Polyelectrolyte layer-by-layer films to explore mammalian and bacterial cell control

I. physical-chemistry

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Biomaterials

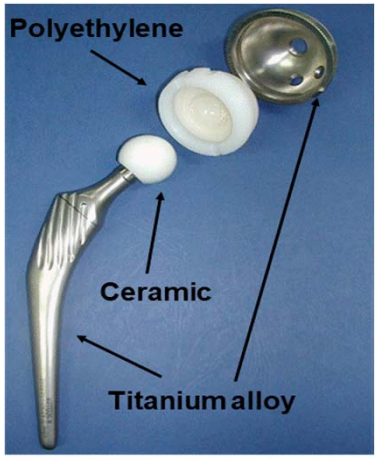
Definition agreed upon the Conference of the European Society for Biomaterials in 1986

Biomaterials = a non-viable material used in a medical device used in the diagnosis, the cure, the treatment or the prevention of disease. They are thus intended to interact with biological system.

Types of biomaterials

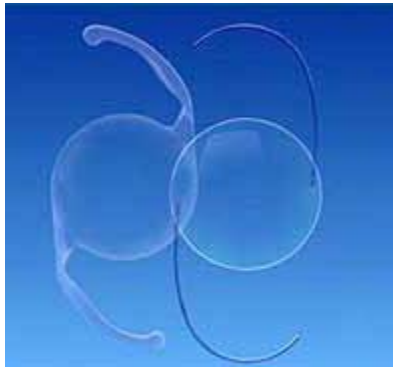
- Titanium and alloys
- Ceramics
- Synthetic and natural polymers

Hip implant



©Implants Industrie

Artificial Lens



Issues at the implantation

Bacterial and fungal infection at the site of implantation

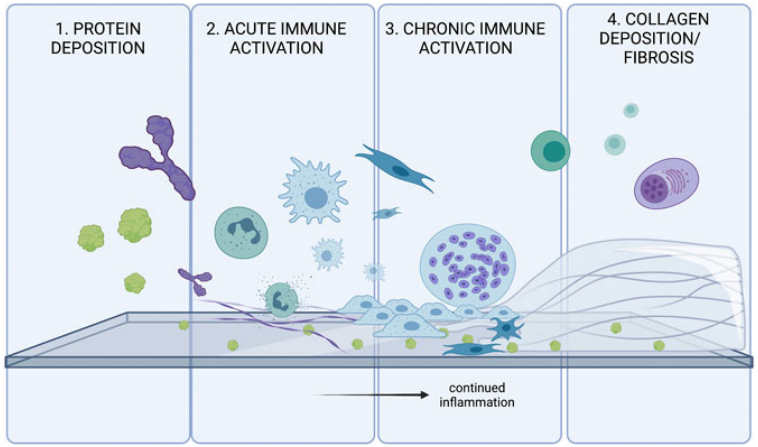
Treatment by antibiotics ⇨ pathogen resistance ⇨ biofilm formation

Nosocomial infections ⇨ major public health issue (medical and financial)

(5% of hospitalized patients, 4000 deaths / year in EU)

GMS Krankenhaushyg. Interdiszip. 2011, 6 , 1

Foreign body reaction



Collagen layer encapsulation (Fibrosis)

⇨ Colonization by fibroblasts: no specific cells

⇨ Loosening of the implant

Front. Bioeng. Biotechnol., 2021, DOI: 10.3389/fbioe.2021.730938

Which properties for biomaterials ?

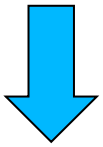
- To prevent bacterial infections

Control of bacterial adhesion / proliferation ⇨ prevent biofilm formation

- To favor biointegration

Control the cell adhesion and be specific on the biomaterial surfaces

Control of materials/environment interactions



Tool: surface functionalization

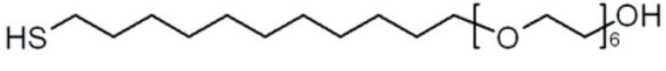
Physisorption

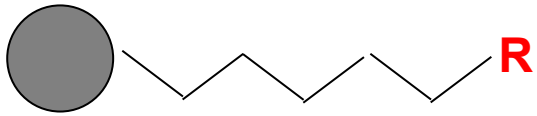
Self Assembled Monolayer (SAM)

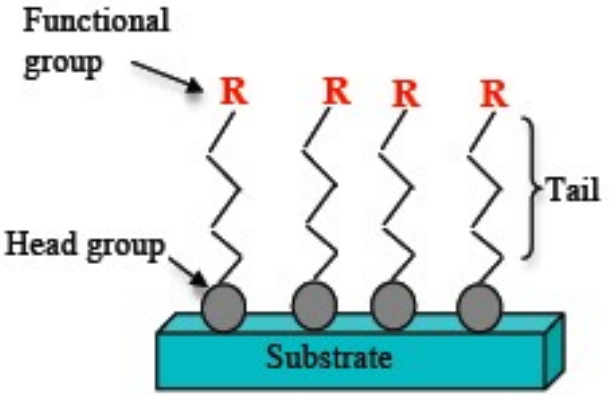
DDT

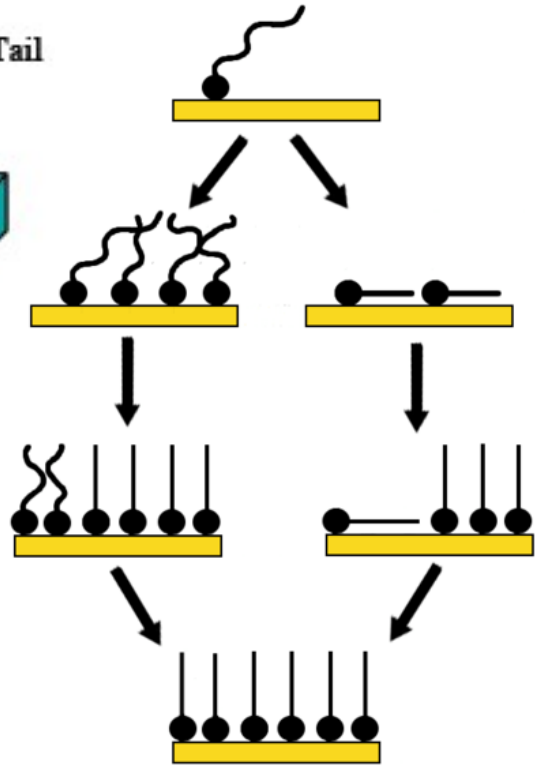
MUA

EG6OH







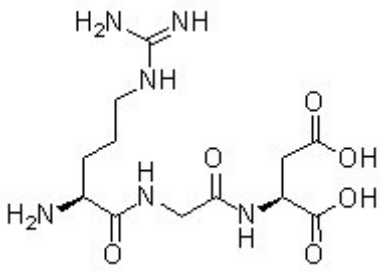


Exple: thiols affinity for gold **=> Self-assembly**

Advantage: control of the surface density of molecules
Drawback: specific to gold surfaces

RGD moities

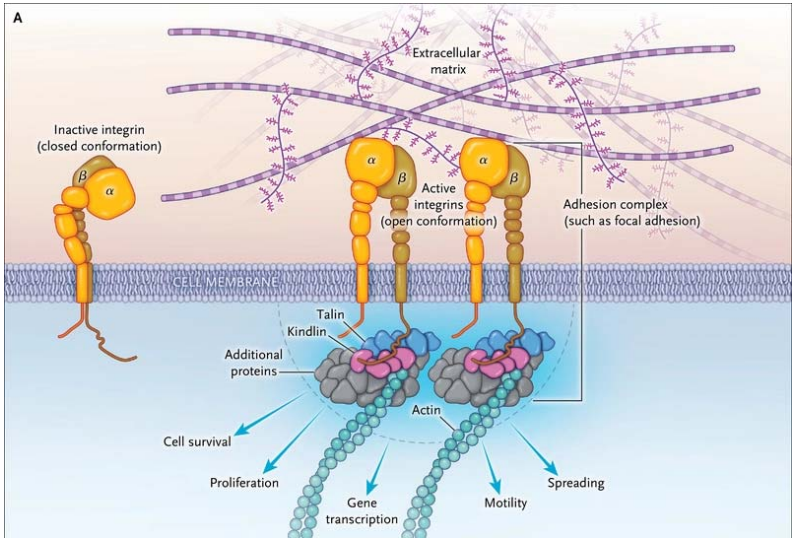
RGD : arginine-glycine-aspartic acid tripeptide

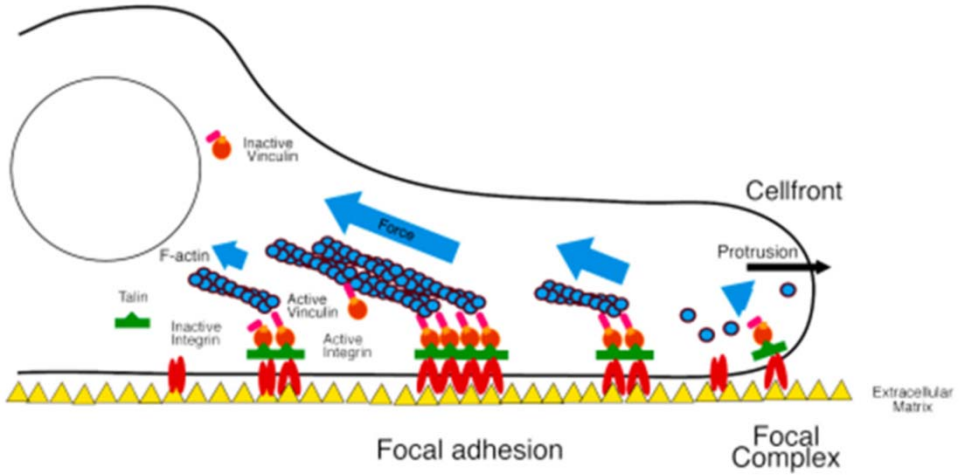


- Recognized by integrins of cells as attachment point
- Facilitate cellular adhesion
- Present in collagen, fibronectin, laminin etc… (extracellular matrix)

Integrins (protein receptor)

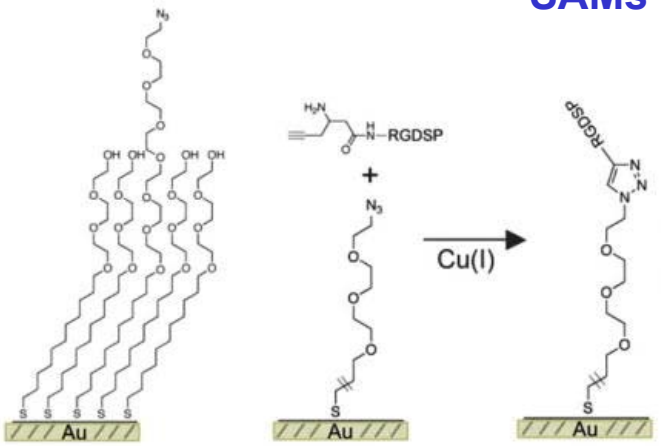
- Integrin = ligand used by some cells for adhesion and cell signaling

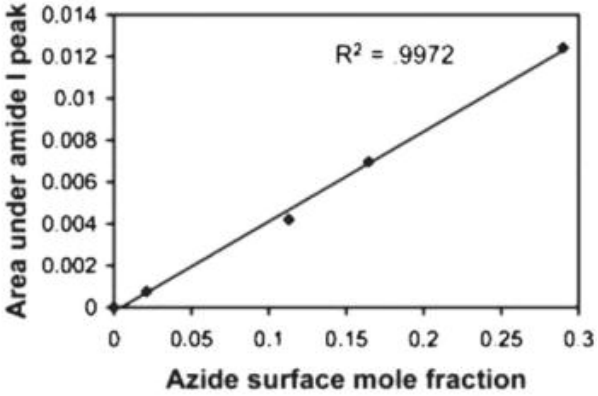




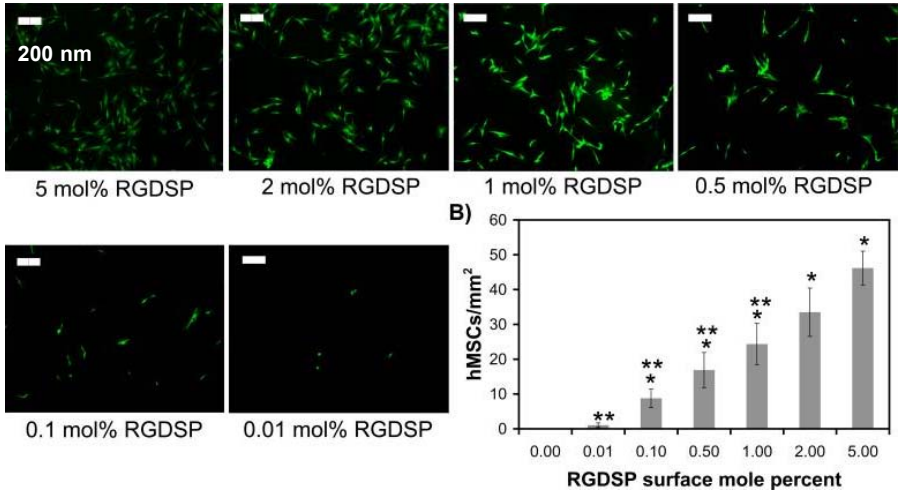
Physisorption

SAMs with different density of RGD

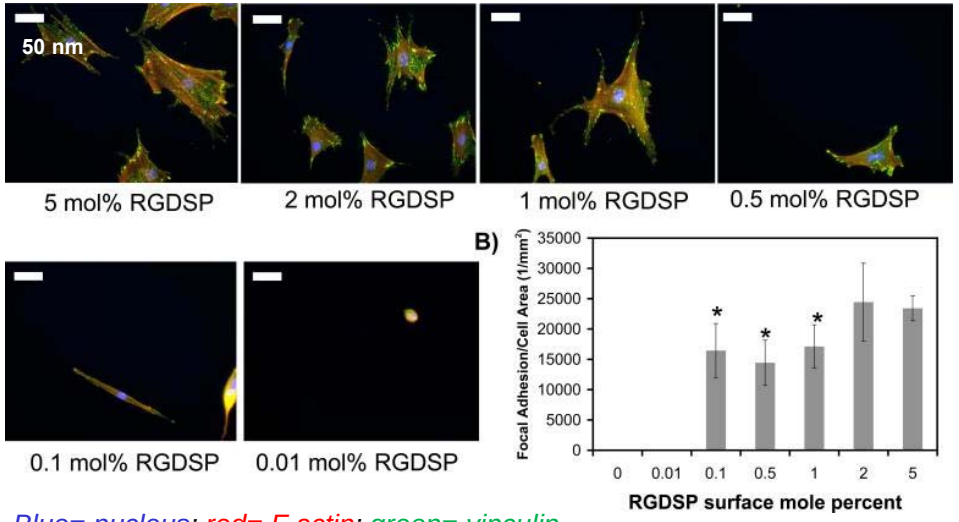




Human mesenchymal stem cells on SAMs with different density of RGD



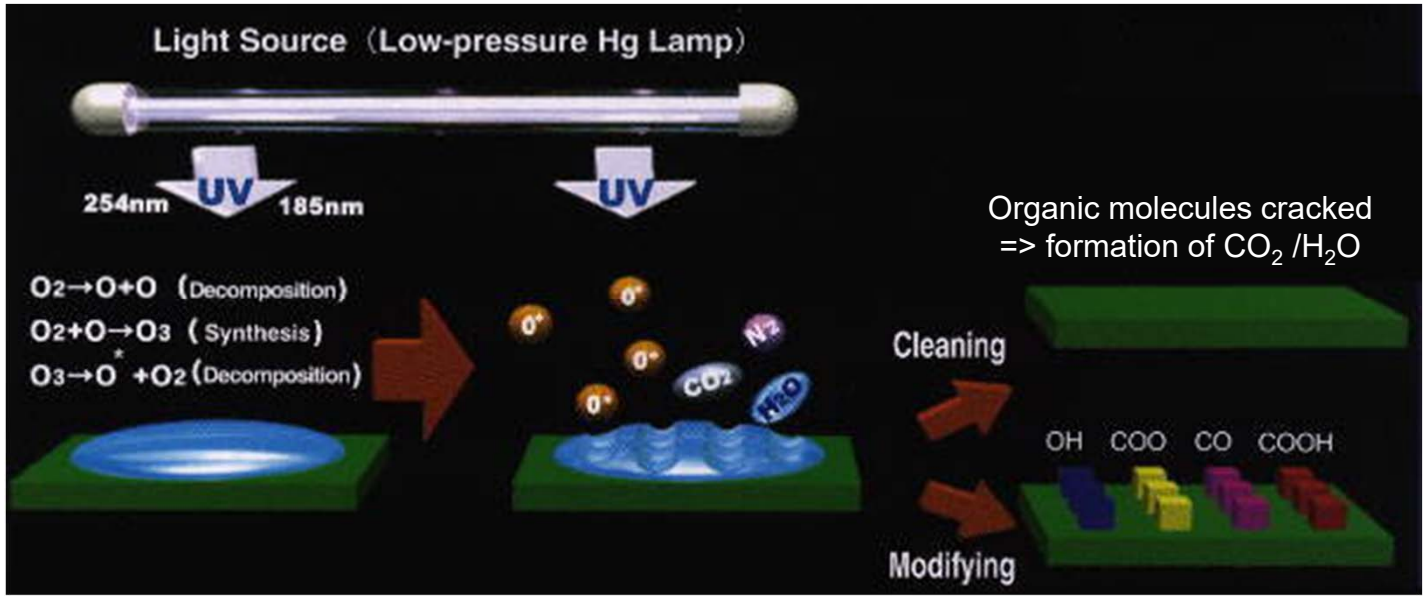
Calcein cell-permeant dye for cell viability



Blue= nucleus; red= F actin; green= vinculin

Chemical grafting

Physical treatment: UV-ozone, O_2 plasma or gamma

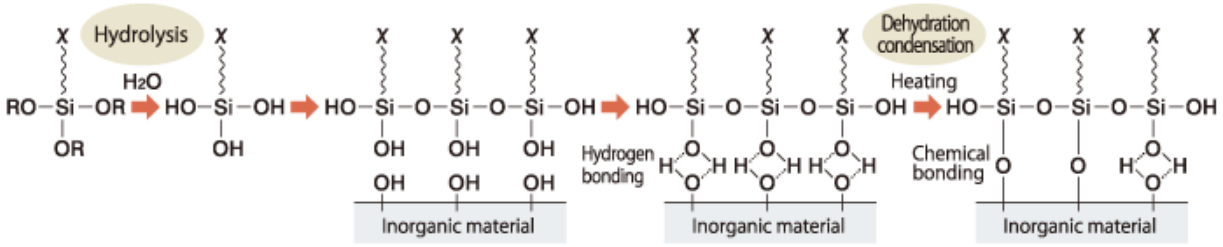


Decomposition of O_2
=> formation of O_3

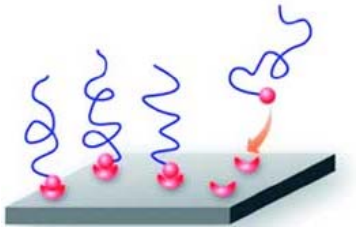
Decomposition of O_3
=> formation of activated O^* ⇨ formation of OH, COO*, CO* and *COOH

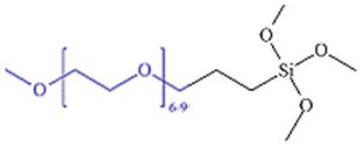
Chemical grafting

- After activation by UV or plasma → formation of OH groups
- OH react with silanes (SiR_3-X avec R= -Cl, $-OCH_3$, $-OCH_2CH_3$)



Grafting to
SiR_3-polymer

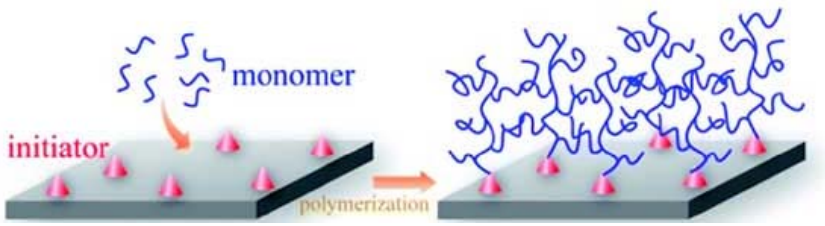


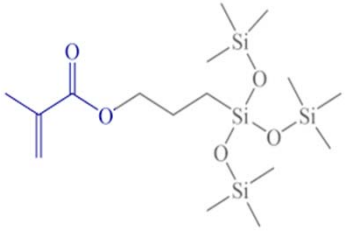


Anti-adsorbant
towards protein

2-(methoxypolyethyelenoxy)$_6$-propyl(trimethoxysilane)

Grafting from
SiR_3-monomer

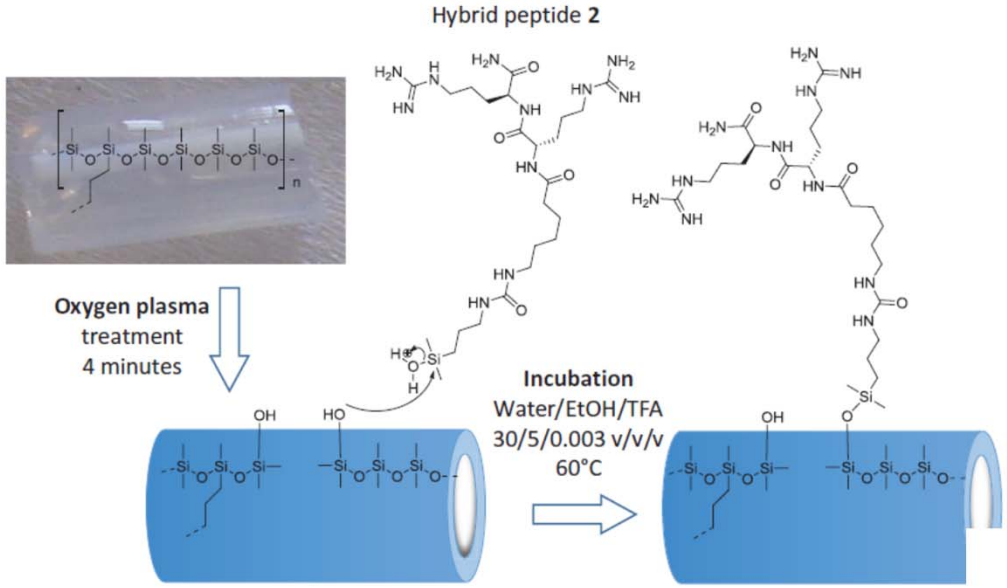




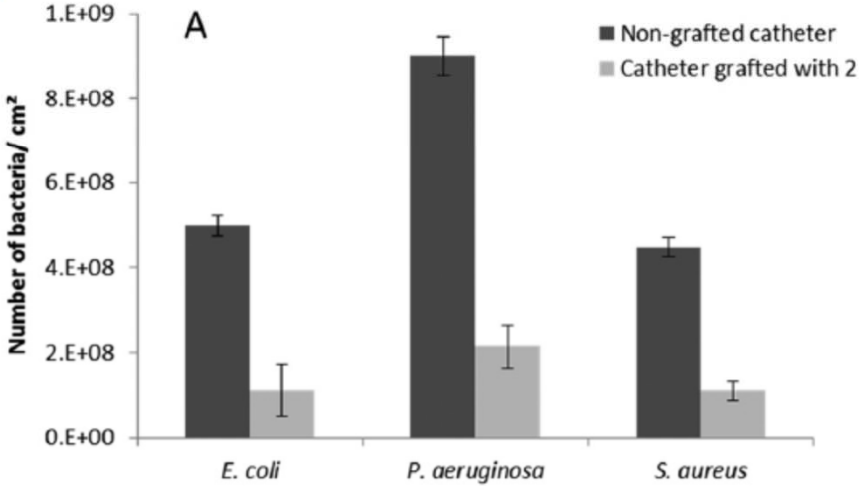
Site for further polymerization

Methacryloxypropyltris(trimethylsiloxy)silane

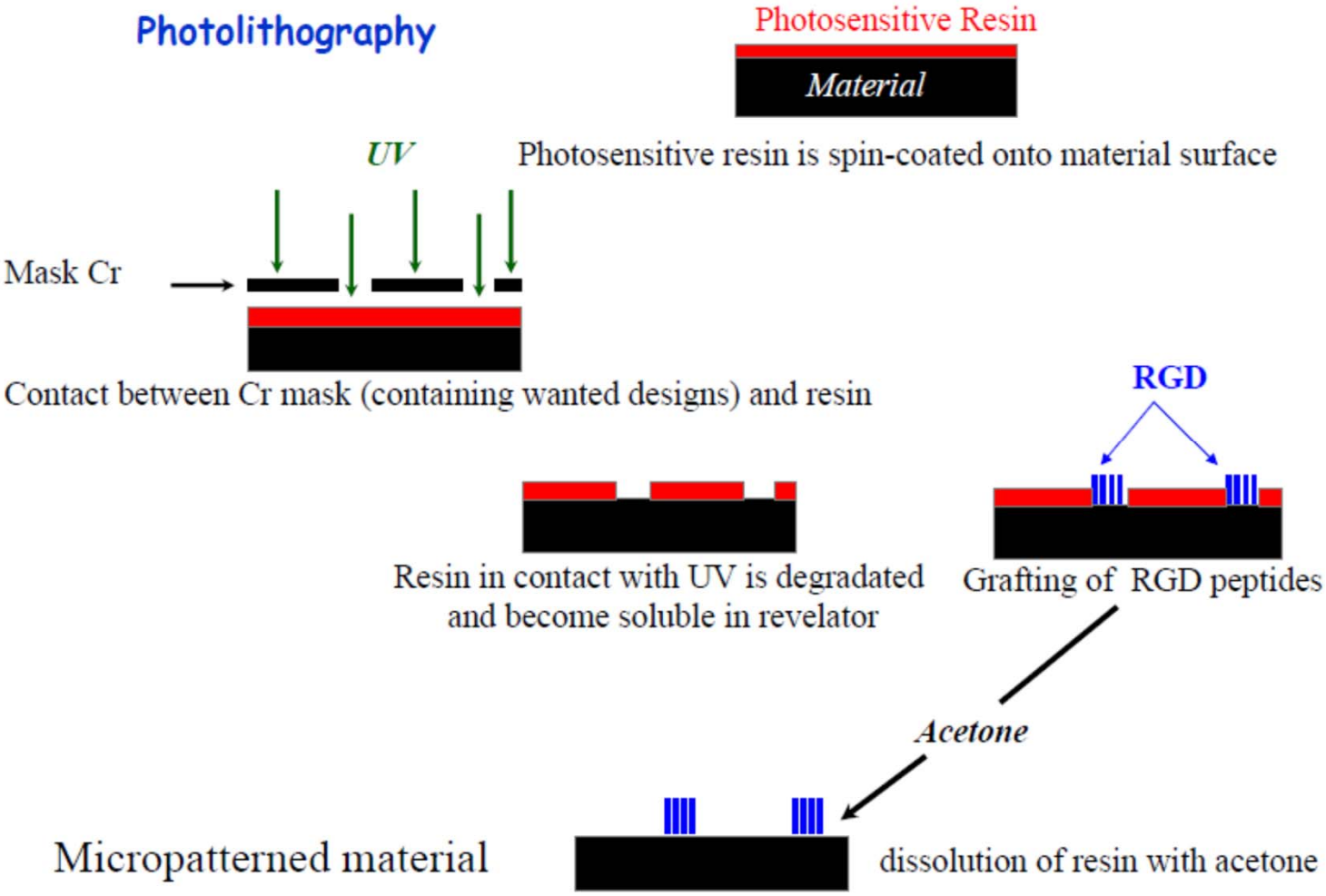
Chemical grafting



Antibacterial property

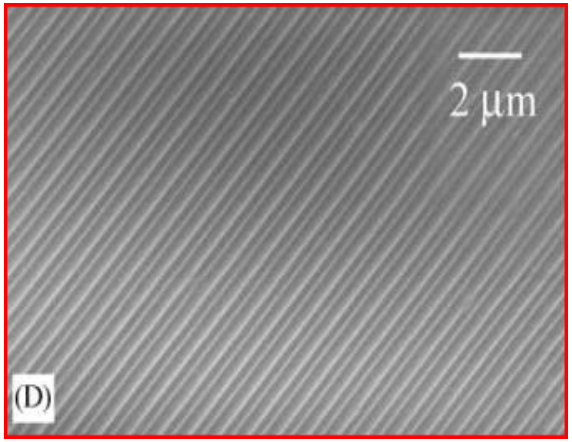


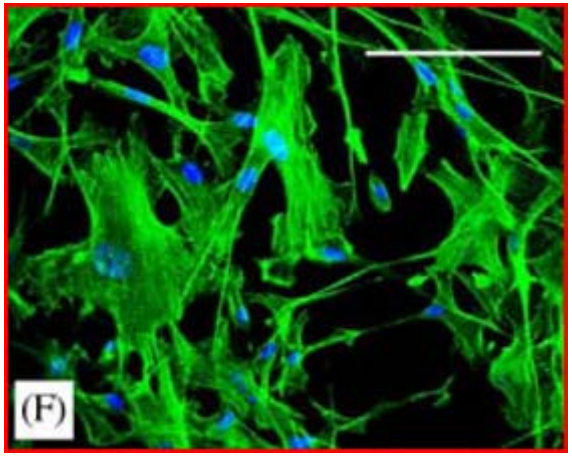
Patterning by chemical grafting

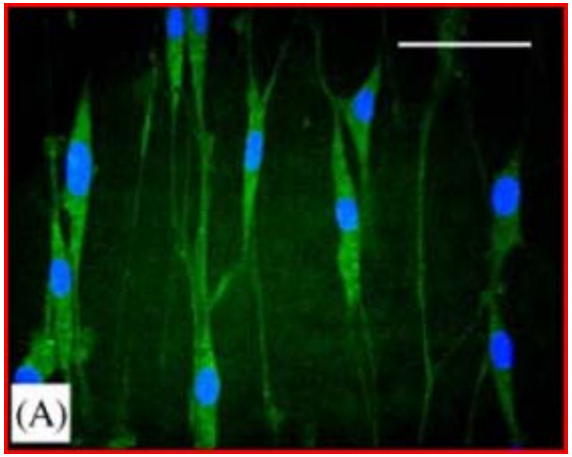


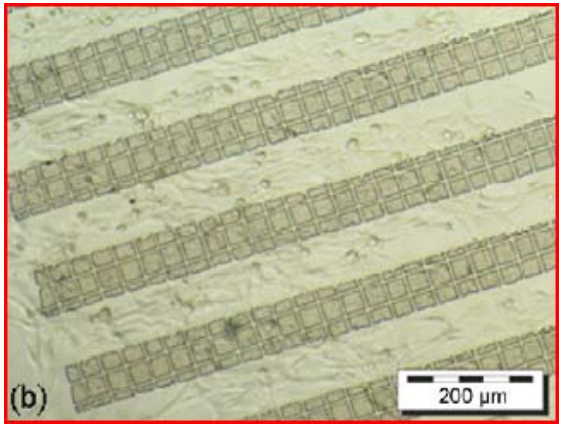
Patterning of RGD by chemical grafting

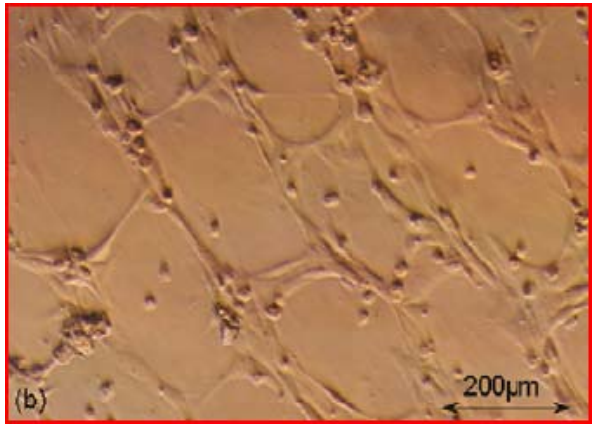
Cellular orientation and network

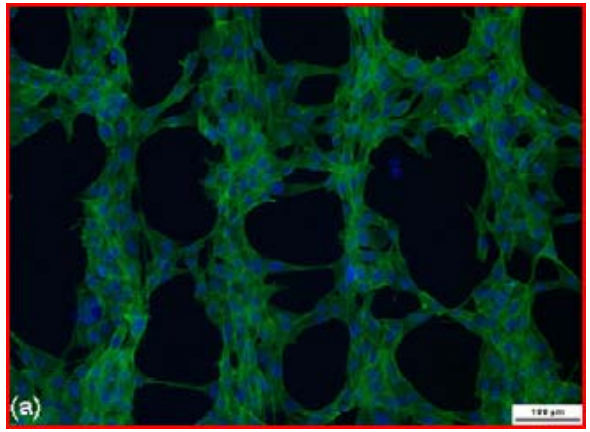










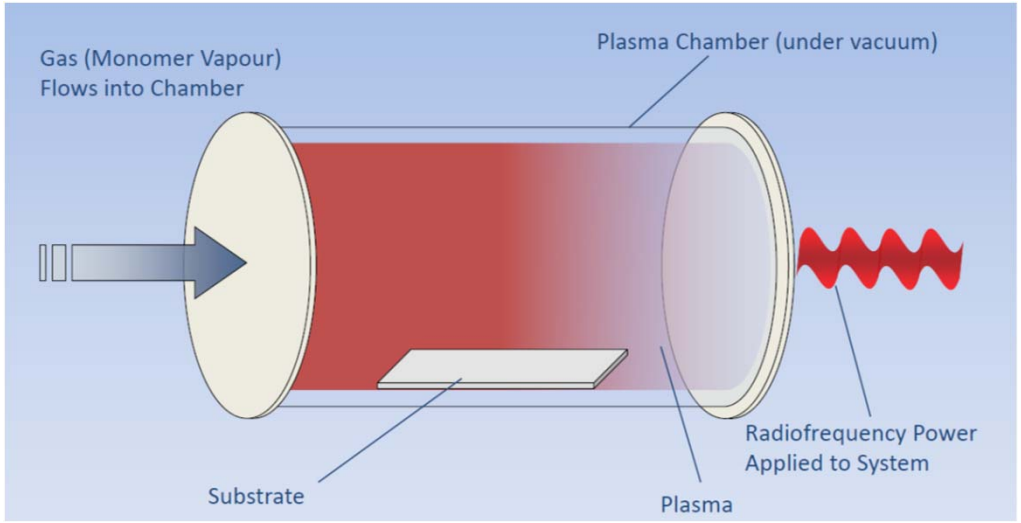


Plasma polymerization

Plasma or radiation (γ or UV) generates surface free radicals that initiate chain polymerizationPlasma (ions, free radicals, electrons) radical formation

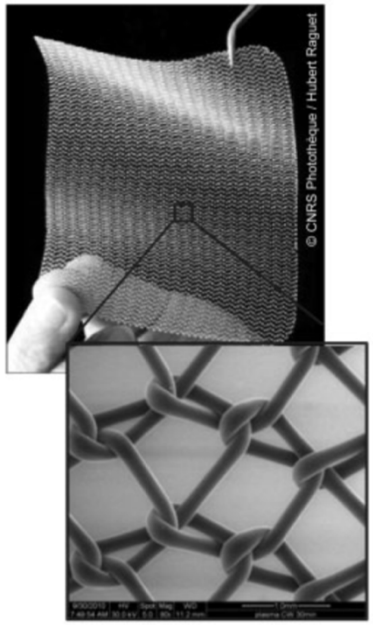
One step,
no solvent (green)
Common double bonded monomers, but also siloxane
Dense 3-D network of insoluble polymers
Adhesive coating
All types of surfaces

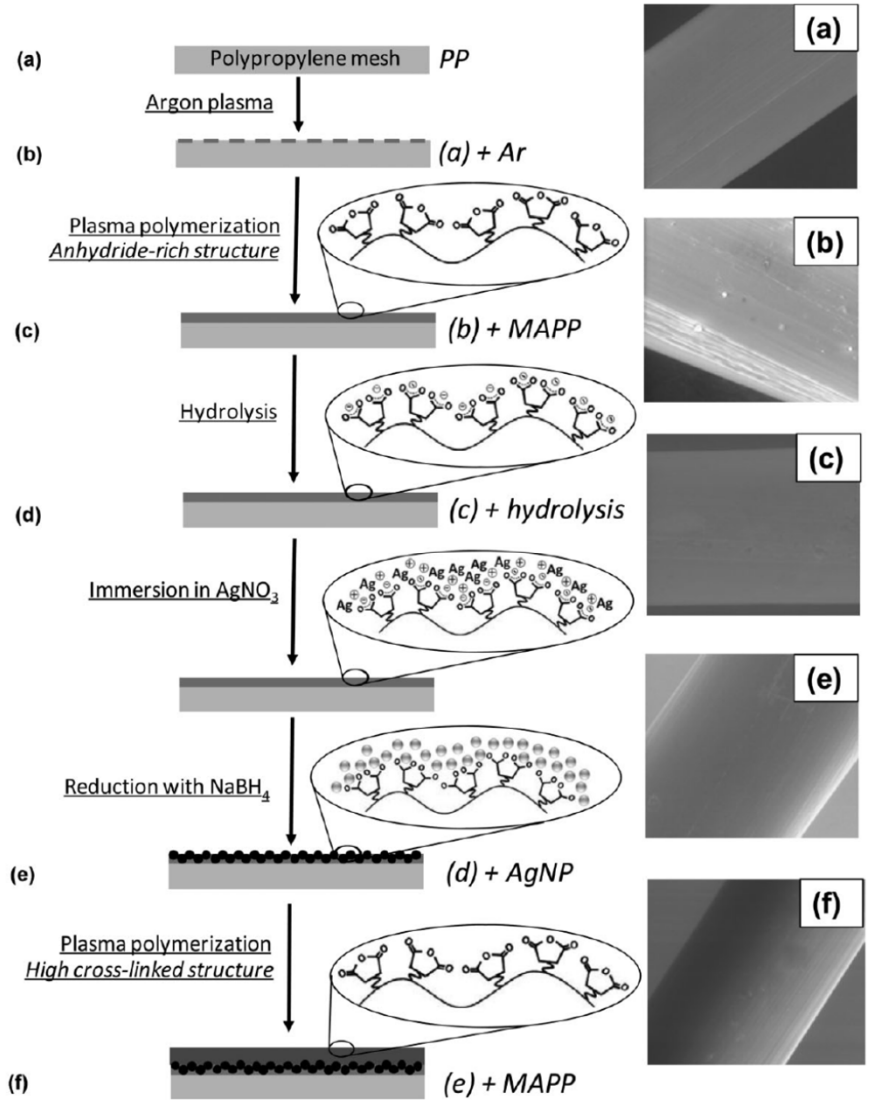
Disadvantages:
- vacuum process- complex procedure
- poorly controlled Mw
- presence of monomer
- homopolymer not bound to the matrix



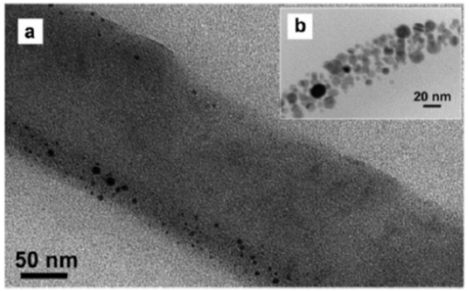
Plasma polymerization

Polypropylene mesh (PP)



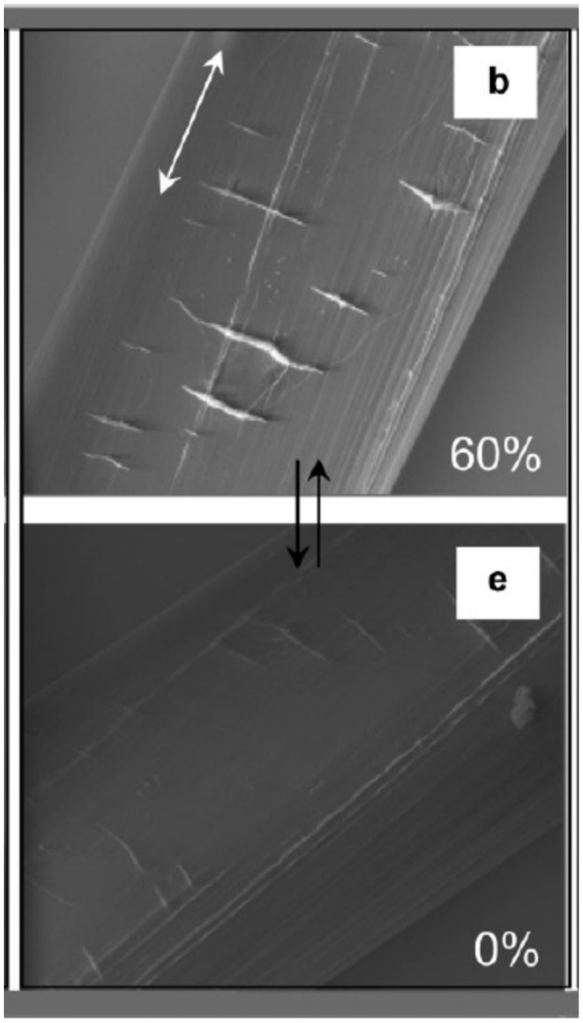


TEM
2 – 20 nm Ø Ag NP

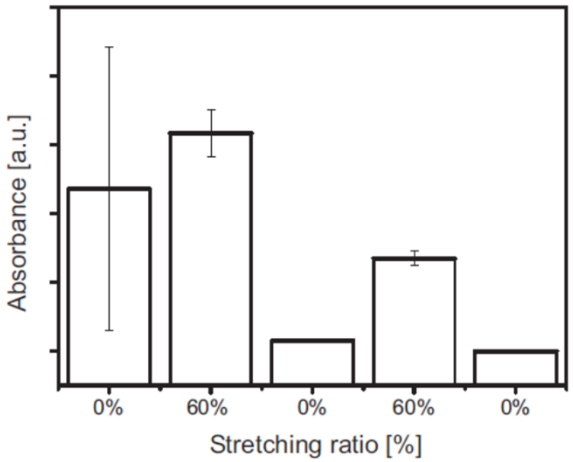


Plasma polymerization

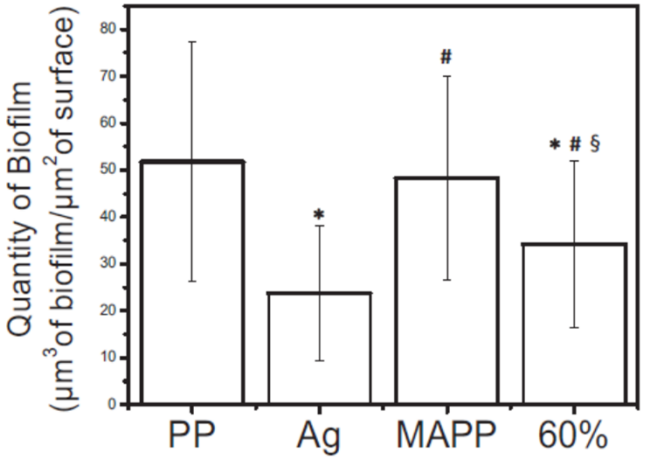
Cracks open/close

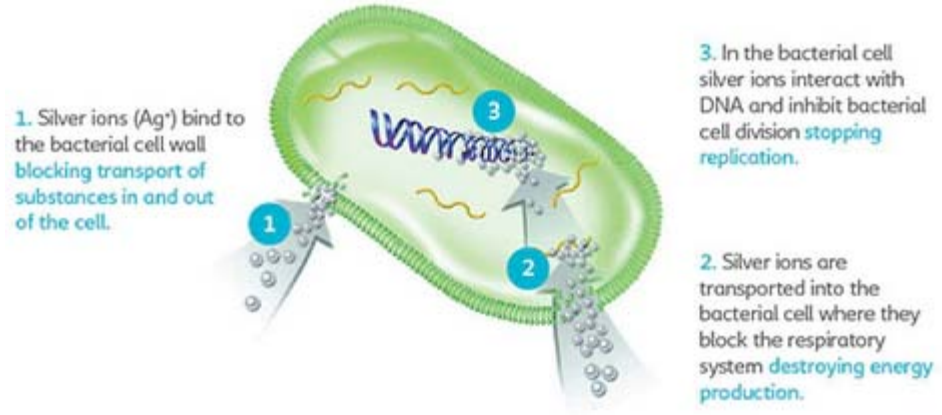


Release of Ag+ under stretching



Biofilm quantification

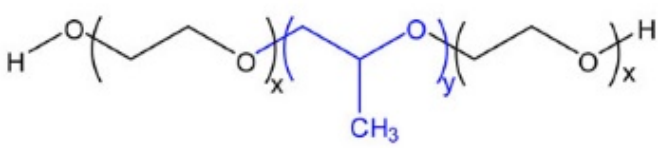


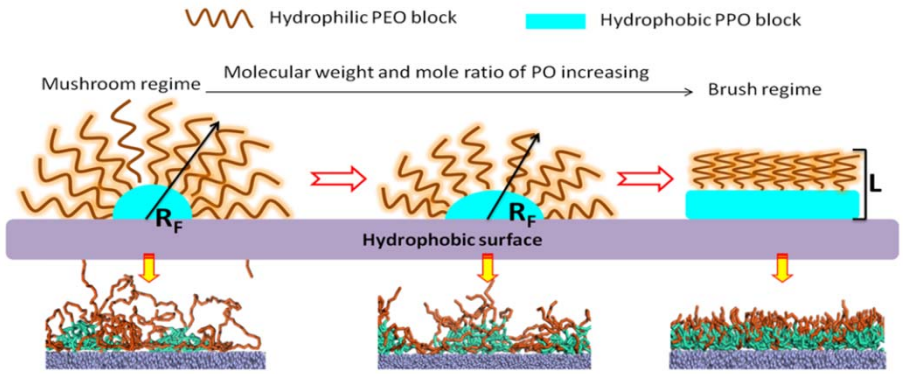


coloplast.com/products

Physisorption of amphiphilic macromolecules

- PEO-PPO-PEO (polyethylene oxide, commercial poly(propylene oxide)
- low toxicity and compatibility with aqueous systems
- adsorption on hydrophobic surfaces due to PPO/substrate interactions
- applications: paints, filtration and drug delivery





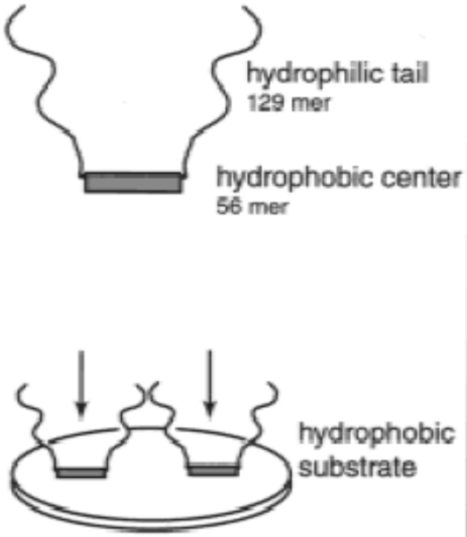
Langmuir 2016, 32, 11375−11385

Disadvantages

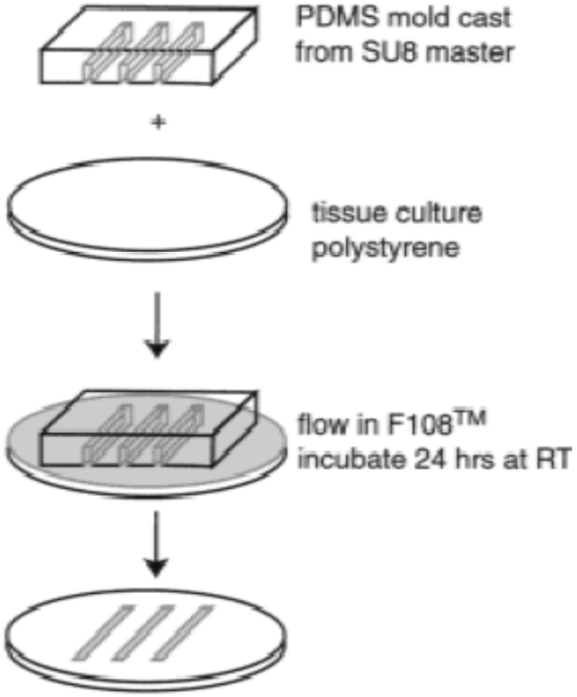
- Low surface density (steric hindrance)
- No covalent rearrangement by the cells

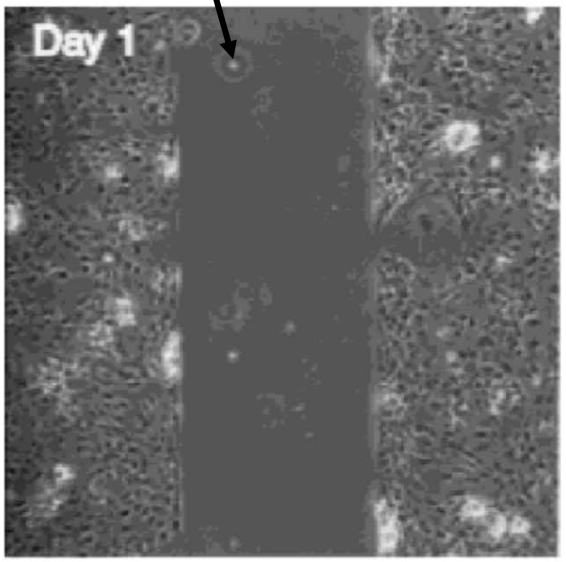
Physisorption of amphiphilic macromolecules

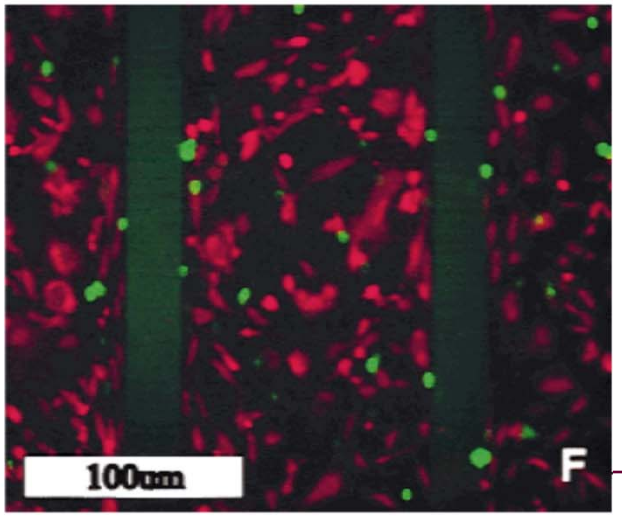
a. $(PEO)_m$ $(PPO)_n$ $(PEO)_m$ triblock polymer F108™



b. Microfluidic deposition of F108™ on tissue culture polystyrene





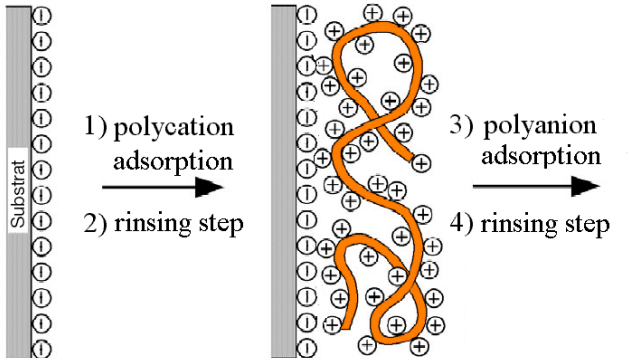


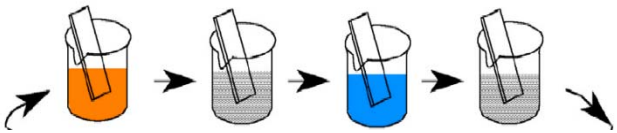
Liu et al. J Biomed Mater Res 2002, 60, 126

Polyelectrolyte multilayers

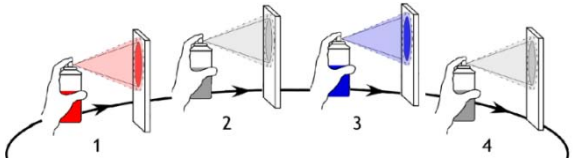
Polyelectrolyte multilayers

Alternated deposition of polycation and polyanion





Decher, G. *Science* **1997**, 277, 1232

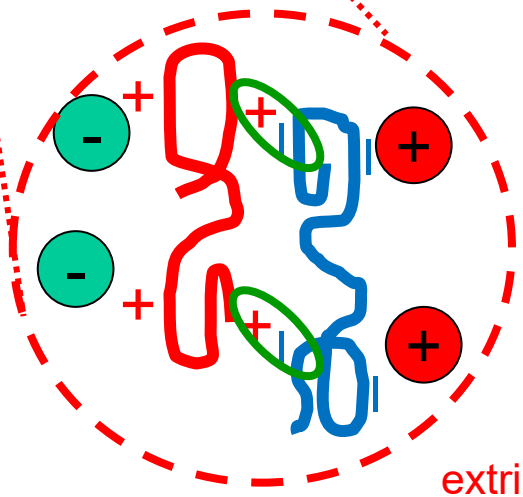


Schlenoff *et al. Langmuir* **2000**, 16, 9968

Park *et al. Scientific Report* **2018**, 3365

Overcompensation of the charges

Compensation of the charges inside the film



intrinsic by the polyelectrolytes

extrinsic by the ions

Schlenoff *et al. JACS* 1998, 120, 7626

History of Layer-by-Layer

- 1966 : Iler (du Pont de Nemours, USA)

first fabrication of multilayers of charged inorganic colloids by consecutive adsorption

JOURNAL OF COLLOID AND INTERFACE SCIENCE 21, 569–594 (1966)

MULTILAYERS OF COLLOIDAL PARTICLES*

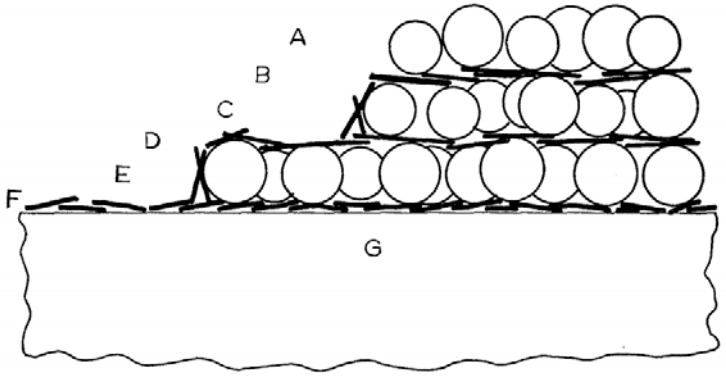
R. K. Iler

The Industrial & Biochemicals Department, E. I. du Pont de Nemours & Co., Wilmington, Delaware

Received March 24, 1965

ABSTRACT

A new technique has been developed by which alternate layers of positively and negatively charged colloidal particles, such as silica and alumina, can be deposited from sols onto a smooth surface such as glass. By this means films of controlled, uniform thickness can be built up, showing interference colors. By using uniform colloidal particles such as silica or polystyrene latex about 100 mμ in diameter as a visible indicator film, the adsorption of invisibly small particles, polyvalent ions, surfactants, and water-soluble polymers can be simply observed and studied.



A, C, E : silica particles
B, D, F : boehmite fibrils or cationic polymers

Limitations of the techniques to work with polymers

History of Layer-by-Layer

- 1966 : Iler (du Pont de Nemours, USA)

Colloidal particles LbL

- 1980 : Fromhertz (Karl-Friedrich-Bonhoeffer-Institut, Germany)

proposed to assemble proteins and linear polyelectrolytes into multilayers

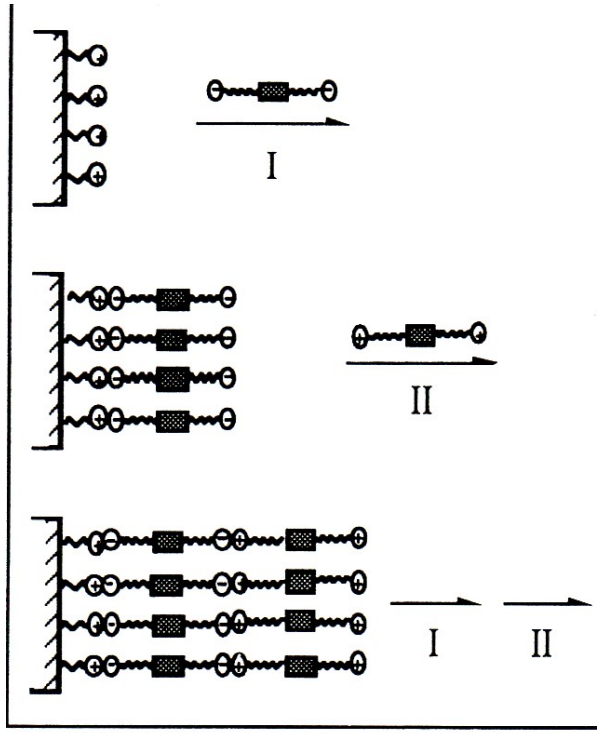
mention "*electrostatic assemblies at interfaces...by alternant binding of proteins and oppositely charged polyelectrolytes*". He also mentioned the charge reversal accompanying a protein adsorption: "..., *the number of charges located in the monolayer of bound protein exceeds the number of charges of the headgroup plane considerably. The bound layer of protein forms thus another adsorptive plane of electrostatic type with a reversed charge....*" **but no experimental proof !**

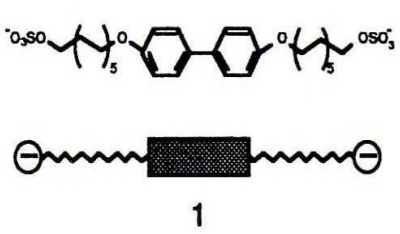
P. Fromherz, *in Electron Microscopy at Molecular Dimensions, W. Baumeister and W. Vogell, Eds. (Springer-Verlag, Berlin, 1980), 338-349.*

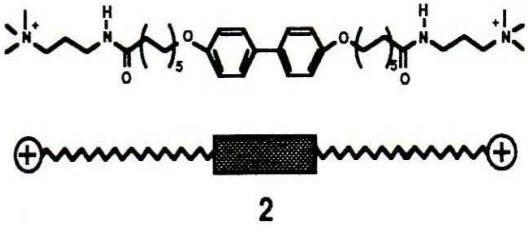
History of Layer-by-Layer

- 1991 : Hong and Decher

Alternated adsorption of anionic and cationic bolaforms (surfactants with two charged heads)







Makromolekulare Chemie-Macromolecular Symposia **1991**, *46*, 321.

History of Layer-by-Layer

- 1991 : Hong and Decher

Alternated adsorption of anionic and cationic bolaforms (surfactants with two charged heads) *Makromolekulare Chemie-Macromolecular Symposia* **1991**, *46*, 321.

- 1992 : Decher, Hong and Schmitt

Thin Solid Films, *210/211* (1992) 831–835

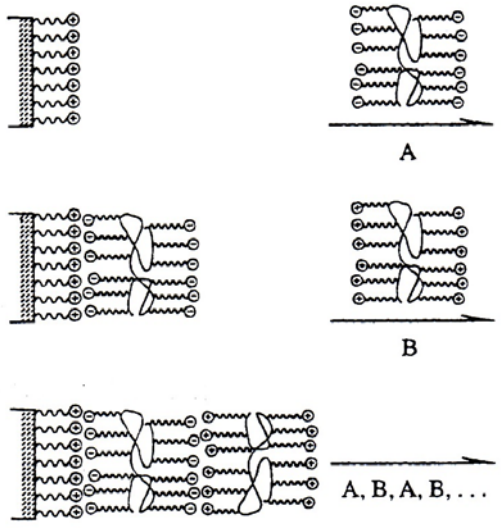
Buildup of ultrathin multilayer films by a self-assembly process: III. Consecutively alternating adsorption of anionic and cationic polyelectrolytes on charged surfaces

G. Decher, J. D. Hong and J. Schmitt

Institut für Physikalische Chemie, Johannes Gutenberg-Universität, Welder Weg 11, D-6500 Mainz (Germany)

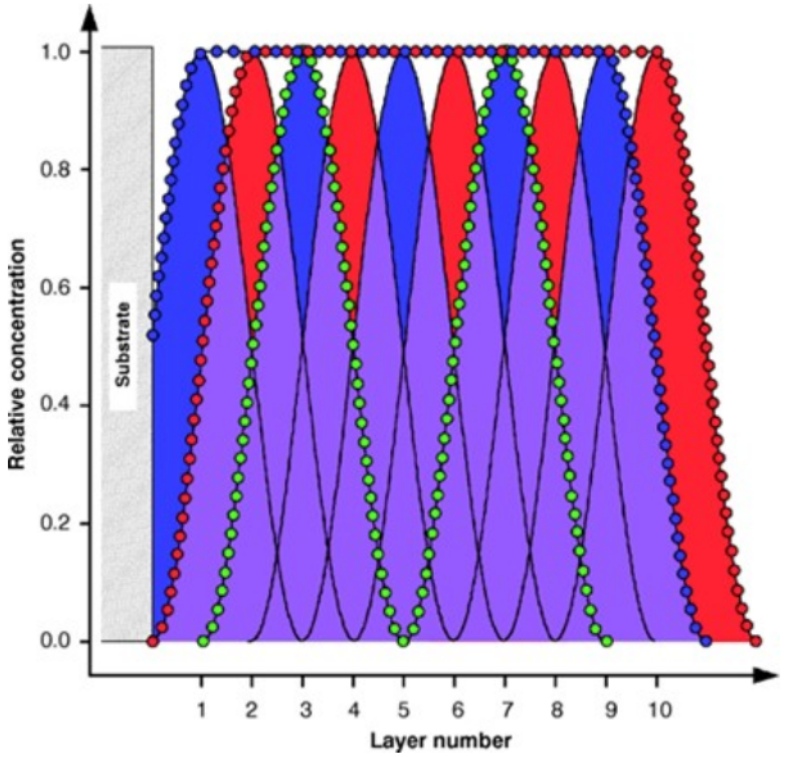
Abstract

A solid substrate with a positively charged planar surface is immersed in a solution containing an anionic polyelectrolyte and a monolayer of the polyanion is adsorbed. Since the adsorption is carried out at relatively high concentrations of polyelectrolyte, a large number of ionic residues remain exposed to the interface with the solution and thus the surface charge is effectively reversed. After rinsing in pure water the substrate is immersed in the solution containing a cationic polyelectrolyte. Again a monolayer is adsorbed but now the original surface charge is restored. By repeating both steps in a cyclic fashion, alternating multilayer assemblies of both polymers are obtained. The buildup of the multilayer films was followed by UV/vis spectroscopy and small angle X-ray scattering (SAXS). It is demonstrated that multilayer films composed of at least 100 consecutively alternating layers can be assembled.



Linear growing multilayers

Decher, **Science, 277**, 1232 (1997)

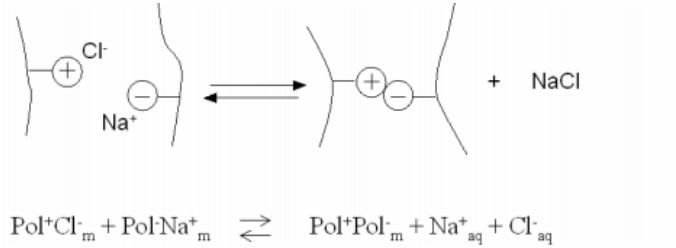


Black curve:
Concentration profile of each layer.

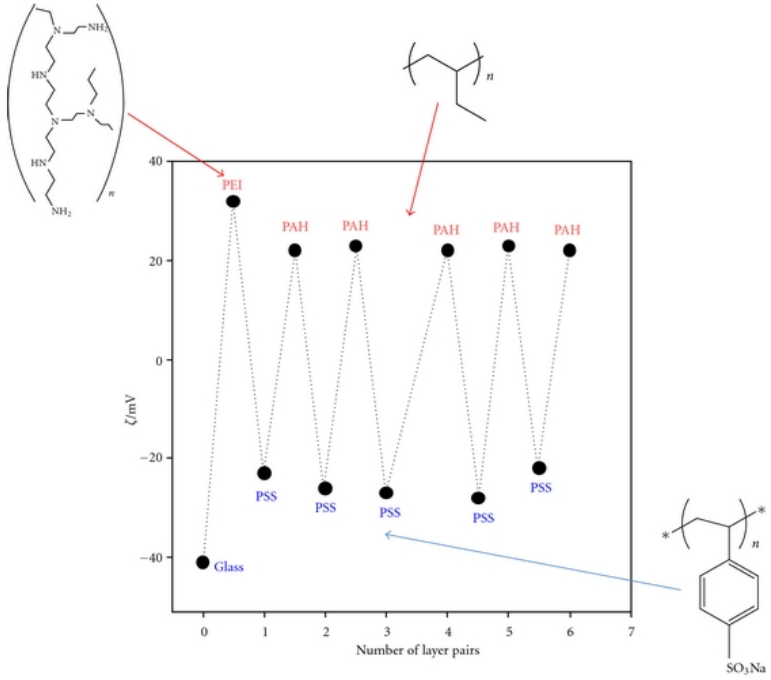
Blue (Red) dots: Total concentration profile of anionic (cationic) groups from all layers.

Green dots:
Concentration profile of a labeling group applied to every fourth layer.

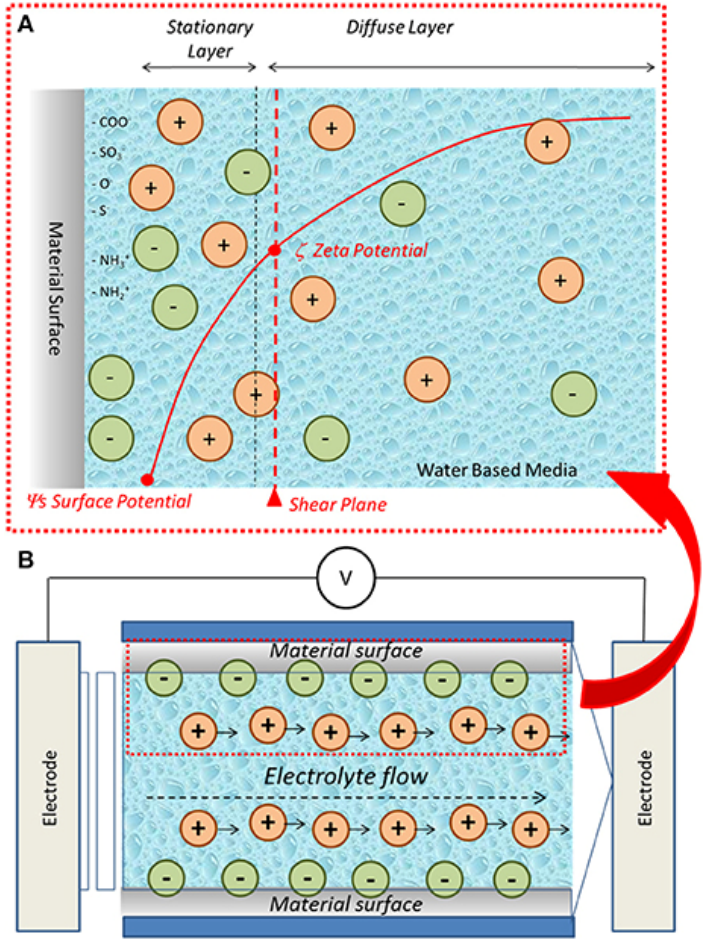
Driven force of the buildup



Zeta potential : electrostatic charges of the surface



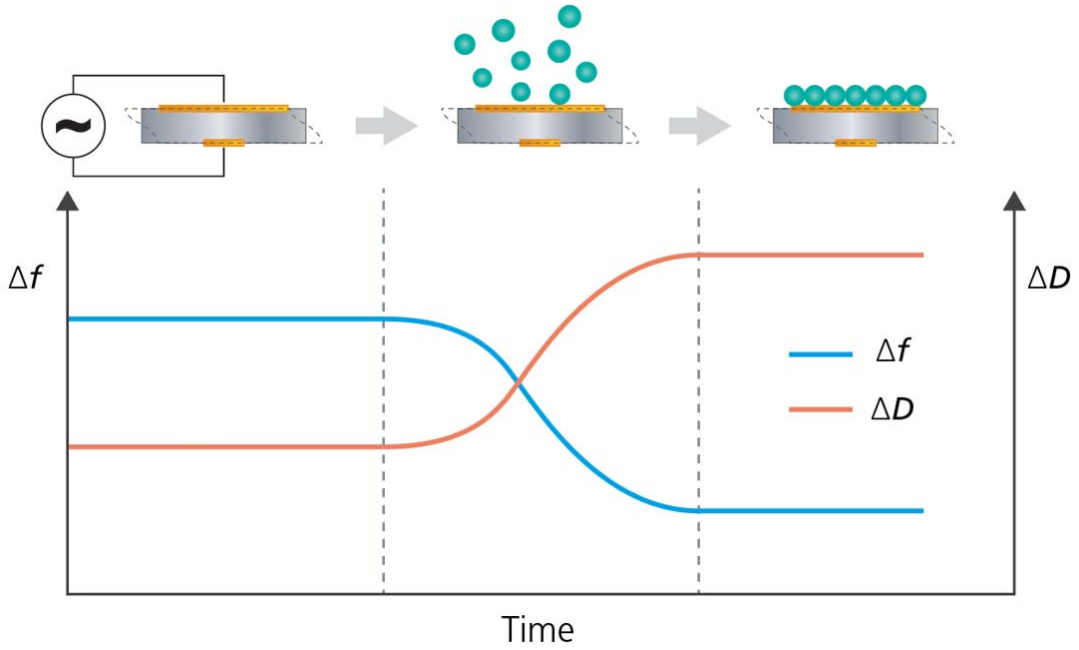
Ladam et al., **Langmuir** 2000, 16, 1249



Front. Bioeng. Biotechnol 2018, 6, 60

Quartz crystal microbalance

- **Viscoelastic technique**



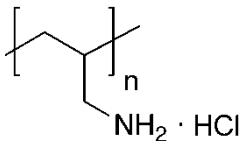
biolinscientific.com

- **Decrease of the frequency proportional to the mass (1st approximation)**

Linear growing multilayers

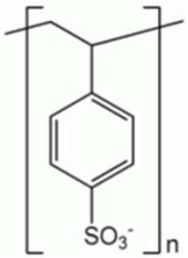
Poly(allylamine)

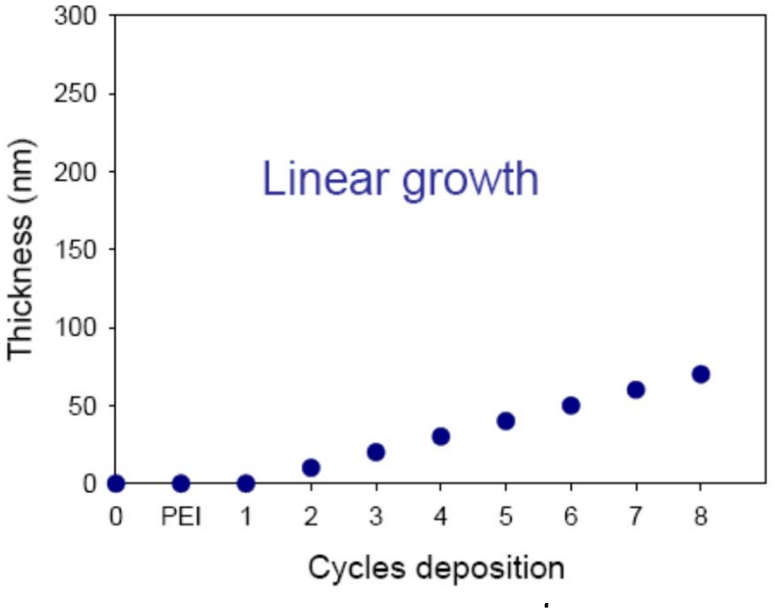
PAH



Poly(styrene sulfonate)

PSS



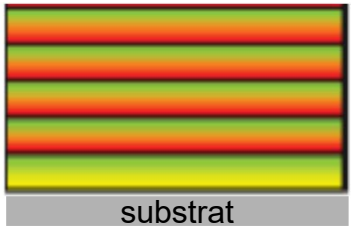


Successive adsorption of monolayers (weak interdiffusion)

Thin films (10 – 100 nm)

Dense and stratified structures

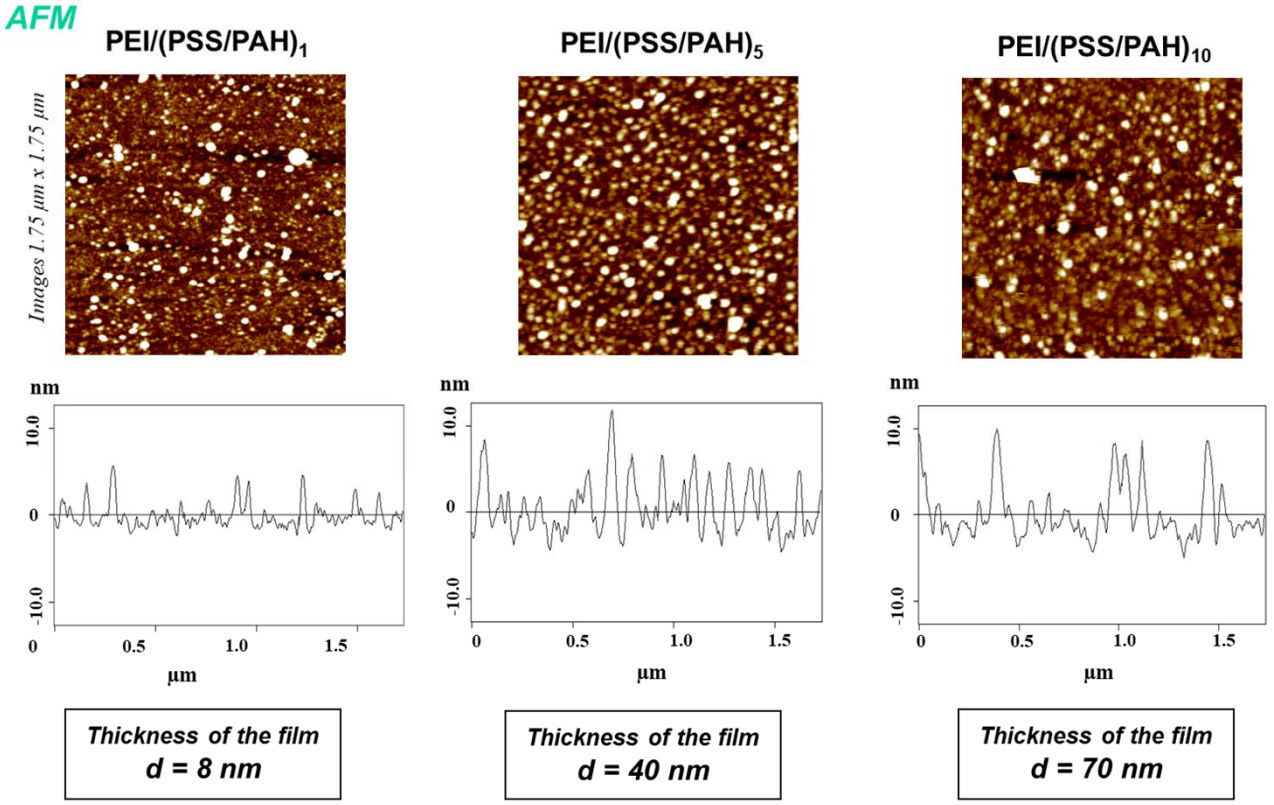
Impermeable to polyelectrolytes and proteins (barrier)



Decher, G. *Science* 1997, 277, 1232

Linear growing multilayers

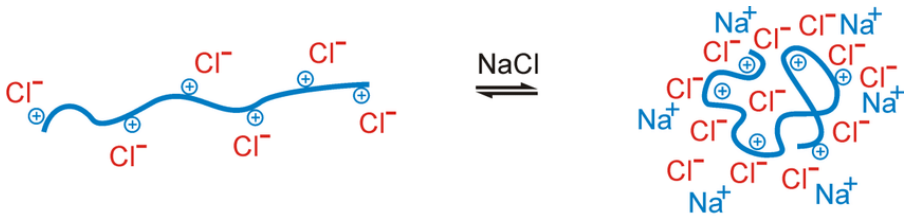
Topography by Atomic Force Microscopy



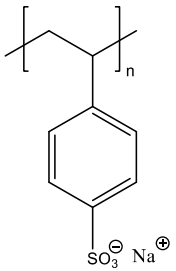
☞ *"Grains" with lateral size ranging from 30 to 80 nm*

Linear growing multilayers

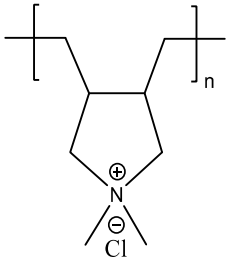
Influence of the salt concentration

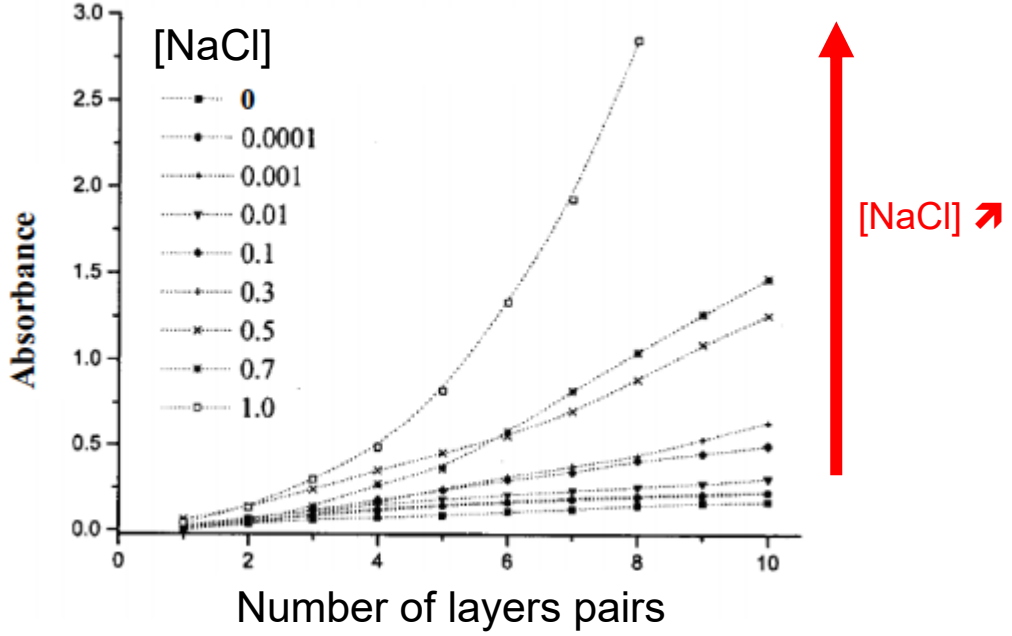


PSS



PDADMA



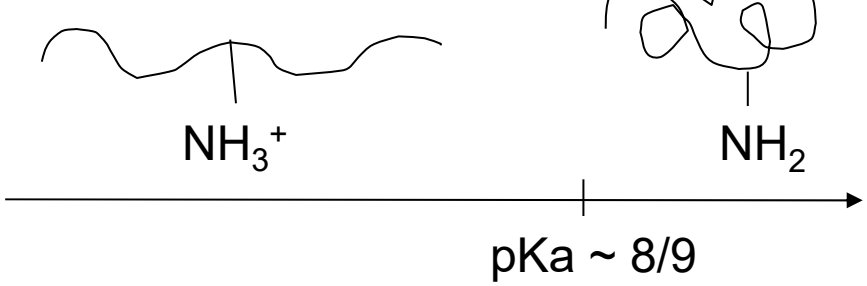


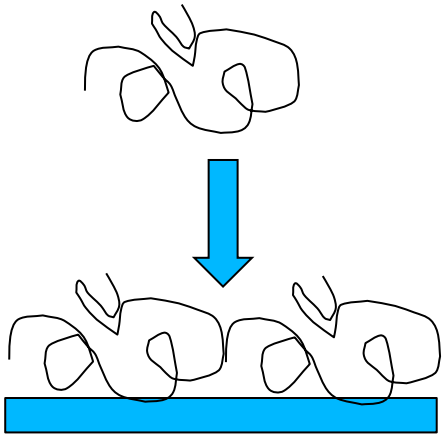
Mc Aloney Langmuir 2001, 17, 6655

Linear growing multilayers

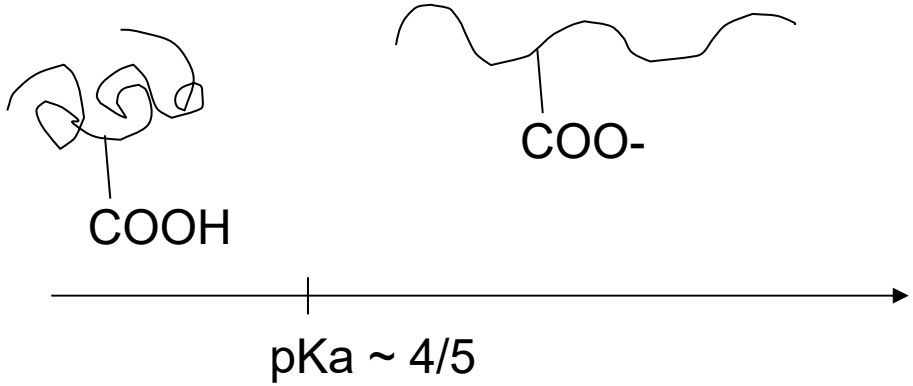
Influence of the pH

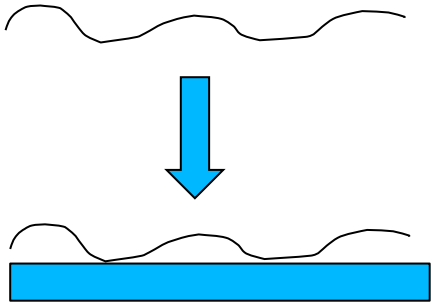
PAH





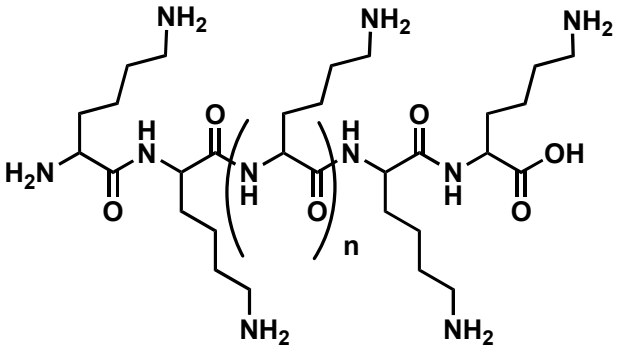
PAA



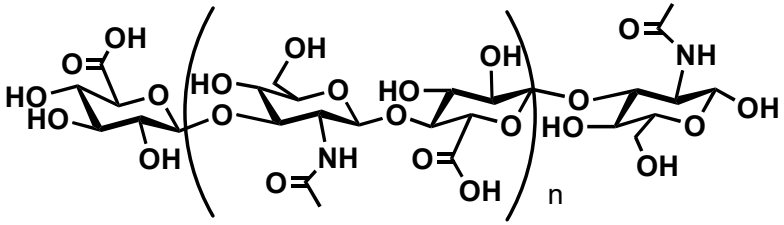


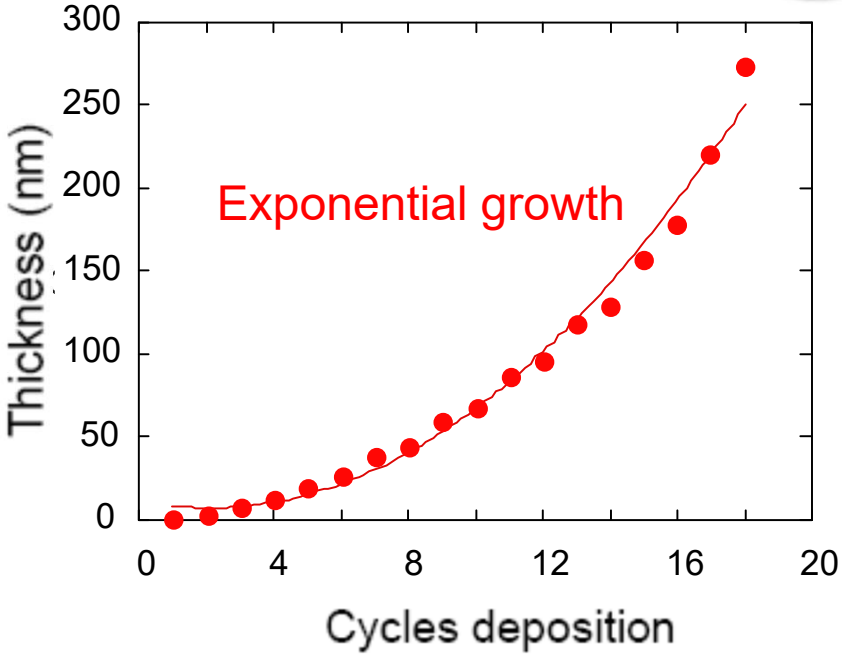
Exponential growing multilayers

Poly(L-Lysine) (PLL)



Hyaluronic acid (HA)





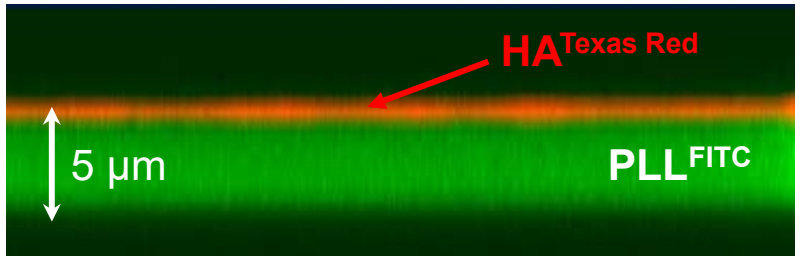
Diffusion of at least one of the polyelectrolytes

Thick films (1 – 10 µm)

Hydrated

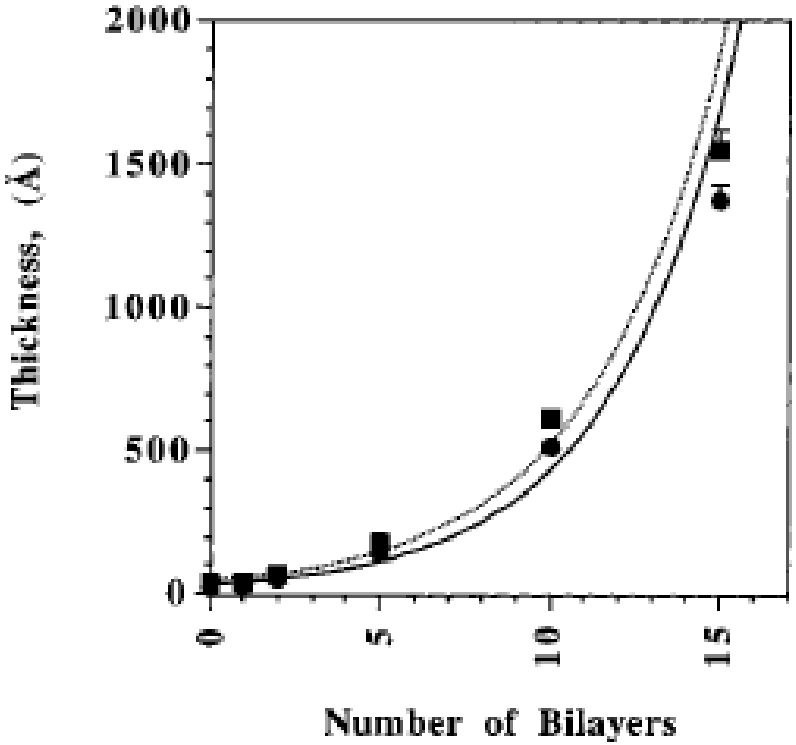
Permeable to polyelectrolytes and proteins (reservoir)

Confocal microscopy z-section



Exponential growing multilayers

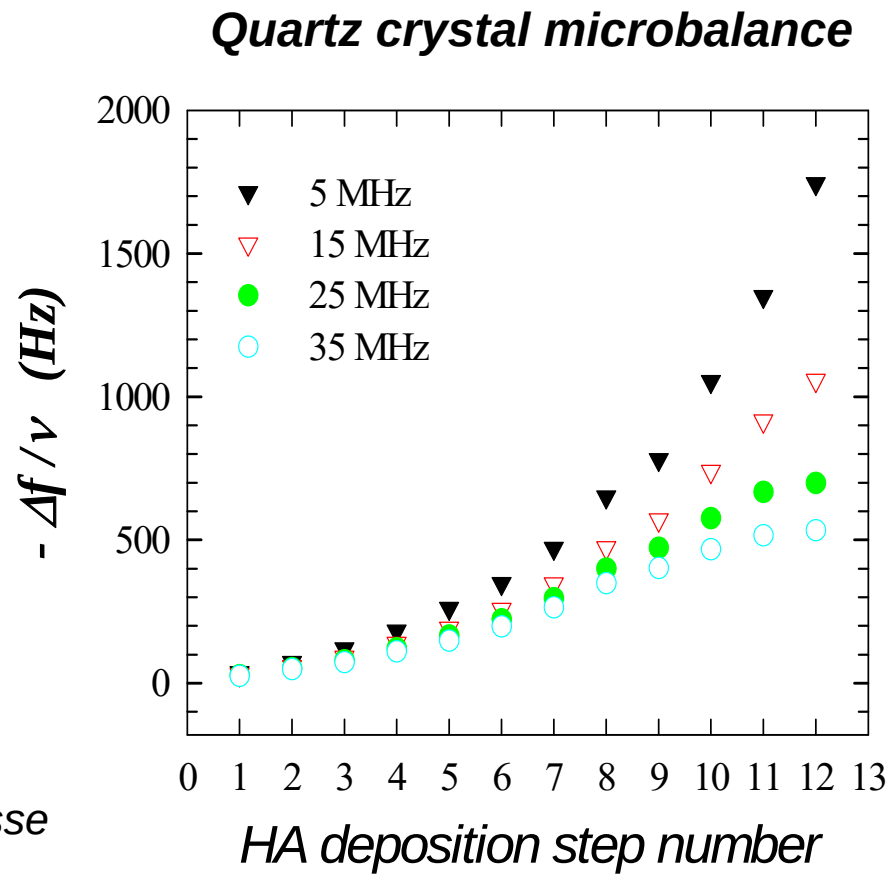
- 1999 : J. Hubbell *et al.*: Poly(lysine)/alginate first exponentially growing film



Langmuir **15**, 5355 (1999)

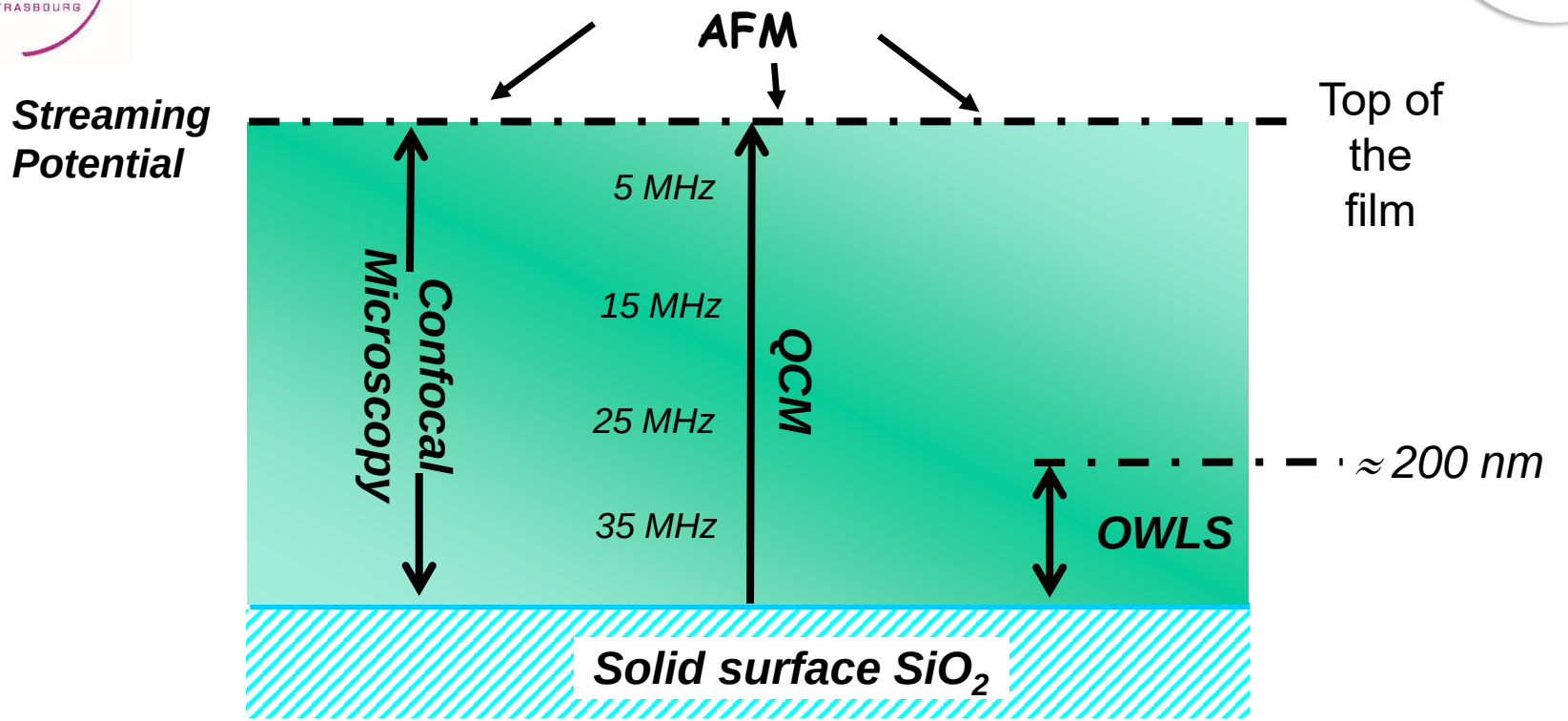
Exponential growing multilayers

- 2001 : Poly(lysine)/hyaluronic acid second exponentially growing film



$-\Delta(f)/\nu \Rightarrow$ masse

Methods *to characterize these films :*



AFM (Atomic Force Microscopy) → ***Topography***

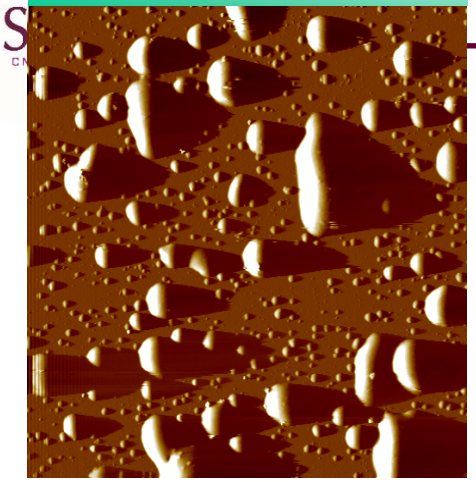
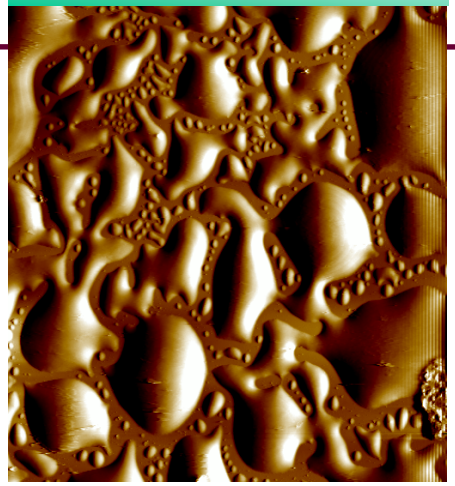
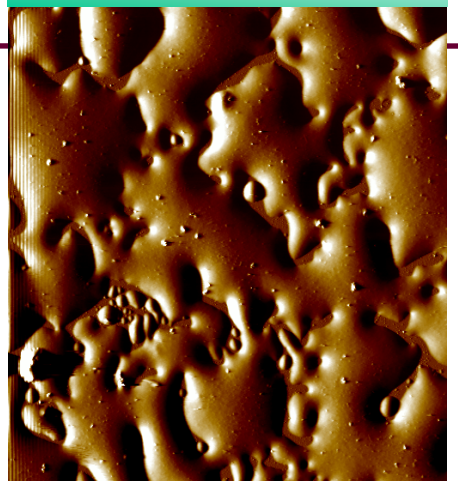
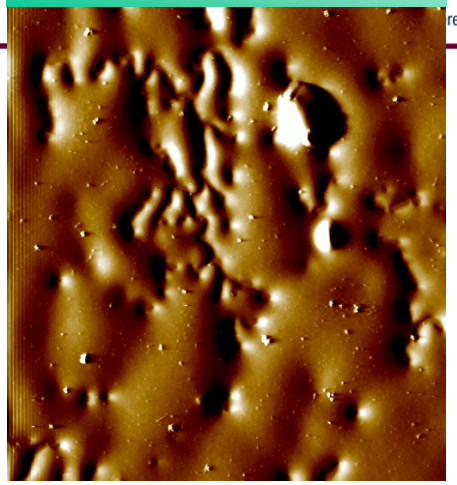
... all performed in liquid conditions

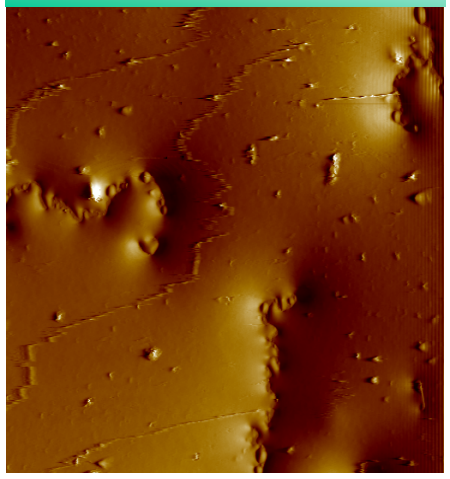
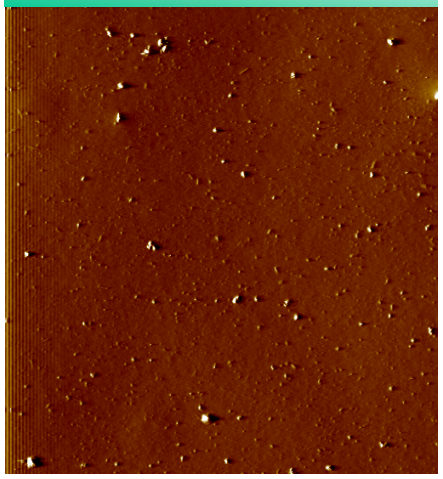
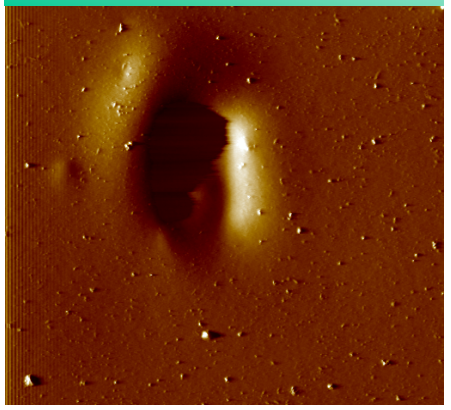
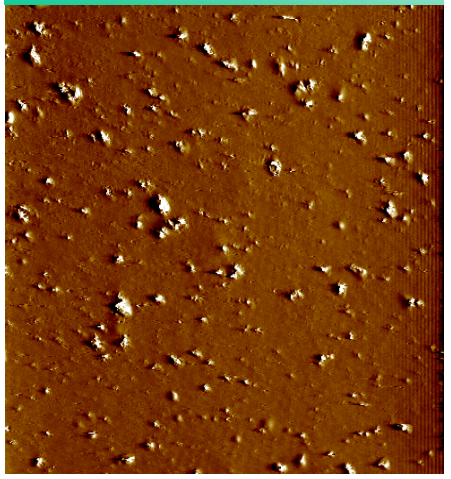
OWLS (Optical Waveguide Lightmode Spectroscopy)
QCM (Quartz Crystal Microbalance) → ***~ Mass and thickness***

Confocal microscopy → ***Image of a Z section*** ***(with fluorescently labelled polyelectrolyte)***

Streaming Potential → ***Zeta Potential*** *of the surface*

AFM *(Images 50 µm x 50 µm)*

(PLL / HA) $_1$	(PLL / HA) $_2$	(PLL/HA)$_4$	(PLL / HA) $_6$
			

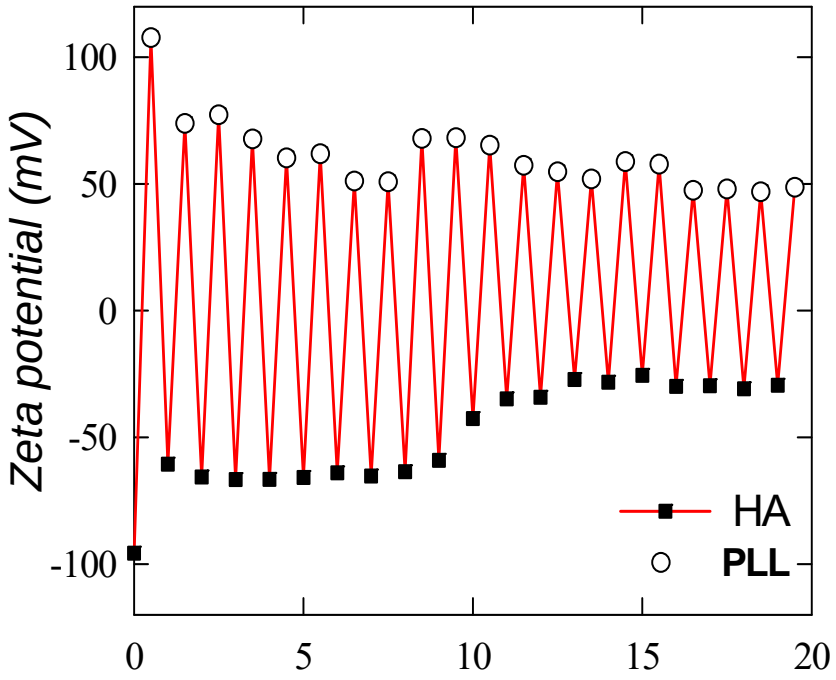
(PLL / HA) $_8$	(PLL / HA) $_8$ - PLL	(PLL / HA) $_8$ - PLL *after central scratch :* *≈ 1µm thickness*	(PLL / HA) $_{10}$
			

(PLL/HA)$_1$: formation of "islets" and "islands" probably resulting from the interaction of PLL with HA.

(PLL/HA)$_2$ → (PLL/HA)$_8$: then coalescence of structures.

(PLL/HA)$_8$-PLL : formation of an almost uniform film with heights ≈ 1 µm. "Gel" like material

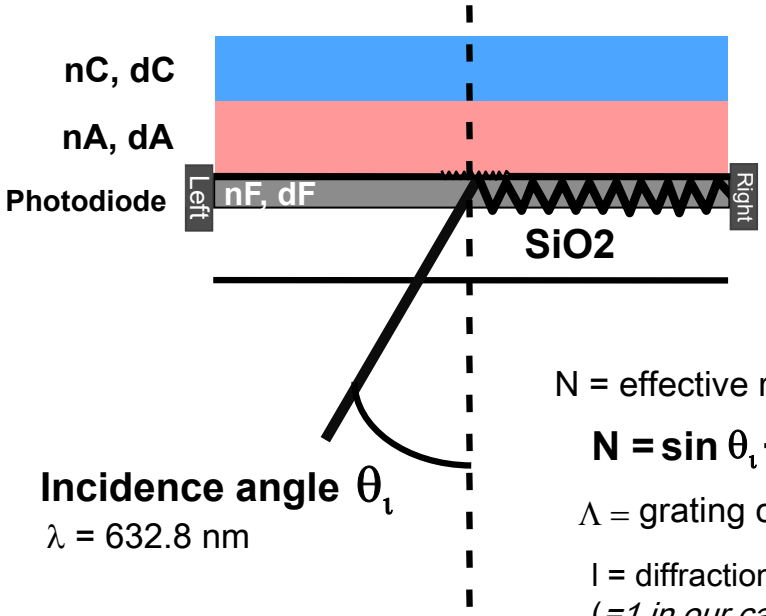
Streaming Potential



Zeta potential alternates in sign

Absolute values ~ constant

Optical Waveguide Lightmode Spectroscopy (OWLS)

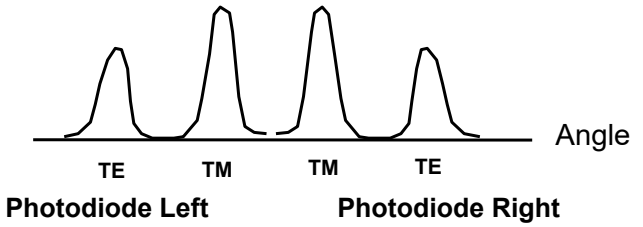


N = effective refractive index

$$N = \sin\theta_i + l\,\lambda/\Lambda$$

Λ = grating constant

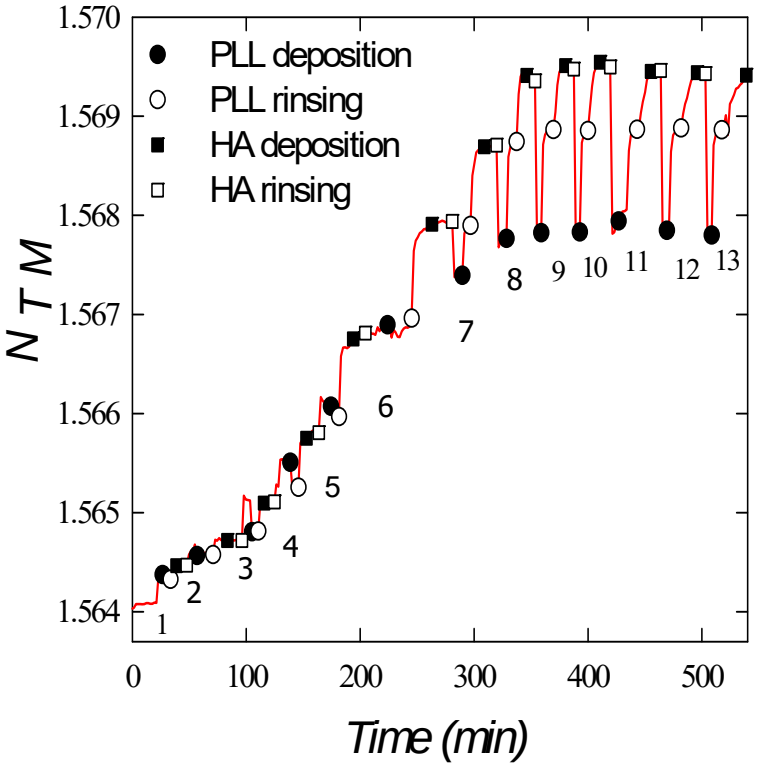
l = diffraction order
(*=1 in our case*)

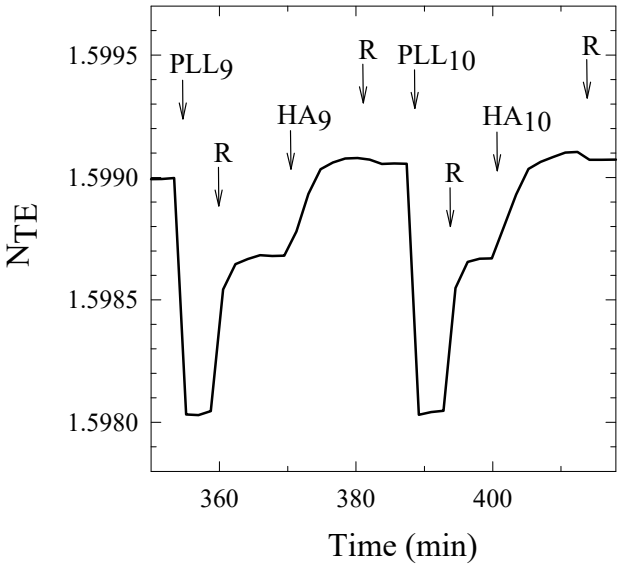


⟶ One incoupling angle for TE and TM mode

⟹ **Determination of n_A, d_A**

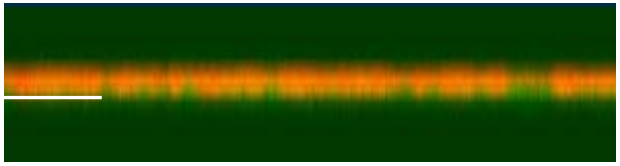
Optical waveguide lightmode spectroscopy



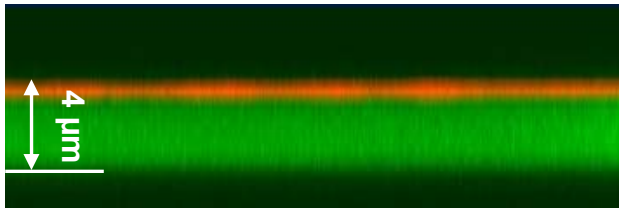


Confocal Microscopy : vertical sections

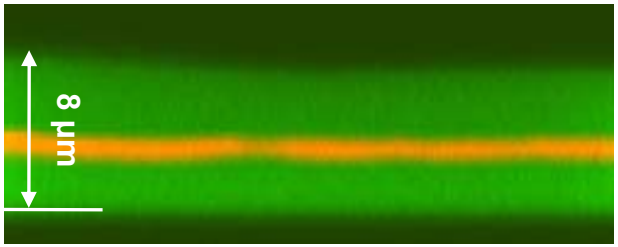
Use of fluorescent labelled polyelectrolytes : HA labelled with Texas Red (red) and PLL with FITC (green)



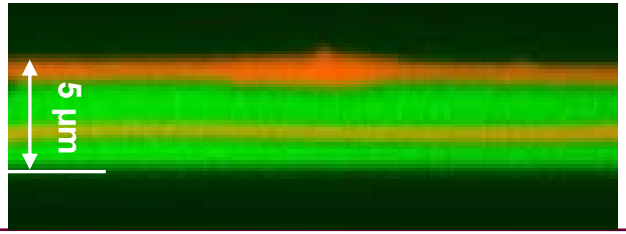
$(PLL/HA)_{10}$-PLL_{11}
with HA_{10}-TR and PLL_{11} –FITC



$(PLL/HA)_{19}$-PLL_{20}
with PLL_{19} –FITC and HA_{20}-TR



$(PLL/HA)_{25}$
with PLL_{19} –FITC and HA_{19}-TR

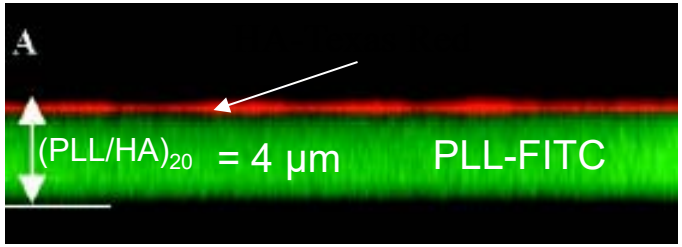


$(PLL/HA)_{20}$
with PLL_{14} –FITC and HA_{14}-TR and HA_{20}-TR

Mechanism of buildup

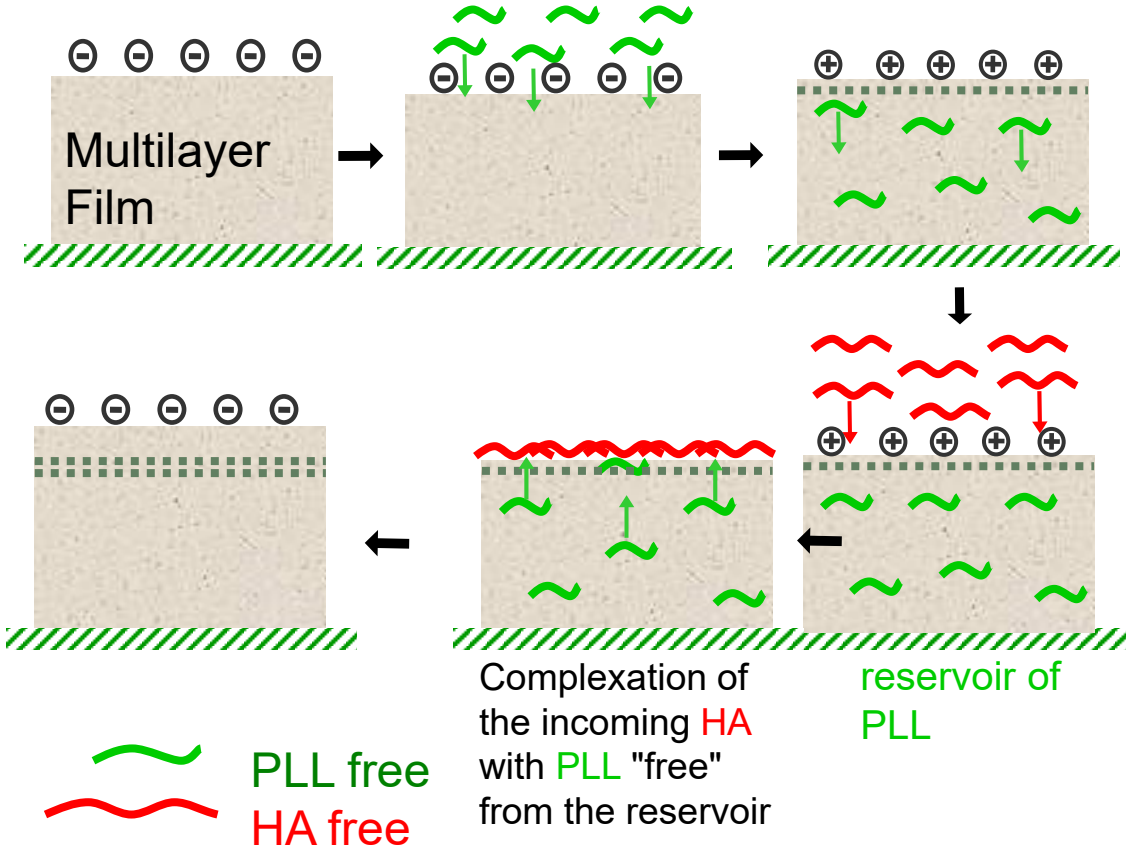
Buildup mechanism : exponential growth due to polypeptide diffusion

(Poly(L-lysine)/hyaluronic acid)$_{20}$



- Thickness proportional to the reservoir
- Exponential growth

Picart *et al.* 2002. *PNAS*, 99, 12535



Mechanism of buildup

- ***PLL chains can diffuse***

  into the film (z direction) when the PLL solution is deposited and fraction of free PLL chains can diffuse out of the film and interact with HA chains at the outer limit of the multilayers when HA is deposited

  + swelling processes

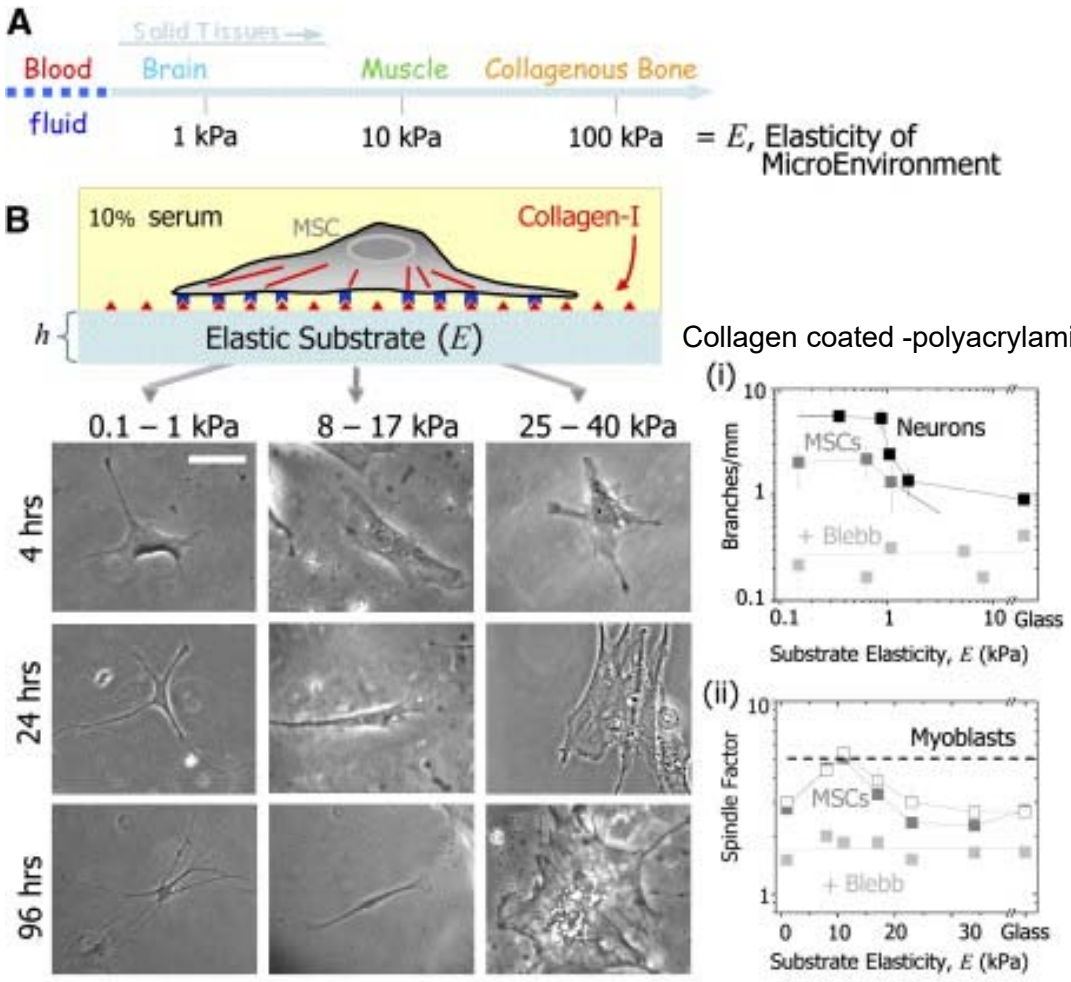
- ***2 build up regimes :***
  - a first, **from $(PLL/HA)_1$ to $(PLL/HA)_8$** = formation of islets and islands which grow and coalesce

  - a second **after $(PLL/HA)_8$**, once a homogenous and viscoelastic film is formed : **stratified** film structure of HA layers after this step

Picart C et al. ***Langmuir*** 2001; 17:7414-7424. and ***Proc. Natl. Acad. Sci. USA*** 2002; 99:12531-12535.

Influence of the mechanical properties

Mechanical properties : Young modulus (E_{film}) => stem cells differentiation



Soft => neuronal cells

Rigid => bone cells

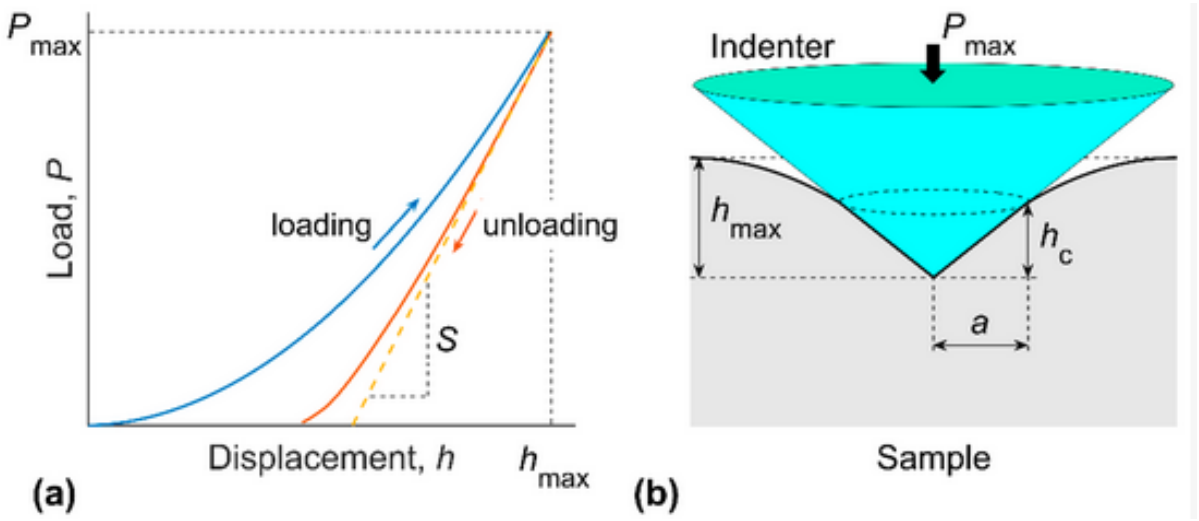
Comparaison linear vs exponential

Mechanical properties : Young modulus (E_{film})

PSS/PAH : 535 kPa stiff film

PLL/HA : 8 kPa soft film

Nanoindentation on micrometric films

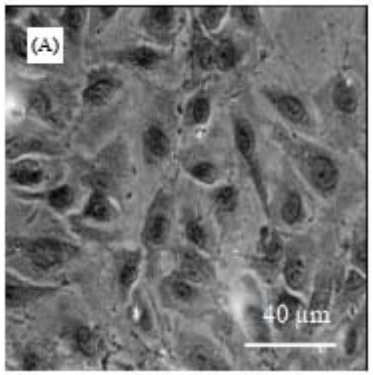


E_{film} proportionnal to the slope S

Cell behavior on PSS/PAH vs PLL/HA

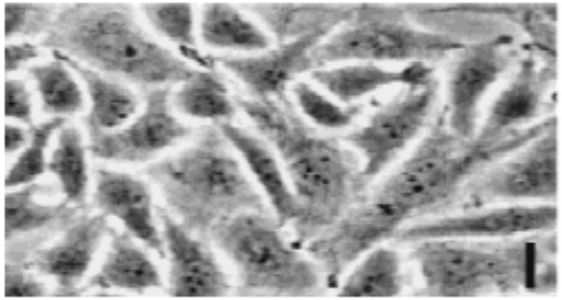
PSS/PAH: Very good adhesion and viability

Human vascular endothelial cells



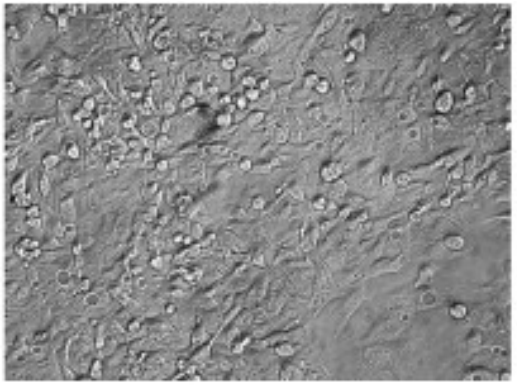
C. Boura et al. *Biomaterials* **2003**, 24, 3521

Human osteoblasts like cells



Mhamdi *et al. Mater Sci Eng* **2006**, 26, 273

Human hepatocellular carcinoma

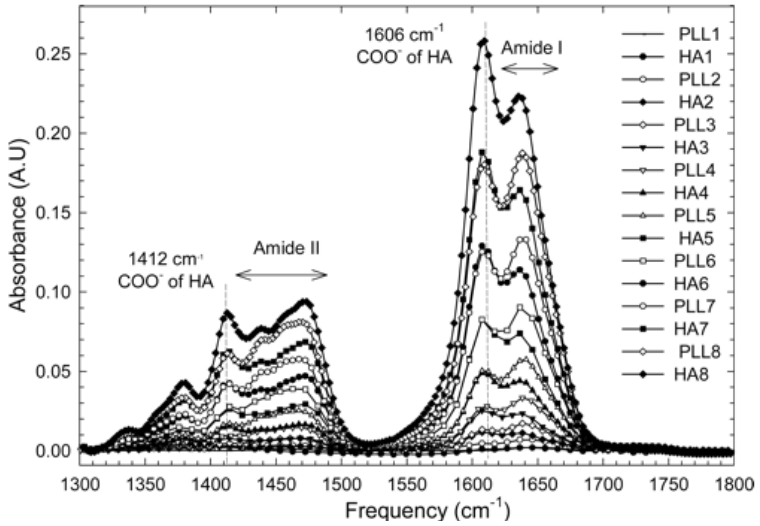


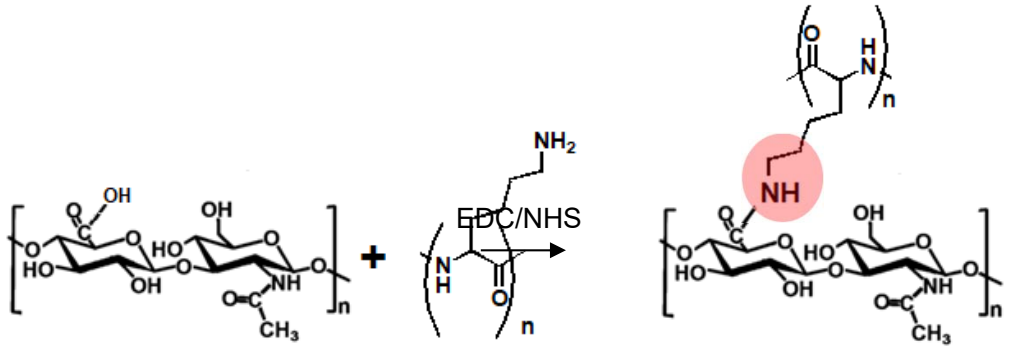
Wittmer *et al. Biomaterials* **2006**, 29, 4082

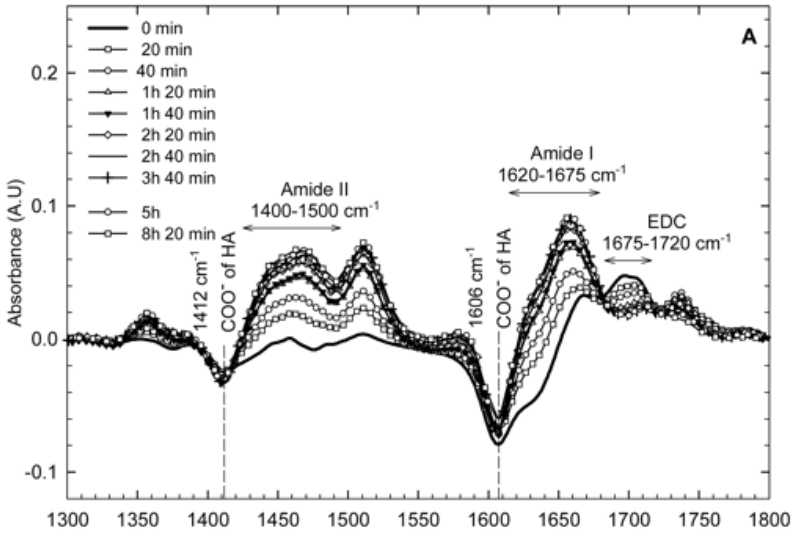
PLL/HA : no adhesion and cytotoxicity

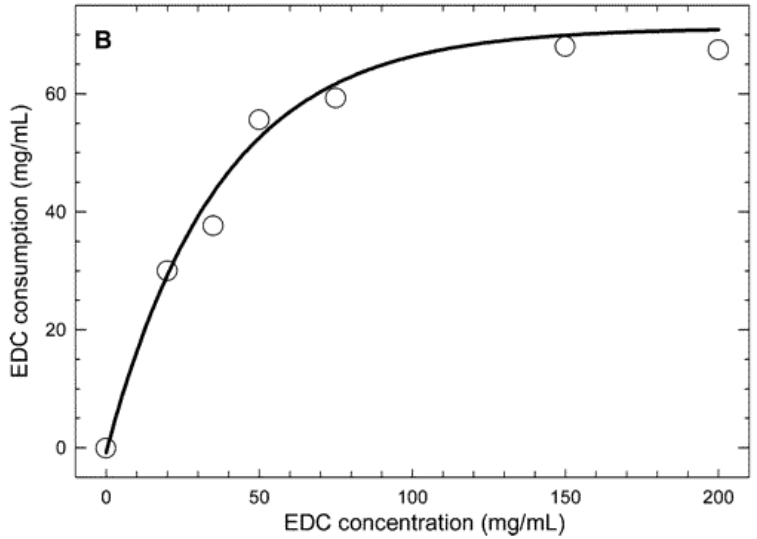
Cell behavior on PLL/HA

PLL/HA : stiffening by crosslinking









Schneider et al. Langmuir, 22, 2006, 1193

Cell behavior on crosslinked PLL/HA

Chondrosarcoma cells

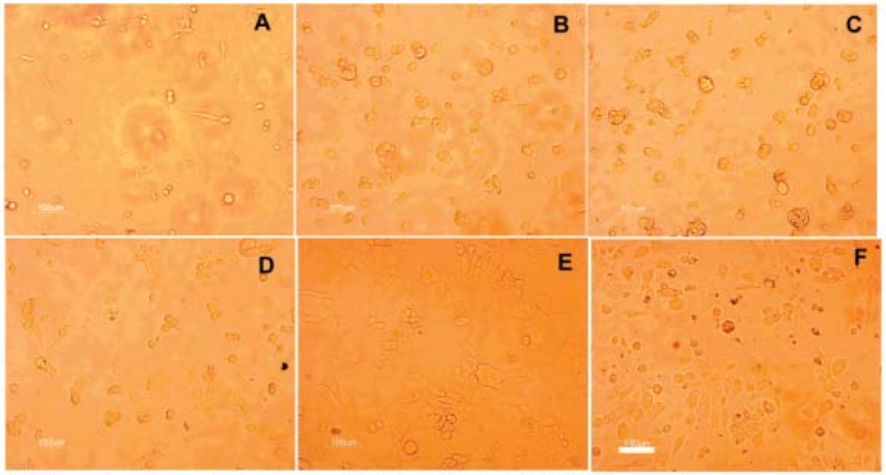
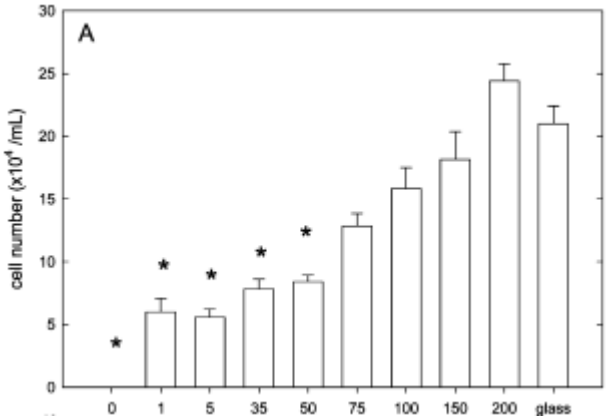
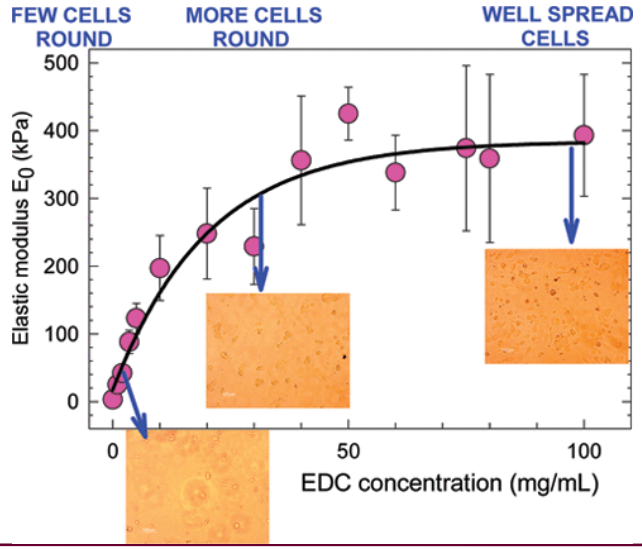


Figure 6. Microscopic observation of chondrosarcoma cells (at day 3) cultured on $(PLL/HA)_{12}$ films cross linked at various EDC concentrations: (A) 1, (B) 5, (C) 20, (D) 35, (E) 100, and (F) 200 mg/mL. Scale bar: 100 μm.

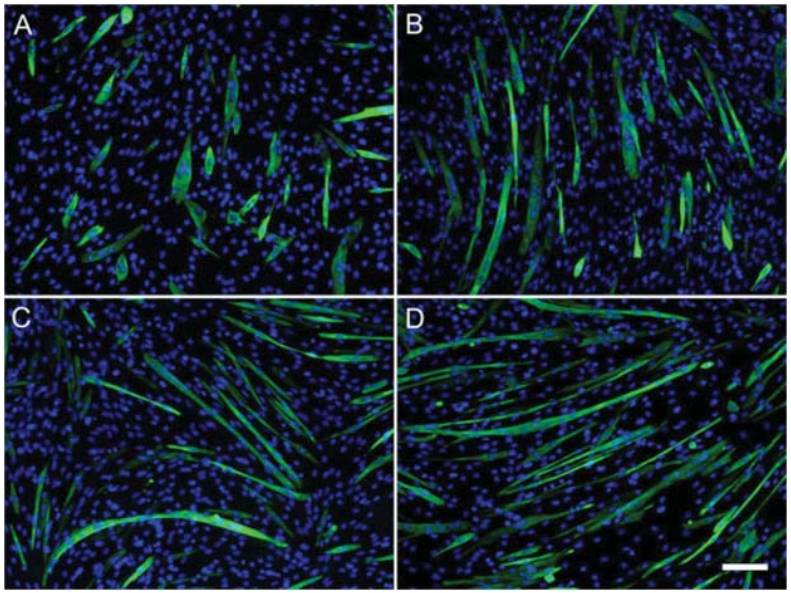




Schneider et al. Langmuir, 22, 2006, 1193

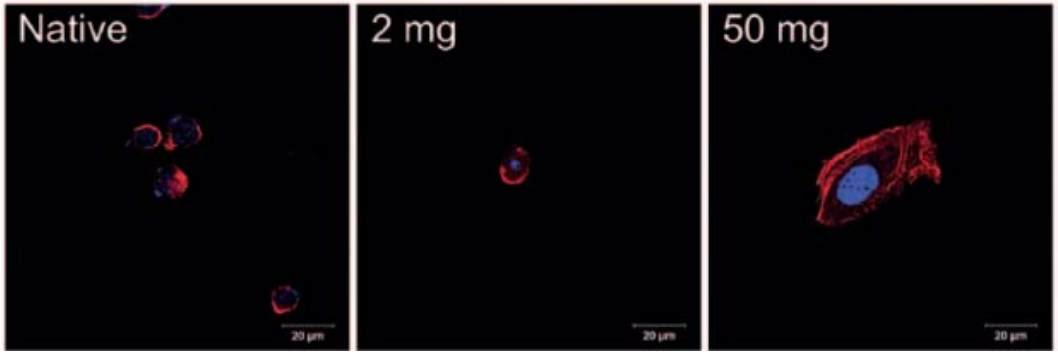
Cell behavior on crosslinked PLL/HA

Myoblasts cells



Ren et al. Adv Funct Mater 2008, 18, 1378

Adipocyte stem cells after 24h



Niepel et al. Int J Art Org 2018, 41, 1

Cell behavior on crosslinked PLL/HA

Adipocyte stem cells after 21 days

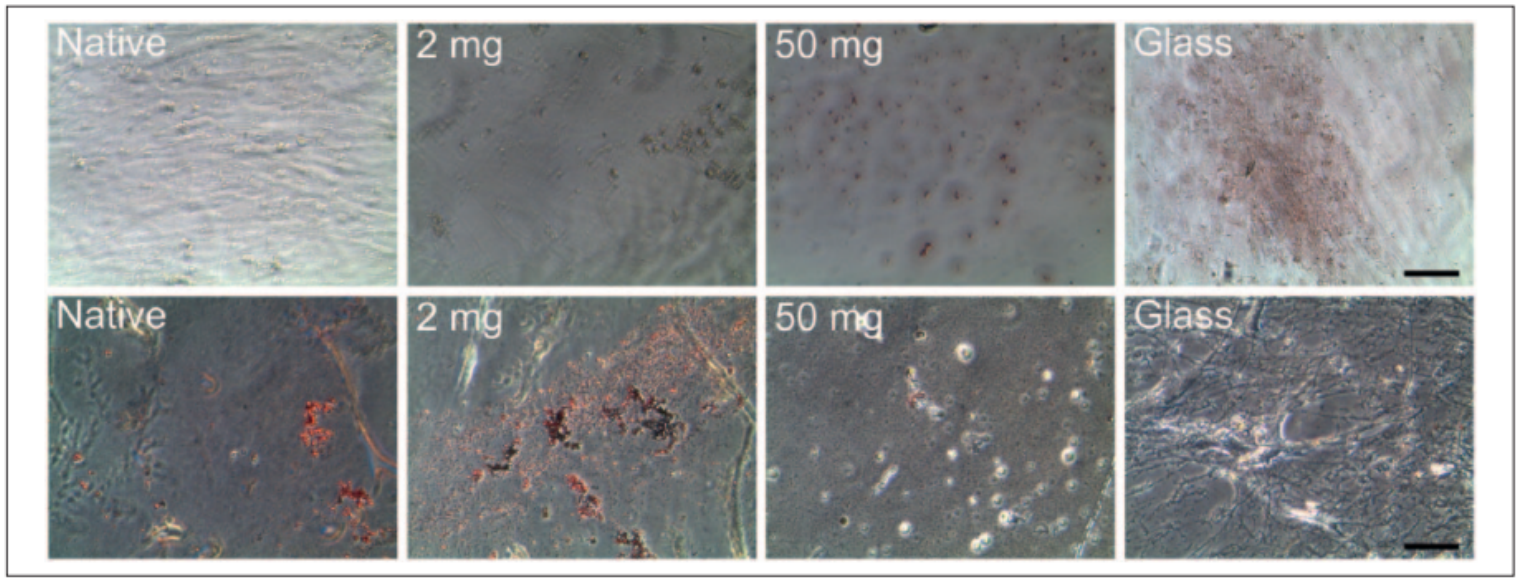
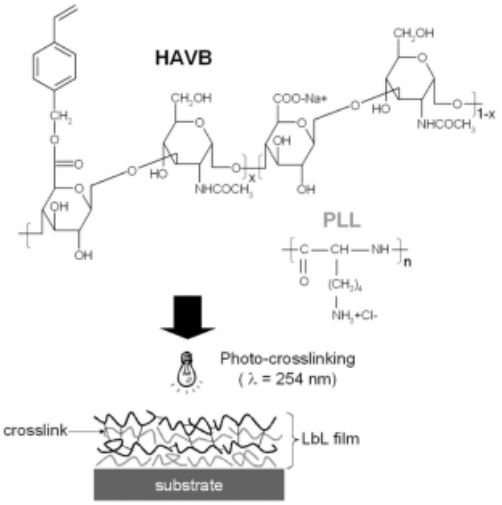


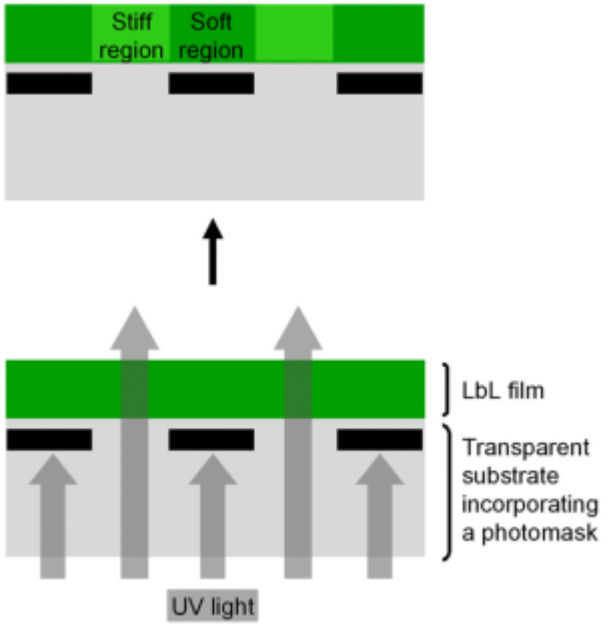
Figure 9. Visualization of osteogenic and adipogenic differentiation. Phase-contrast images of hADSC seeded on native and various cross-linked PEM are displayed after 21 days of cultivation in standard medium at serum conditions. Cells were stained with alizarin red-S solution to investigate the formation of mineralized matrix (upper row) and with oil red solution to investigate the formation of neutral lipid droplets in the cytoplasm (lower row; scale: 100 µm).

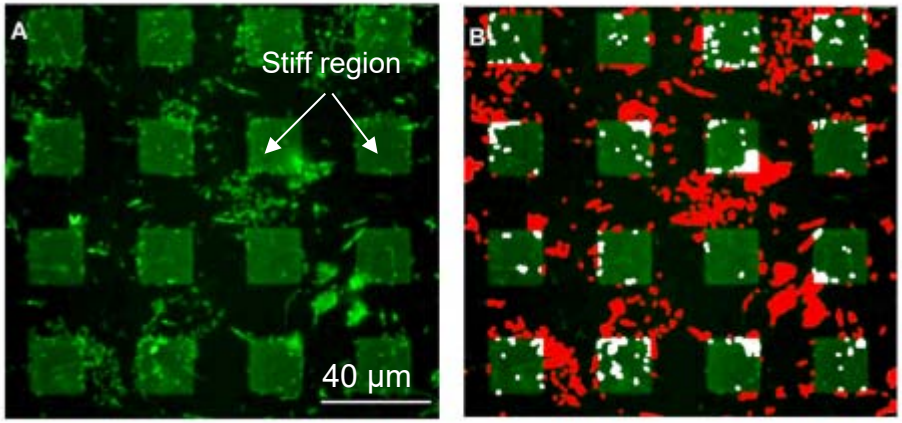
Differentiation into bone cells at high crosslinking degree

Bacteria on PLL/HA



Scheme 1.
Preparation of photo-crosslinked (PLL/HAVB) LbL films.





Soft regions => bacteria colonies

Stiff regions => no bacteria

Polyelectrolyte layer-by-layer films to explore mammalian and bacterial cell control

II. Mammalian cell

Fouzia Boulmedais

Institut Charles Sadron, Strasbourg, France

Biomaterials

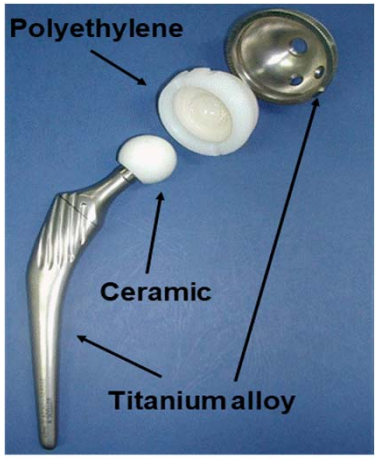
Definition agreed upon the Conference of the European Society for Biomaterials in 1986

Biomaterials = a non-viable material used in a medical device used in the diagnosis, the cure, the treatment or the prevention of disease. They are thus intended to interact with biological system.

Types of biomaterials

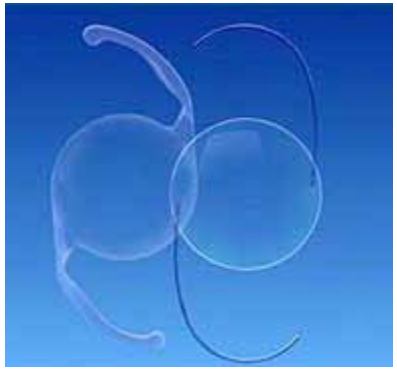
- Titanium and alloys
- Ceramics
- Synthetic and natural polymers

Hip implant



©Implants Industrie

Artificial Lens



Success but some failures

~75 years of research and development

Intraocular lenses (PMMA, silicone) but 25%-50% reoperation rate

Hip / knee prostheses (titanium, steel, PE) but still a 10-15 yr lifetime

Vascular Grafts (Teflon, Dacron) but no healing

Heart Valves (carbon, fixed tissue) but calcification or thrombosis

Stimulatory Electrodes (platinum, iridium) but electrode encapsulation

Catheters (silicone, PVC, PEU, Teflon) but infection (1,000's of deaths/yr)

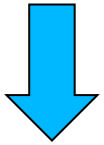
The surface dictates the biological reaction

Which properties for biomaterials ?

- To favor biointegration

Control the cell adhesion and be specific on the biomaterial surfaces

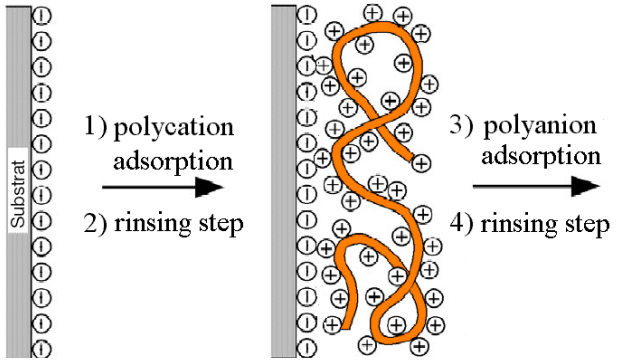
Control of materials/environment interactions

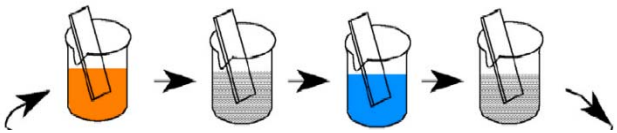


Our tool: surface functionalization by polyelectrolyte multilayers

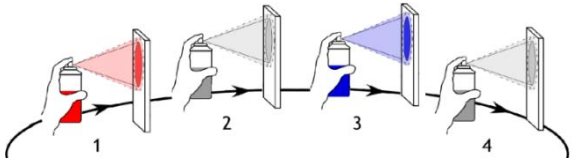
Polyelectrolyte multilayers

Alternated deposition of polycation and polyanion





Decher, G. *Science* **1997**, 277, 1232

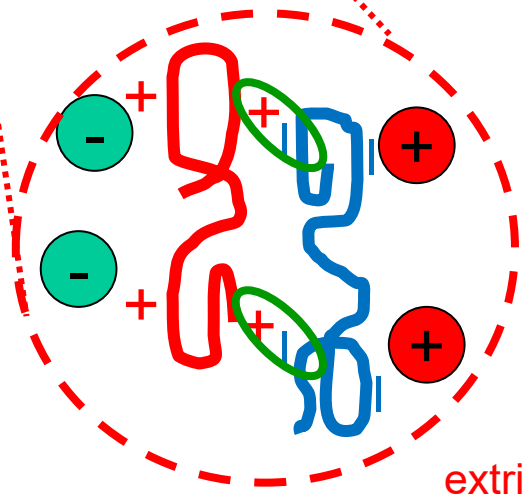


Schlenoff *et al. Langmuir* **2000**, 16, 9968

Park *et al. Scientific Report* **2018**, 3365

Overcompensation of the charges

Compensation of the charges inside the film



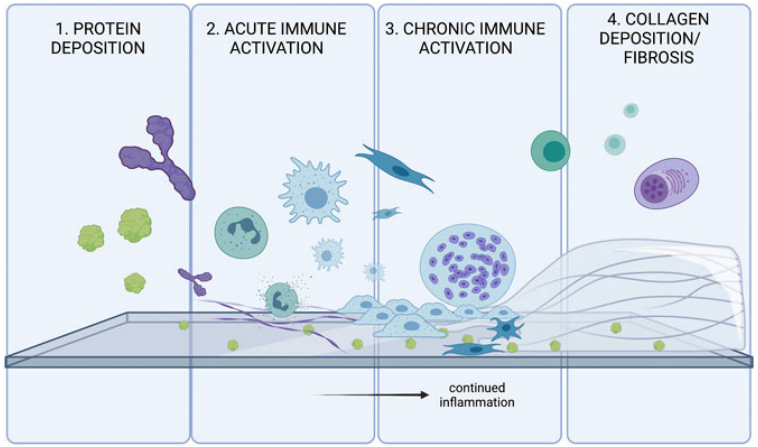
intrinsic by the polyelectrolytes

extrinsic by the ions

Schlenoff *et al. JACS* 1998, 120, 7626

Issues at the implantation

Foreign body reaction



Collagen layer encapsulation (Fibrosis)

⇨ Colonization by fibroblasts: no specific cells

⇨ Loosening of the implant

Front. Bioeng. Biotechnol., 2021, DOI: 10.3389/fbioe.2021.730938

Lots of works have been done to prevent the first step (protein adsorption)
to prevent the following steps

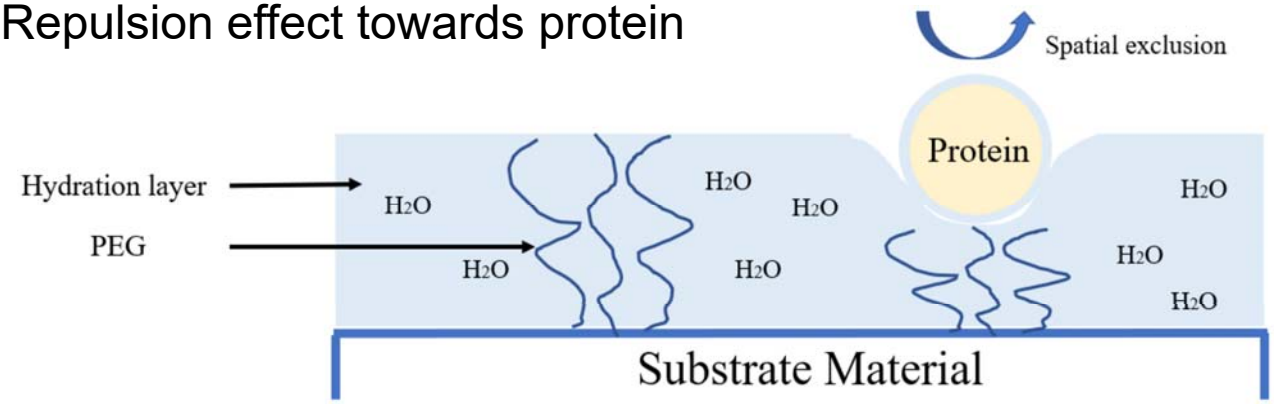
Prevention of protein adsorption

Typical polymer used

Poly(ethylene glycol) (PEG) or poly(ethylene oxide) (PEO): $-(CH_2-CH_2O)_n-$

Formation with H-bonds with water
High hydrophilicity
Non immunogenic

=> Repulsion effect towards protein



Largely used in pharmaceutical formulation to obtain furtivity of nanovectors

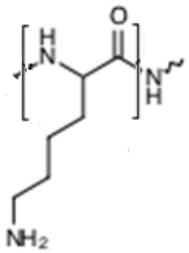
Prevention of protein adsorption

Physical adsorption of polycations

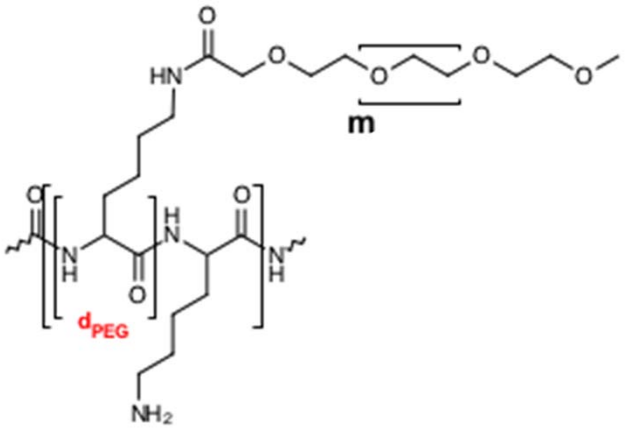
- Generally negatively charged surfaces
- Monolayer of PLL (poly(L-lysine) used for cell adhesion

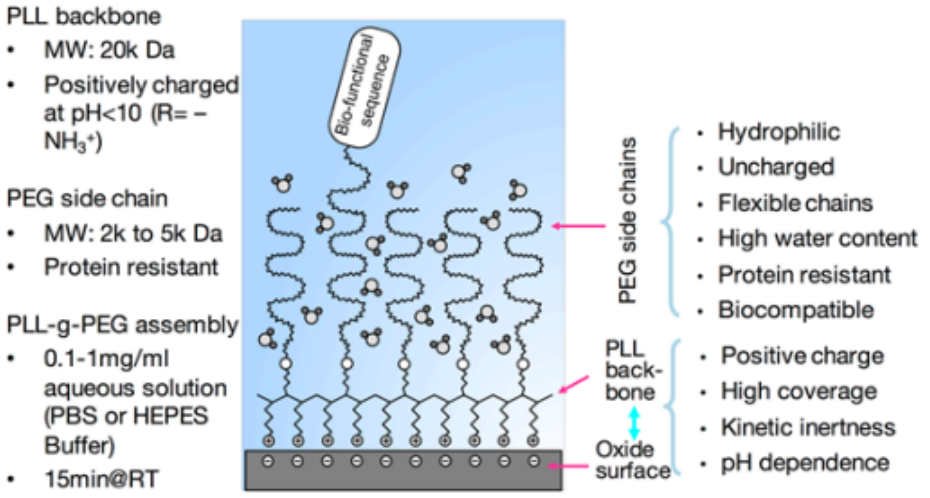
- PLL-g-PEG (polyethylene glycol) used to prevent adsorption of proteins and cell adhesion

PLL



PLL-g-PEG





www.susos.com

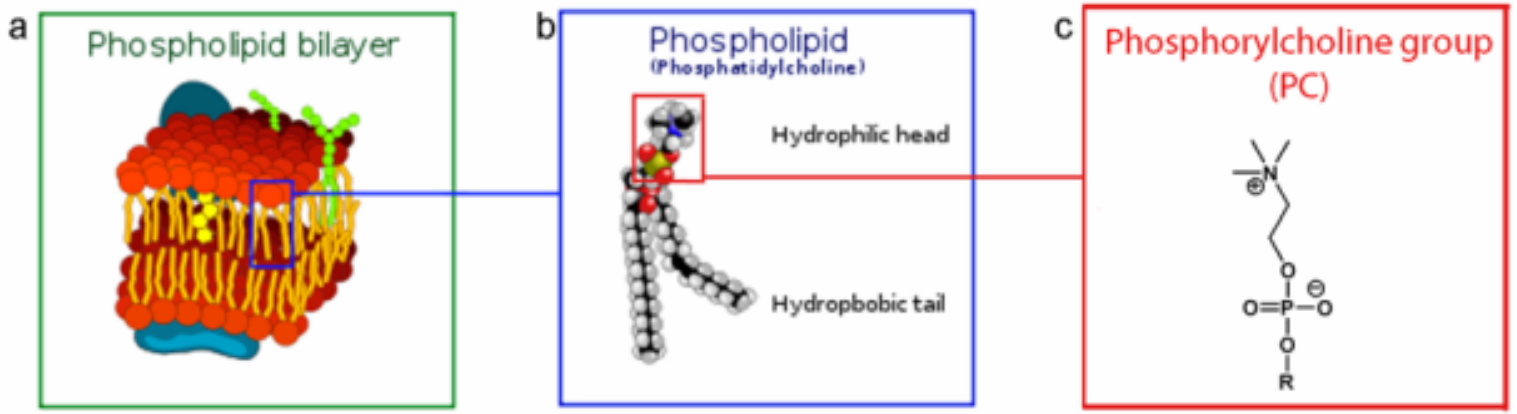
Prevention of protein adsorption

Phosphoryle choline (PC)

- Zwitterionic group present on the surface of cell membranes (phospholipid membrane)

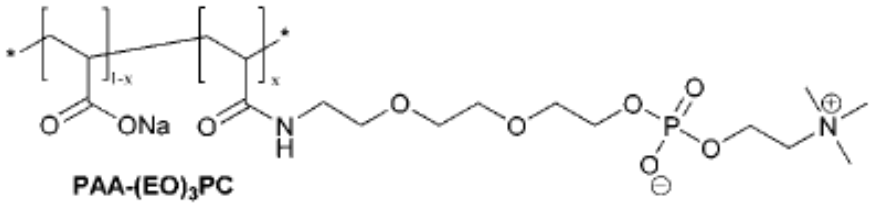
Inner leaflet of erythrocyte membrane has negatively charged lipids => blood coagulation

Outer leaflet bears more lipids with phosphorylcholine head => no coagulation

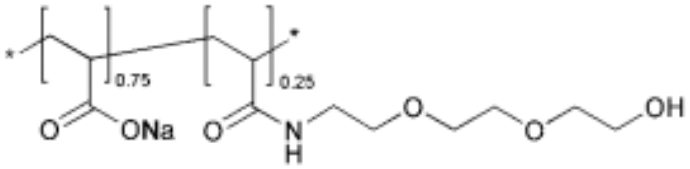


Prevention of protein adsorption

Synthesis of polyelectrolyte bearing phosphoryle choline (PC)

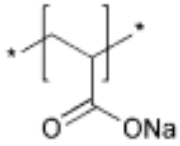


PAA-$(EO)_3$PC

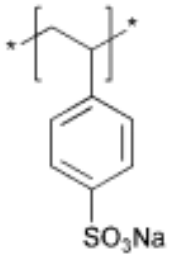


PAA-$(EO)_3$

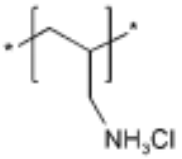
for comparison



PAA



PSS

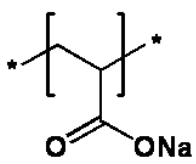


PAH

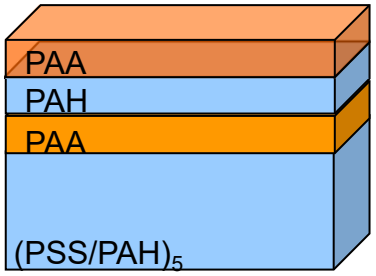
Reisch *et al. Langmuir* **25**, 3610 (2009)

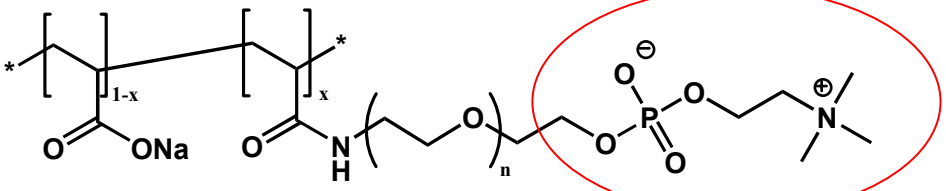
Prevention of protein adsorption

Protein adsorption on PEI-$(PSS/PAH)_5$ ended by



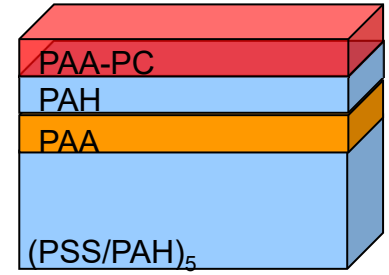
PAA

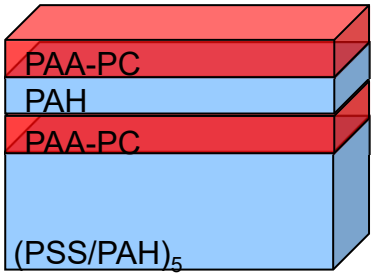


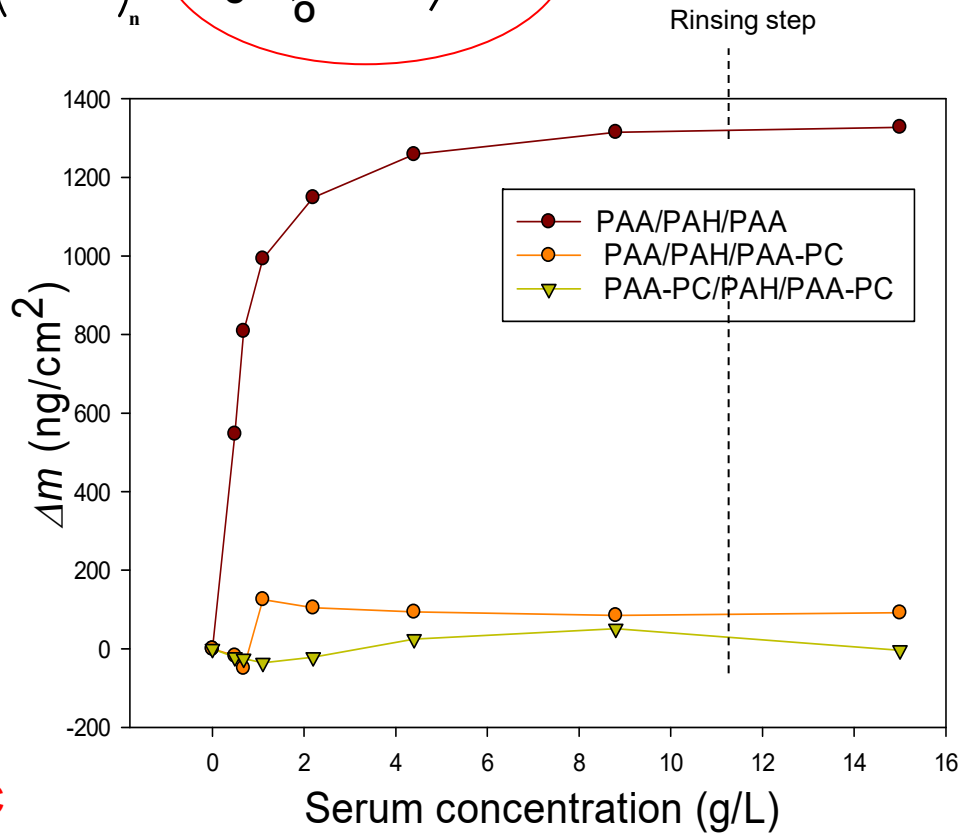


PC

n=2: PAA-$(EO)_3$PC



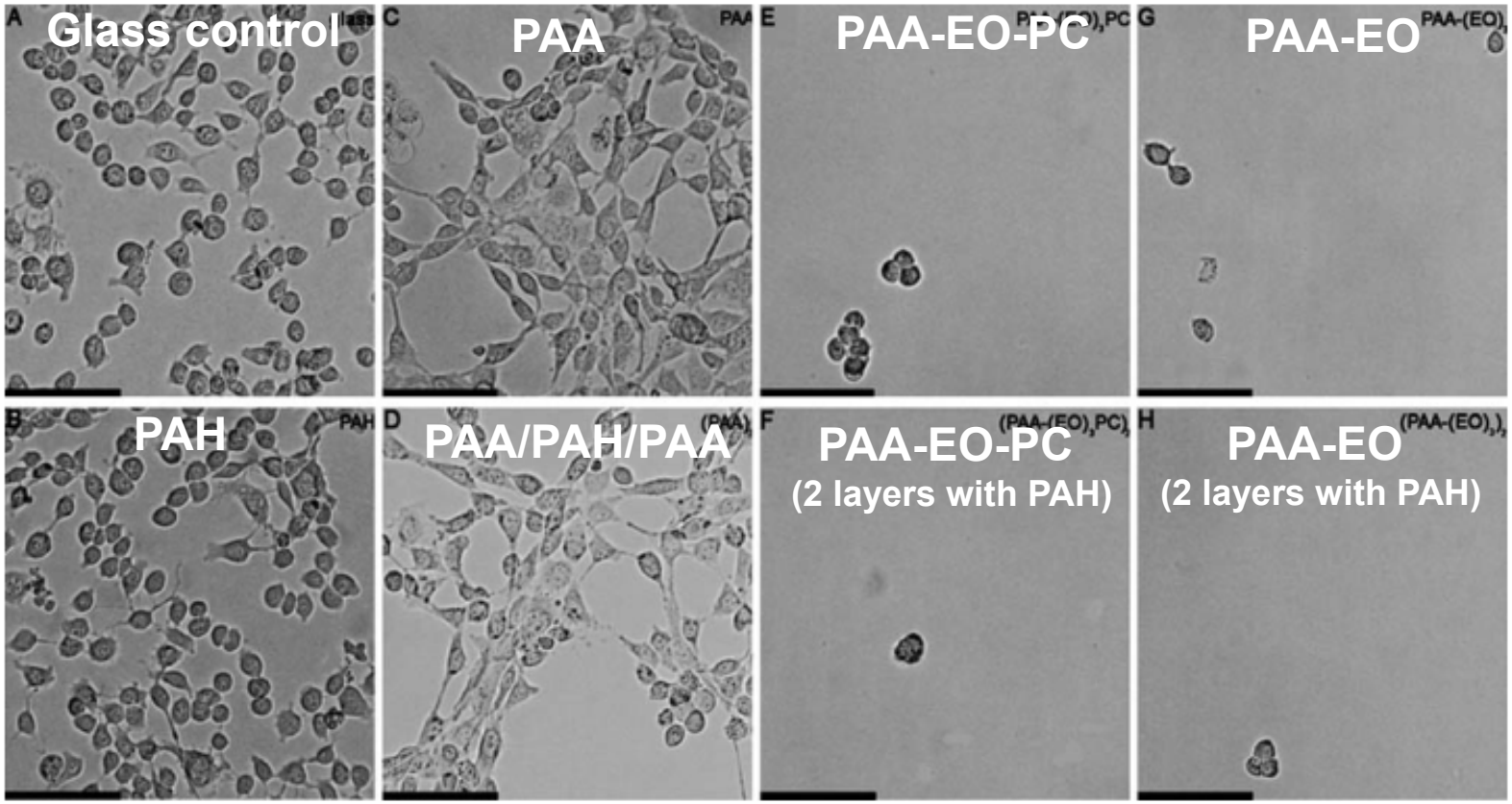




No protein adsorption on 2 layers PAA-EO_3-PC

Prevention of cell adhesion

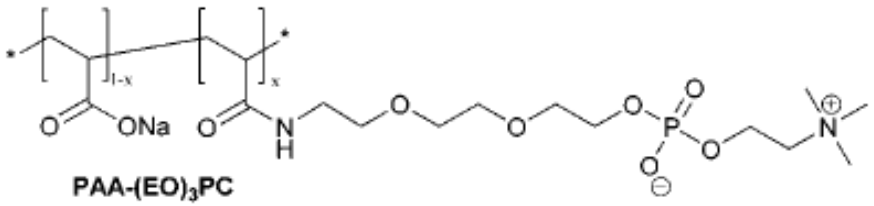
NIH3T3 cells after 48 h of culture on PEI-$(PSS/PAH)_5$ ended by



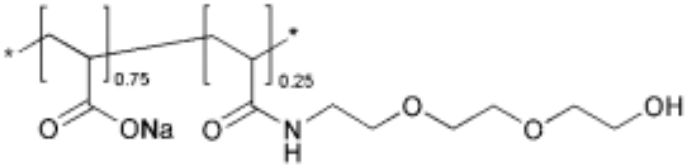
Cell adhesion reduction on films ended by PAA-EO-PC and PAA-EO

Prevention of cell adhesion

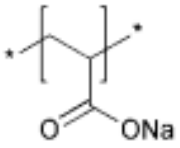
- Phosphoryle choline (PC)



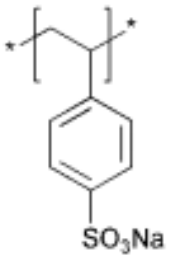
PAA-$(EO)_3$PC



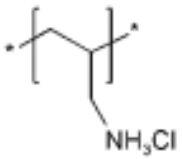
PAA-$(EO)_3$



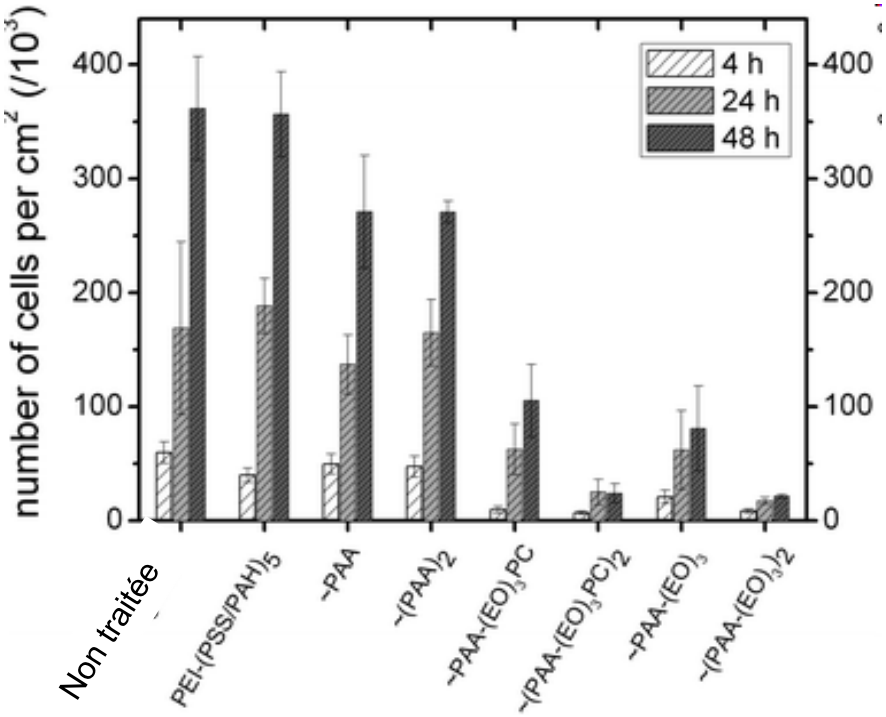
PAA



PSS



PAH



No protein adsorption

Cell adhesion reduction of 95%

Reisch *et al. Langmuir* **25**, 3610 (2009)

Polysaccharide based films

Strategy

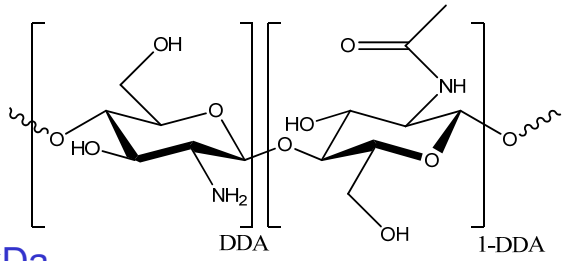
Polysaccharides

- biocompatible
- biodegradable
- largely used in biomedical application

Polysaccharides used

Polycation

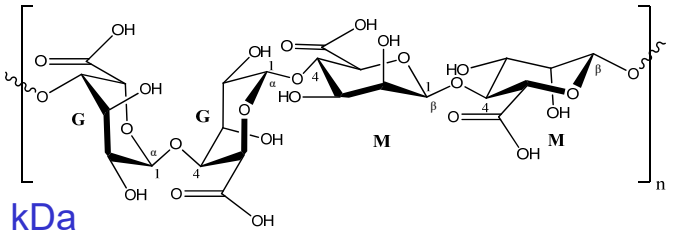
Chitosan (CHI)



Mw = 260 kDa
DDA = 83%

Polyanion

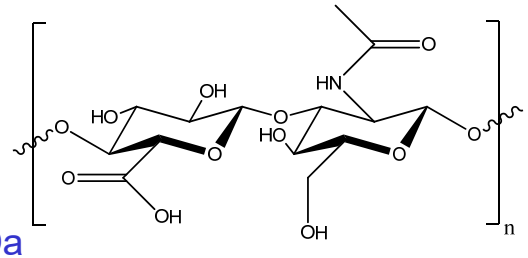
Alginate (ALG)



Mw = 70 kDa

Polyanion

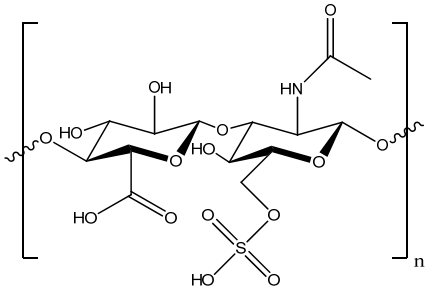
Hyaluronic acid (HA)



Mw = 420 kDa

Polyanion

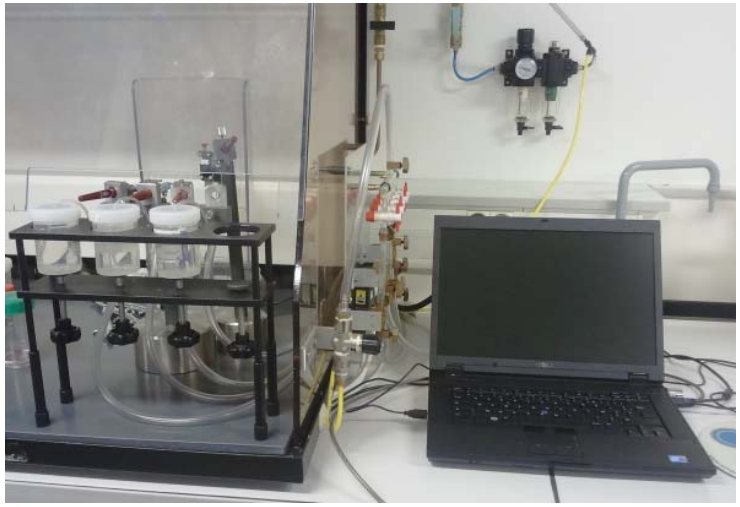
Chondroïtin sulfate (CS)

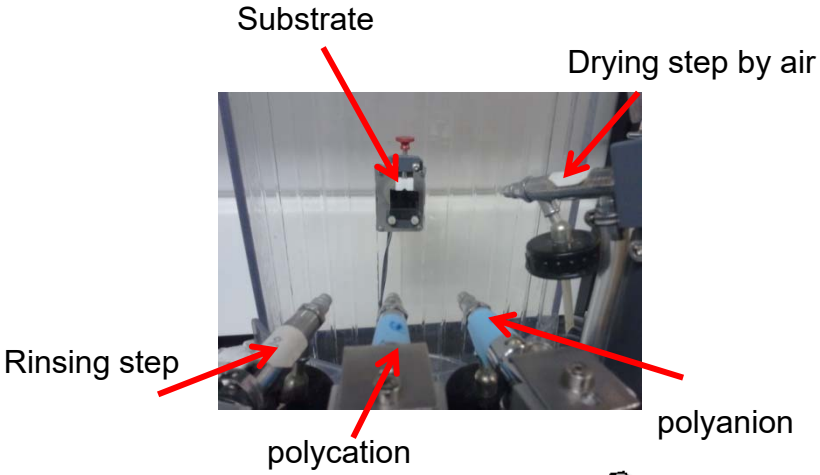


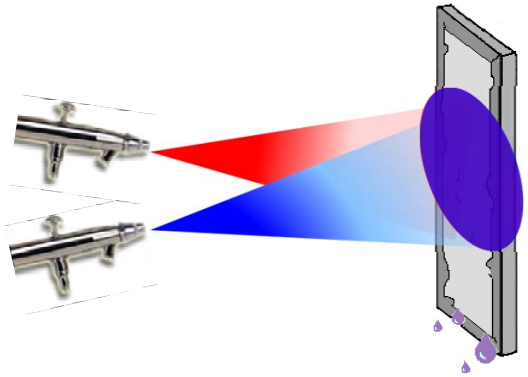
Mw = 70 kDa

Process

Spray process: simple et fast

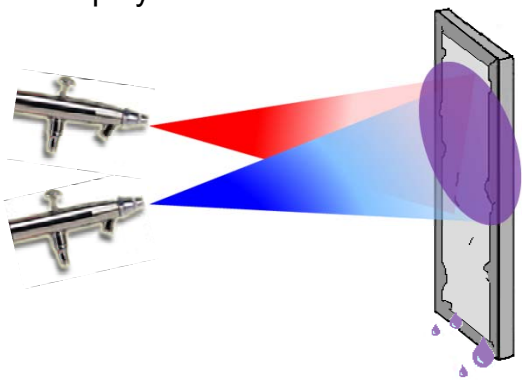






Alternated spray

Schlenoff, J. *et al., Langmuir* , 2000. **16** : 9968

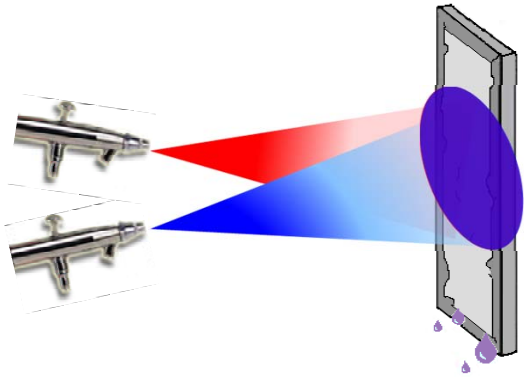


Simultaneous spray

Porcel, C. *et al., Langmuir*, 2005. **21** : 800
Lefort, M. et al., *Ang. Chem. Int. Ed.* 2010. **49:** 10110

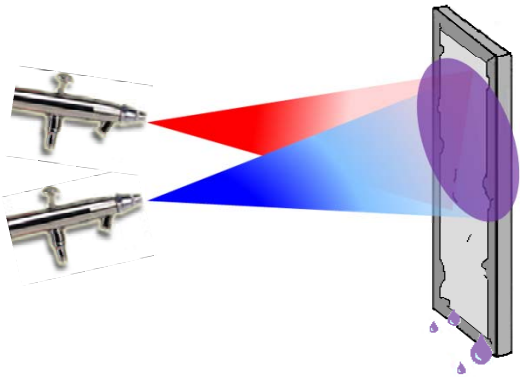
Polysaccharide films obtained by spray

Spraying process: Simple and fast for surface coating



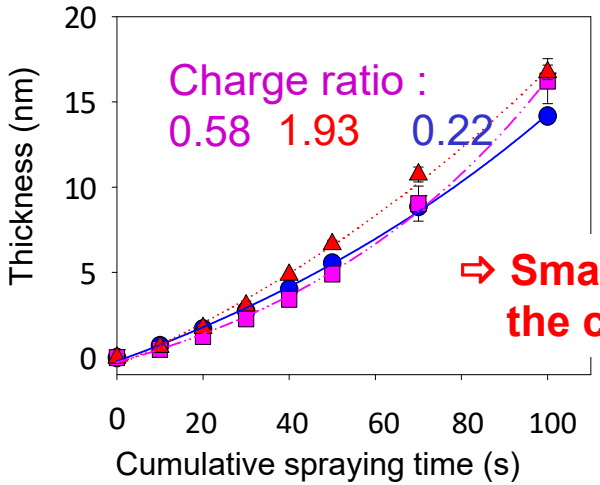
Alternated spraying

Schlenoff, J. *et al., Langmuir* , 2000. **16** : 9968

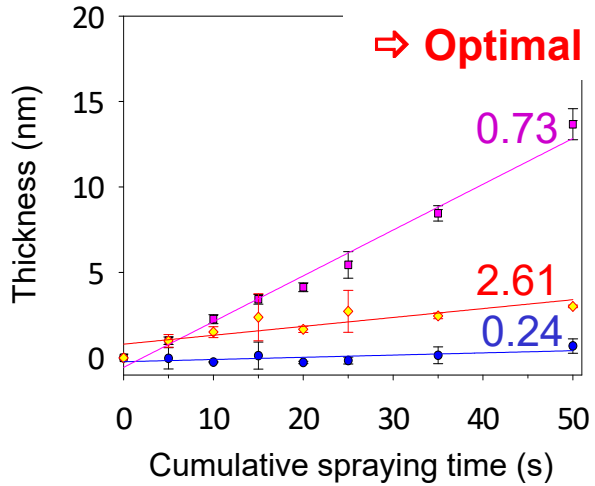


Simultaneous spraying

Porcel, C. *et al., Langmuir*, 2005. **21** : 800
Lefort, M. et al., *Ang. Chem. Int. Ed.* 2010. **49:** 10110

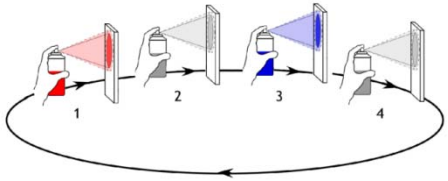


⇨ **Small influence of the charge ratio**

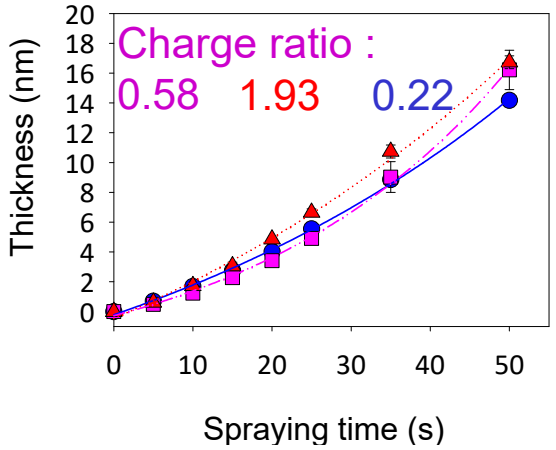


⇨ **Optimal charge ratio**

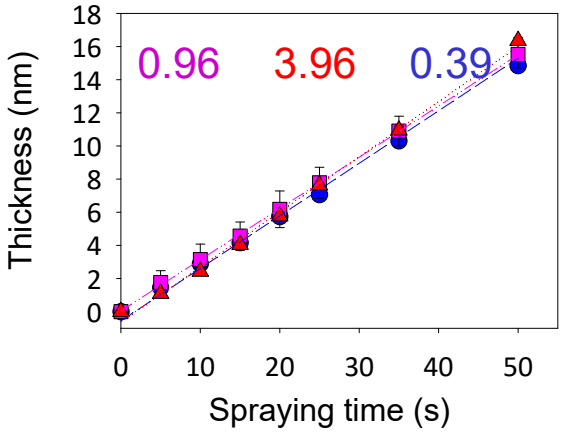
Polysaccharide films obtained by spray



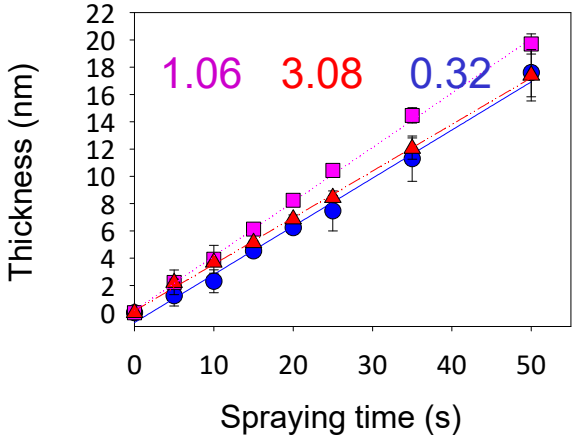
CHI/HA

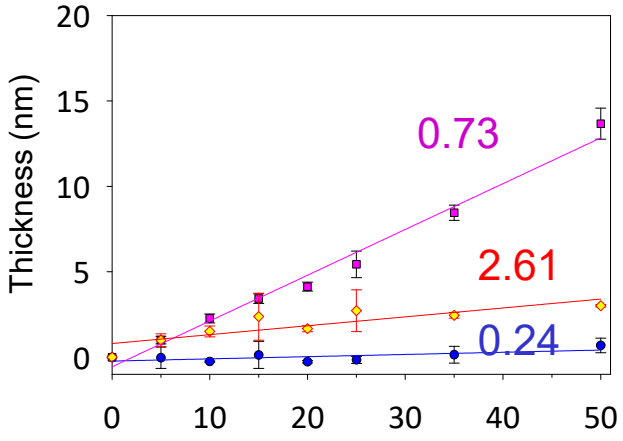


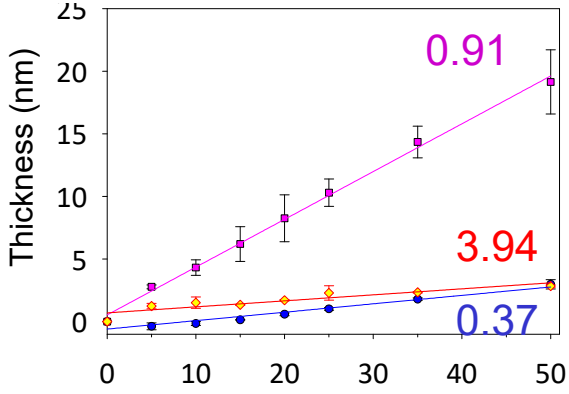
CHI/ALG

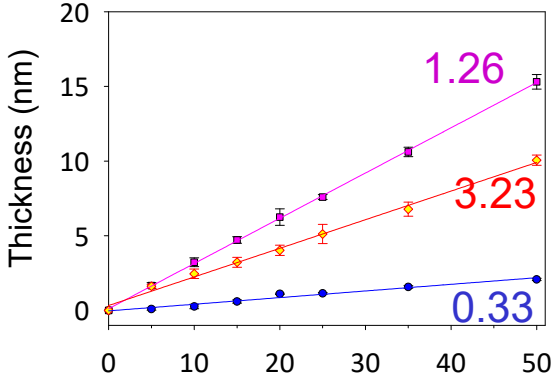


CHI/CS

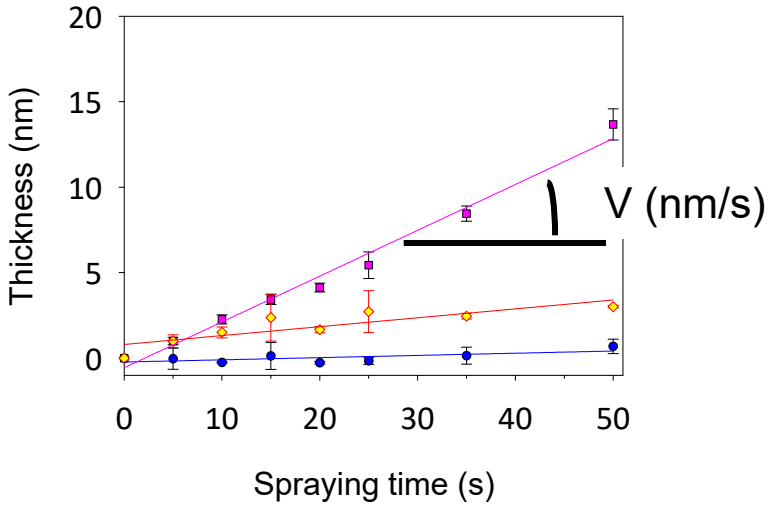


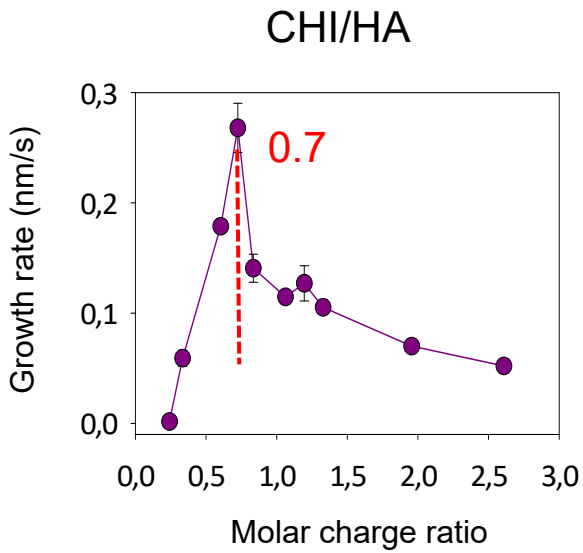


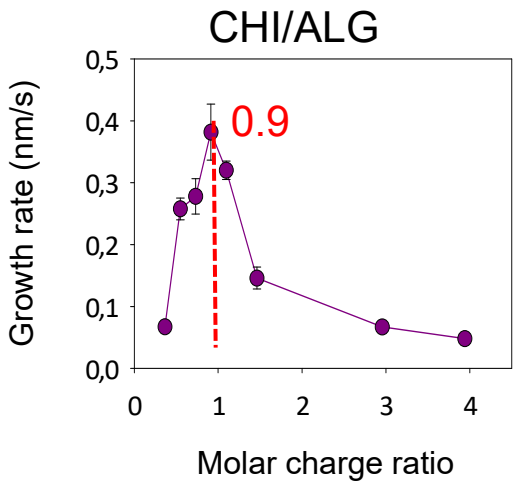


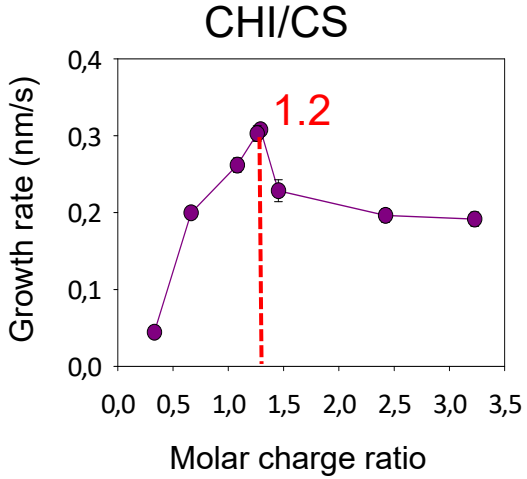


Polysaccharide films obtained by spray



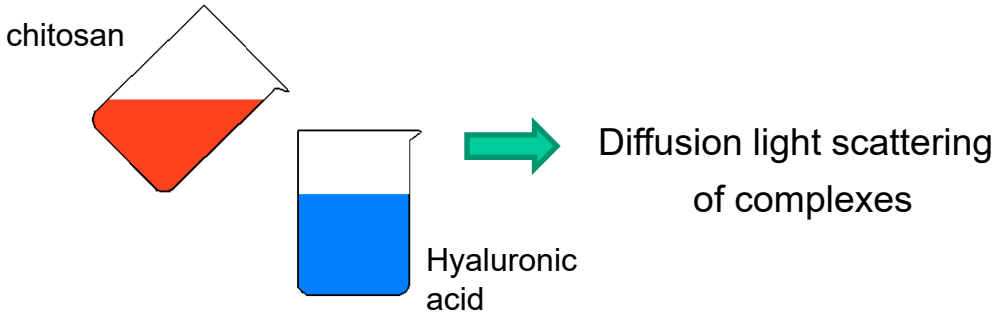


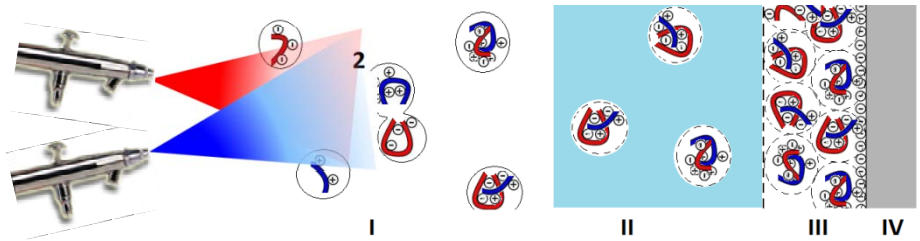




Polysaccharide films obtained by spray

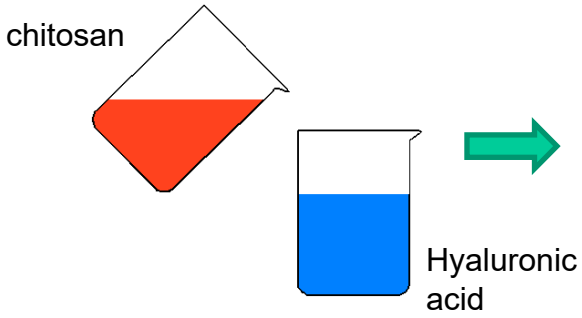
Chitosan/Hyaluronic acid



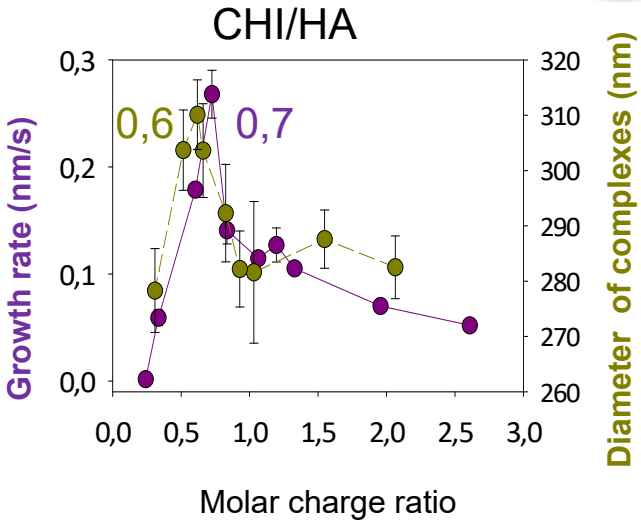


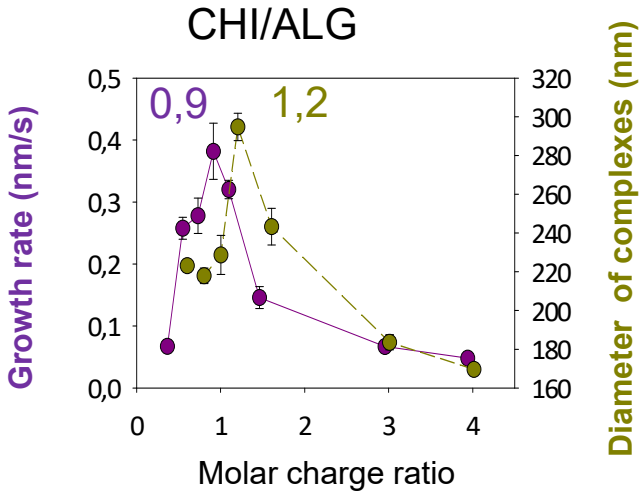
Polysaccharide films obtained by spray

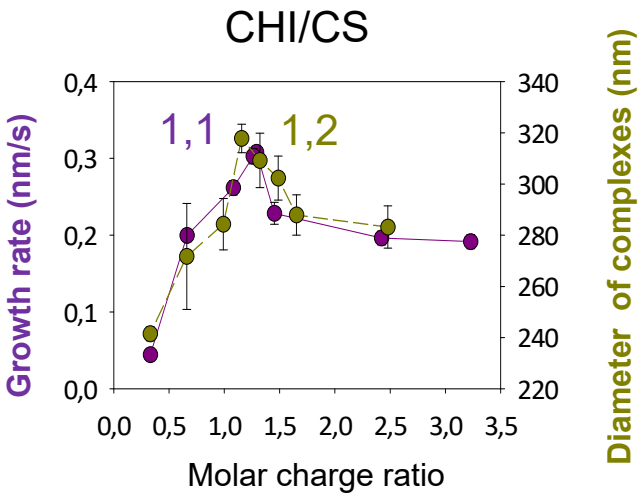
Chitosan/Hyaluronic acid



Diffusion light scattering of complexes

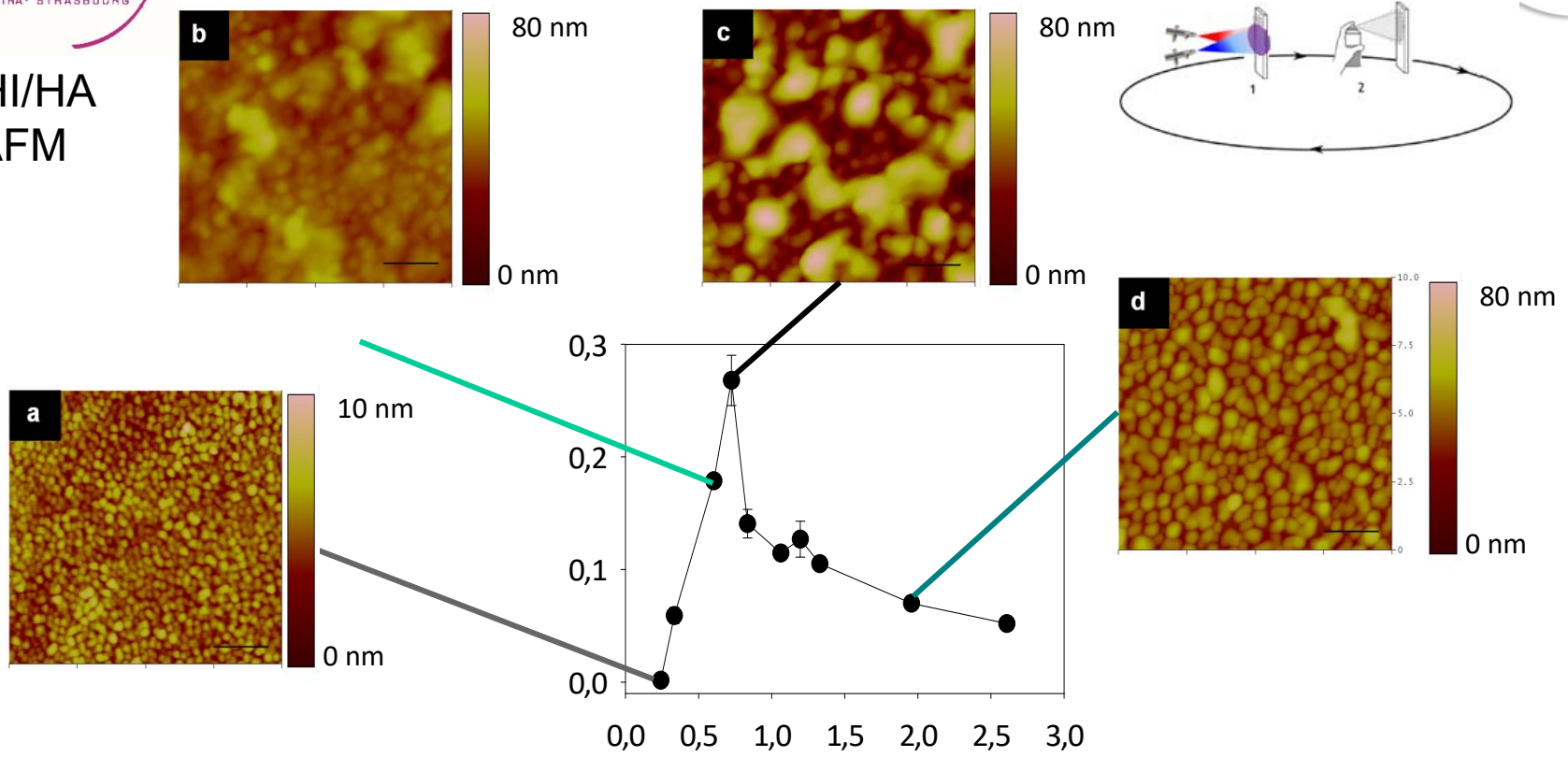






Polysaccharide films obtained by spray

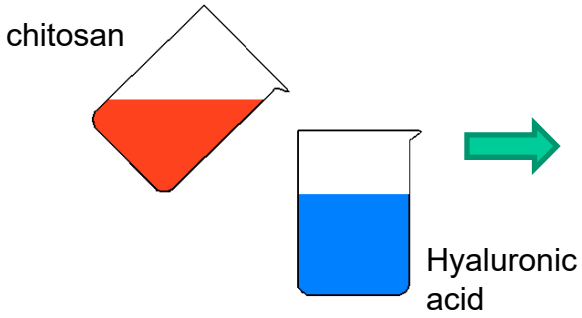
CHI/HA
AFM



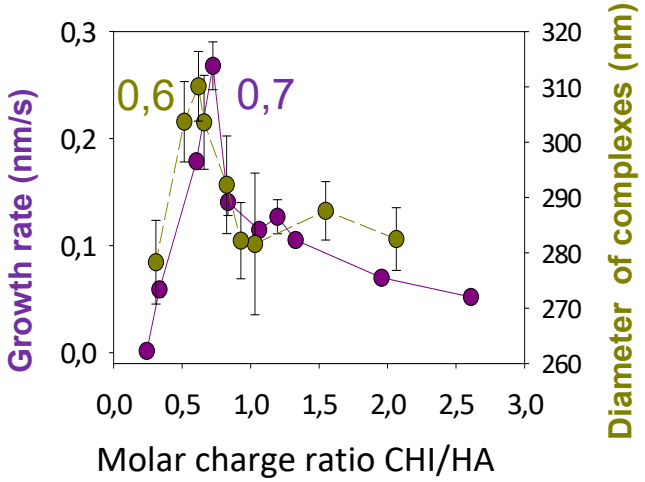
	Molar charge ratio	Roughness(nm)	Diameter of complexes (nm)
CHI/HA	0.22	1	278 ± 7
	0.54	7	304 ± 9
	0.58	15	310 ± 6
	1.93	7	283 ± 6

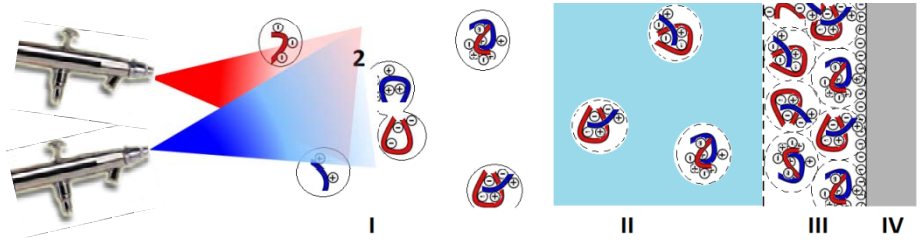
Polysaccharide films obtained by spray

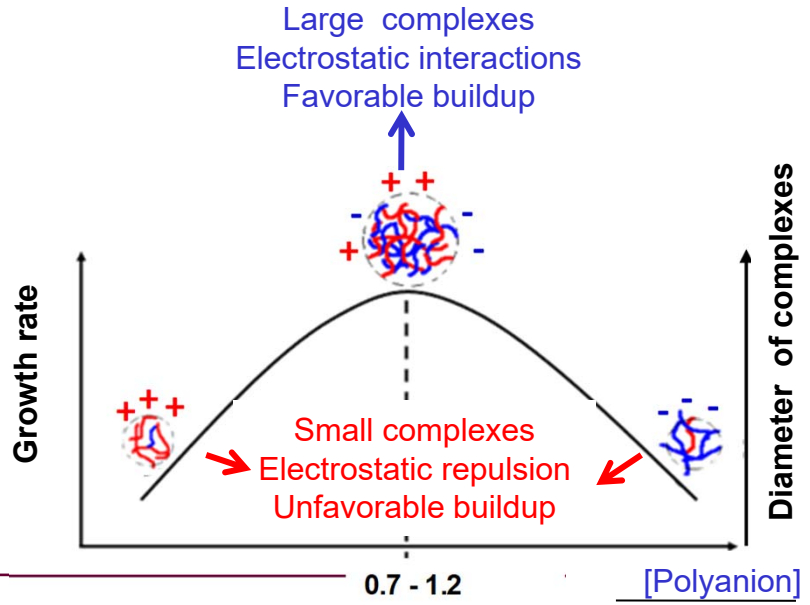
Chitosan/Hyaluronic acid



Diffusion light scattering of complexes



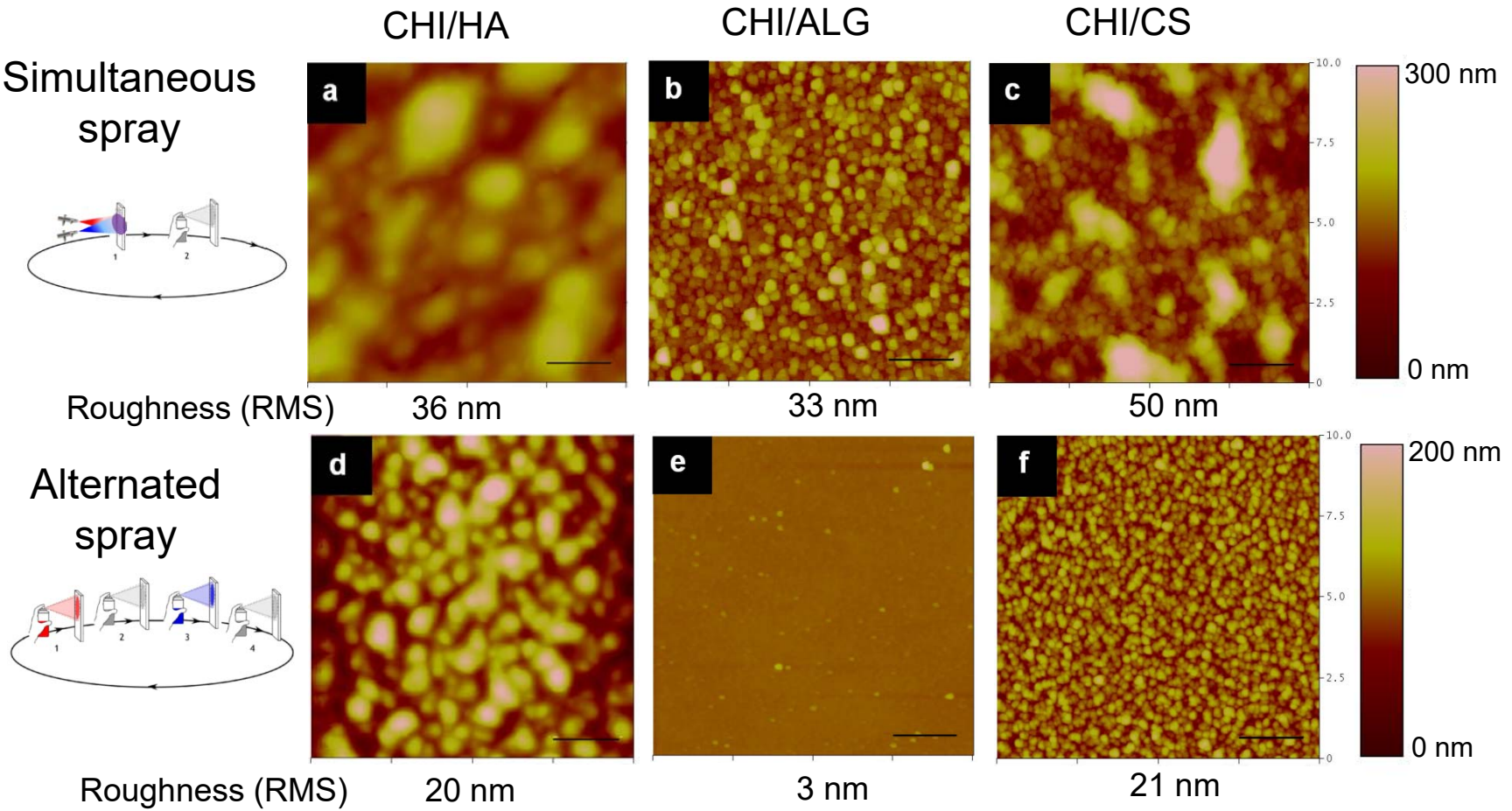




Cado G. et al. , *Langmuir,* 2012, 28, 8470

Polysaccharide films obtained by spray

AFM images of 90 nm (± 10 nm) thickness films

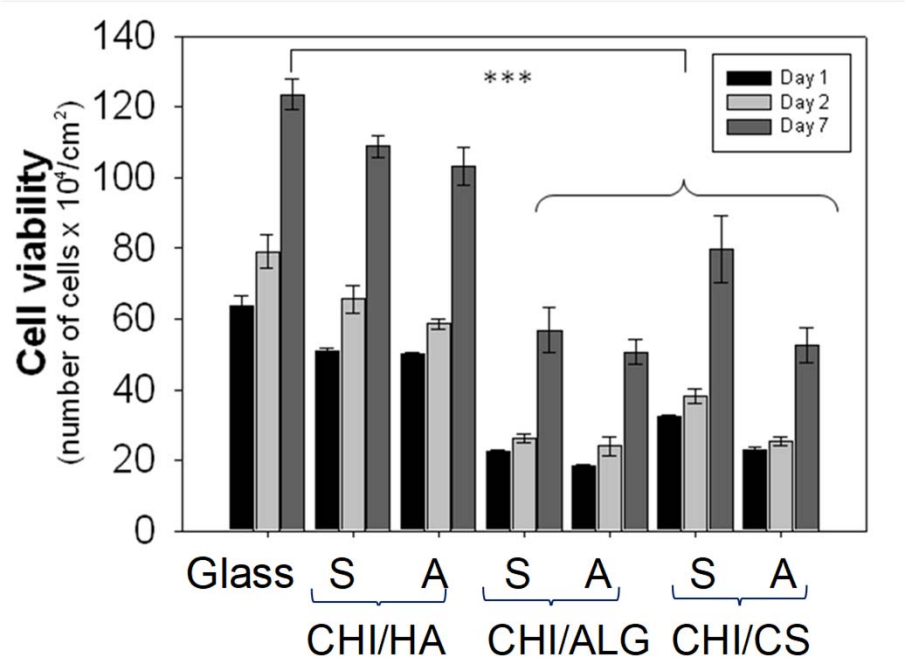


Rougher films obtained by simultaneous spray
Influence on cell adhesion ?

Polysaccharide films as biomaterial coating

Cytotoxicity tests with human gingival fibroblasts

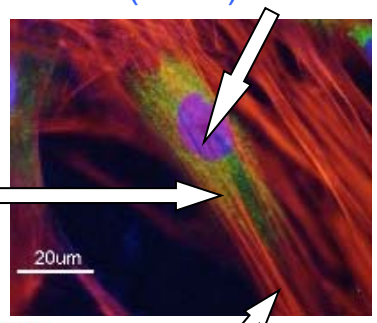
Film thickness : 100 ± 20 nm

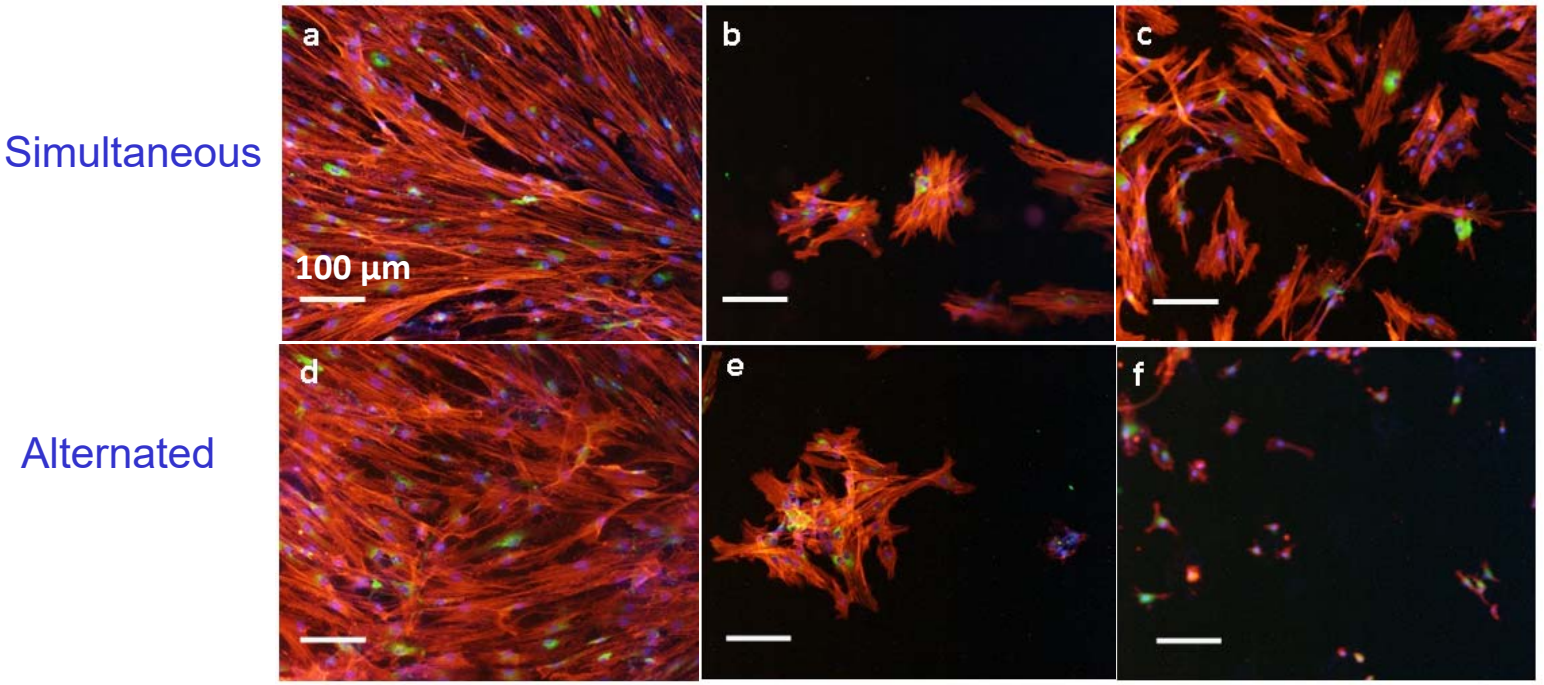


Polysaccharide films as biomaterial coating

Cell viability : better with CHI/HA

Cell adhesion: Immunofluorescent labelling after 12 days of culture



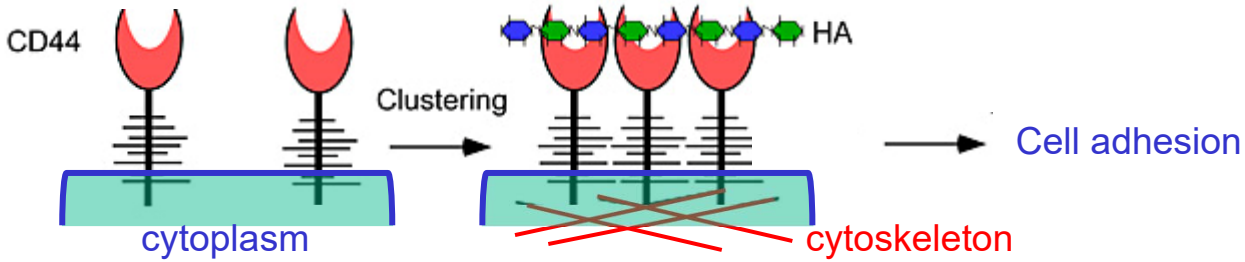


Best results for CHI/HA films : elongated cells and collagen production

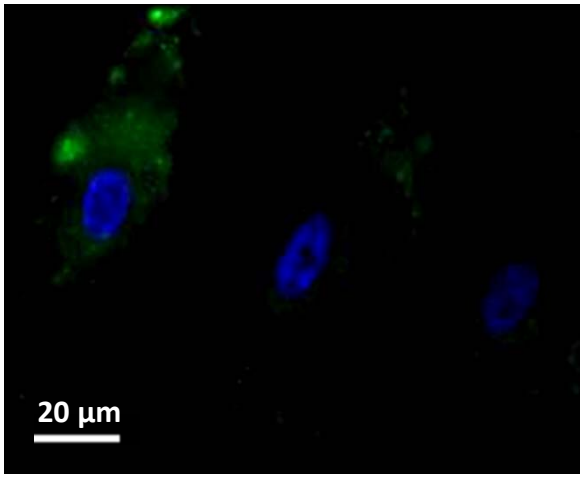
Polysaccharide films as biomaterial coating

HA : ligand of CD44 receptor of cells

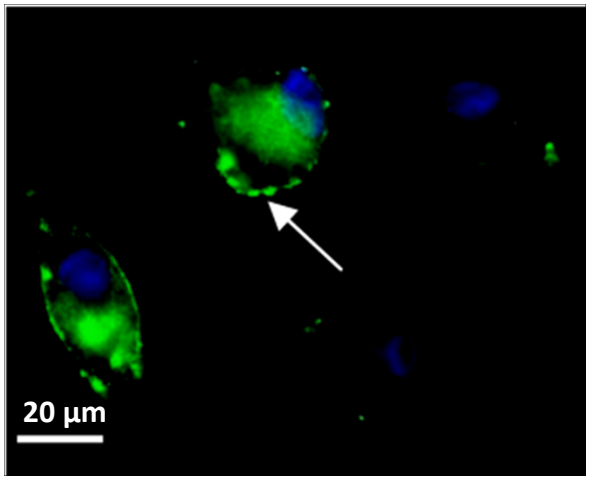
Fluorescent labelling of CD44 after 24h of culture



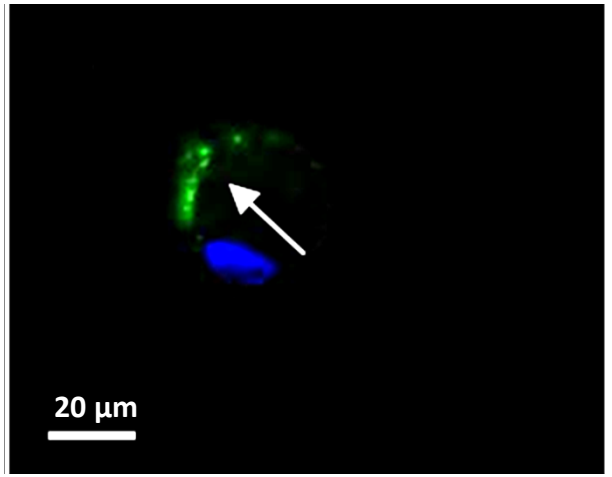
Glass



CHI/HA simultaneous



CHI/HA alternated

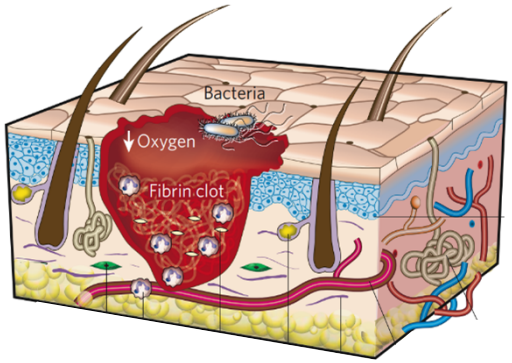


Cluster formation of CD44 receptor

Better adhesion and proliferation on CHI/HA

Wound healing

① Inflammation

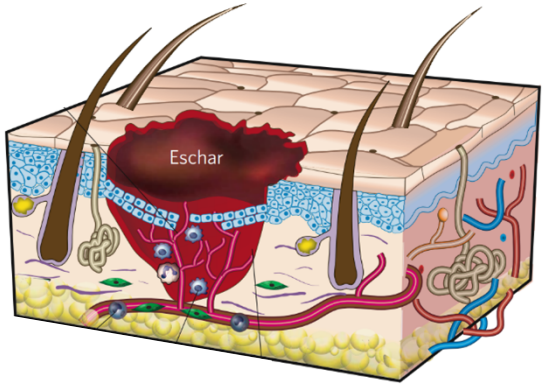


Coagulation in the wound

Immune response against infections

Elimination of devitalized tissue

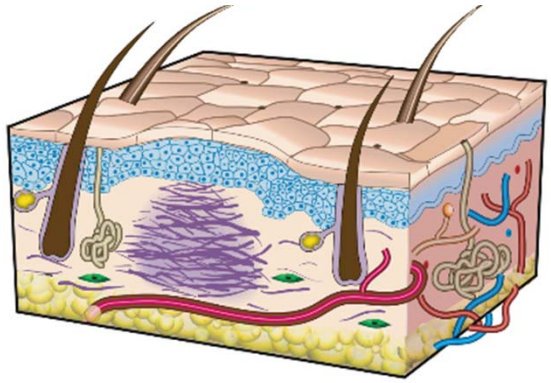
② Proliferation



Repair of the dermis

Creation of new vessels

③ Remodellling



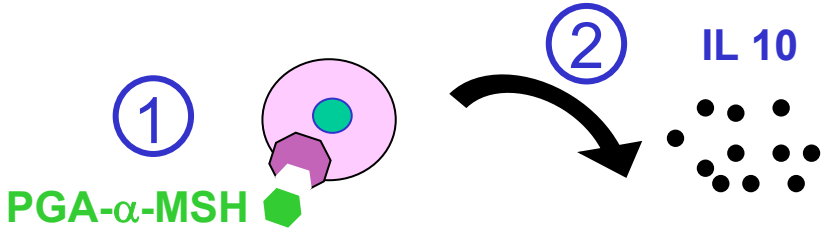
Three perfectly orchestrated phases for an effective healing process

Chronic wounds : blocked in the inflammation phase => no healing

Anti-inflammatory activity

Functionnalisation by the α-MSH : anti-inflammatory hormone

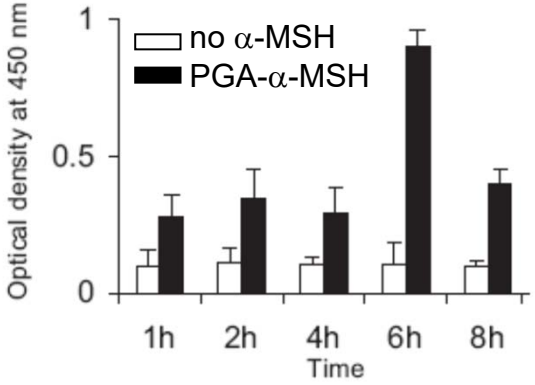
Monocytes
Cells of the immune system



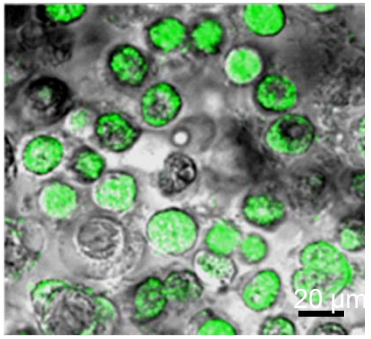
Interleukine 10:

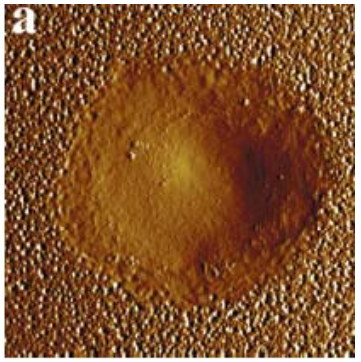
Molecule which inhibites the inflammation / favor the reconstruction of tissues

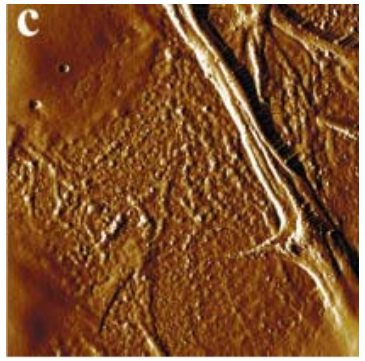
Production of IL10 : in solution



Internalisation / morphology of the cells

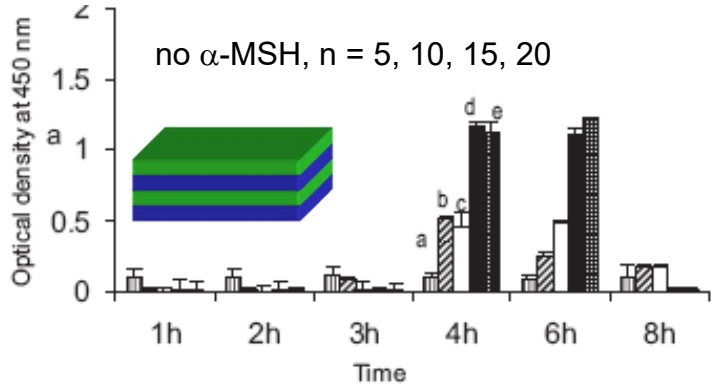






Films without α-MSH **Film with α-MSH**

Production of IL10 : $(PGA-\alpha-MSH/PLL)_n$



Activity of the hormone grafted as alone in solution

Presence of fibers protusion from cells = cellular activity

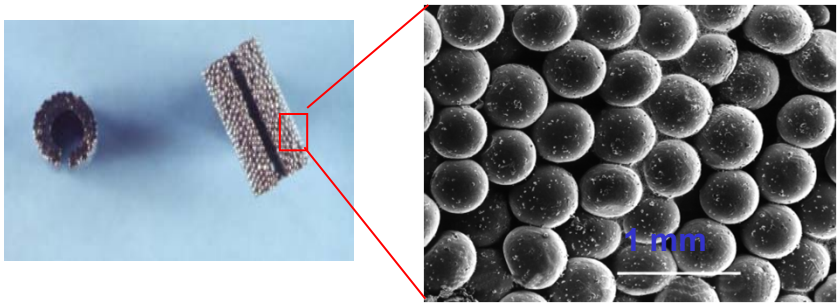
Acide polyglutamique (PGA) α-melanocortin hormone (α MSH)

Jessel *et al. Adv. Mater.* **16**, 1507 (2004)

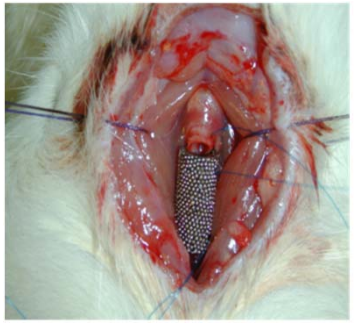
Anti-inflammatory activity

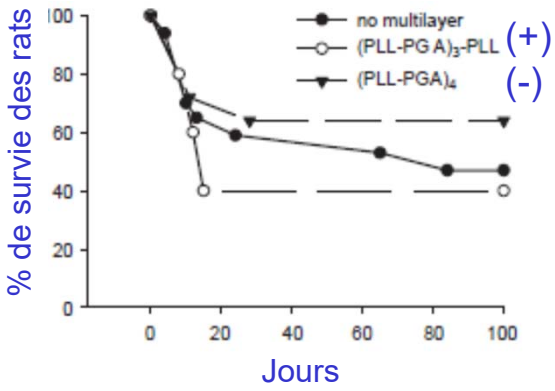
in vivo study

Tracheal implant based on titanium beads

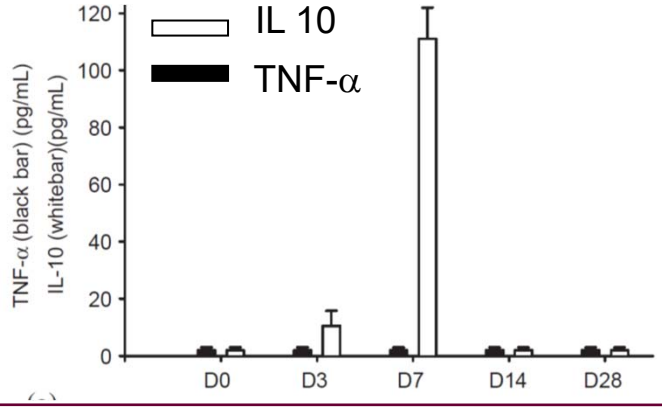


Implantation in the rat model for 3 months





Production of cytokines due to PGA-αMSH



Activity of the hormone *in vivo*

Good biointegration of the implant

Schultz *et al. Biomaterials* **26**, 2621 (2005)

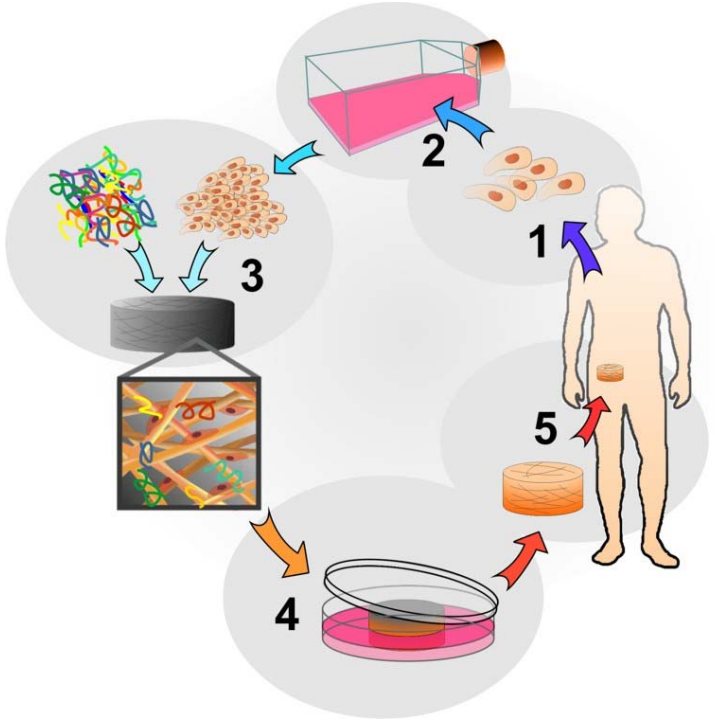
Tissue engineering

Tissue engineering

Challenge : to repair or replace portions of or whole tissues

Definition: Technics and methods of engineering and life science

To develop biological substitutes
to restore, maintain, or enhance tissues and organs



Tools:

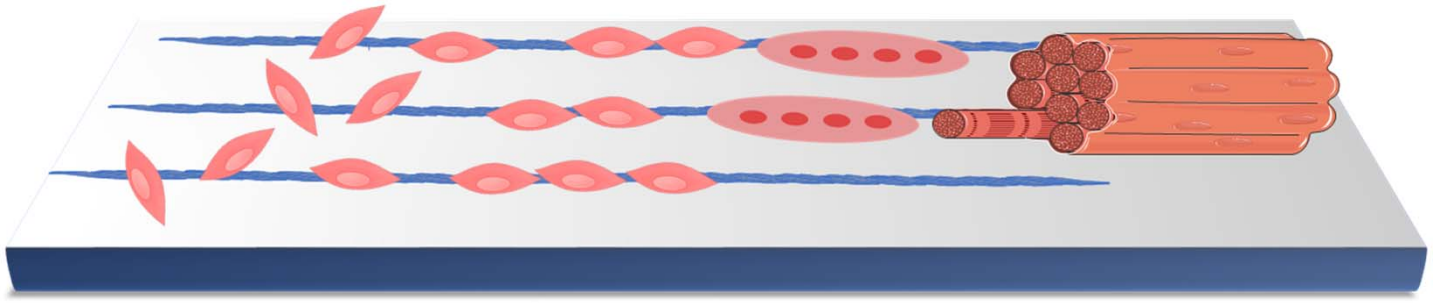
- Cells (autologous, stem cells,…)
- Substrate (3D, 2D platform)
- Culture media, growth factor…

http://www.centropede.com/UKSB2006/ePoster/images/background/TE_model_large.jpg

Muscle tissue engineering

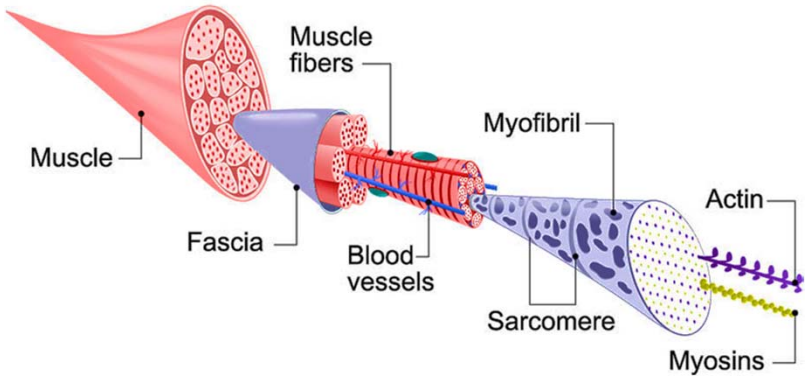
How to guide cells to obtain anisotropic muscle tissue *in vitro*,

such as muscle fibers?



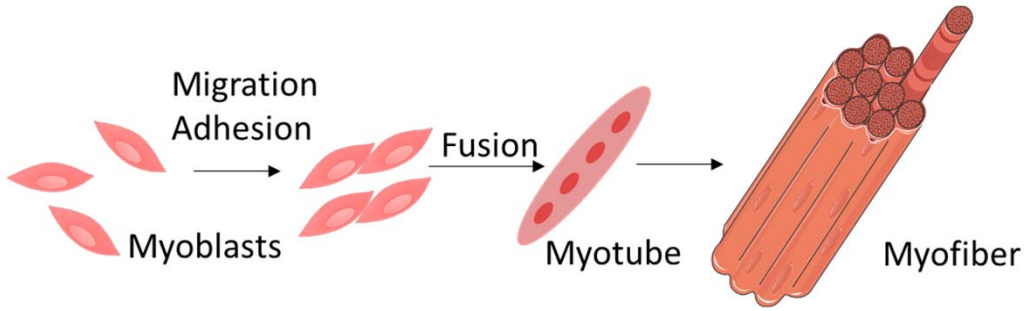
Anisotropic muscle tissue

Anisotropic tissue made of oriented myofibrils



M. Beldjilali-Labroet et al., *Materials*, 2018, 11, 1116

Myofibrils = several oriented myotubes



orientation & differentiation

of myoblasts needed

Abmayr et al, *Development* 2012, 139, 641

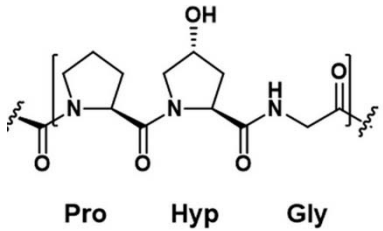
Choice of macromolecule and method

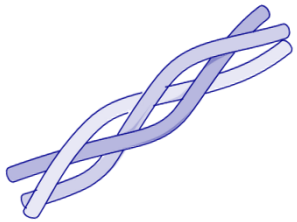
H-bonding collagen based films

Collagen Type I (COL)

25-35% protein content of human body

act as nucleation sites for cell proliferation

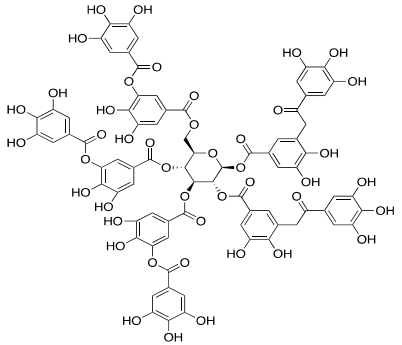




Tannic acid (TA)

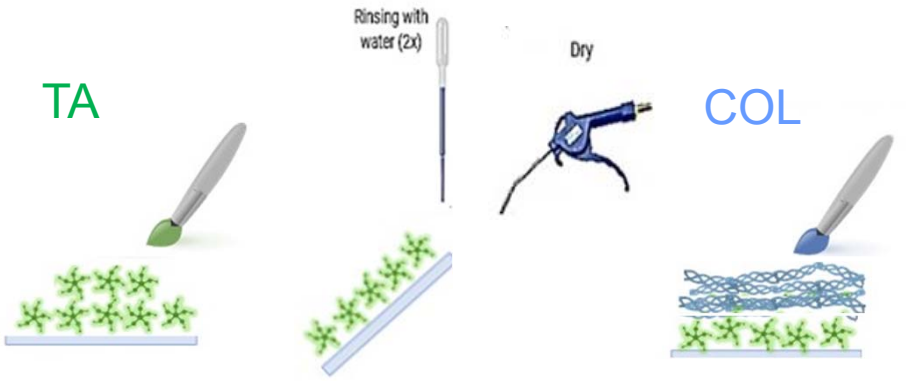
Natural polyphenol

Antibacterial, antioxidant



Brushing method to orient COL

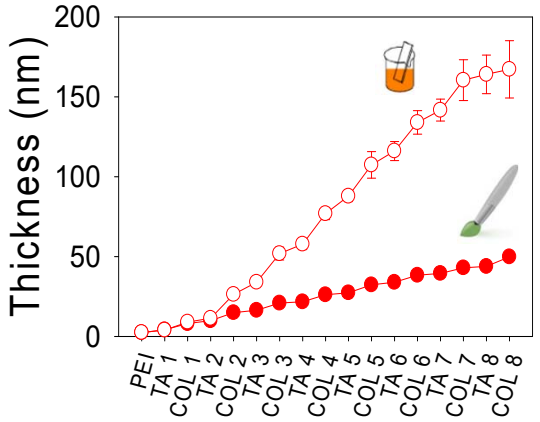
Setup with paintbrush



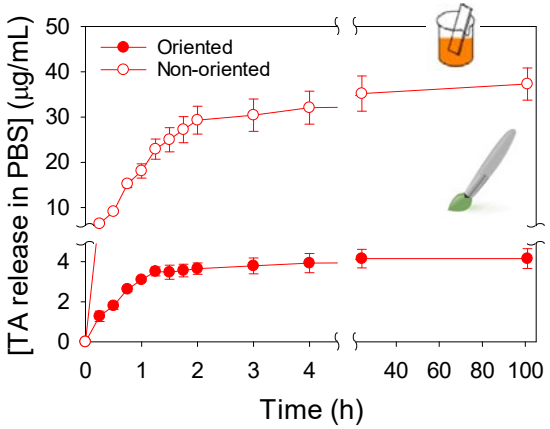
Iqbal et al. *ACS Nano 2022,* 10.1021/acsnano.2c06329

H-bonding collagen oriented films

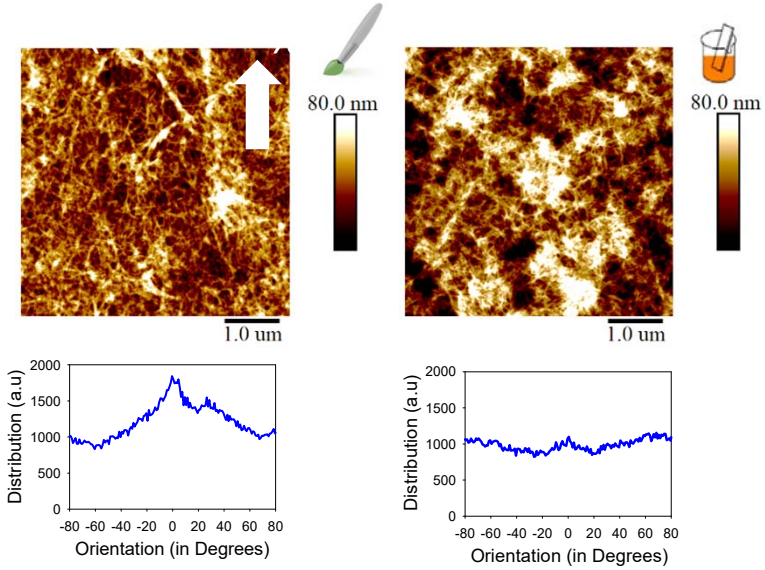
Film thickness (ellipsometry) buildup at pH 4



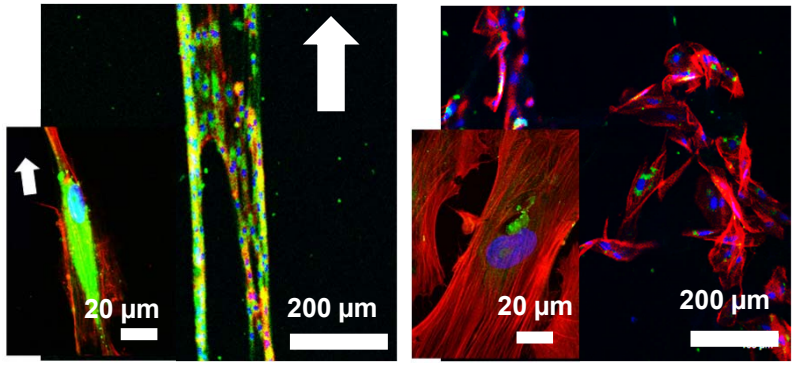
TA release in physiological medium



Topography of $(TA/COL)_8$ (AFM)



Human myoblasts after 12 days



Actin (red), Nuclei (blue), differentiation marker Myosin (green)

Brushing COL/TA

➥ COL orientation

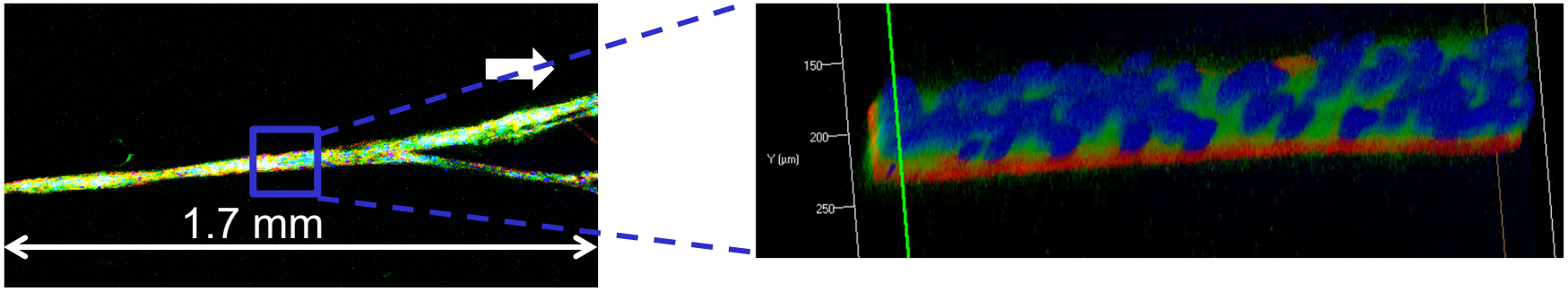
TA release in physiological medium

➥ Myoblasts orientation

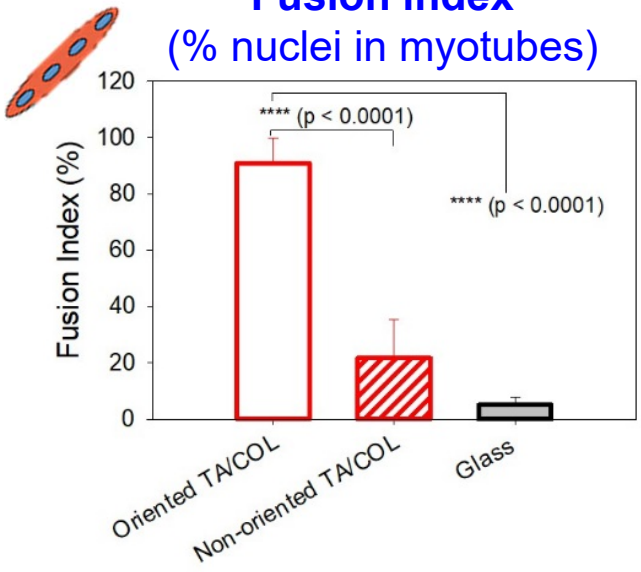
➥ Multinucleated myofibers

➥ Differentiation (myosin)

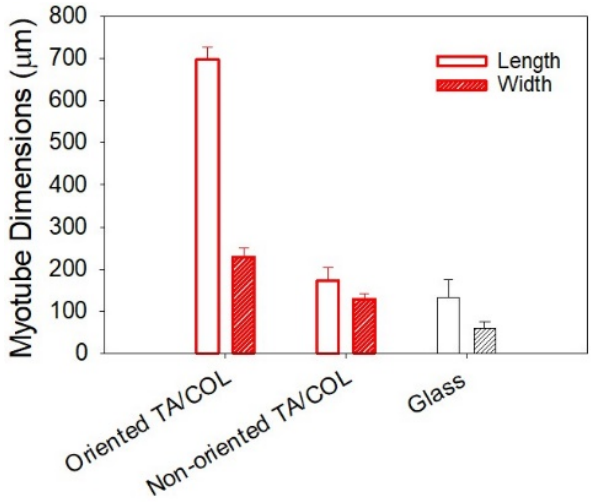
Differentiation characteristics



Fusion index
(% nuclei in myotubes)



Myotubes dimension



Brushed TA/COL films

90% of nuclei in myotubes

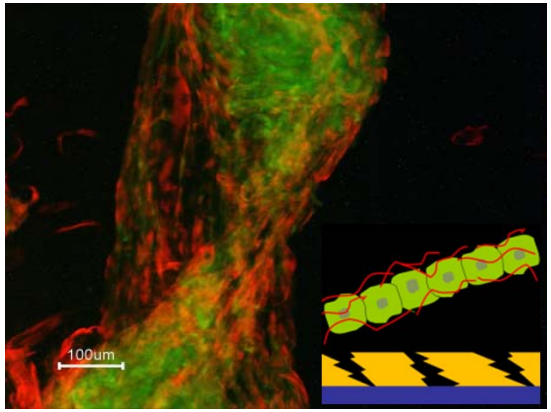
Myotubes of 700 µm long and 220 µm width

Application of brushed TA/COL films: *in-vitro* development of patient muscle fibers for pharmacology

Polymeric platform for cell sheet engineering

Two strategies for two applications

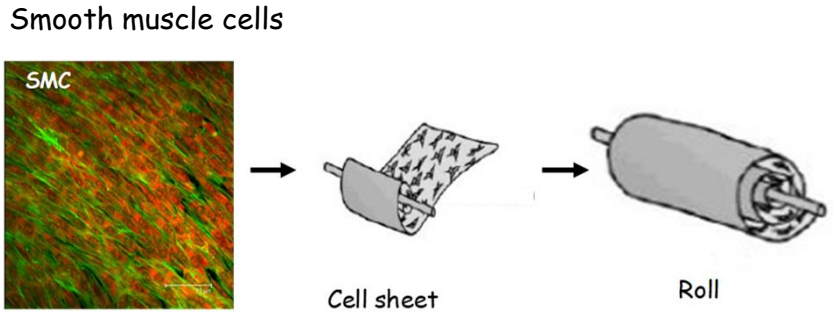
Wound dressing:
Autologous explant of fibroblast cells



Chemical detachment
of cellularized PEMs

A. Chassepot. *et al*. Chem Mater 2012, 24, 930

Blood vessel reconstruction:
Smooth muscle cells sheet



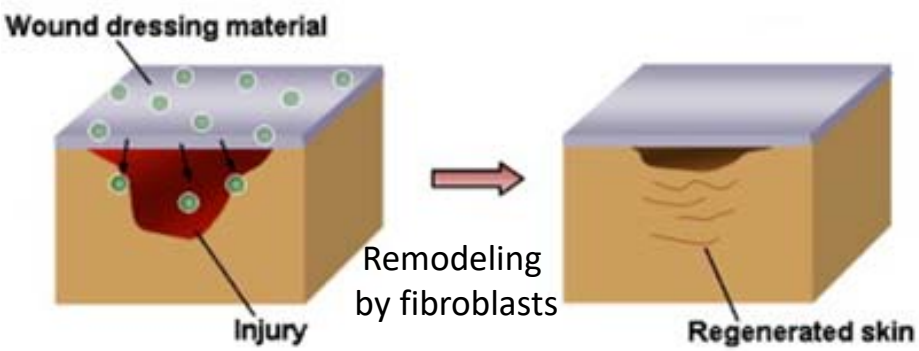
Mechanical peeling off
a cellularized alginate membrane

H. Kerdjoudj. *et al*. Soft Matter 2011, 7, 3621

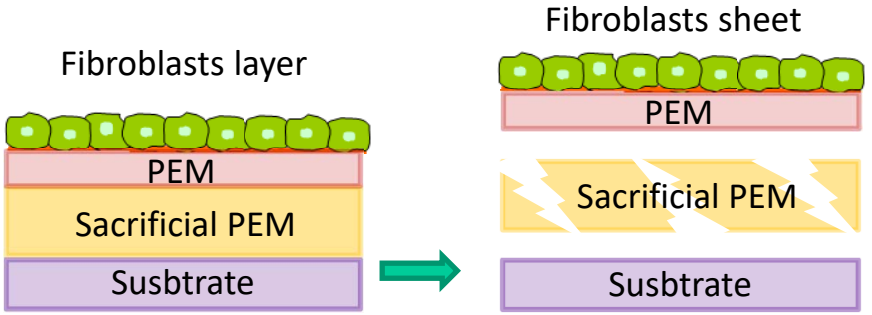
Wound dressing

Development of Human Gingival Fibroblasts (HGF) sheets

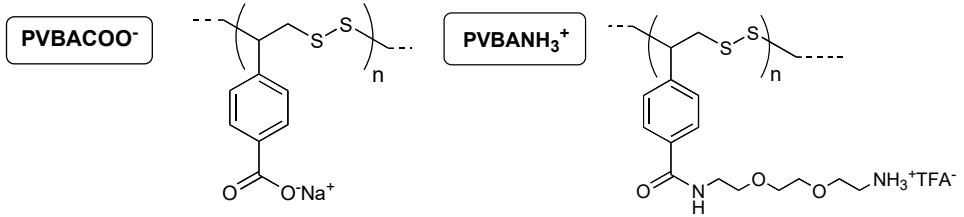
Cell therapy for wound repair



Strategy: chemically detachable PEM

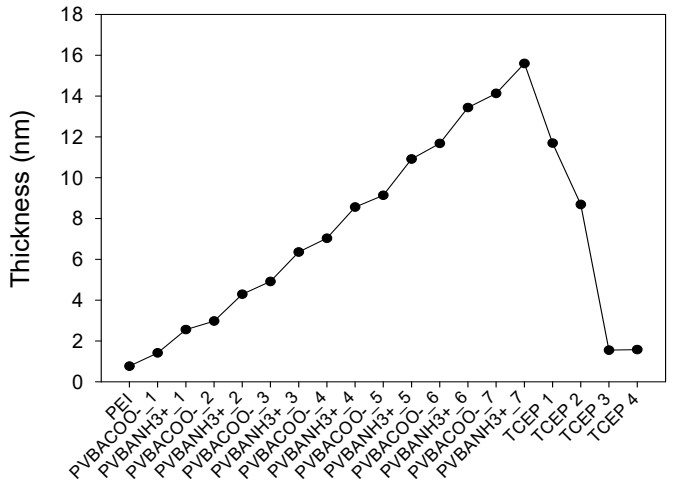


Synthesis of disulfide containing polyelectrolytes



TCEP: Tris(2-carboxyethyl)phosphine

Buildup and dissolution in contact with TCEP



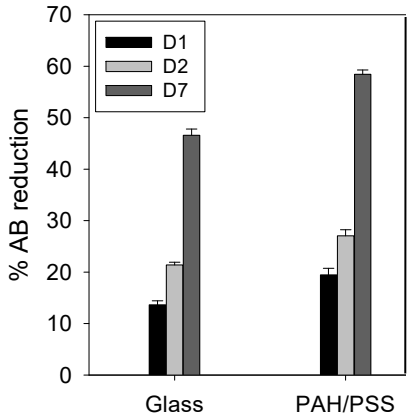
A. Chassepot. *et al*. Chem Mater 2012, 24, 930

Autologous explant for wound healing

Development of Human Gingival Fibroblasts (HGF) sheets

Cell culture on PSS/PAH films

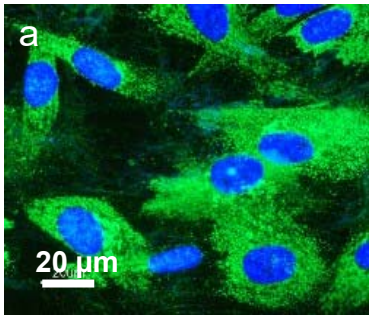
Metabolic activity



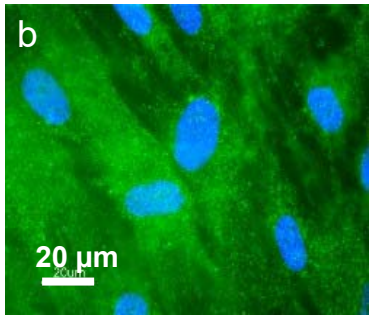
Good viability of HGFs

Immunolabeling

Collagen I

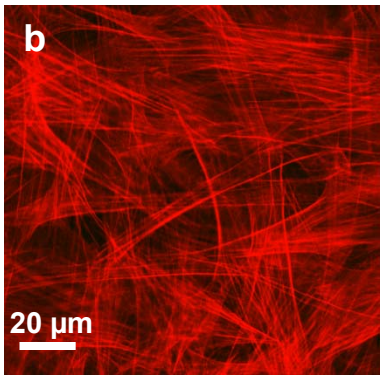


MMP 2

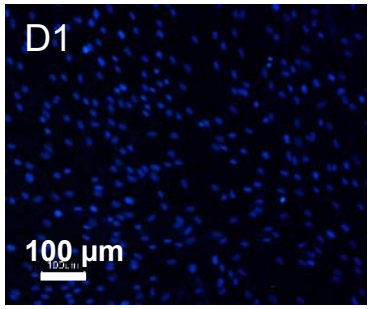


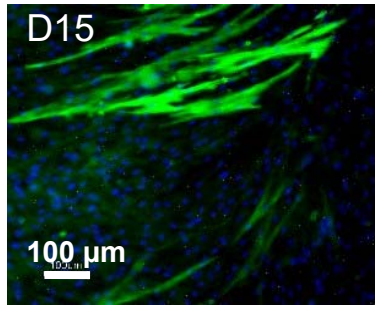
Stability of fibroblasts phenotype over a week

Cytoskeleton visualization



Good cell adhesion



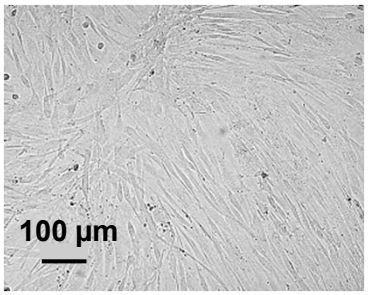


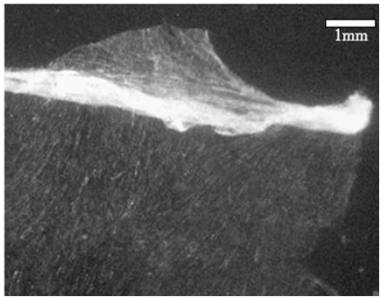
Expression of contractile markers (a-SMA) after 15 days

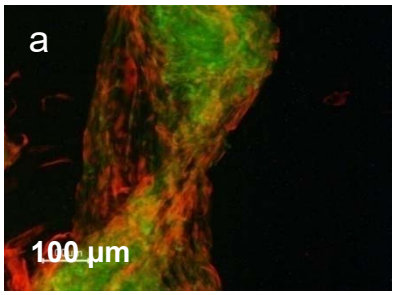
Autologous explant for wound healing

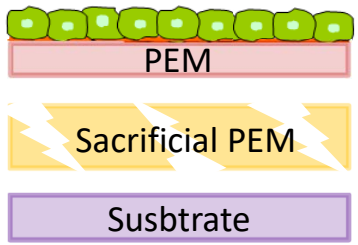
Development of Human Gingival Fibroblasts (HGF) sheets

Cell culture on Sacrificial PEM - PSS/PAH films and contact with TCEP



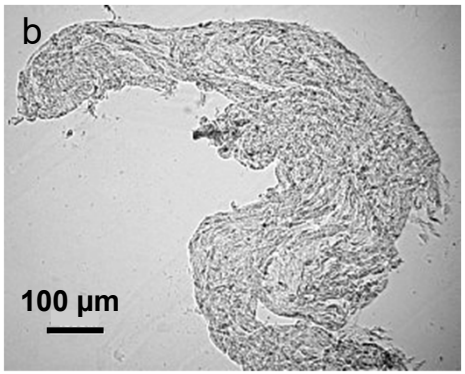


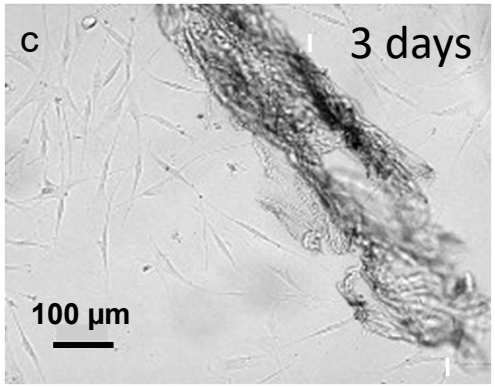


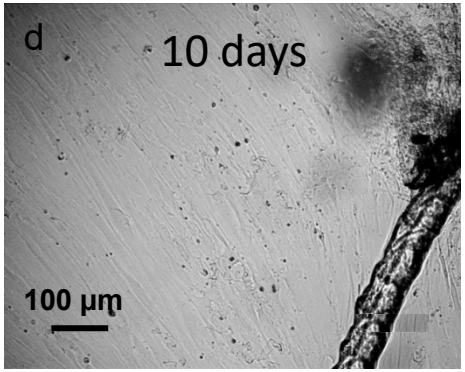


Chemical detachment of the cell sheet

Culture of a cell sheet fragment -> colonization of the substrate



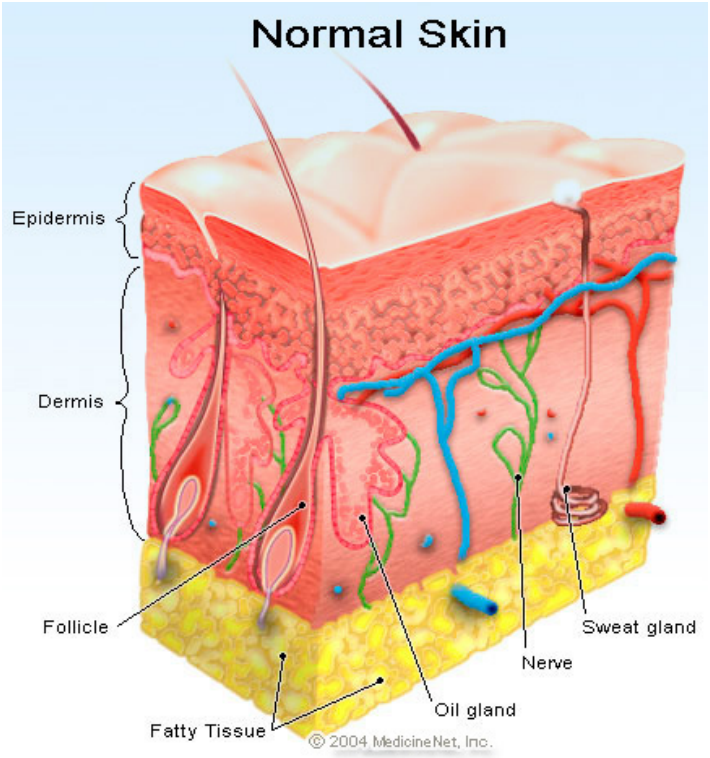




Colonization of the substrate from the fibroblasts sheet

Autologous explant for wound healing

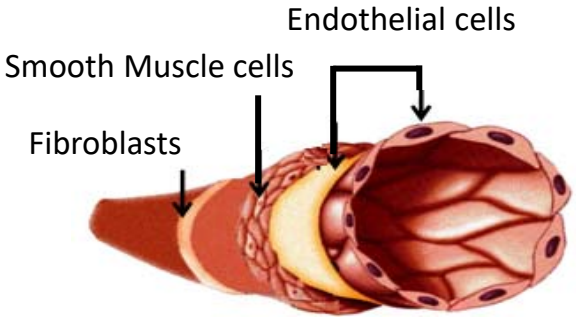
Challenge: Keratynocytes colonization of the cellularized wound dressing



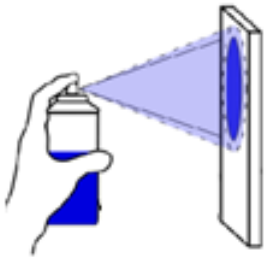
Towards blood vessel reconstruction

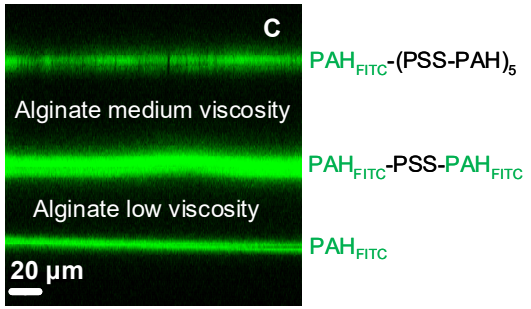
Development of Smooth Muscle Cell (SMC) sheets

Native blood vessel

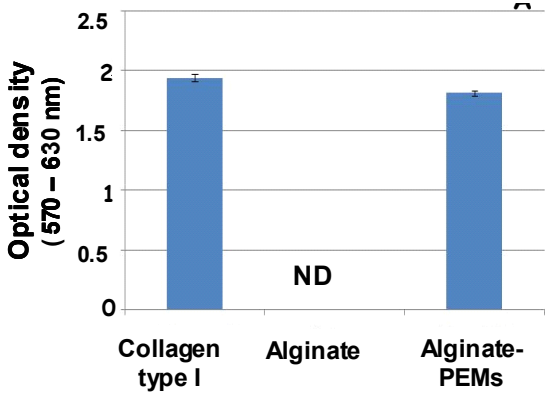


Strategy: Alginate-PEM membrane obtained by spray



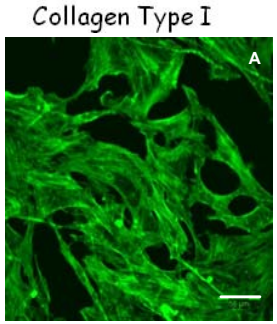


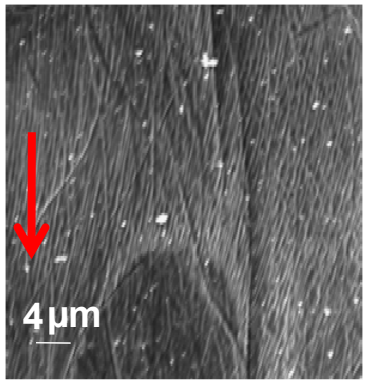
Metabolic activity (Alamar Blue® test)

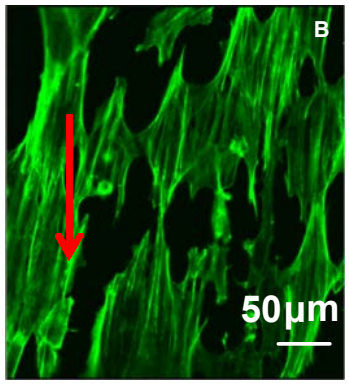


Good SMCs viability

Cytoskeleton visualization (F actin labeling)







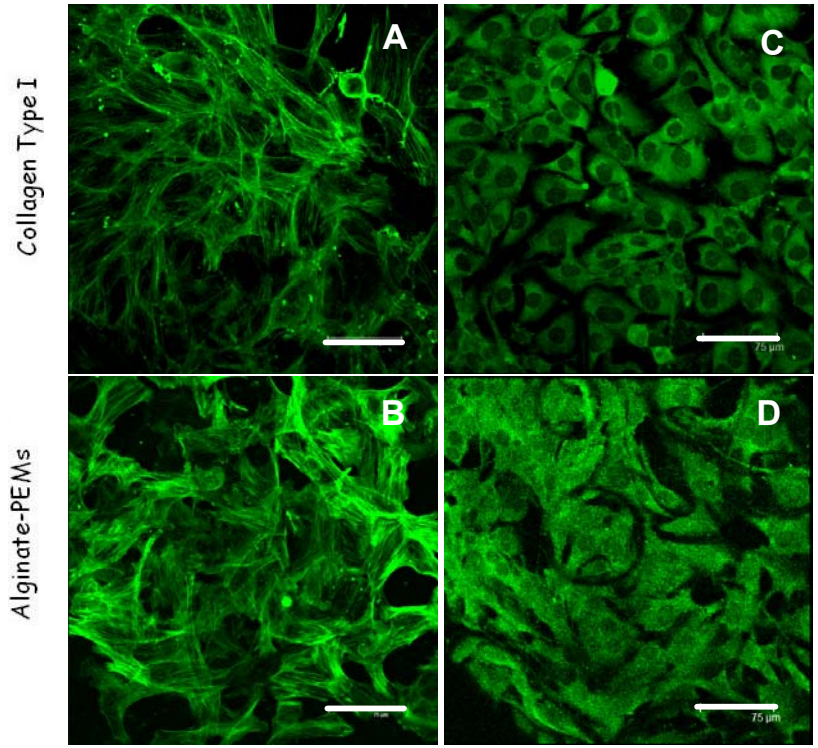
Micro-structured surface → Cells alignment

Towards blood vessel reconstruction

Development of Smooth Muscle Cell (SMC) sheets

Immunolabeling of contractile marker

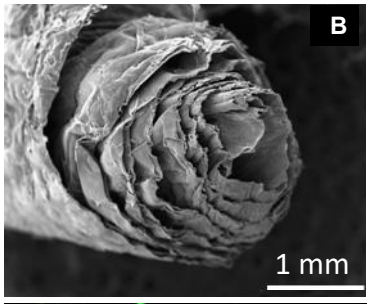
α- SM actin SM myosin heavy chain



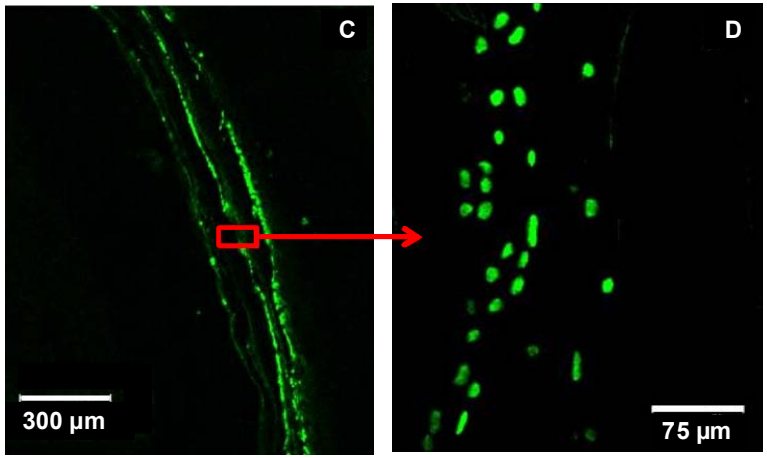
Stability of the SMCs phenotype over a week

Towards a blood vessel

Peeling and rolling of the membrane



Nuclei labeling

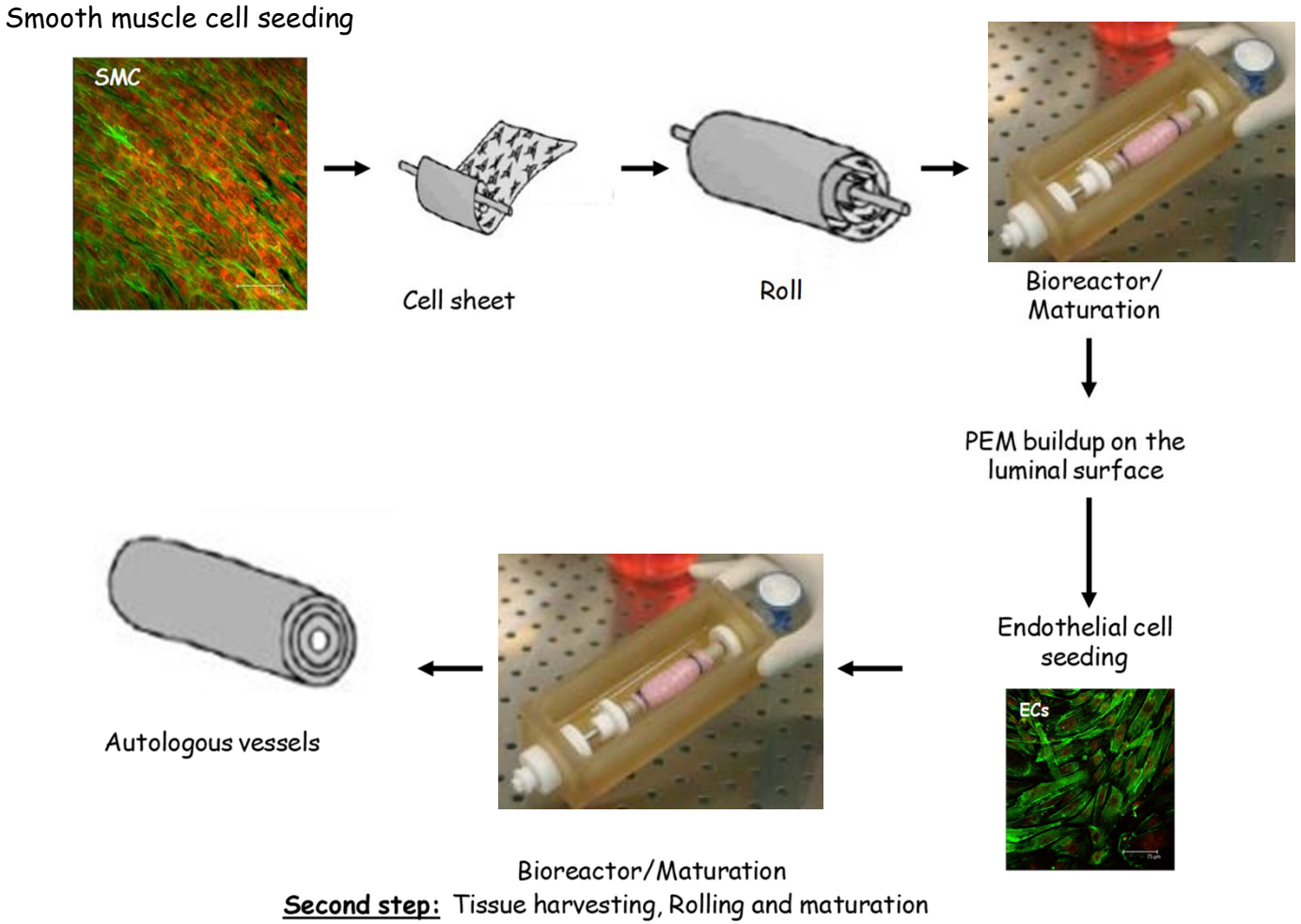


Smooth muscle cellularized tube

Towards blood vessel reconstruction

Challenge:

balance between the membrane degradation/extracellular matrix production of cells



Second step: Tissue harvesting, Rolling and maturation

Stem cell differentiation

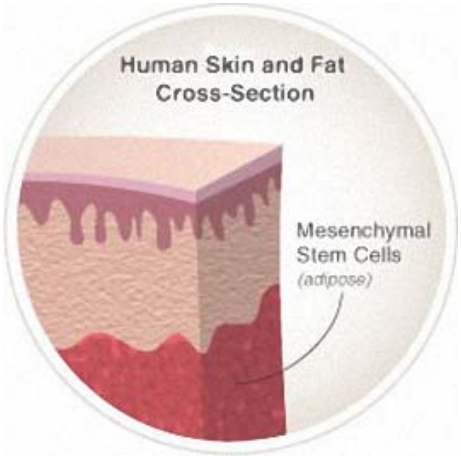
Interest in Stem cells

Stem cells are interesting to use for tissue engineering

- non-immunogenic
- self-renew
- good proliferation
- differentiation into different specialized cells

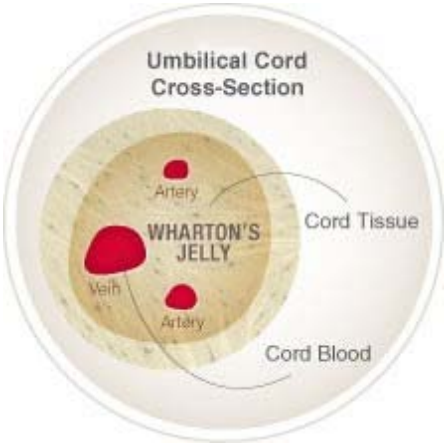
Bone Marrow

Adipose Tissue



Tooth Pulp

Umbilical Cord
(Wharton's Jelly)



Stem cell differentiation

How to differentiate them ?

- specific biomolecules in solution (growth factors, hormones, proteins…)
- substrate of culture (mechanical properties, topography)
- chemistry of the substrate

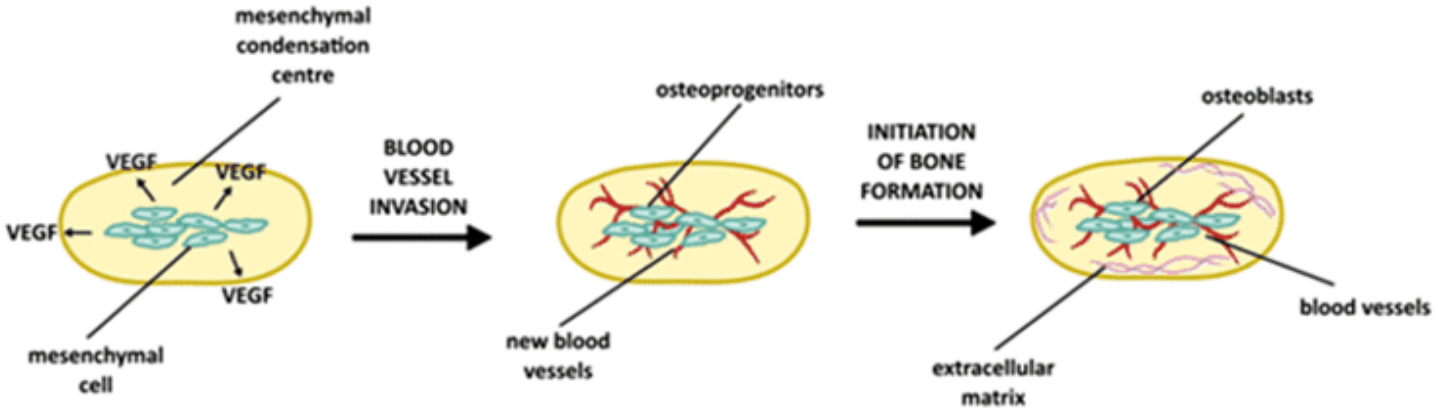
Materiobiology: material-guided cell behavior

Li et al. Chem. Rev. 2017, 117, 4376

Bone regeneration issue

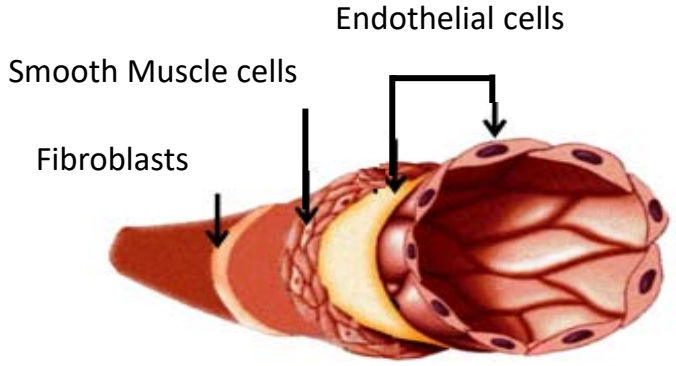
Bone = richly vascularized connective tissue

Delivery of O_2, nutrients, biomolecules necessary for appropriate bone regeneration



Filipowska, J. et al. Angiogenesis (2017) 20, 291.

Native blood vessel



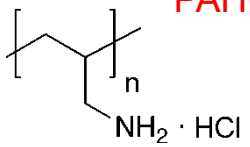
2 types of cells are necessary :

- Osteoblasts (bone)
- Endothelial cells (blood vessel)

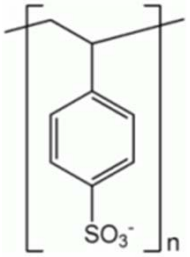
Stem cells differentiation in endothelial cells

BM (Bone marrow) and WJ (Wharton Jelly)

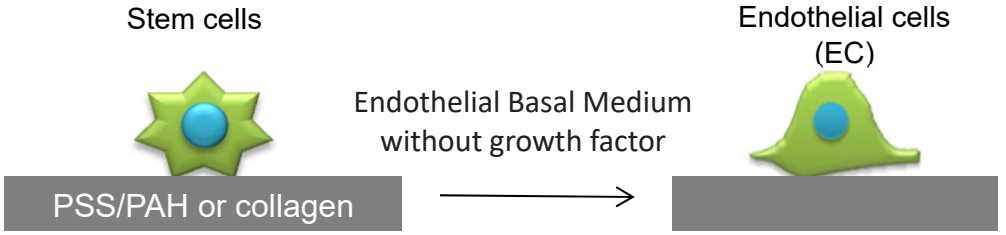
Poly(allylamine) PAH

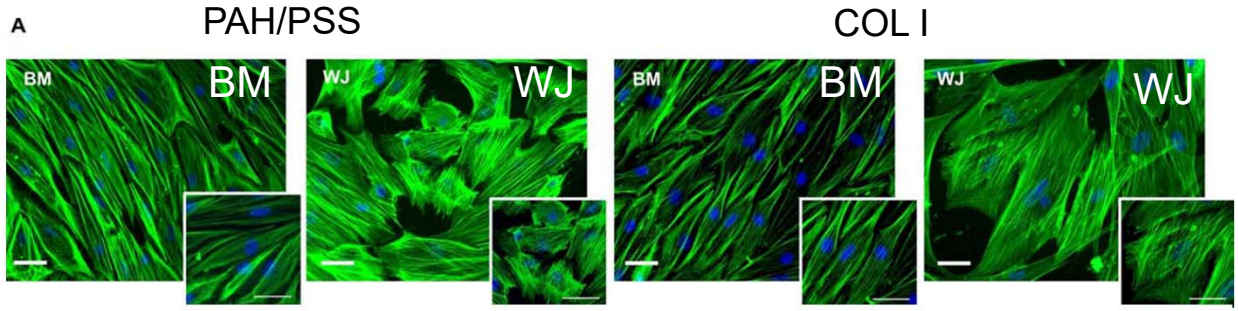


Poly(styrene sulfonate) PSS

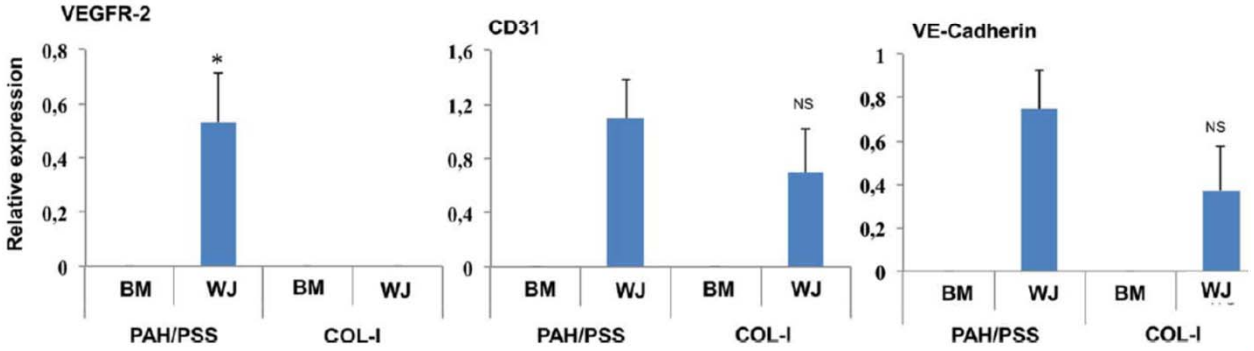


Thickness 15 nm





After 2 weeks, change of shape



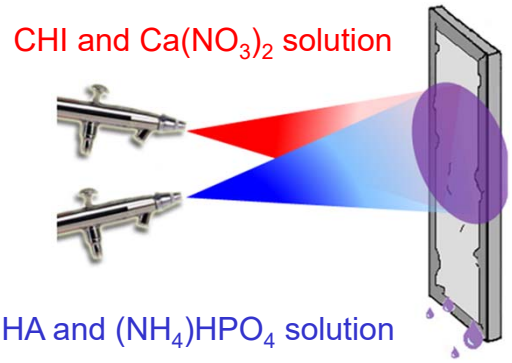
More expression of EC proteins for WJ cultured on PSS/PAH

Rammal et al. J. Biomed. Mater. Res 2017, 105, 292

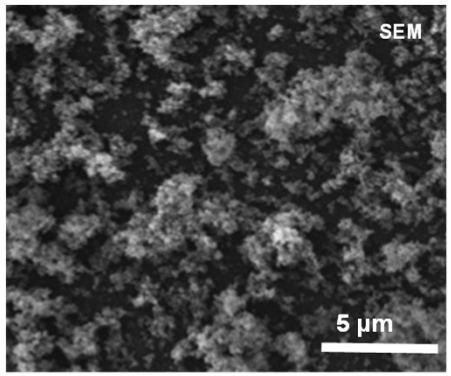
Polyelectrolytes Team

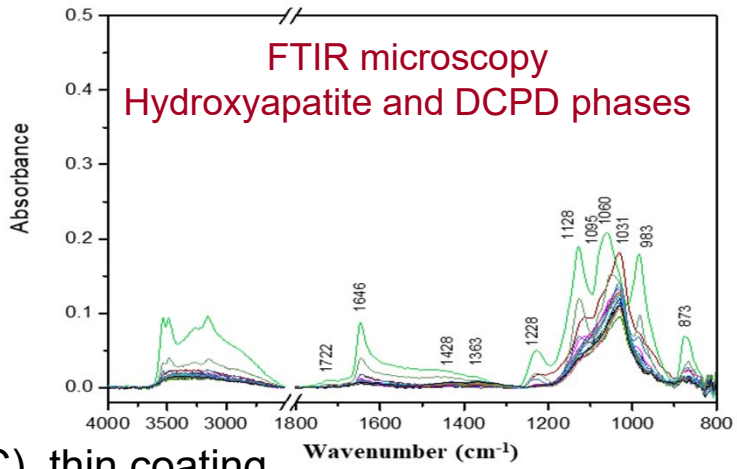
Stem cells differentiation in osteoblasts

Simultaneous spray of salt solutions



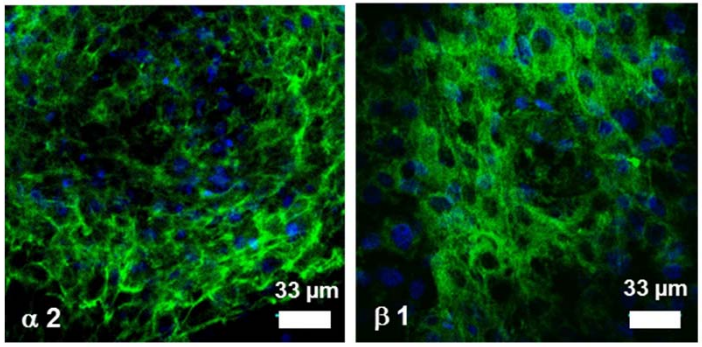
50 cycles thickness of 1.2 µm



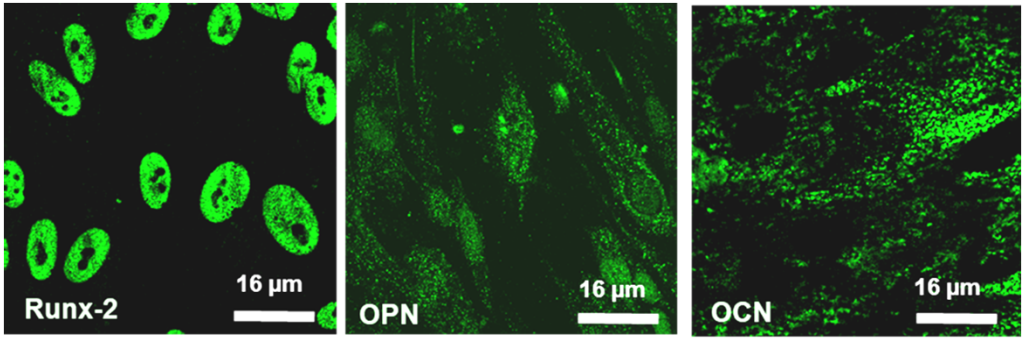


Coating obtained in mild conditions (pH, T°C), thin coating

Wharton jelly cultivated on the hybrid coating without growth factor



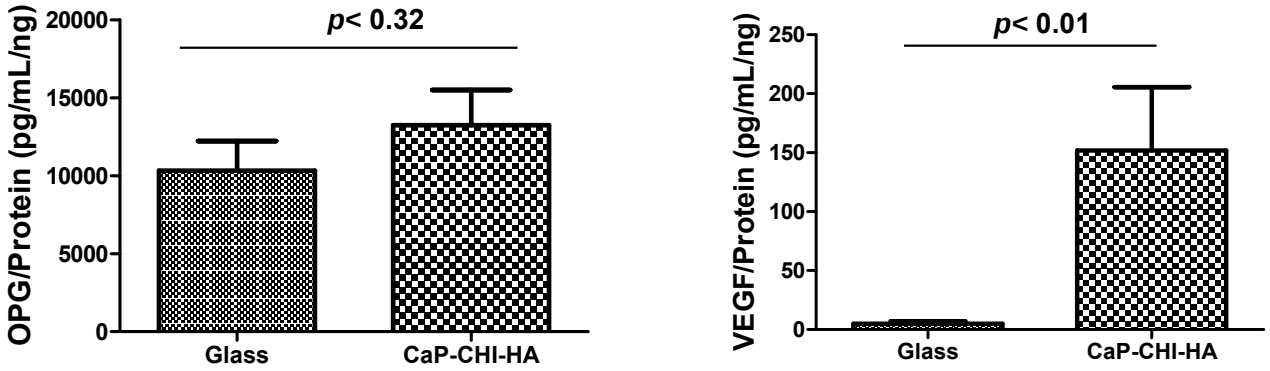
Strong adhesion (presence of integrin) after 5 days



Expression of bone specific markers after 5 days

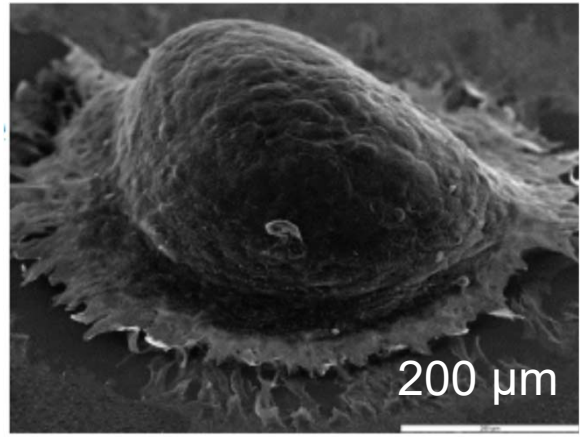
Stem cells differentiation in osteoblasts

Expression of bone specific markers



Secretion of OPG (block bone resorption) and VEGF (favour vascularisation)

Formation of a bone nodule after 28 days



Assembly of cells in 3D structure
Formation of mineralized collagen matrix

Polyelectrolyte layer-by-layer films to explore mammalian and bacterial cell control

III. Bacterial cells

Fouzia Boulmedais

Institut Charles Sadron, Strasbourg, France

Biomaterials

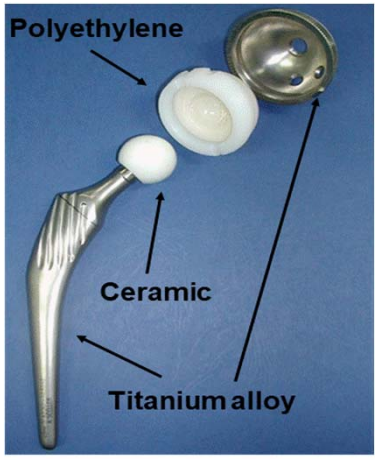
Definition agreed upon the Conference of the European Society for Biomaterials in 1986

Biomaterials = a non-viable material used in a medical device used in the diagnosis, the cure, the treatment or the prevention of disease. They are thus intended to interact with biological system.

Types of biomaterials

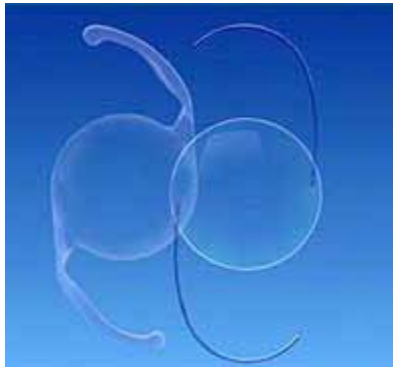
- Titanium and alloys
- Ceramics
- Synthetic and natural polymers

Hip implant



©Implants Industrie

Artificial Lens



Issues at the implantation

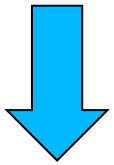
Bacterial and fungal infection at the site of implantation

Treatment by antibiotics ⇨ pathogen resistance ⇨ biofilm formation

Nosocomial infections ⇨ major public health issue (medical and financial)

(5% of hospitalized patients, 4000 deaths / year in EU)

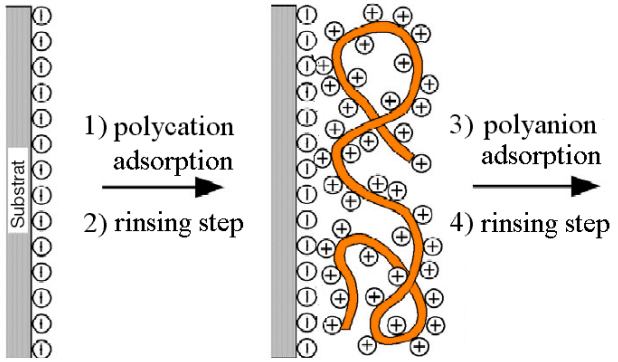
GMS Krankenhaushyg. Interdiszip. 2011, 6 , 1

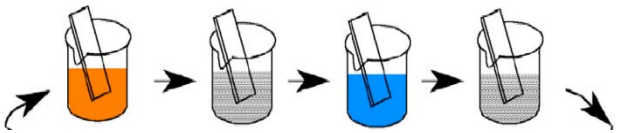


Solutions to prevent bacterial proliferation

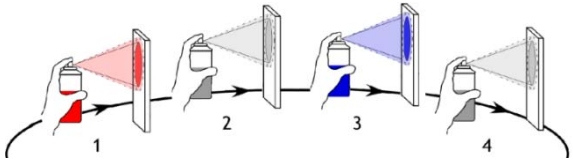
Polyelectrolyte multilayers

Alternated deposition of polycation and polyanion





Decher, G. *Science* **1997**, 277, 1232

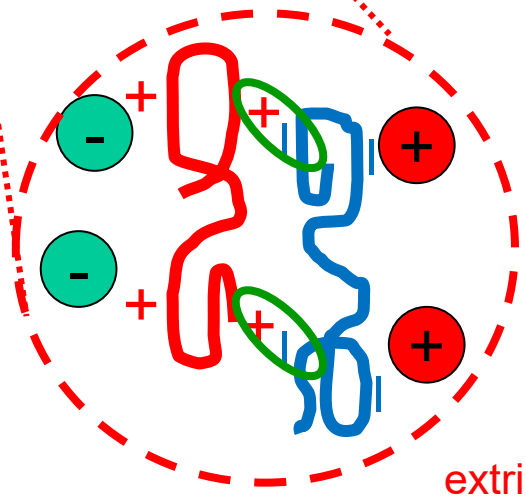


Schlenoff *et al. Langmuir* **2000**, 16, 9968

Park *et al. Scientific Report* **2018**, 3365

Overcompensation of the charges

Compensation of the charges inside the film



intrinsic by the polyelectrolytes

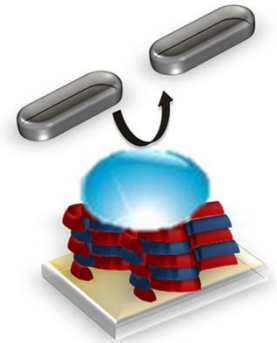
extrinsic by the ions

Schlenoff *et al. JACS* 1998, 120, 7626

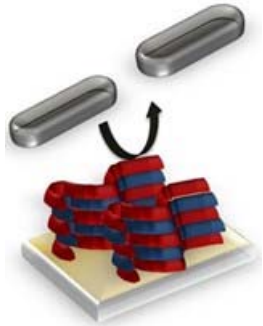
Strategies to prevent bacterial infections

Antibacterial polyelectrolyte multilayers

Anti-adhesive



Inhibition of the close approach due to high film hydration



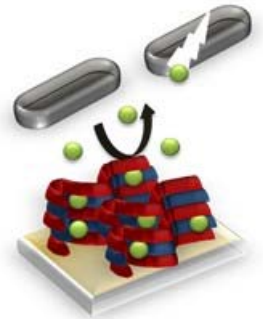
Specific stiffnesses to decrease the bacterial attachment

Contact-killing

Contact with highly positively charged surface

Contact with immobilized antimicrobial agents

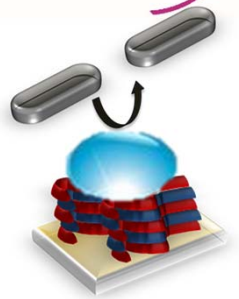
Release-killing



Diffusion of the antibacterial agent through the film

Degradation of the film to release the antibacterial agent

Anti-adhesive multilayers



Highly hydrated films
prevent bacterial adhesion

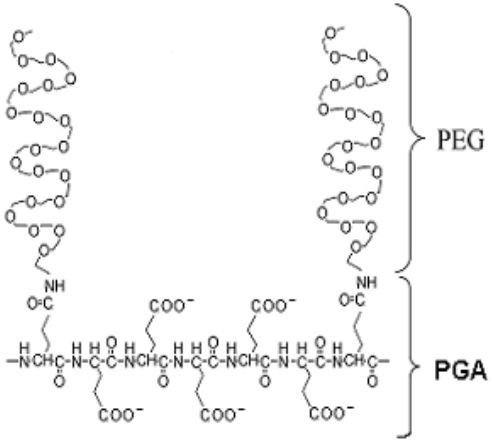
Synthesis of pegylated polyanion

PLL/PGA-g-PEG multilayers

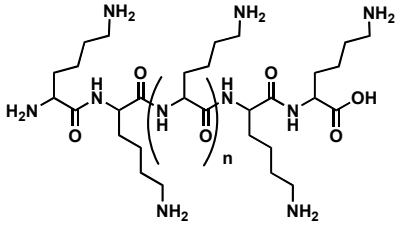
Escherichia Coli

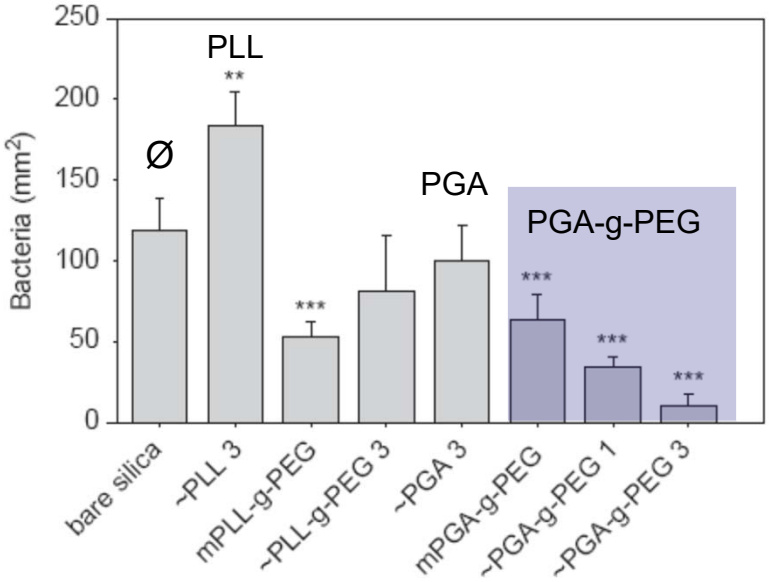
contact time 30 min and rinsing step

PGA-g-PEG
20% grafting ratio



PLL
poly(L-lysine)



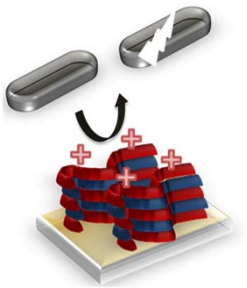


PGA: poly(L-glutamic acid)
PEG: poly(ethylene glycol)

92% decrease of bacterial adhesion

No protein adsorption

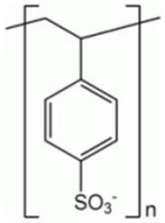
Contact-killing multilayers



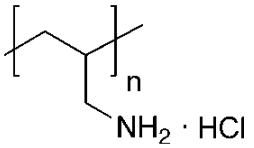
Highly positively charged films
are bactericidal

physico-chemical approach
by acid activation of free amino groups

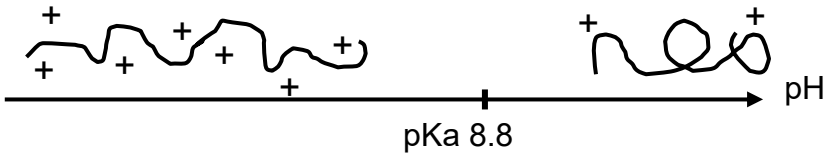
PSS
poly(styrene sulfonate)



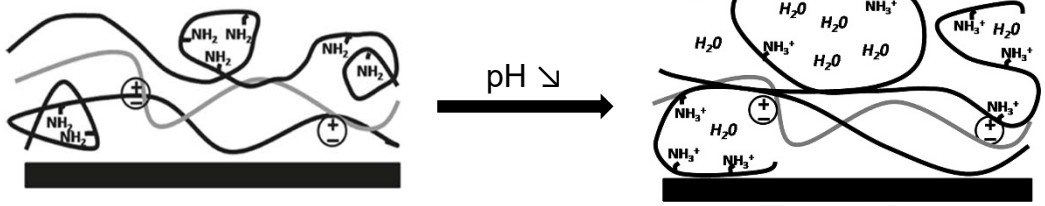
PAH
poly(allylamine hydrochloride)



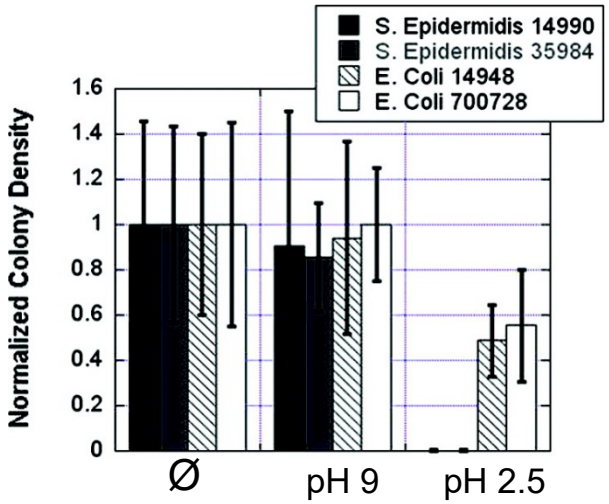
PAH in solution



PAH/PSS film built at pH 9



E. Coli and S. Epidermis
12 h bacteria contact at pH 7



Bactericidal effect for pH post-treated films

Release-killing silver ions based coating

Release killing

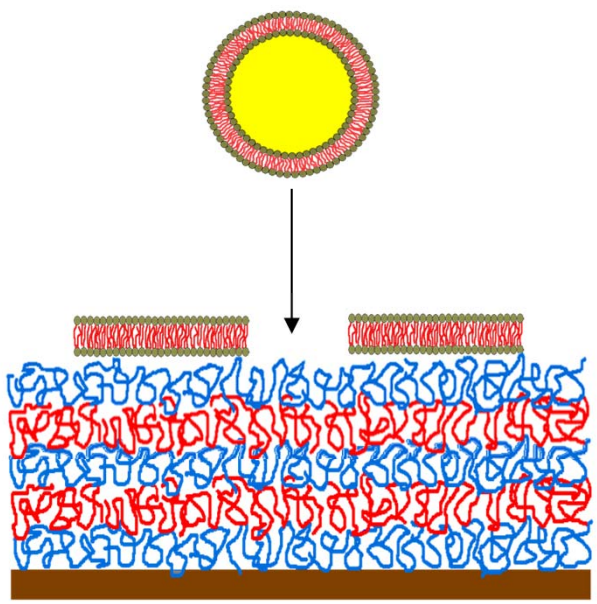
Silver nanoparticles: efficient antibacterial agent due to the presence of

Ag+ in equilibrium with Ag, disturb the membrane of bacteria

but toxic and meant to be limited in use by EU

Idea : Liposomes used as a cargos by insertion into LbL films

Problem : Liposomes are disrupted upon adsorption on polyelectrolyte LbL films



Cassier, T. et al. (1999), ***Coll. Surf. B***, 15, 215.

Moya, S. et al. (2000), ***Macromolecules***, 33, 4538.

Richter, R.P. et al. (2006), ***Langmuir***, 22, 3497.

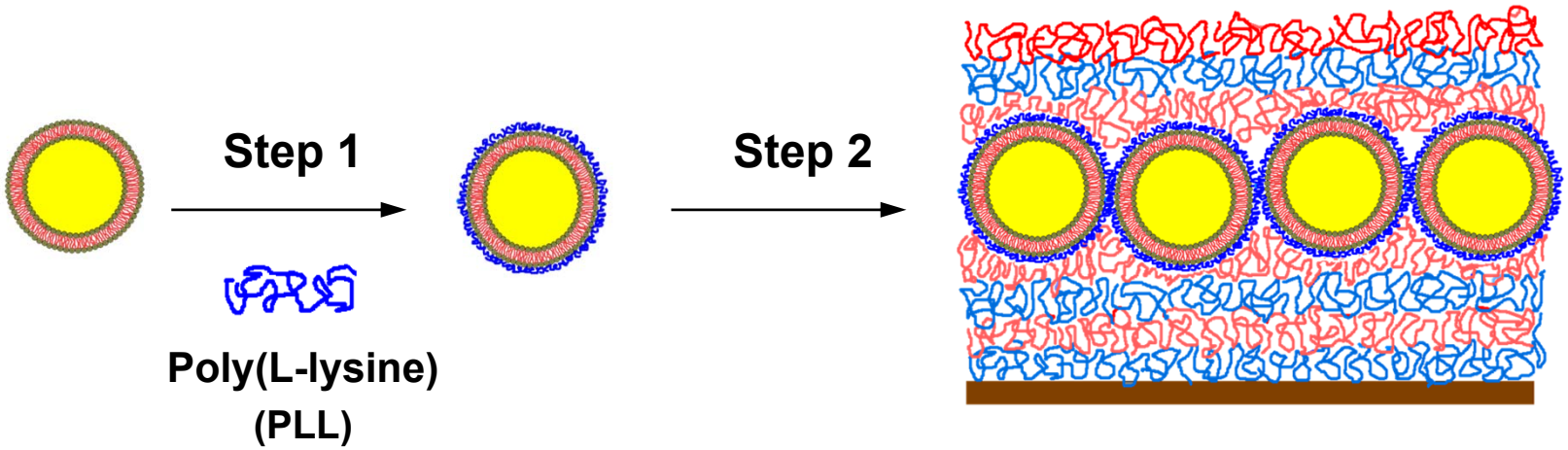
Strategy of liposomes integration into multilayers

Step 1. Coating of liposomes by a poly(L-lysine) (PLL) layer

⇨ vesicles keep their integrity after coating

Step 2. Adsorption of PLL-liposomes on multilayer films

⇨ coated vesicles keep their integrity when adsorbed on the film

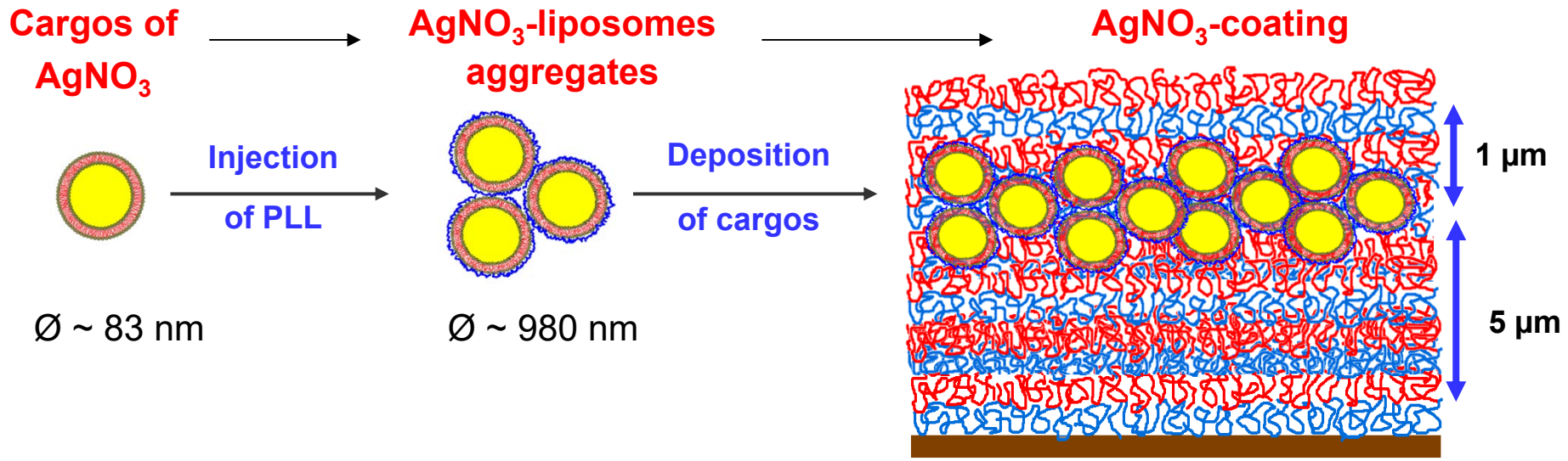


Volodkin D. *et al.* (2007), ***J. Control. Release***, 117, 111.

Volodkin D. *et al. (2008),* ***Soft Matter***, 4, 122

Controlled release of bactericidal agent by a surface

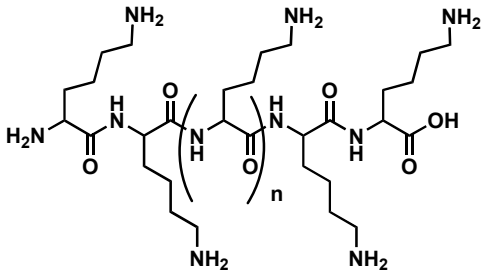
Proposed strategy : local silver ions release from multilayers coating



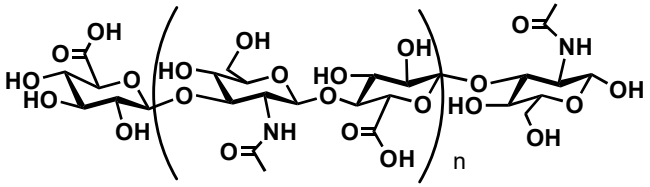
Gel-like multilayer film: a perfect matrix for liposomes deposition

Poly(L-Lysine)/Hyaluronic acid (PLL/HA) multilayer films

Poly(L-lysine) PLL

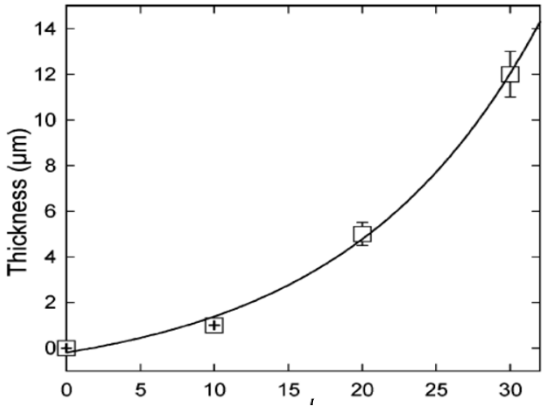


Hyaluronic acid (HA)

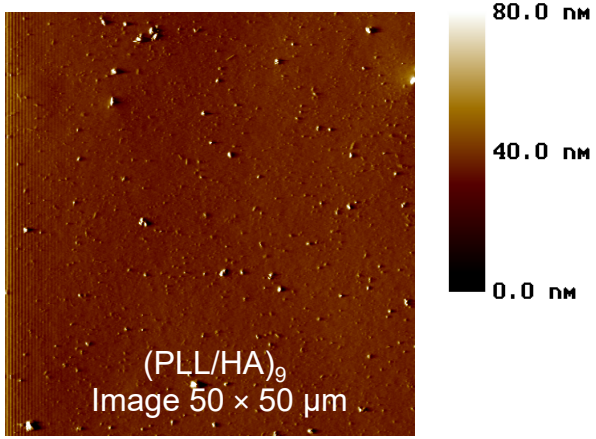


- Micrometric films
- Smooth gel-like films

Thickness of PLL/HA films



Atomic Force Microscopy



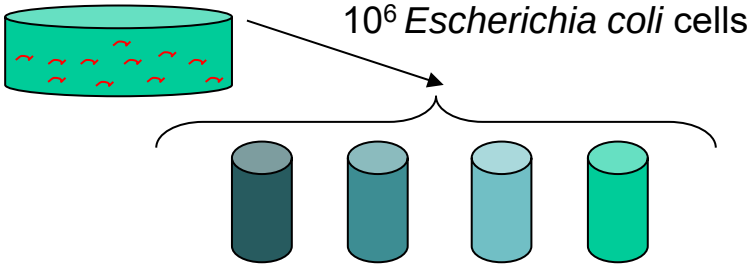
Biological interest: no adhesion of mammalian cells

Richert *et al.* (2004), ***Biomacromolecules***, 5, 284 and 1908

Lavalle *et al.* (2001), ***Langmuir***, 17, 7414

$AgNO_3$ in solution / $AgNO_3$-liposomes aggregates

$AgNO_3$ in solution



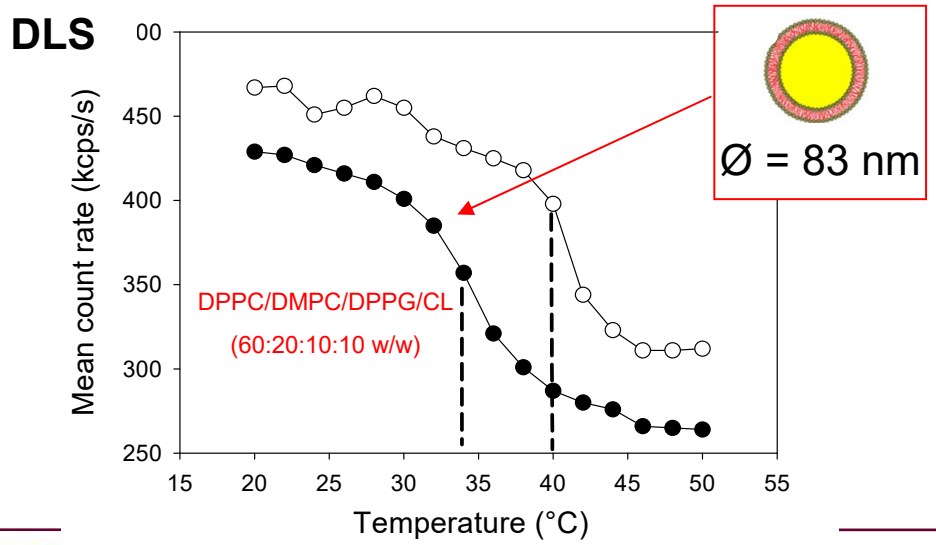
Minimal Inhibitory concentration (MIC)

[$AgNO_3$] that inhibits the visible growth of a bacteria after overnight incubation at 37°C

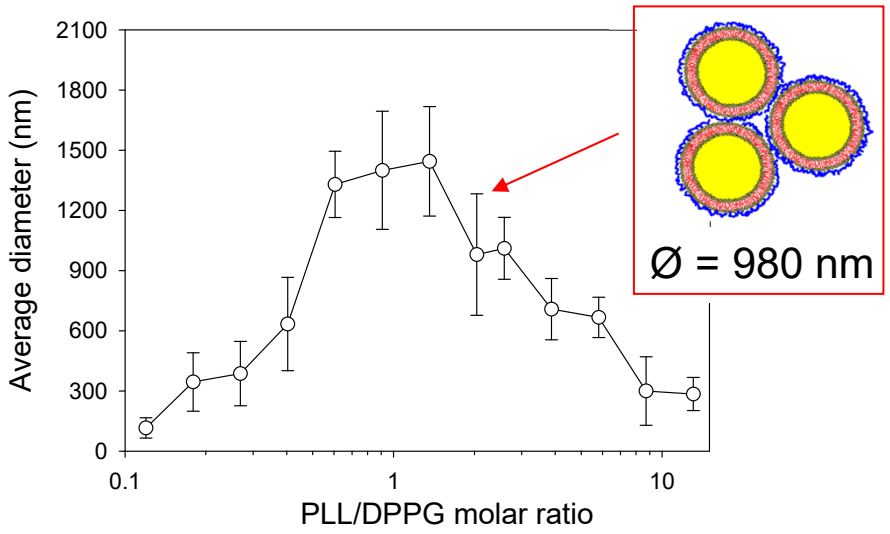
MIC of $AgNO_3$: 22 µg/mL

$AgNO_3$-Liposomes : with encapsulation of 1 M $AgNO_3$

Phase transition temperature at 34°C



$AgNO_3$-liposomes aggregation by PLL



Leaking from liposomes above 34°C

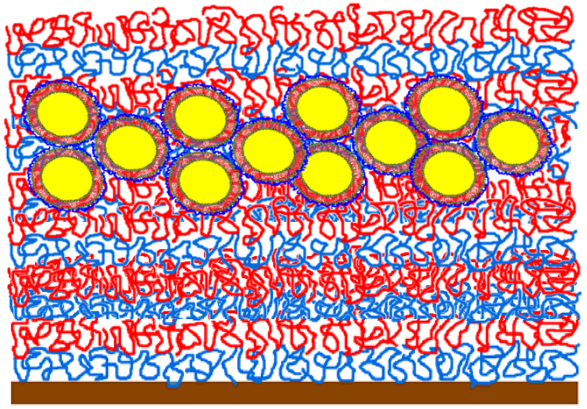
Bactericidal property of the film with embedded $AgNO_3$-liposomes

$AgNO_3$ coating

- deposition of $AgNO_3$-liposomes aggregates
- dried 2h and addition of HA/PLL/HA capping layers

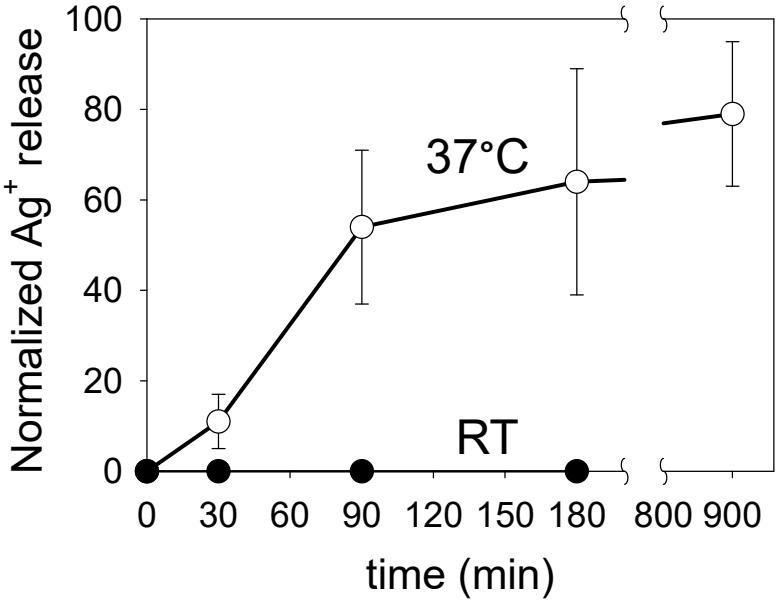
  56% of deposited solution remains on PLL/HA film

  15% of deposited solution remains on bare substrate
- dilution of the deposited solution to tune $AgNO_3$ amount



$AgNO_3$ release from $AgNO_3$ coating

Gravimetry analysis of the supernatant by AgI precipitation

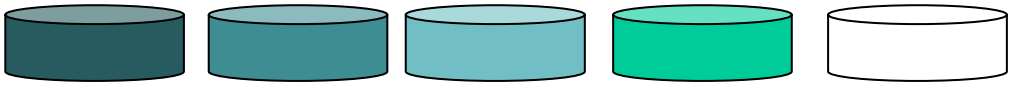


Continuous release of Ag^+ ions at 37°C

Bactericidal property of the film with embedded Ag-liposomes

$AgNO_3$ coating : bactericidal property

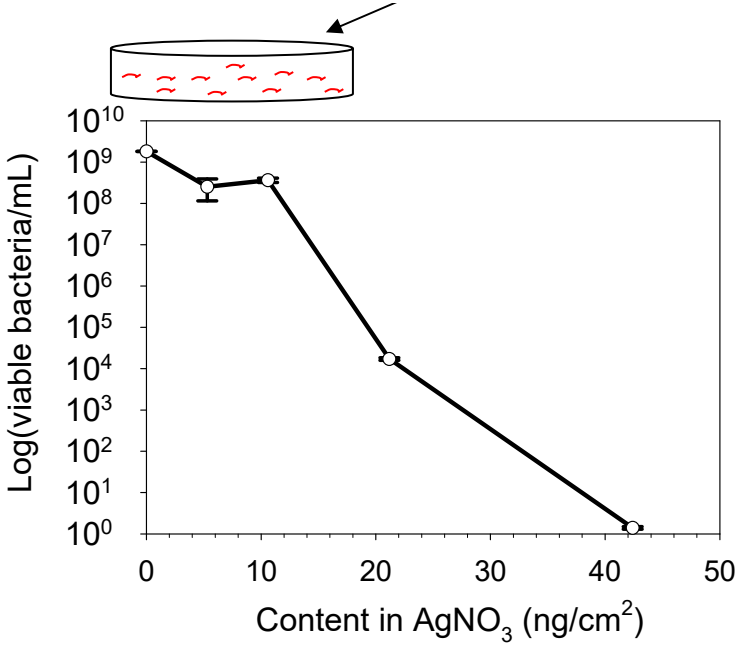
t = 0 : 10^6 *E. coli* cells in contact with $AgNO_3$ coating



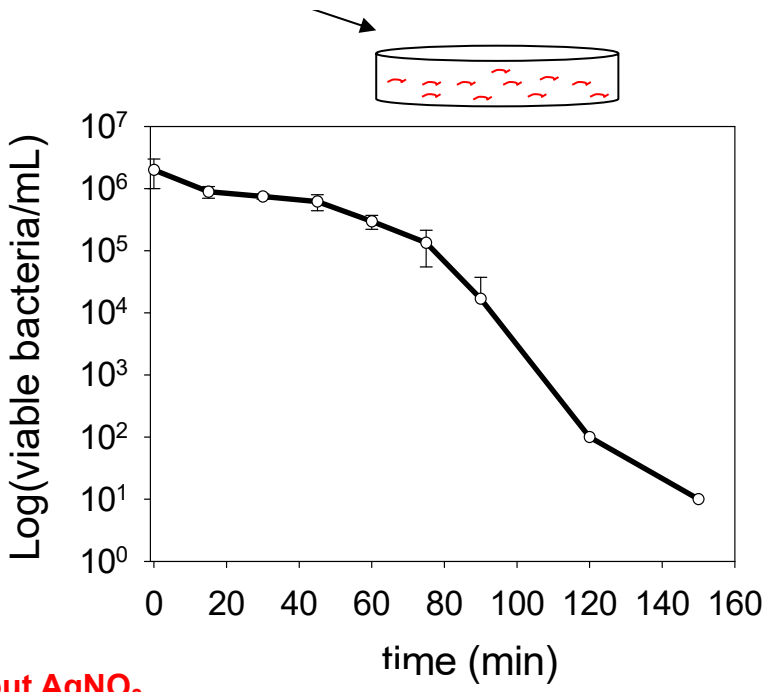
incubation at 37°C

Increased [$AgNO_3$] in $AgNO_3$ coating

after 20h of incubation at 37°C



Aliquots vs time



No bactericidal effect without $AgNO_3$

Complete bacterial death with 42 ng/cm^2 $AgNO_3$

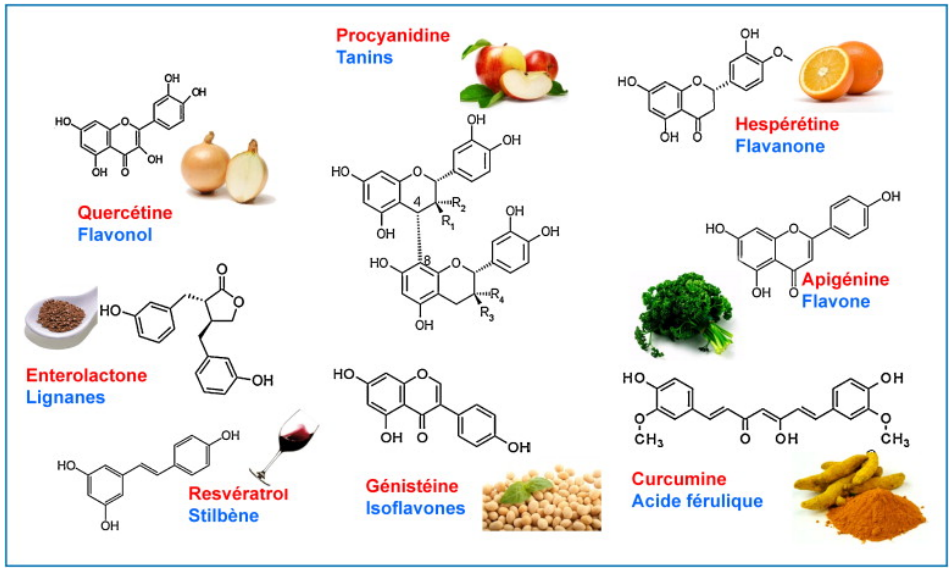
Release-killing polyphenols based coating

Polyphenols

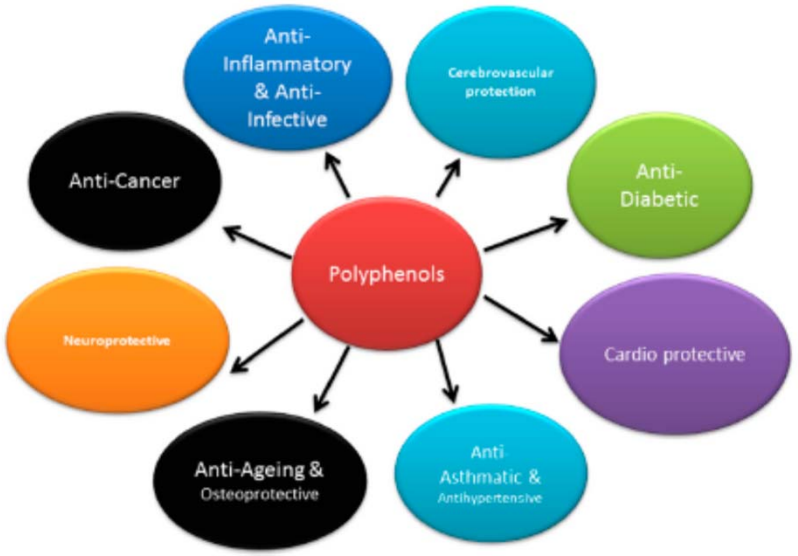
Polyphenols: found largely in fruits, vegetables, tea, coffee, chocolates…

Over 8000 polyphenols identified in nature

Main functions: antioxidant, protection from free radical damage and defense against UV radiation or aggression by pathogens.



Bennetau-Pelissero *Cahiers de nutrition et de diététique* **2014,** 49, 151



Ganesan et al. *Nutrients* **2017**, 9, 455

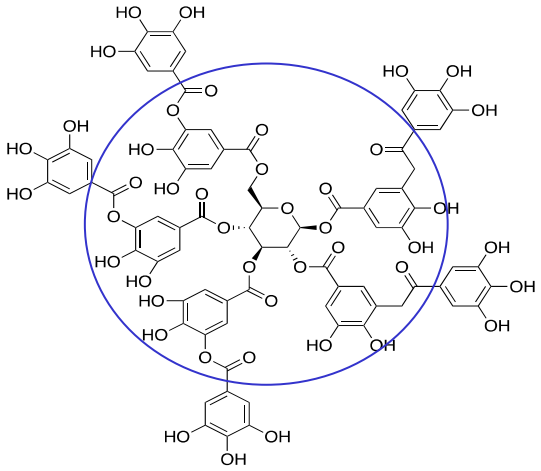
Strategy

Hydrogen-bonding based Layer-by-layer

Tannic acid (TA)

Natural polyphenol present in leaves of many plants and fruits.

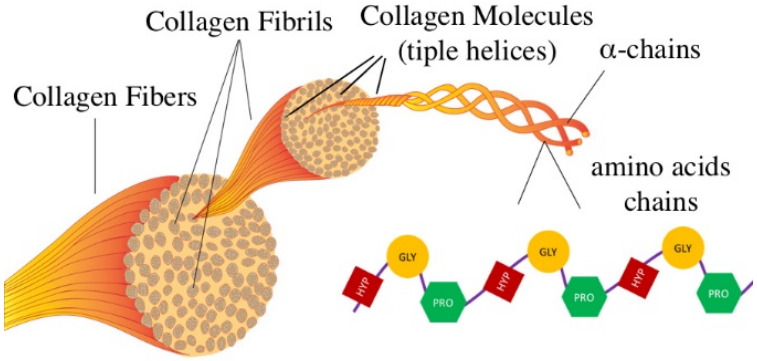
- Antibacterial
- Antioxidant
- Anti-inflammatory & anti-tumor
- Known to complex with protein through H-bonds



Collagen (COL)

Comprises of 25-35% protein content of human body.

- guide fibroblasts
- act as nucleation sites for cell proliferation

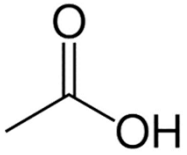


Due to solubility issues of COL => buildup at acidic pH

TA/COL multilayers films

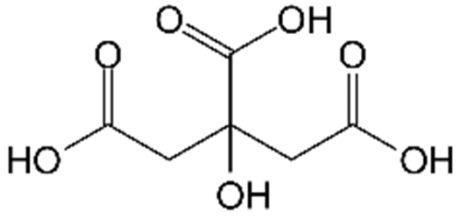
Buildup at pH 4 with two different buffers

Acetate buffer



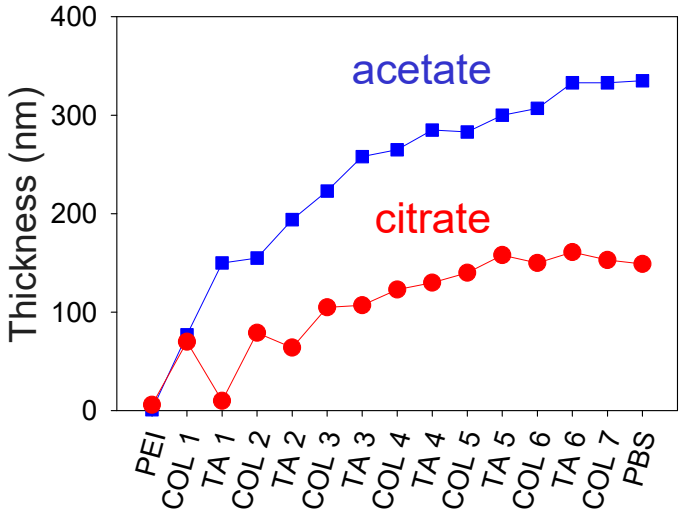
pKa = 4.7

Citrate buffer

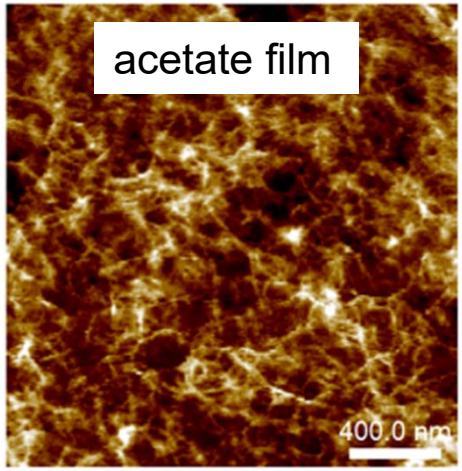


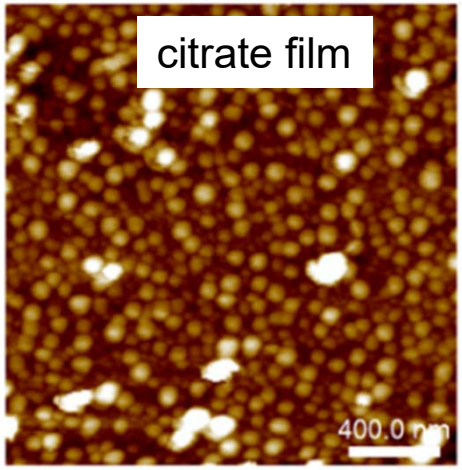
pKa = 3.1, 4.8 and 6.4

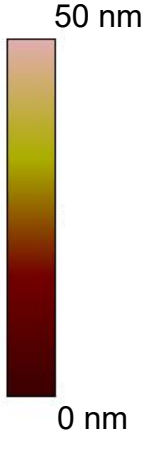
Quartz crystal microbalance



Atomic force microscopy in liquid state





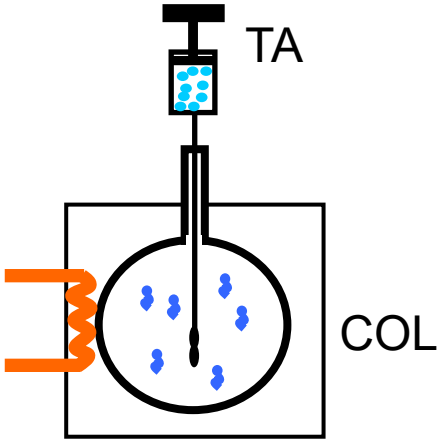


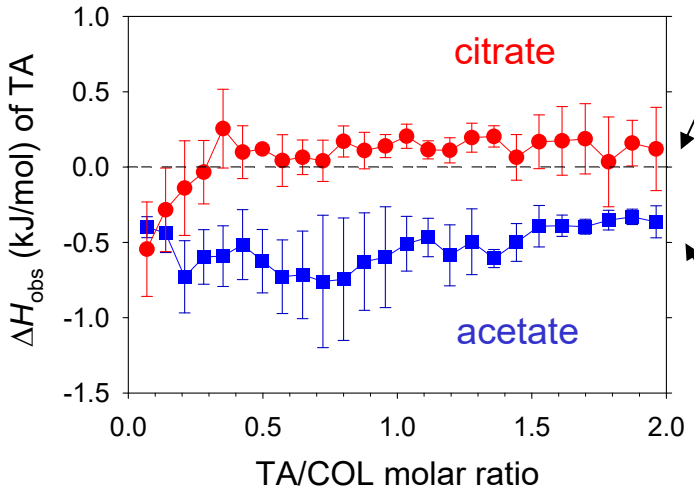
Influence of the buffer on the buildup and topography of the LbL !

Iqbal et al. *ACS Appl. Mater. Inter.* 2020 DOI: 10.1021/acsami.0c04475

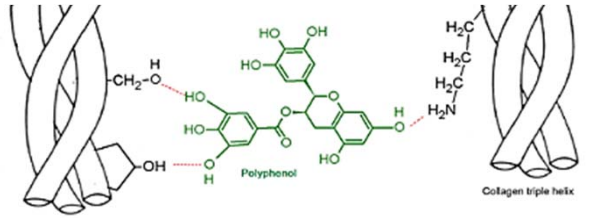
TA/COL complexation

TA/COL complexation by Isothermal calorimetry (ITC)





Citrate: saturation and aggregation



Natarajan et al. PLoS ONE 2015 10, e0127165

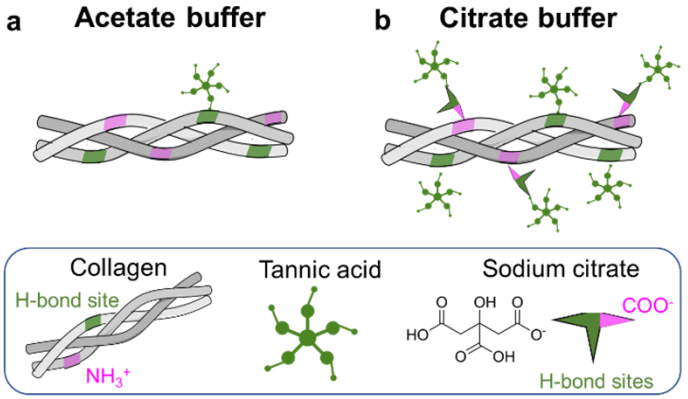
Acetate: exothermic (heat released)

Stronger binding of TA/COL in the presence of citrate

XPS study on TA/COL films

More TA in citrate films than acetate ones
Incorporation of citrate

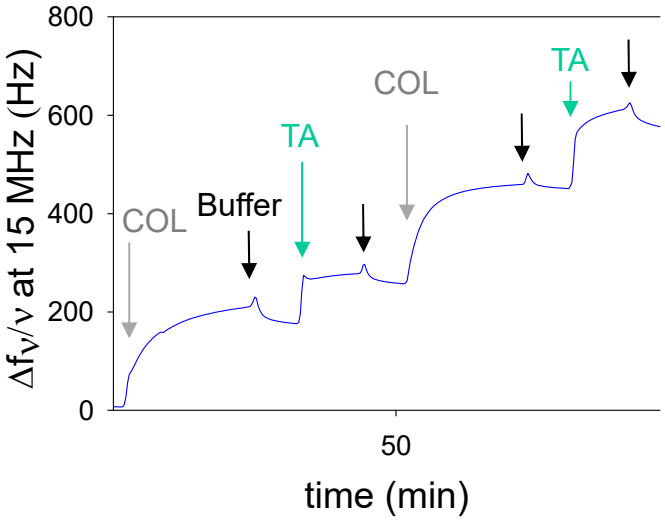
Citrate molecules => ⇗ the number of TA sites on COL



How to explain TA/COL films topography?

In acetate: lower binding of TA/COL and lower amount of TA

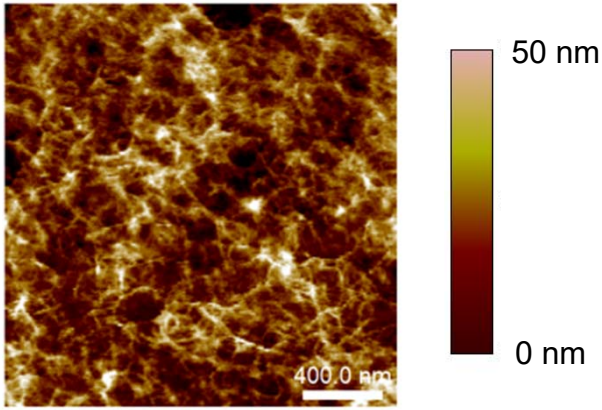
QCM buildup of TA/COL acetate film



Standard LbL buildup

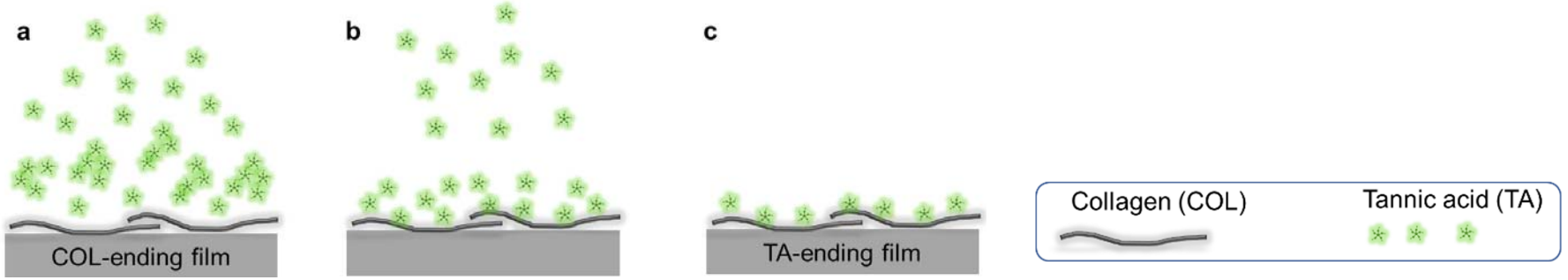
Fibrillar structures

AFM in liquid state



TA adsorption step

Rinsing

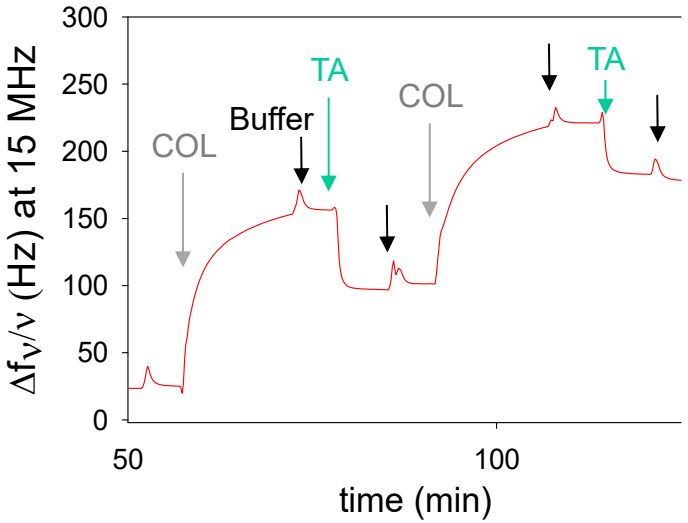


How to explain TA/COL films topography?

In citrate: strong binding of TA/COL and high amount of TA

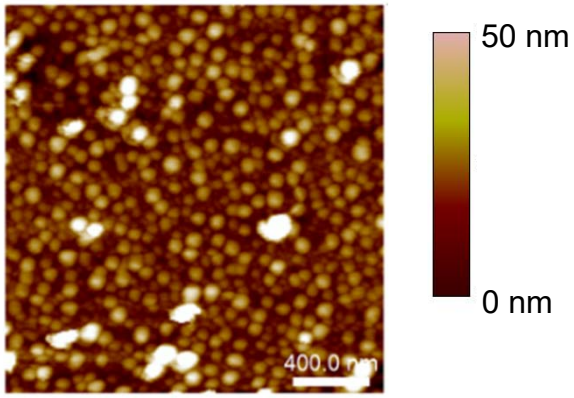
Irregular buildup

QCM buildup of TA/COL citrate film

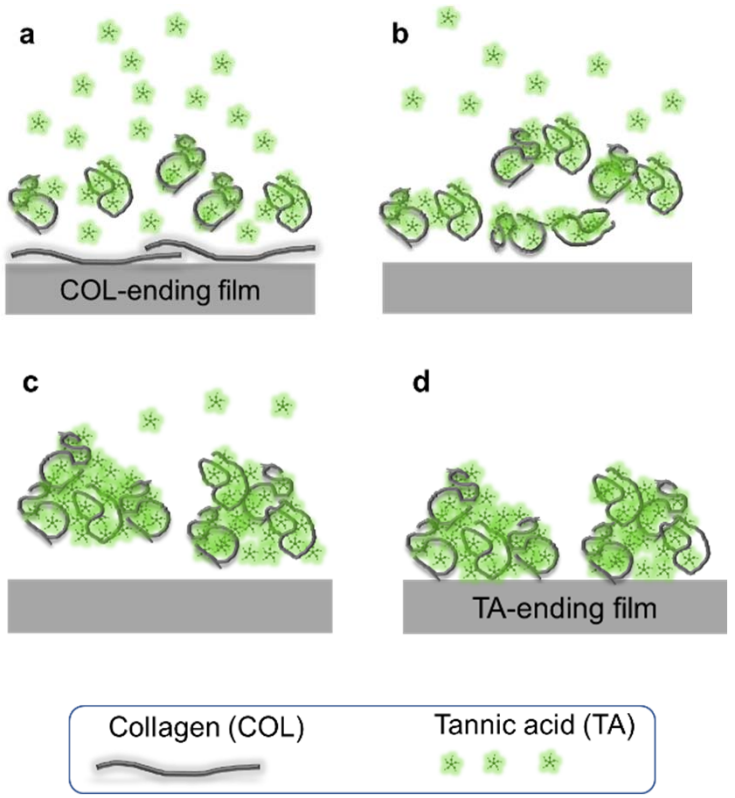


Granular structures

AFM in liquid state

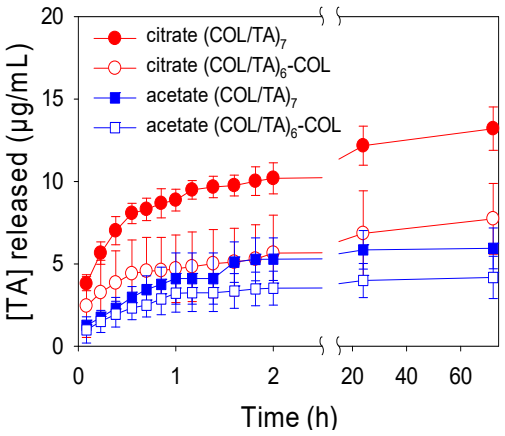


TA adsorption step



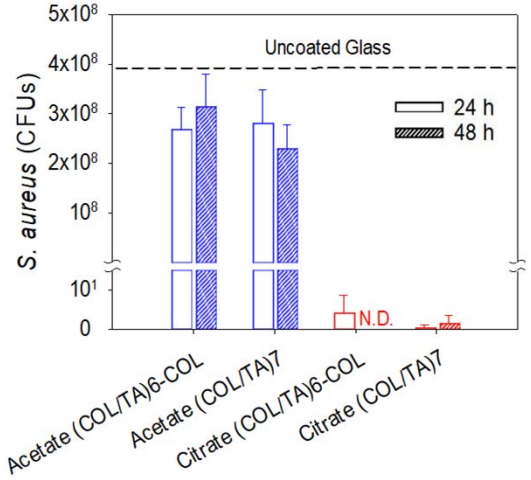
TA release from TA/COL films

TA release in contact with PBS



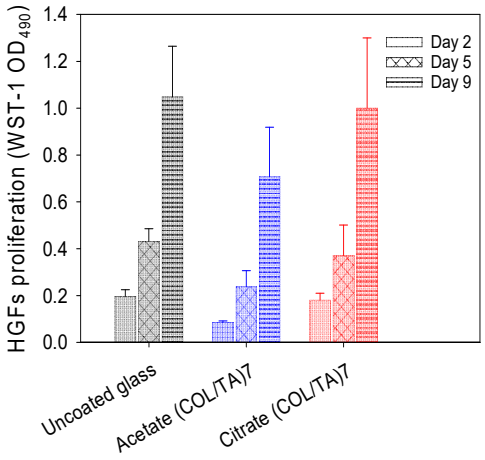
[TA] citrate > acetate

Staphylococcus aureus



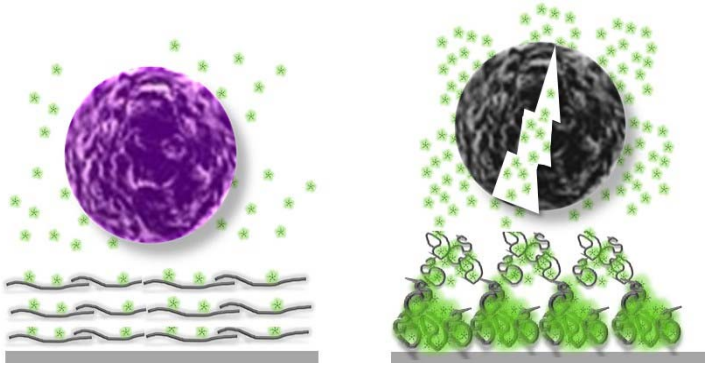
Only citrate films are antibacterial

Human gingival fibroblasts



Absence of cytotocixity

minimal inhibition concentration of TA towards *S. aureus* (100 µg/mL)



Local release-killing !

Iqbal et al. *ACS Appl. Mater. Inter.* 2020 DOI: 10.1021/acsami.0c04475

Polyelectrolytes Team

How to create a self-defensive coating ?

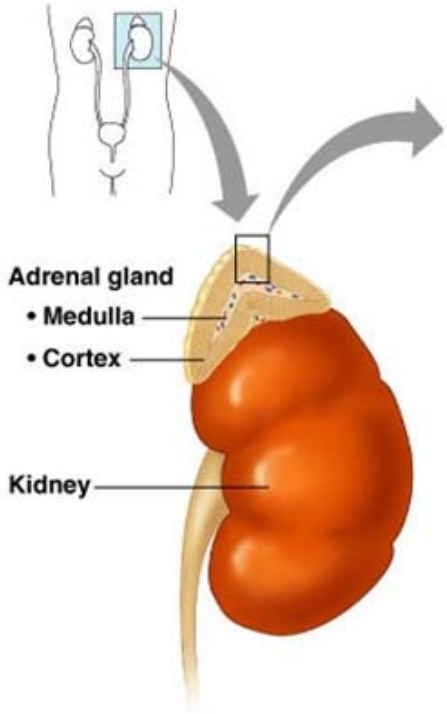
Self-defensive coating

☑ Polysaccharide films as biomaterial coating

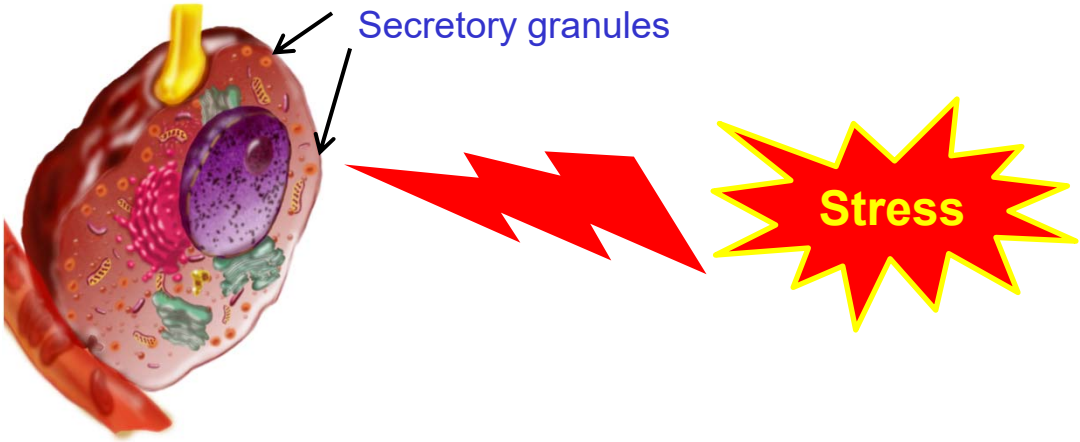
Choice of the antimicrobial compounds ?

Innate immunity of the body: chromogranin A

Adrenal glands



Chromaffin cell



Production of

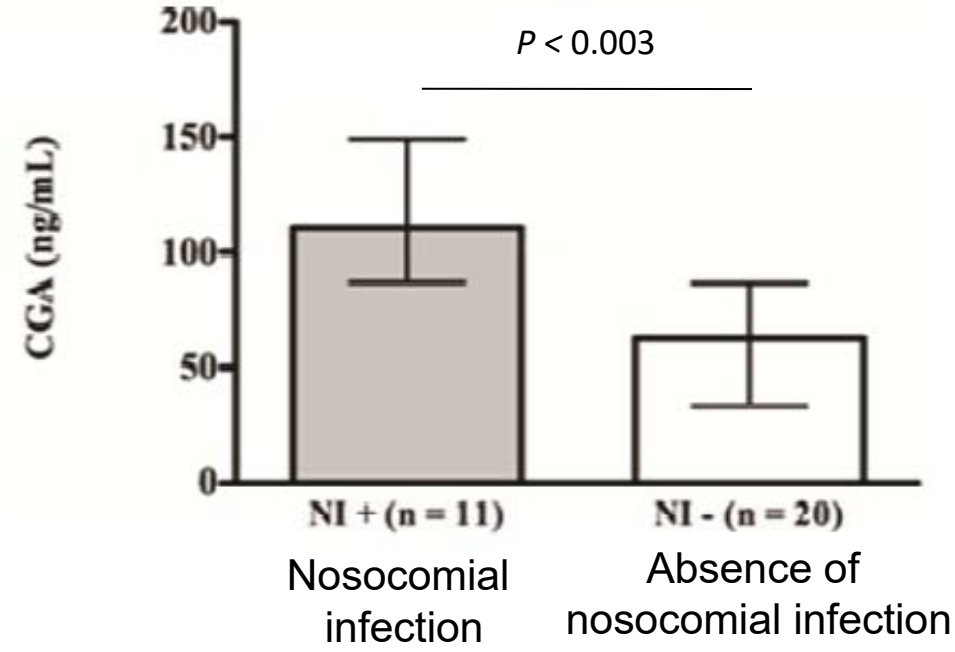
- Catecholamines (0.5-0.6M)
- Prohormones convertases, Cathepsine, Carboxypeptidase…
- Proteins, peptides among them : **nine granins such as chromogranin A**

Chromogranin A as a marker of critical illness

Prospective study of intensive care unit patients by measuring chromogranin A

Measurement from plasma in admission of 31 patients (blunt trauma)

Control (patient with no trauma): CGA = 19.5 ng/mL



High concentration of CGA in admission

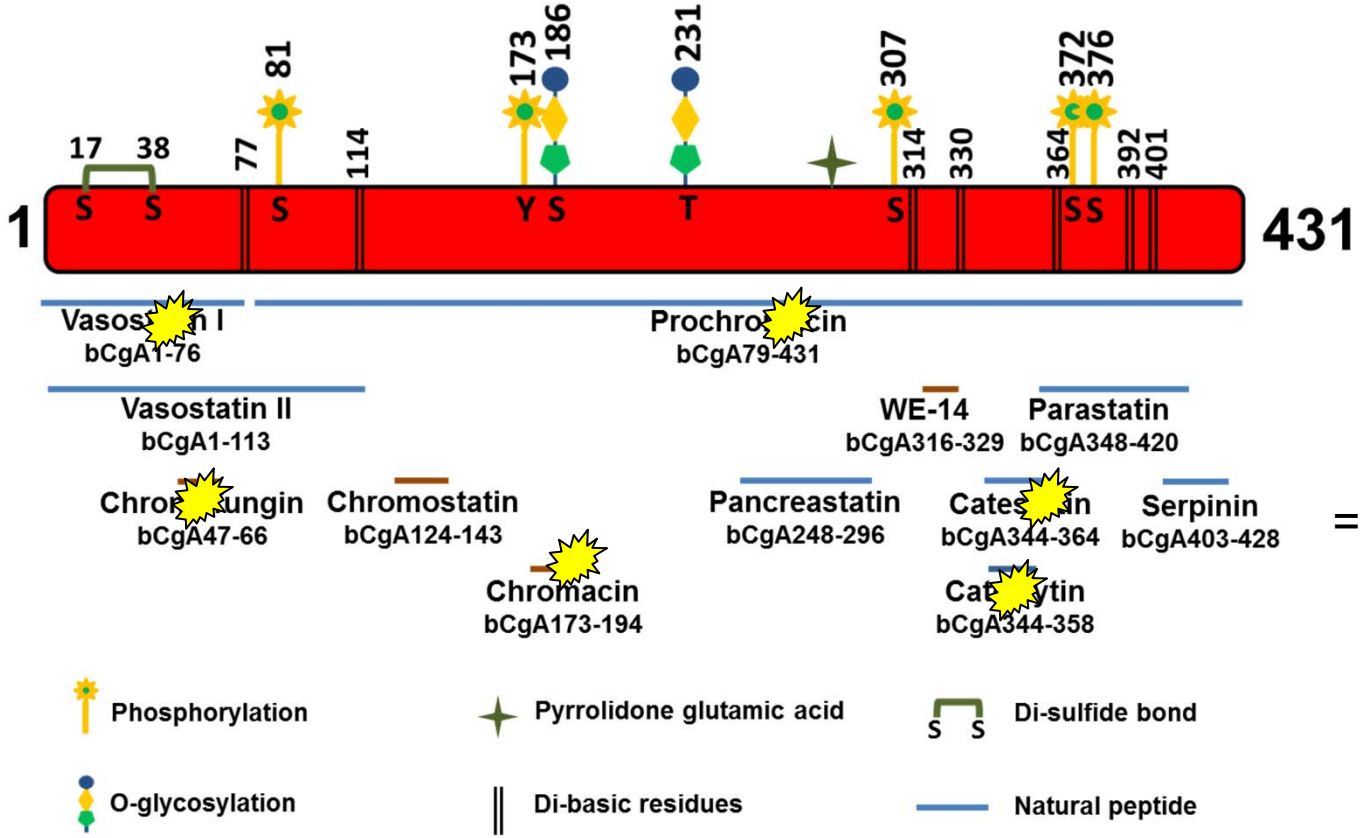
High risk of nosocomial infection

Schneider et al. Shock 2018, 49, 522

Study of M.H Metz-Boutigue (INSERM UMR 1121) and F. Schneider (Intensive care unit at Hautepierre Hospital)

Antimicrobial peptides derived from chromogranin A

CGA usually hydrolyzed by endopeptidases in the body



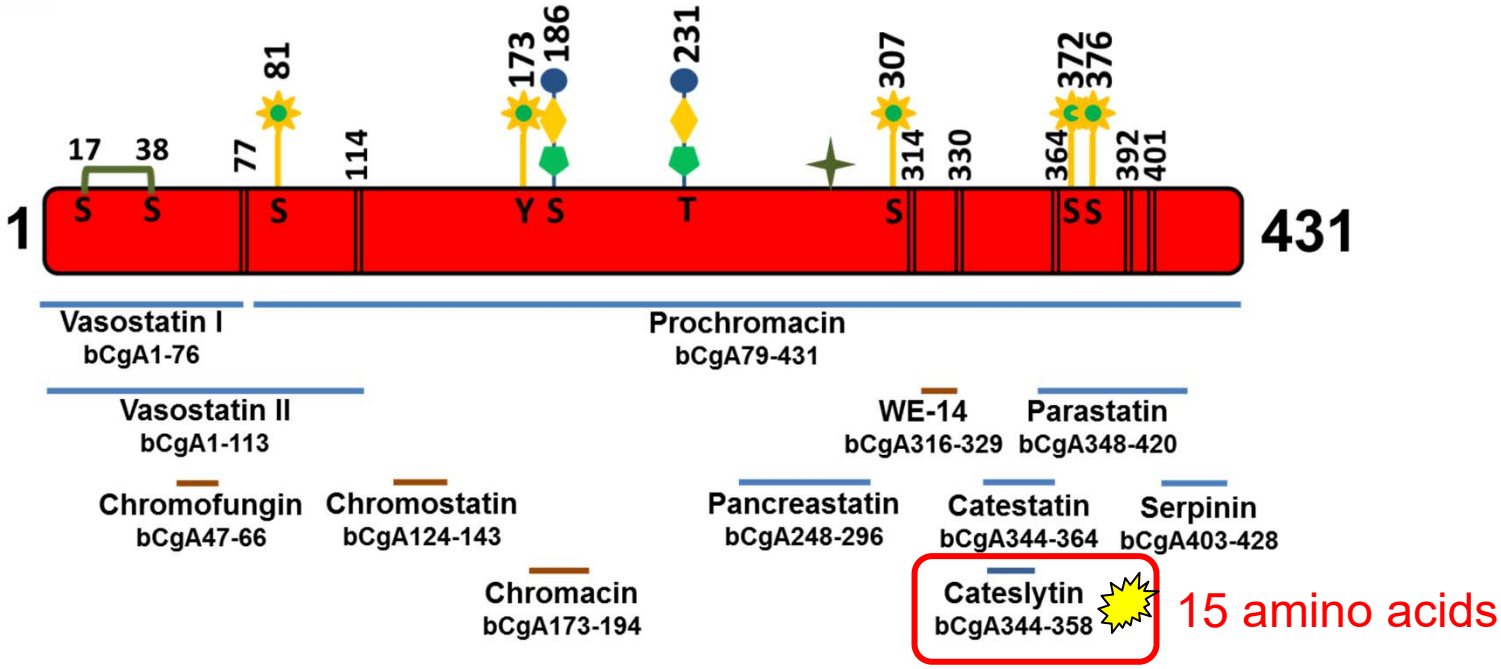
=> antimicrobial peptides

Shooshtarizadeh et al. Regulatory peptides 2010, 165, 102

High concentration of CGA => less antimicrobial peptides to fight against infection

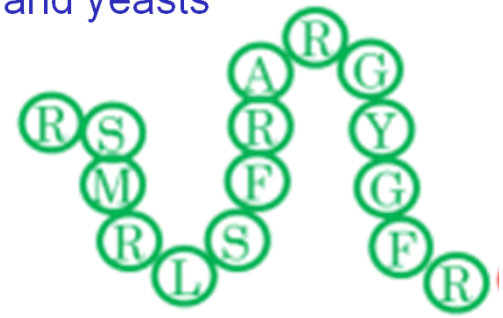
Study of M.H Metz-Boutigue (INSERM UMR 1121) and F. Schneider (Intensive care unit at Hautepierre Hospital)

Choice of AMPs



cateslytin (CTL)

- active (concentration < 100 µM) against bacteria, fungi and yeasts
- resistant against protease of *S. aureus*
- non-toxic for human cells
- stimulate innate immunity



Self-defensive coating

☑ Polysaccharide films as biomaterial coating

☑ Antimicrobial peptide

Choice of the strategy ?

Self-defensive polysaccharide films

Strategy: Release of the peptide only in the presence of the pathogens

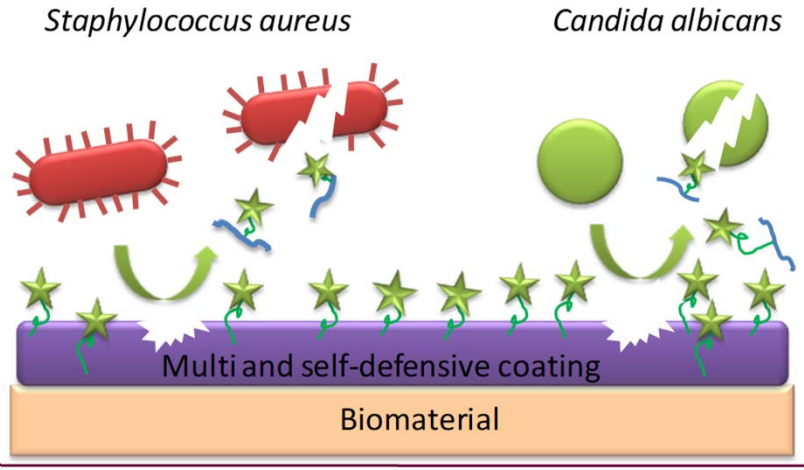
Pathogens:

- *Staphylococcus aureus*: virulent bacteria with high rates of infection and mortality
- *Candida albicans*: common yeast that forms a biofilm (synergy with *S. aureus*)

Pathogens produce hyaluronidase

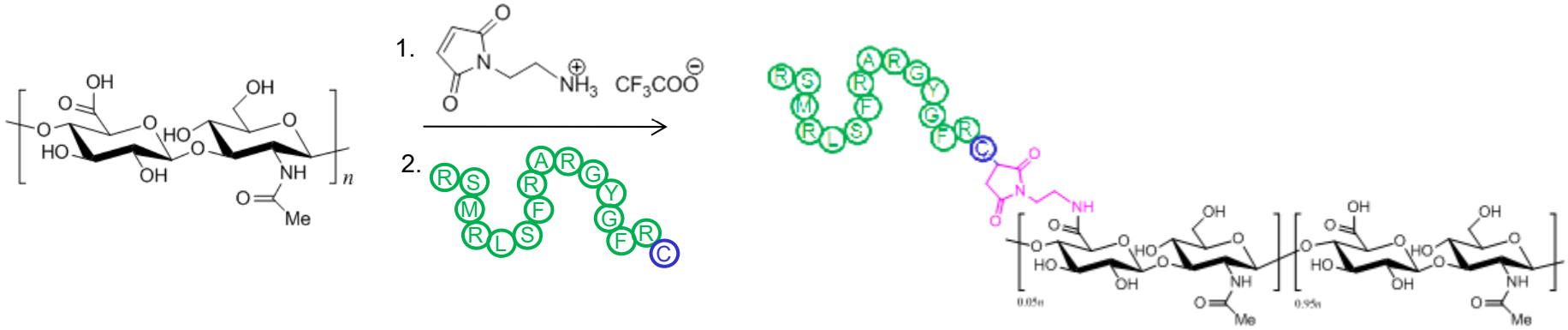
↓

Hyaluronic acid based films: Biomimetic release of the peptide only in the presence of the pathogens

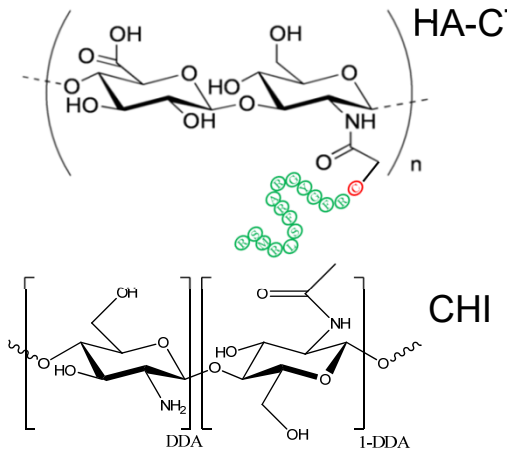


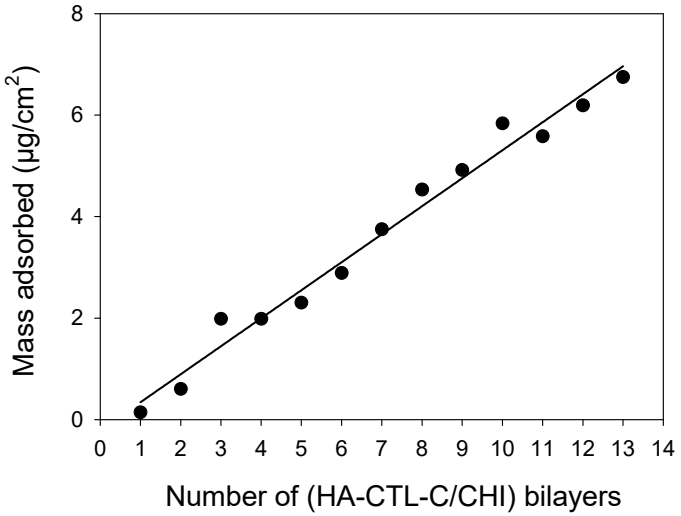
Self-defensive polysaccharide films

Fonctionnalisation of hyaluronic acid by CTL-C



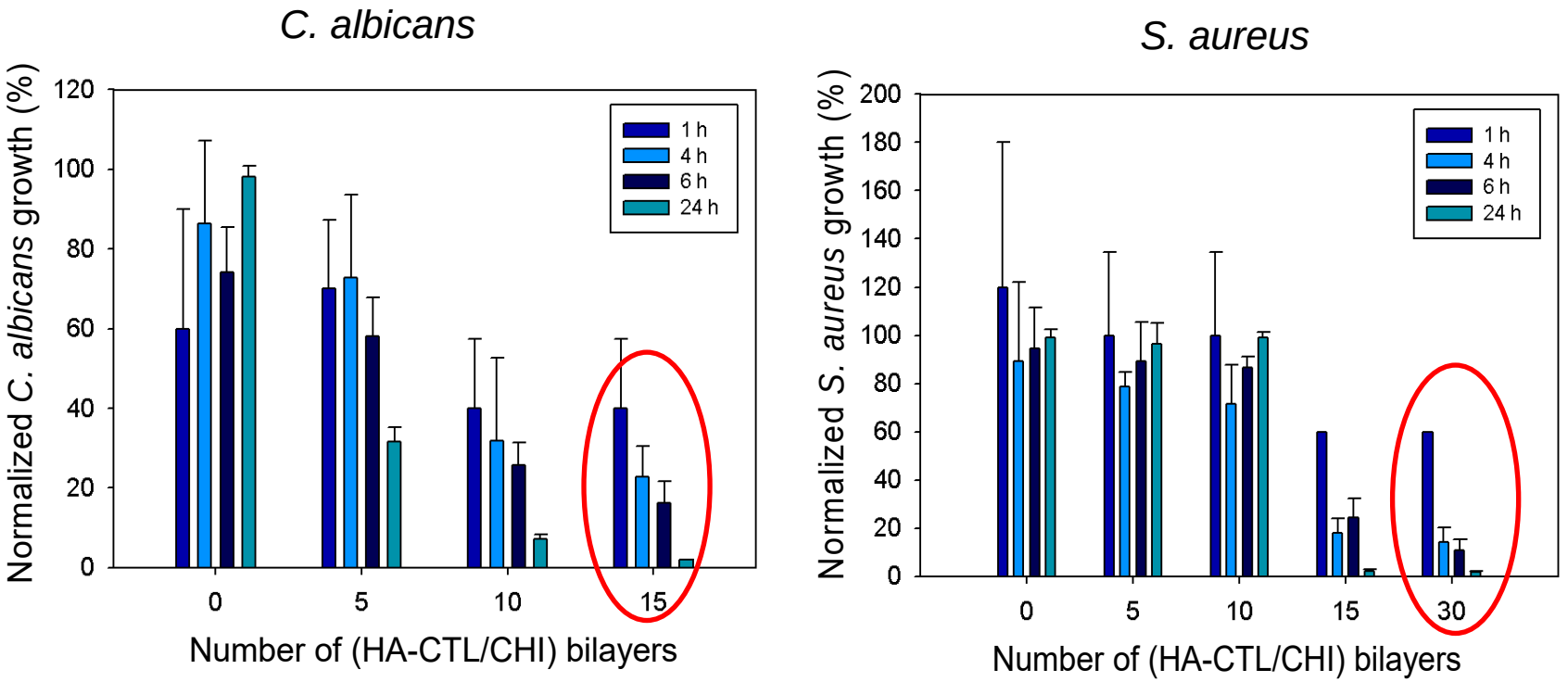
Buildup of CHI/HA-CTL-C films by MP-SPR





Antibacterial and antifungal coating

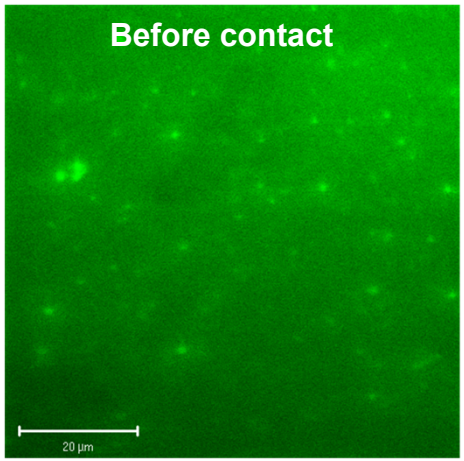
Pathogen tests on $(HA\text{-}CTL\text{-}C/CHI)_n$ multilayer films

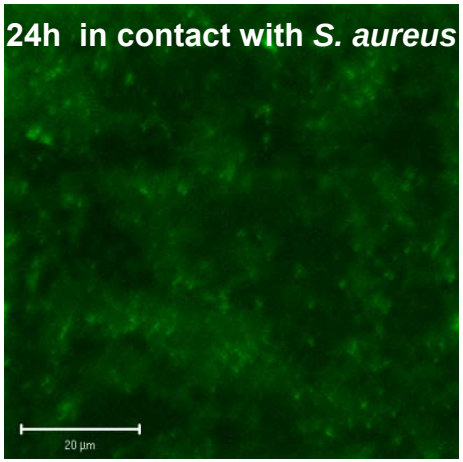


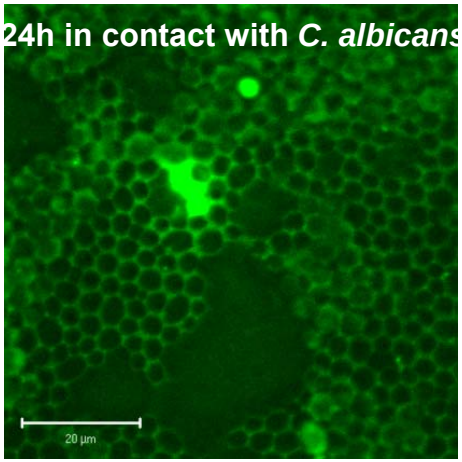
Inhibition after 24h of contact: - *C. albicans*: 15 bilayers
- *S. aureus*: 30 bilayers

Self-defensive polysaccharide films

Confocal microscopy of CHI/HA-FITC films



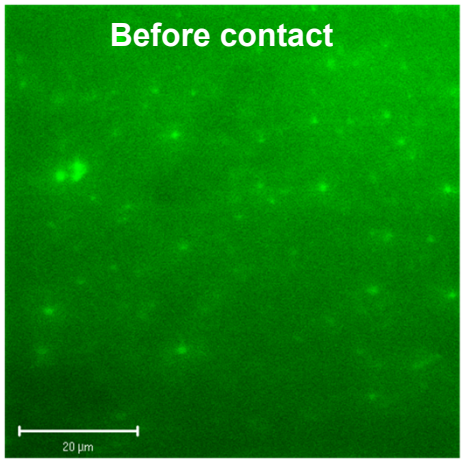


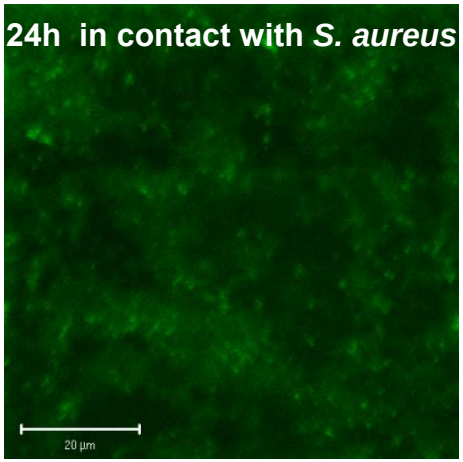


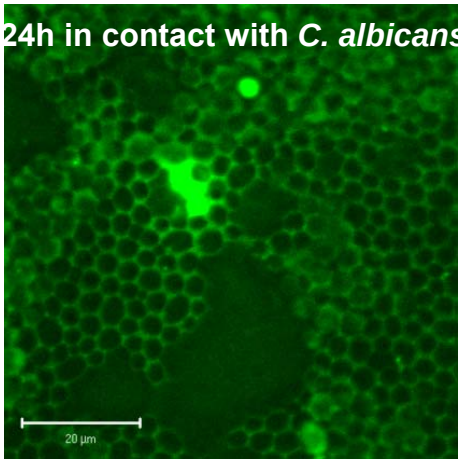
Degradation of the coatings by pathogens

Self-defensive polysaccharide films

Confocal microscopy of CHI/HA-FITC films

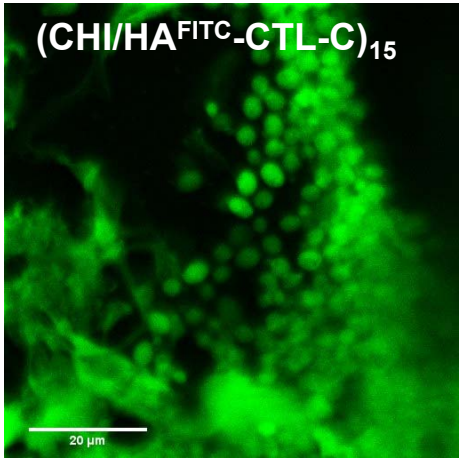


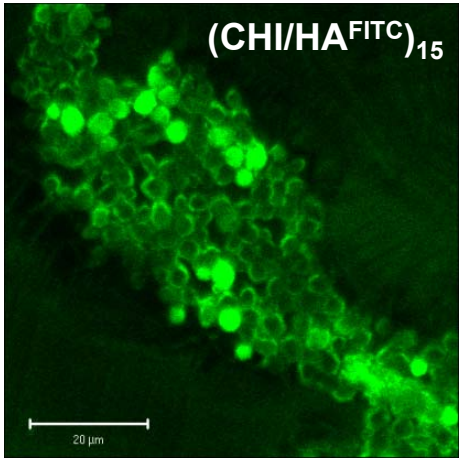




Degradation of the coatings by pathogens

C. Albicans
45 min incubation at 30°C

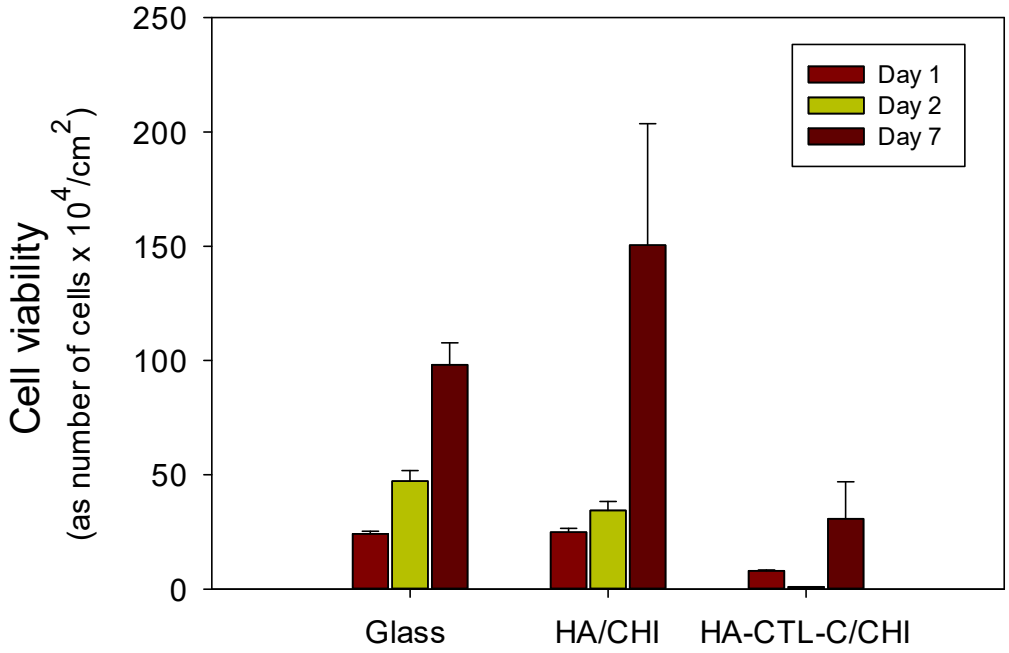




Penetration of HA-CTL-C inside the pathogen

Self-defensive polysaccharide films

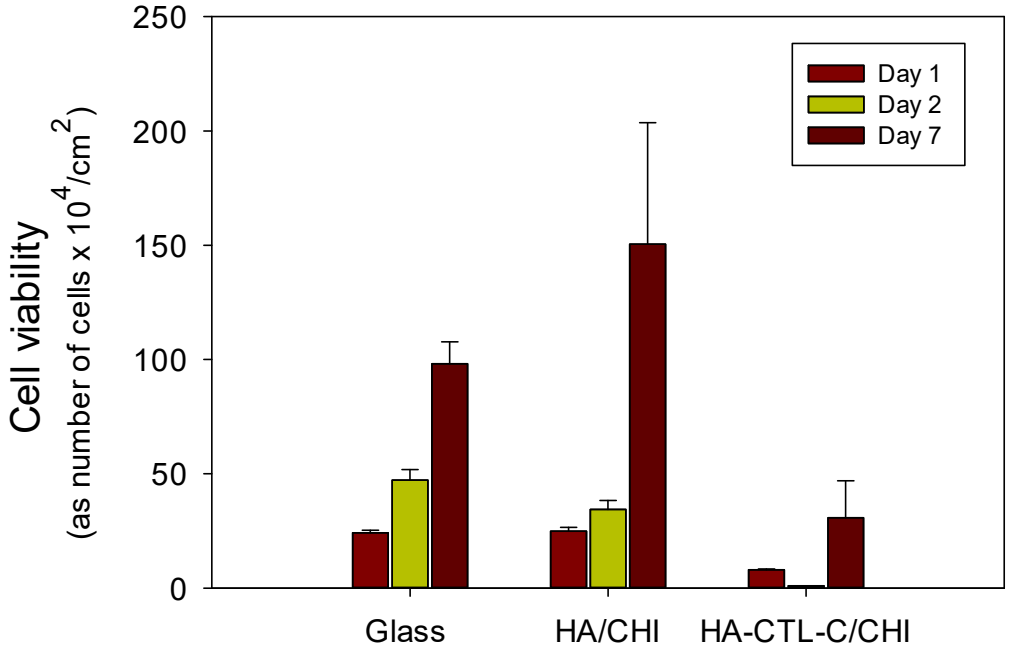
Cytotoxicity tests: Human gingival fibroblasts

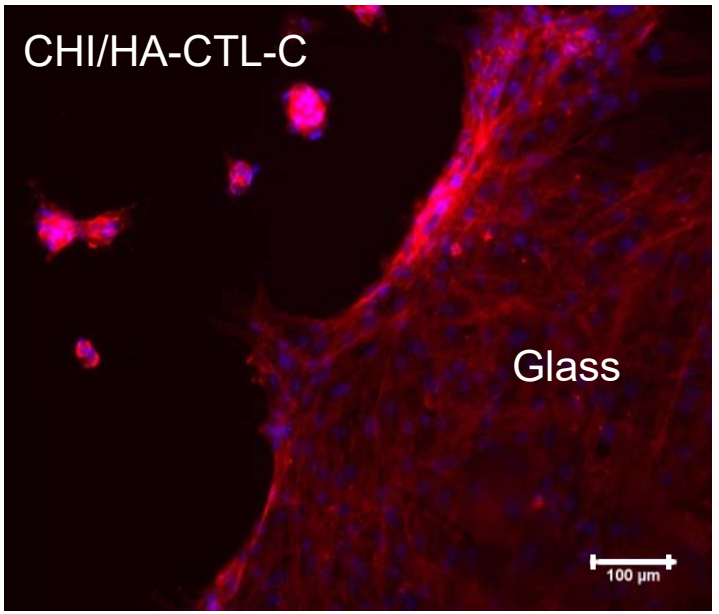


$(HA\text{-}CTL\text{-}C/CHI)_{15}$ no cell adhesion

Self-defensive polysaccharide films

Cytotoxicity tests: Human gingival fibroblasts





Nucleus Cytoskeleton

$(HA\text{-}CTL\text{-}C/CHI)_{15}$ no cell adhesion → non-adherent film

Conclusion

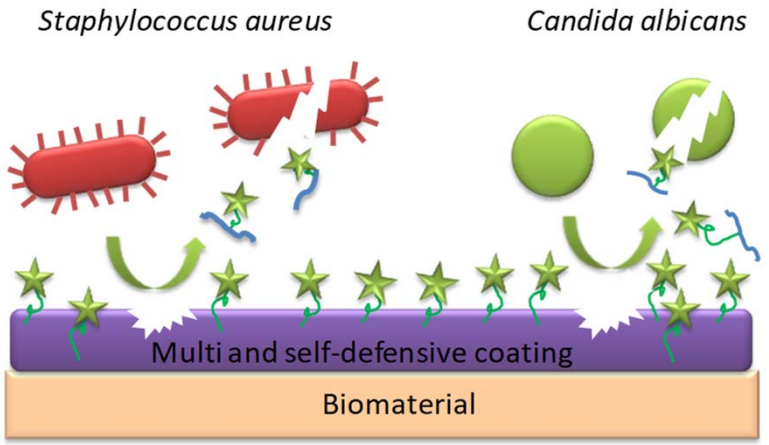
Self-defensive biomaterial coating with embedded antimicrobial peptides

HA/CHI

- biocompatible polymers
- adherent for cells

HA-CTL-C/CHI

- anti-adherent but not cytotoxic
- active against both bacteria and yeasts
- degradable in the presence of pathogens



Application of the films on catheters: temporary implants that are not integrated in the body

Conclusion

Functionalization of surfaces = powerful tool to give targeted properties to a biomaterial

The layer-by-layer method

- simple to implement
- performed in water
- give films with tunable properties depending on the buildup conditions

Highly applicable in biomaterials science