy** (Fluorescence imaging)

RGD-TAMRA/*BMP-2-FITC* micropatterns



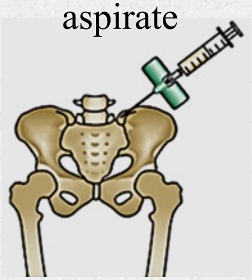


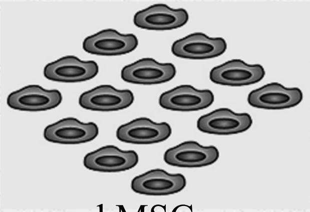


Cell culture Experiments

Methodology: Lineage-specific differentiation

Patient's iliac crest aspirate





hMSCs

BM: α-MEM + 10 % FBS + 1% ATB

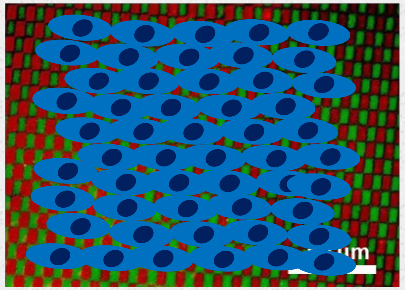
Differentiation markers

Osteoblasts (bone cells)

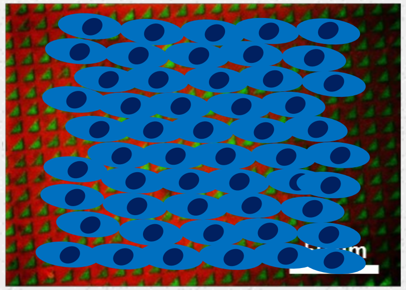
Immunofluorescence staining:
- STRO-1 ⊢
- Runx-2 ✓
- OPN ✓

Colorimetric assay:
- ALP ✓

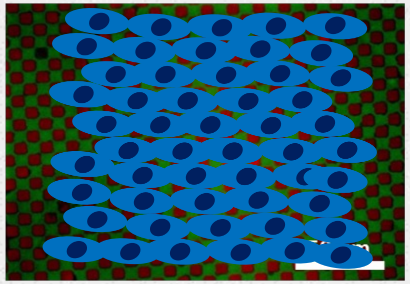
Triangular patterns



Rectangular patterns



Square patterns



4 weeks of culture without osteogenic media

Cell culture Experiments

Results: Stem cells & osteogenic markers expression

H: Homogenous grafting

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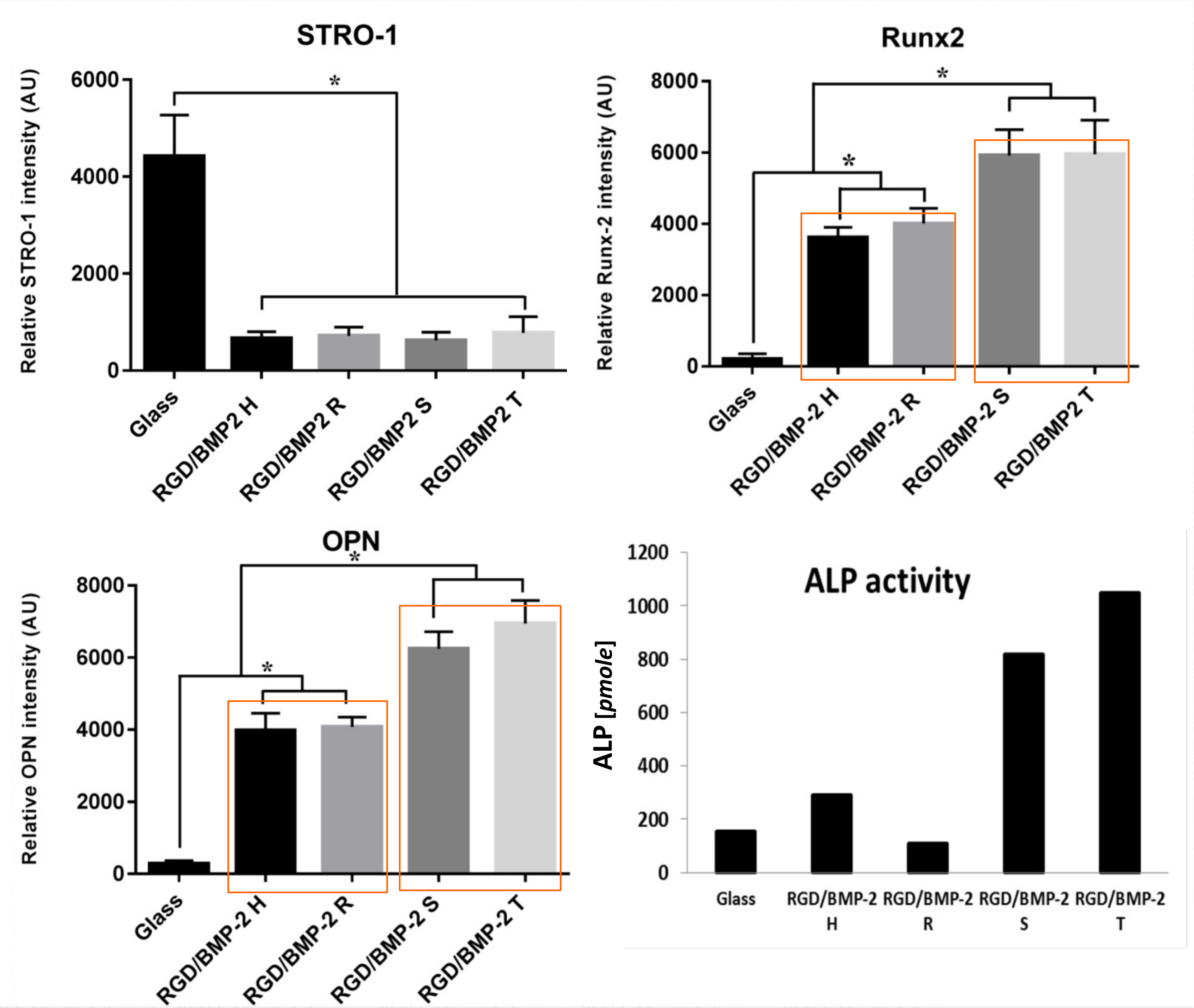


Conclusion

- ✓ The effect of **RGD/BMP-2 crosstalk** on hMSCs osteogenesis was **not affected** in case of ligand distribution as **rectangular micropatterns**.
- ✓ In case of **triangular** and **square micropatterns**, the microscale distribution of **RGD/BMP-2** peptides **accentuated** the osteoblastic phenotype in hMSCs as compared to the random peptide distribution.

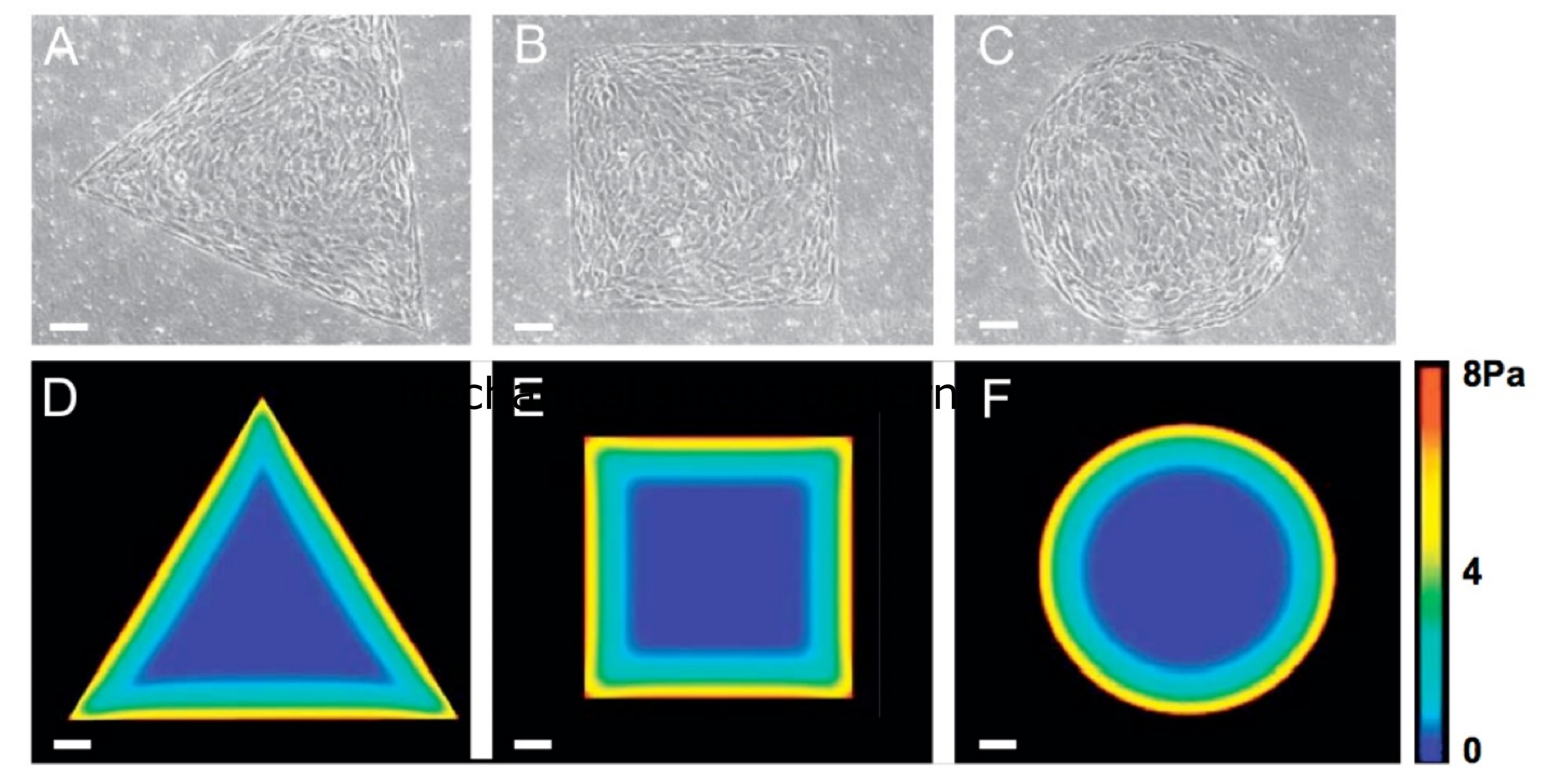
ACS Biomater. Sci. Eng. 2017, 3, 2514–2523

General conclusion

- ✓ We successfully engineered microstructured surfaces with one or more ECM-derived ligands.
- ✓ **RGD** and **BMP-2** peptides, randomly distributed on the material surface, **act synergistically** to enhance hMSCs osteogenic differentiation.
- ✓ The **microscale distribution** of **BMP-2**, **but not RGD** peptide, effectively enhances the osteogenic differentiation.
- ✓ **Ligand crosstalk** and **ligand micropatterning** are two potential parameters that can **cooperate** to improve osteogenesis.

Presenting ECM-derived ligands in a spatially controlled manner is an additional step toward recreating the natural stem cell microenvironment…

Effect of the mechanical properties



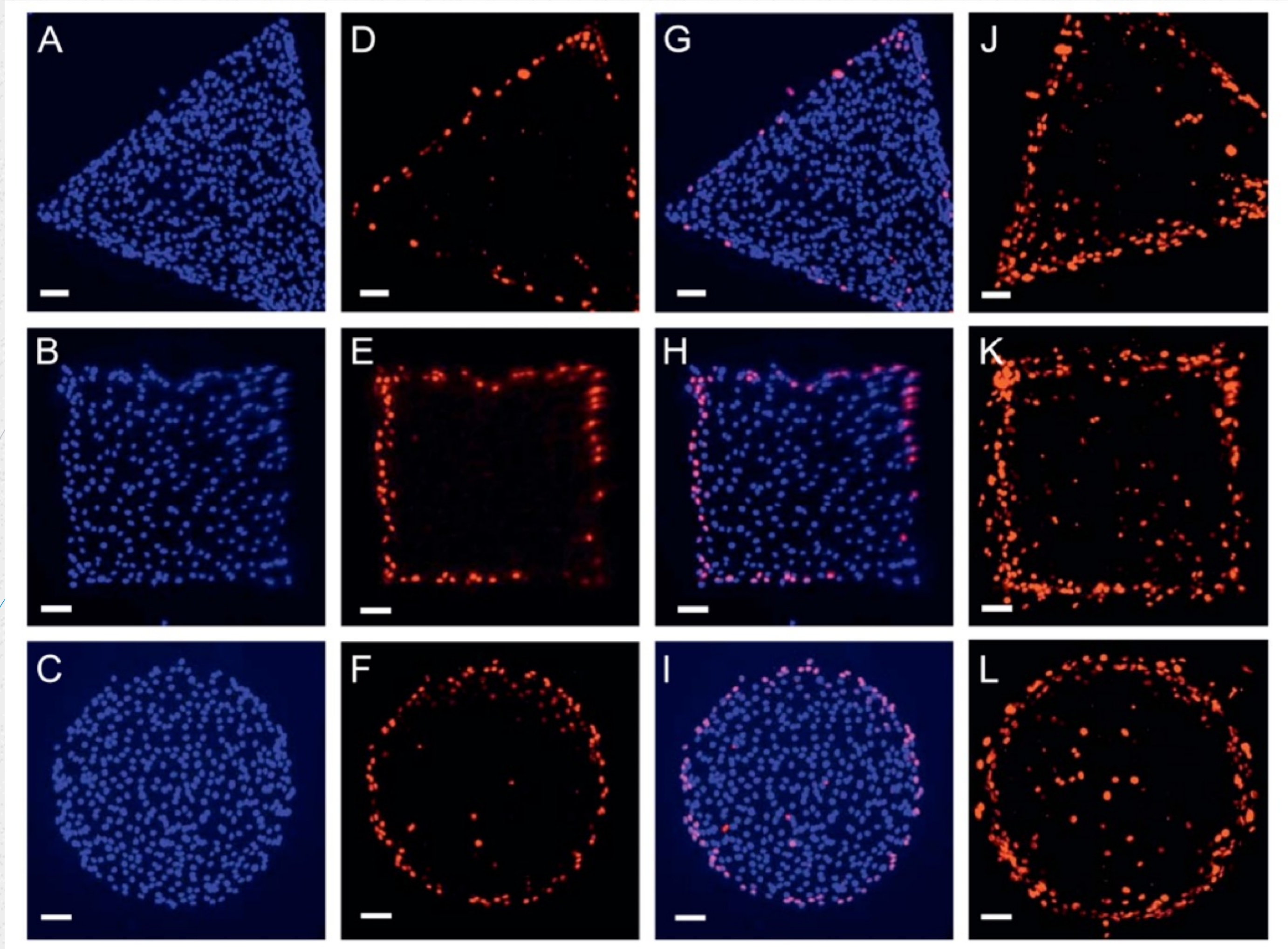
3T3 Fibroblasts proliferation

Mechanical stress pattern

Scale bar=100 μm
Island areas: 490 000 μm^2

B Li, F Li, KM Puskar et al, J Biomech, 2009; **42**: 1622-1627.

Effect of the mechanical properties

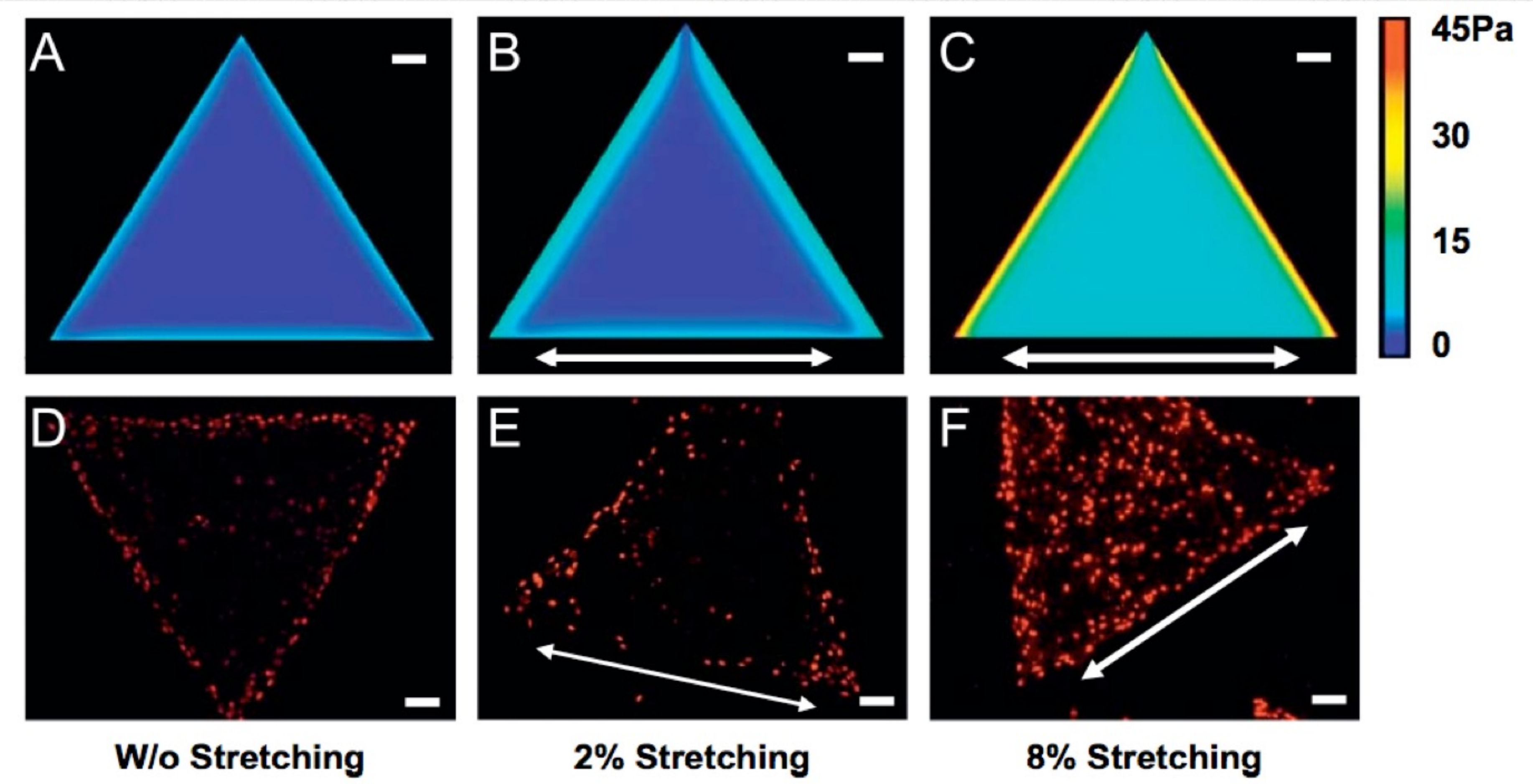


Scale bars=100 μm

Blue: adhering cells
Red: proliferation cells

B Li, F Li, KM Puskar et al, J Biomech, 2009; **42**: 1622-1627.

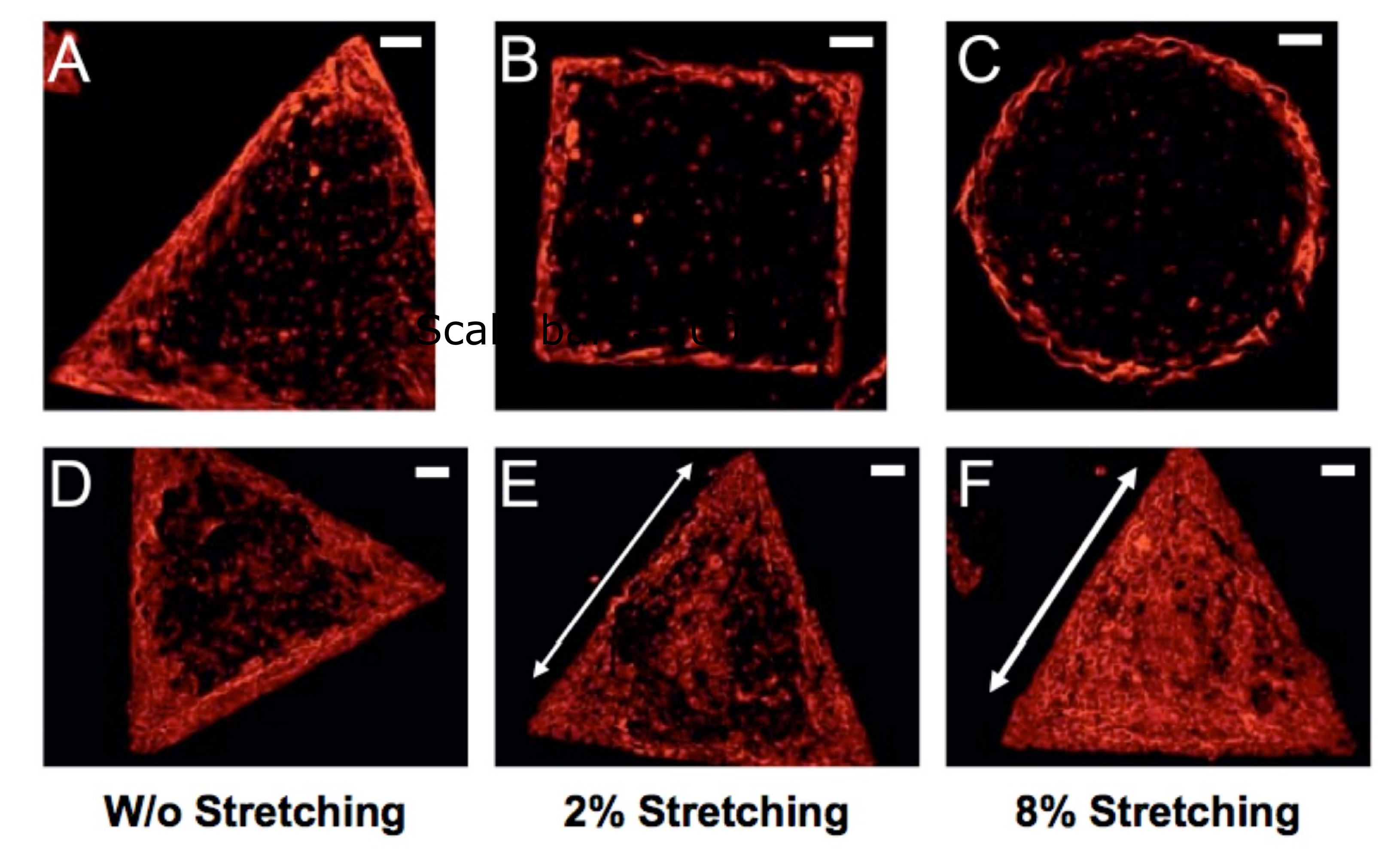
Effect of the mechanical properties



Scale bars=100 μm

B Li, F Li, KM Puskar et al, J Biomech, 2009; **42**: 1622-1627.

Effect of the mechanical properties

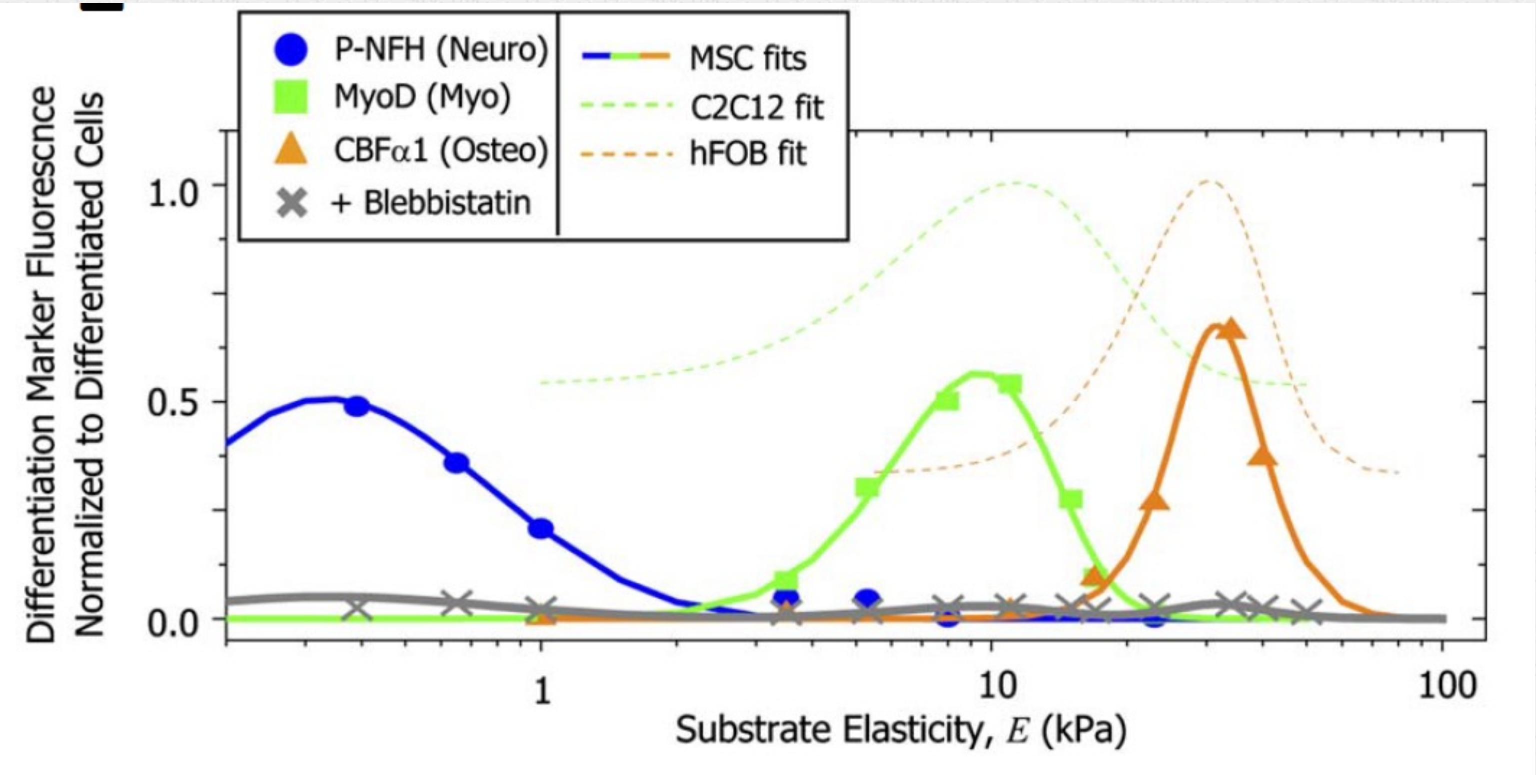


Differentiation into myofibroblasts through detection of α-SMA cellular expression

Scale bars=100 μm

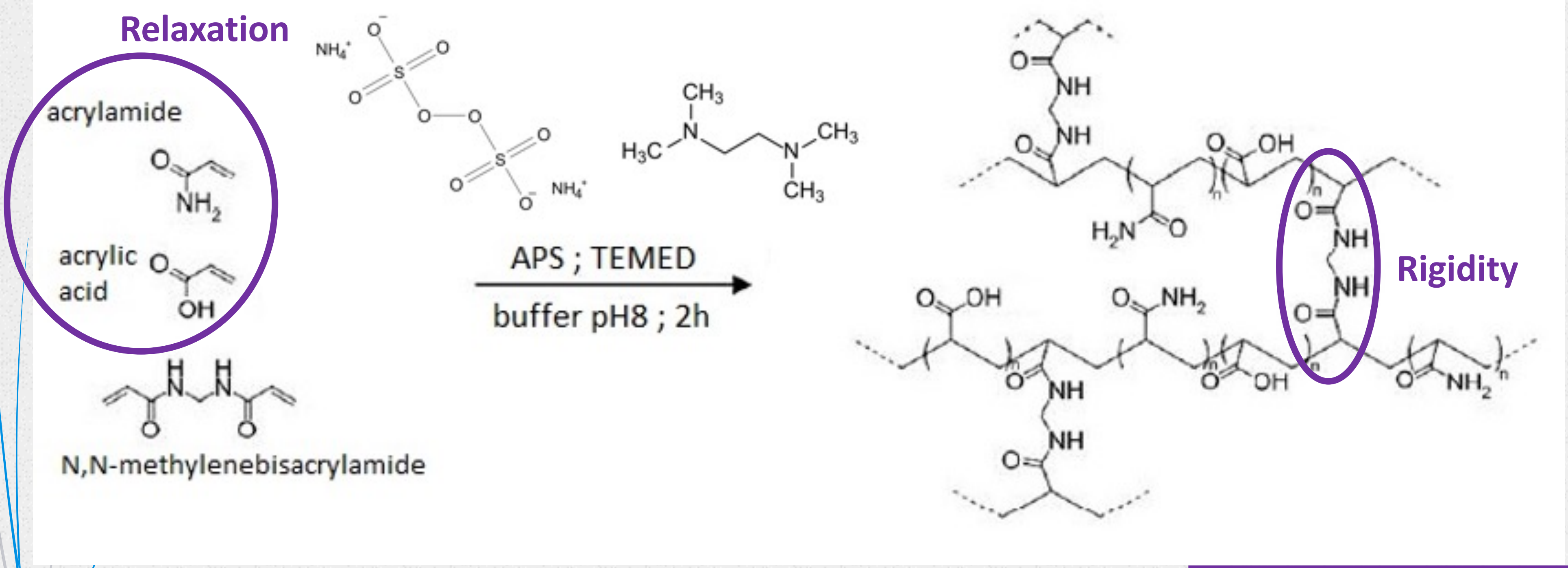
B Li, F Li, KM Puskar et al, J Biomech, 2009; **42**: 1622-1627.

EFFECT OF THE MECHANICAL PROPERTIES



J.R. Tse & A.J. Engler, Current protocols in cell biology, 2010; 47 :10-16.

EFFECT OF BIOMECHANICAL AND BIOCHEMICAL CUES



→ Easy synthesis

→ Modulus ranging from 1 to hundreds of kPa

→ Covalent crosslinking

→ No cell adhesion

RIGIDITY

5 - 150 kPa

RELAXATION

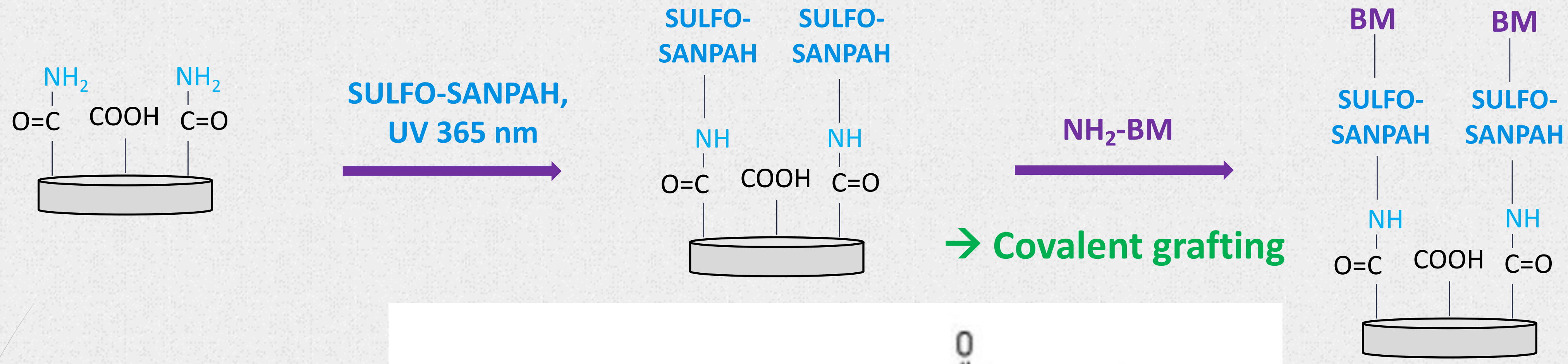
0 – 100%

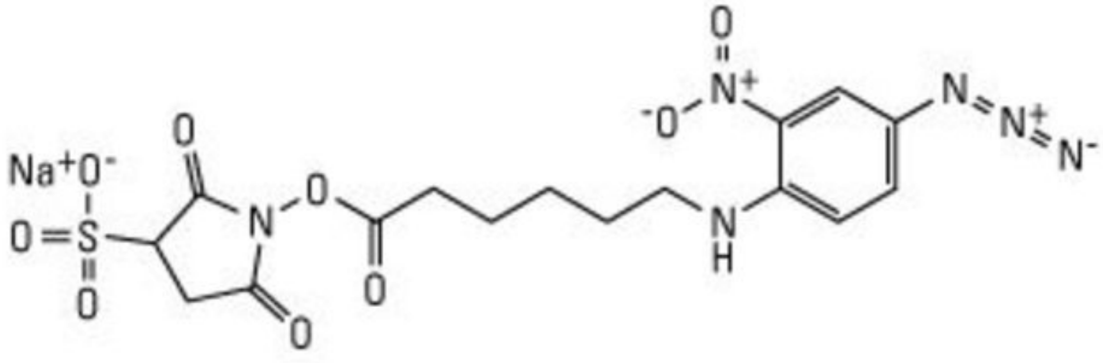
Bis-acrylamide
0.03 - 0.24 - 0.36 - 0.48 w/v%

Acrylamide/acrylic acid
100/0 – 95/5 – 90/10 – 82/18 mol%

O.F. Zouani et al. Biomaterials, 2013; 34: 2157-2166

Effect of biomechanical and biochemical cues





Sulfo-SANPAH

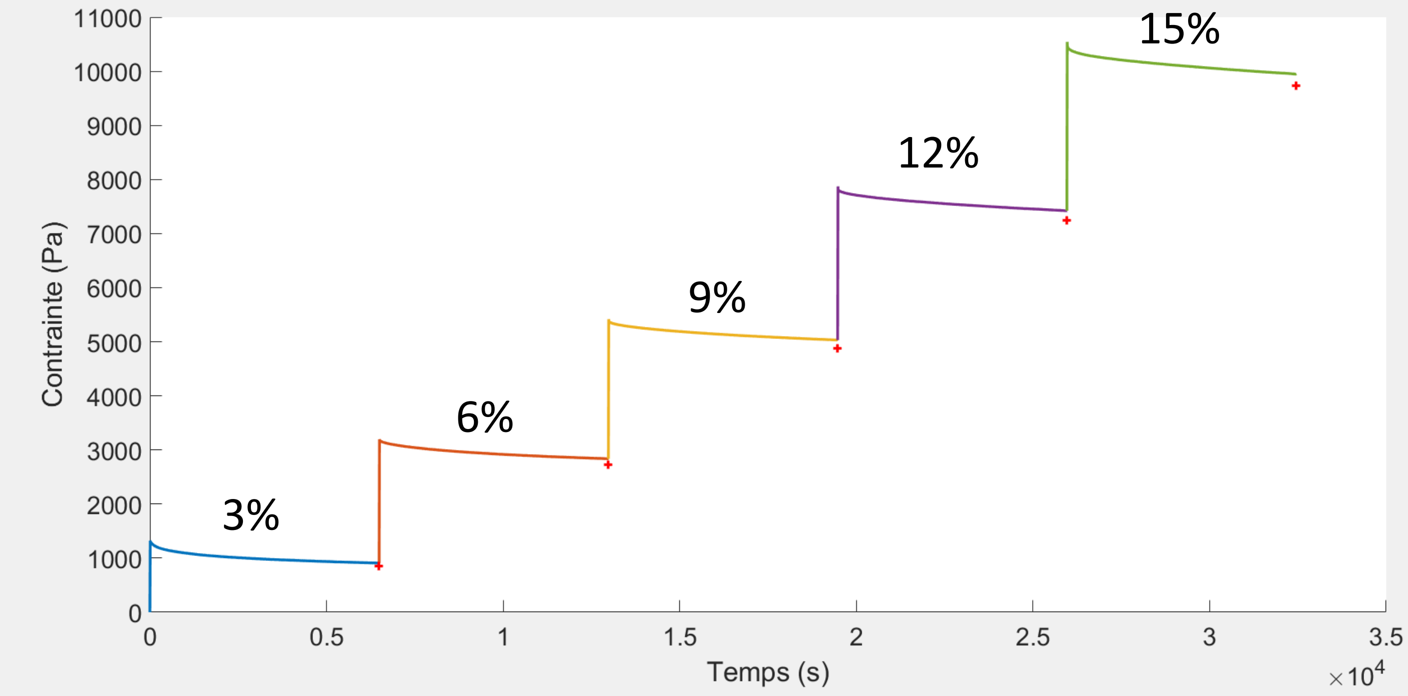
Sulfosuccinimidyl 6-(4'-azido-2'-nitrophenylamino)hexanoate

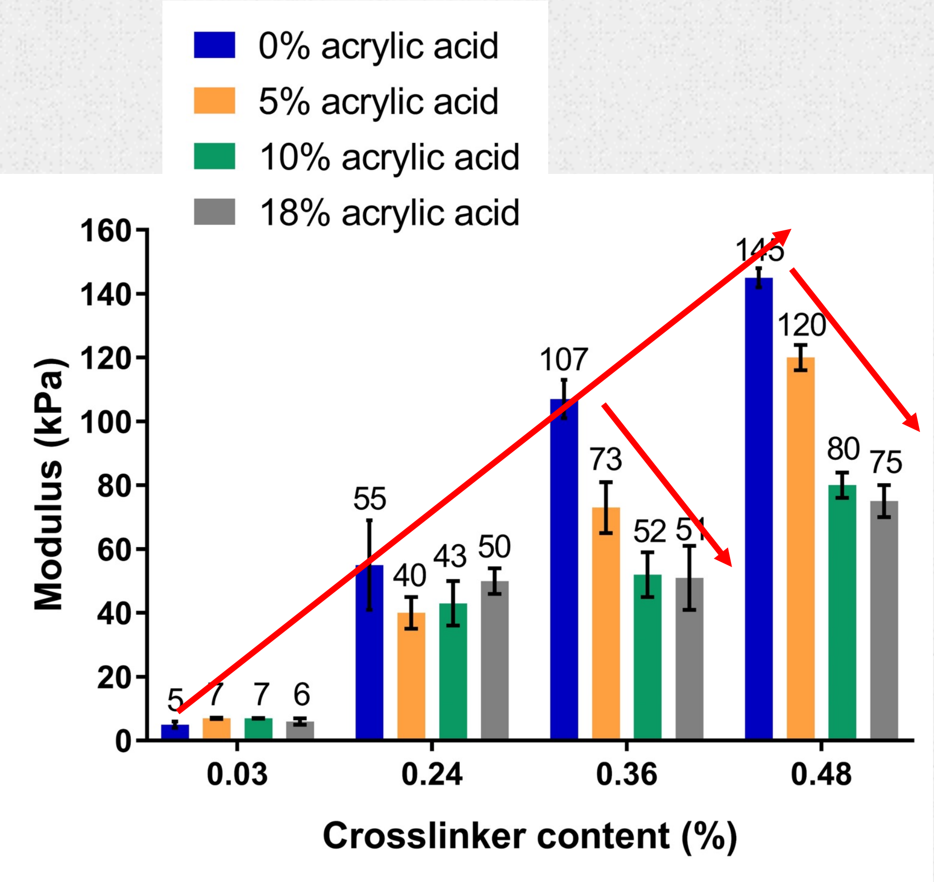
MW 492.40

Spacer Arm 18.2 Å

J.R. Tse & A.J. Engler, Current protocols in cell biology, 2010; 47 :10-16.

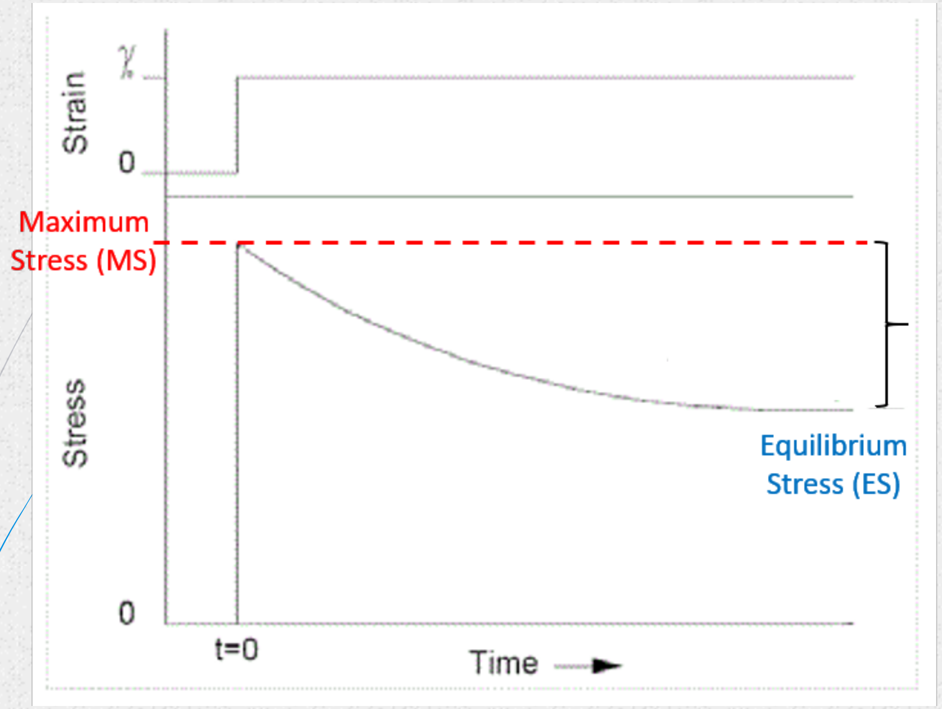
Effect of biomechanical and biochemical cues



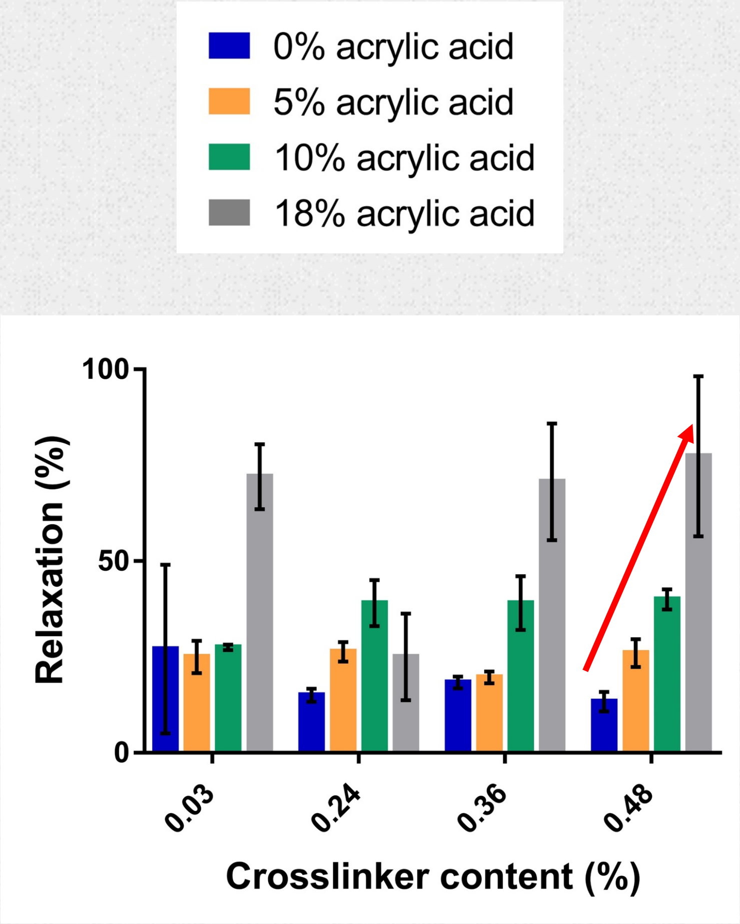


E. Prouvé et al.,
Macromol. Biosci, 2021; 2100069: 1-12
Biomater. Sci., 2022; 10, 4978-4996.

Effect of biomechanical and biochemical cues



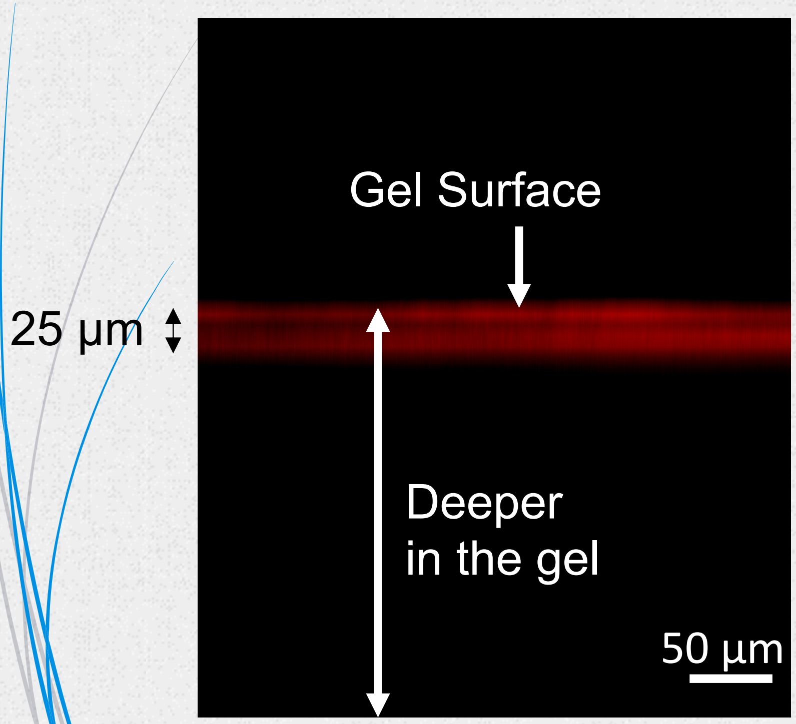
Relaxation degree (%) = $\frac{MS - ES}{MS}$ x 100



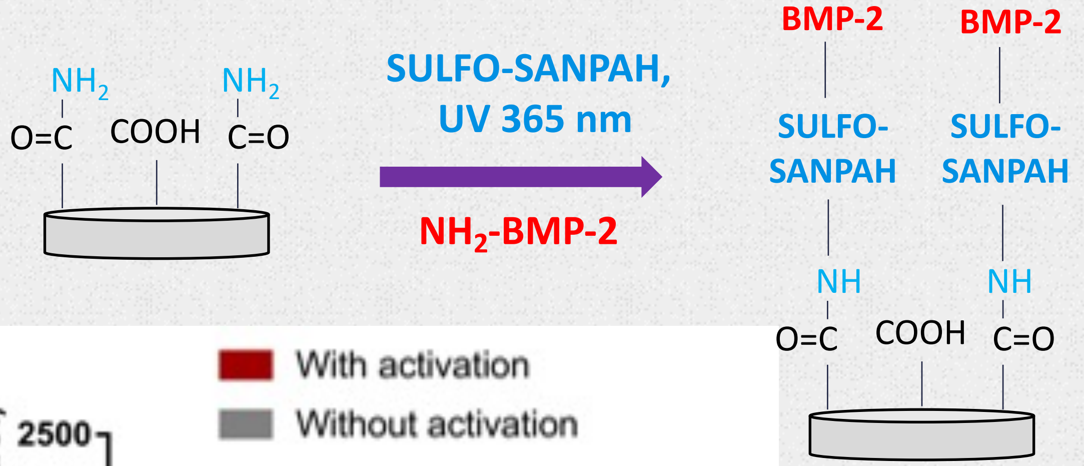
Effect of biomechanical and biochemical cues

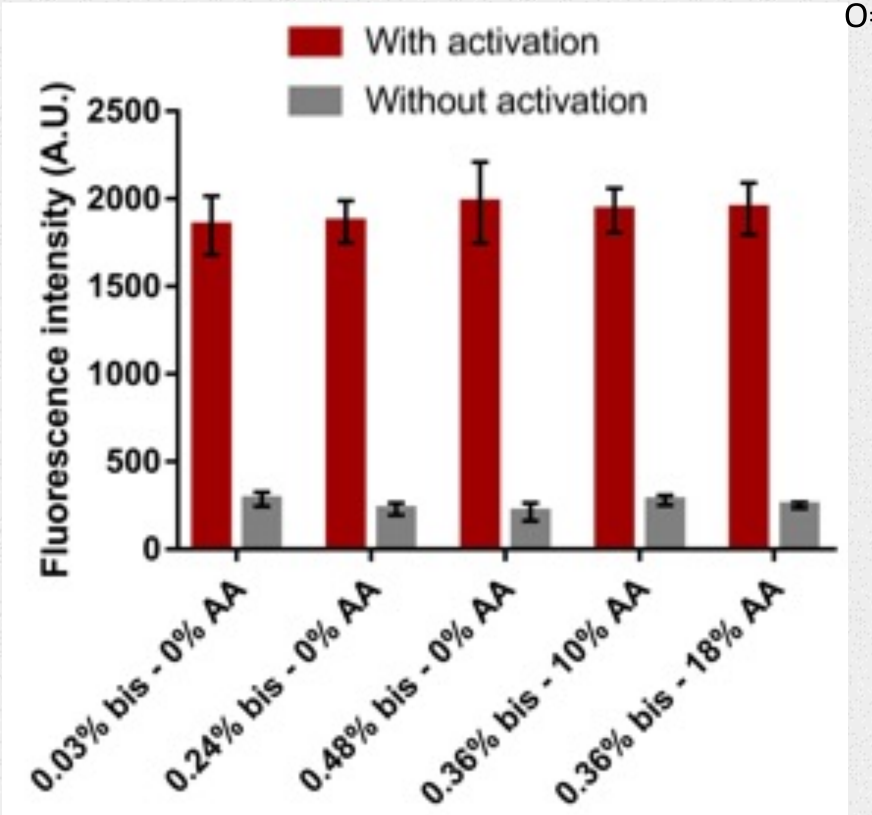
Florescent peptide (TAMRA)

NH_2-BMP-2-



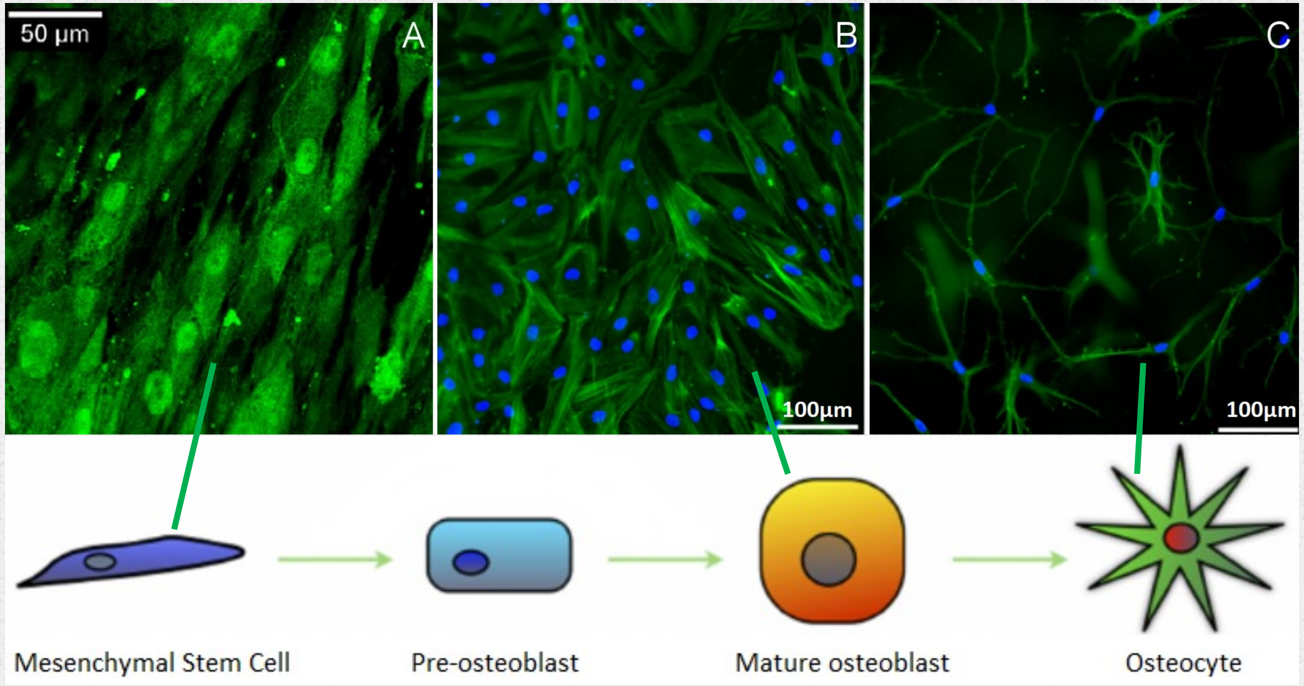
Side view- confocal fluorescence microscopy





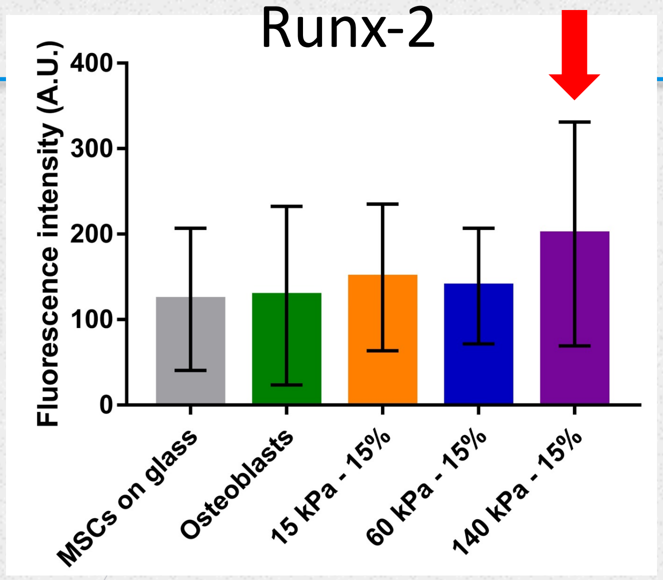
→ Peptide immobilized on gel

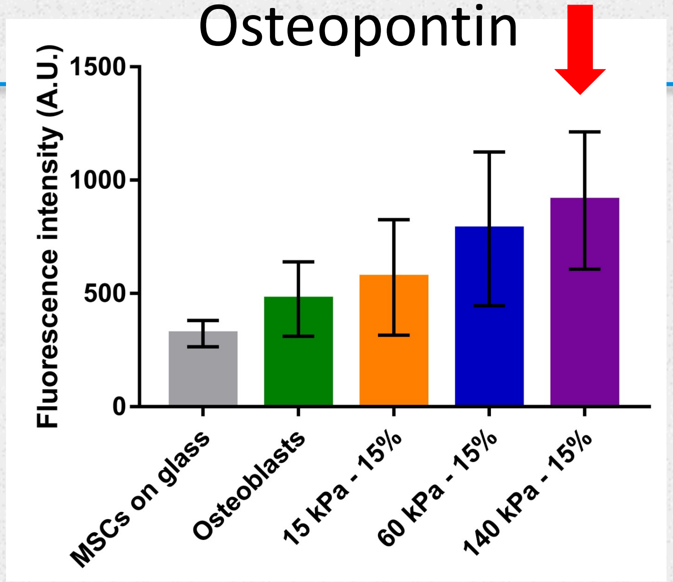
Effect of biomechanical and biochemical cues



Pre-osteoblast	Mature osteoblast	Osteocyte
Runx-2	**Runx-2**	**E11**
ALP	ALP	**DMP-1**
COL1	COL1	PHEX
Osterix	**Osteopontin**	MEPE
	Ostéocalcine	**Sclerostin**
	BSP	

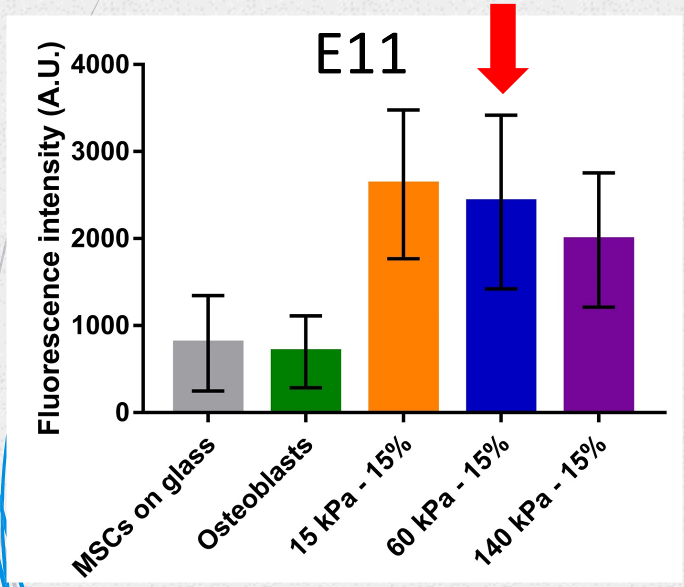
OSTEOBLAST

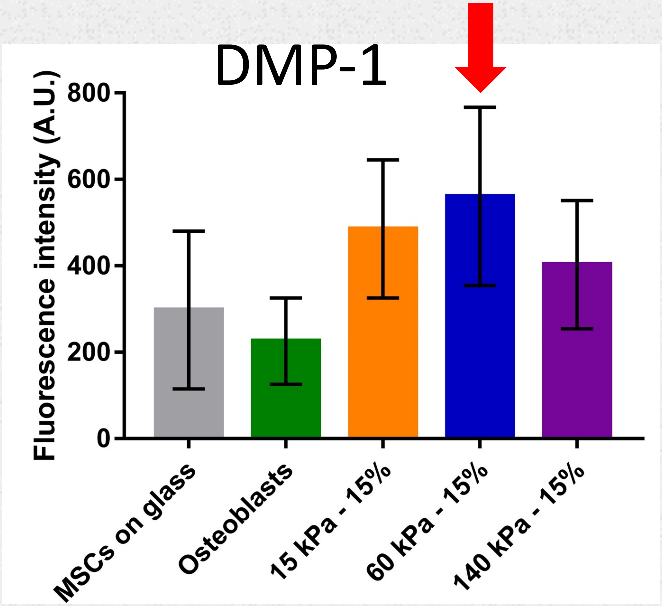


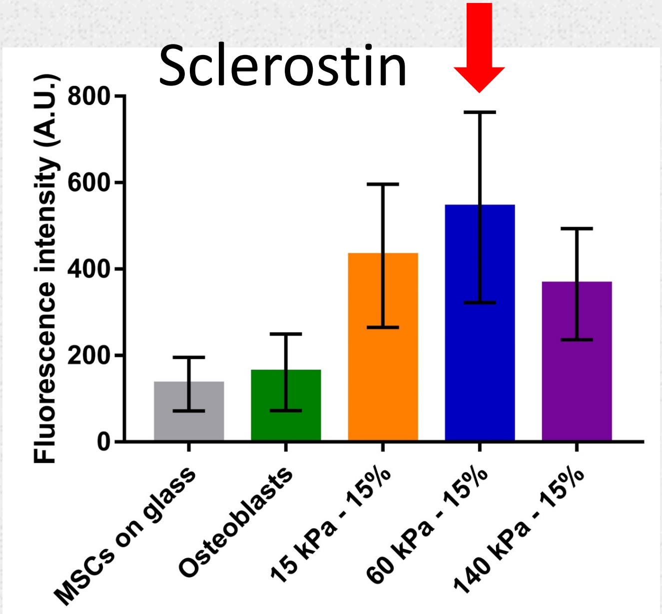


→ **Rigidity 60 kPa**

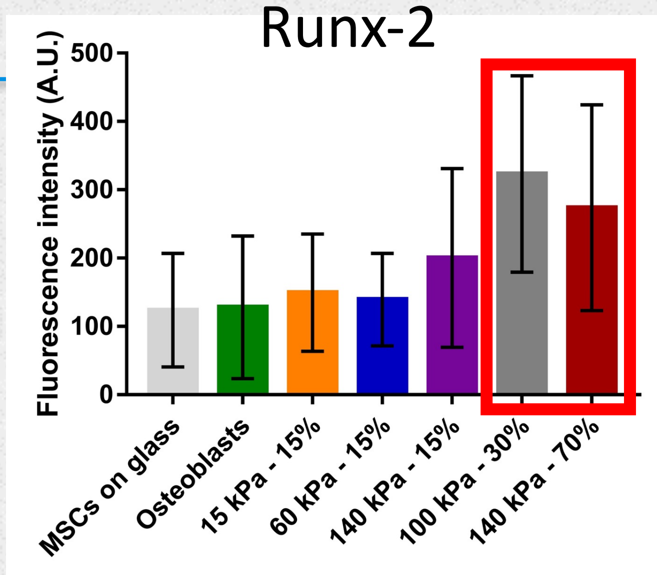
OSTEOCYTE

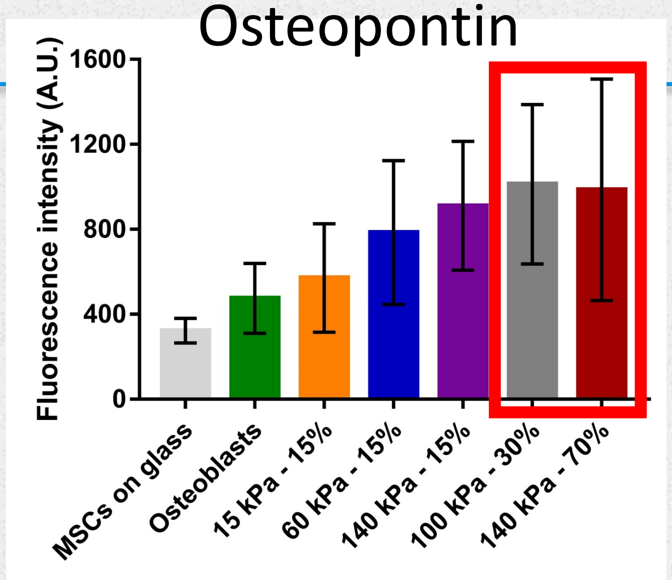






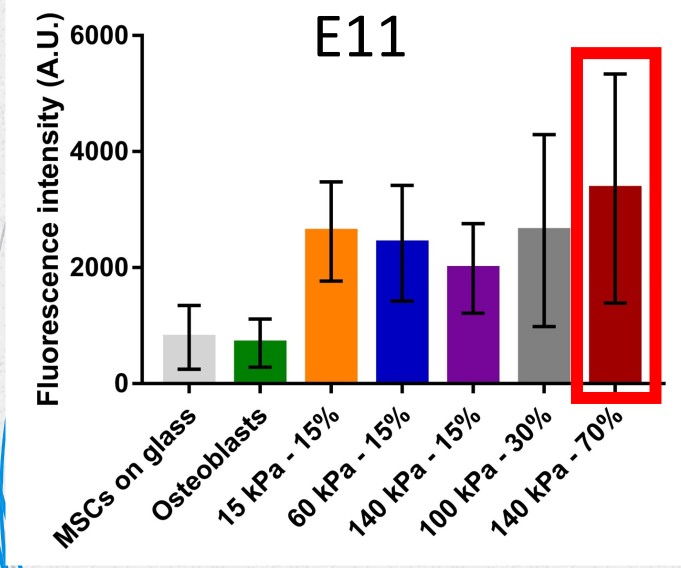
OSTEOBLASTE

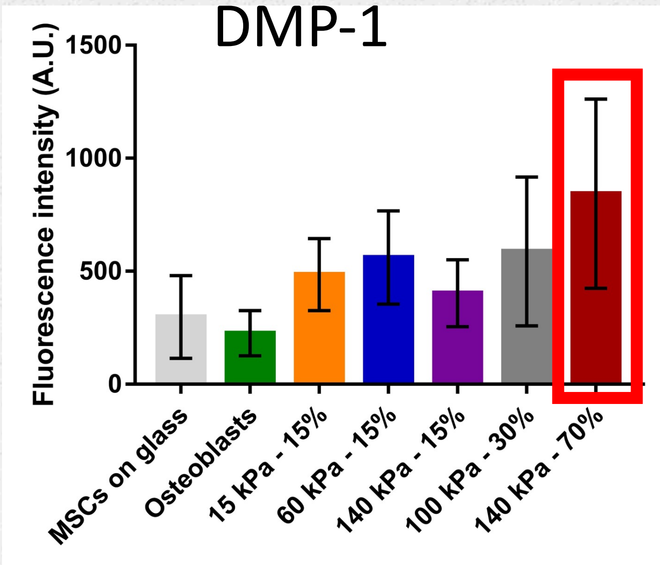


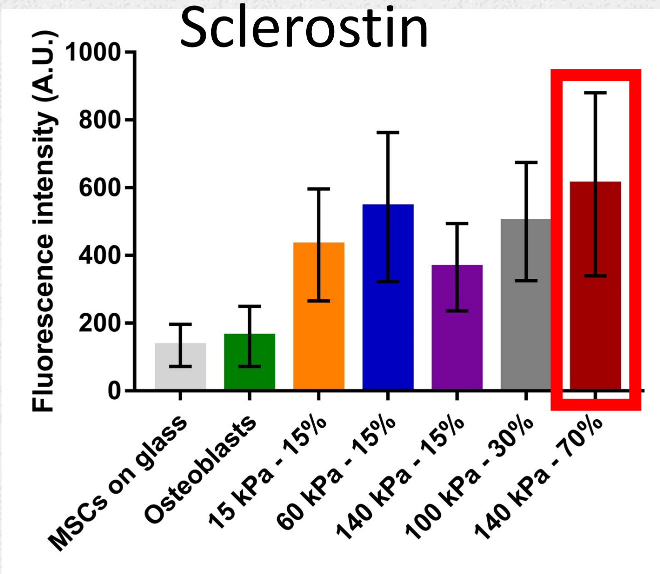


→ **Relaxation 70%**

OSTEOCYTE







The mechanical properties of biomaterials drive MSCs differentiation.

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INVESTISSEMENTS D'AVENIR
RÉGION Nouvelle-Aquitaine
CHU de Québec
UNIVERSITÉ LAVAL
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CHINA SCHOLARSHIP COUNCIL
HORIZON 2020 LE PROGRAMME DE RECHERCHE ET D'INNOVATION DE L'UNION EUROPÉENNE
European Commission ERASMUS MUNDUS
SPARK BORDEAUX
université de BORDEAUX
cqmf centre québécois sur les matériaux fonctionnels
PRIMA
NSERC CRSNG
Mitacs

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