Tailoring surface properties for advanced biomaterials devices and application

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Outline

Motivation

- surface and its role

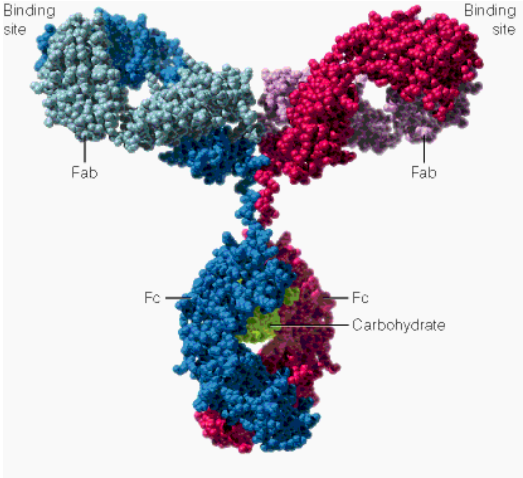
Application

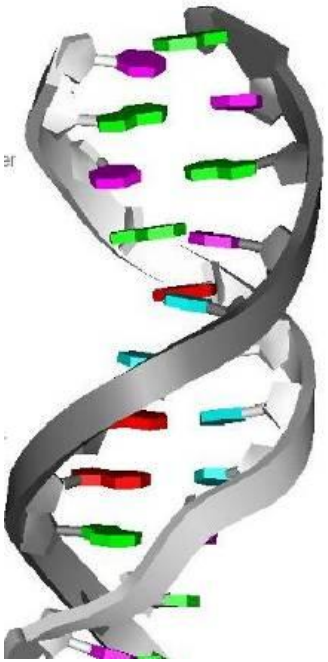
- implantation
- bacteriophage
- drug delivery
- microchip sandwich device
- DNA-on-chip

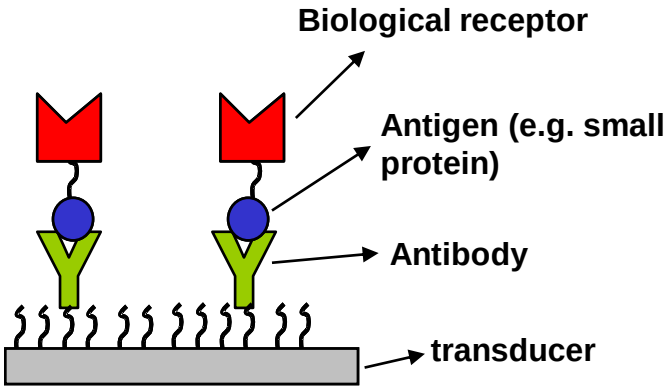
Conclusion

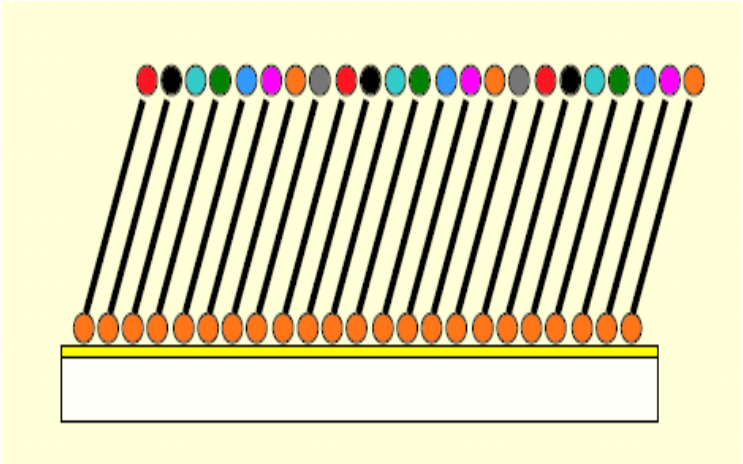
Motivation

- Surface chemical modification and immobilisation processes
- Implantable materials/devices
- Drug delivery systems
- Biochips/biosensors
- Micro-fluidics









Materials utilised for medical devices selected on the basis of mechanical and physical properties

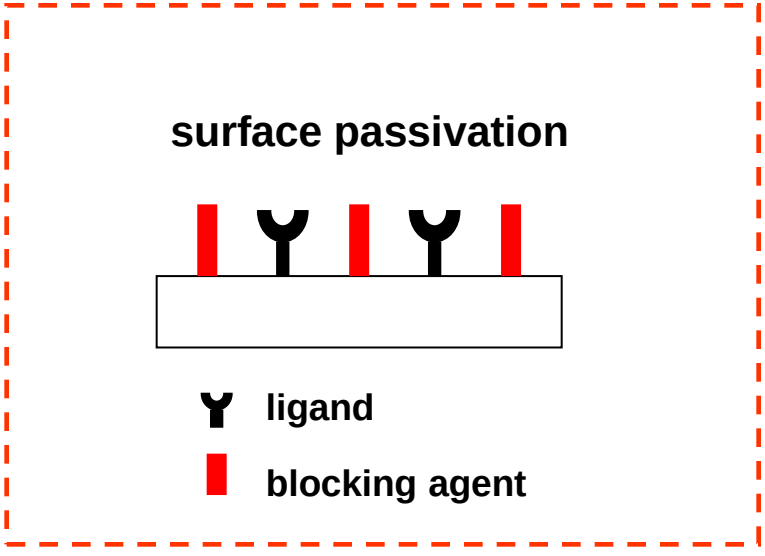
Development of biological functional material stimulating bioactivity and biorecognition

Surface modification processes of materials allow the combination of ideal bulk properties e.g. *tensile strength or stiffness for implants*, *electronic or optical properties for sensors,* with desired surface properties: e.g. *biocompatibility or selectivity to a particular biomolecule*

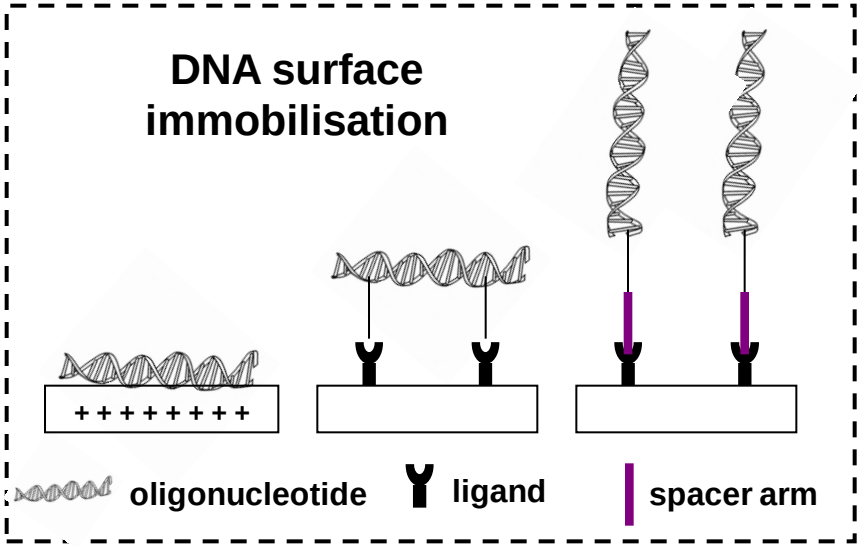
Goal

is to exercise a control over the way in which the body or individual biomolecules respond to the material surface

1) Reduction of the aspecific adhesion



2) Specific immobilisation of biomolecules

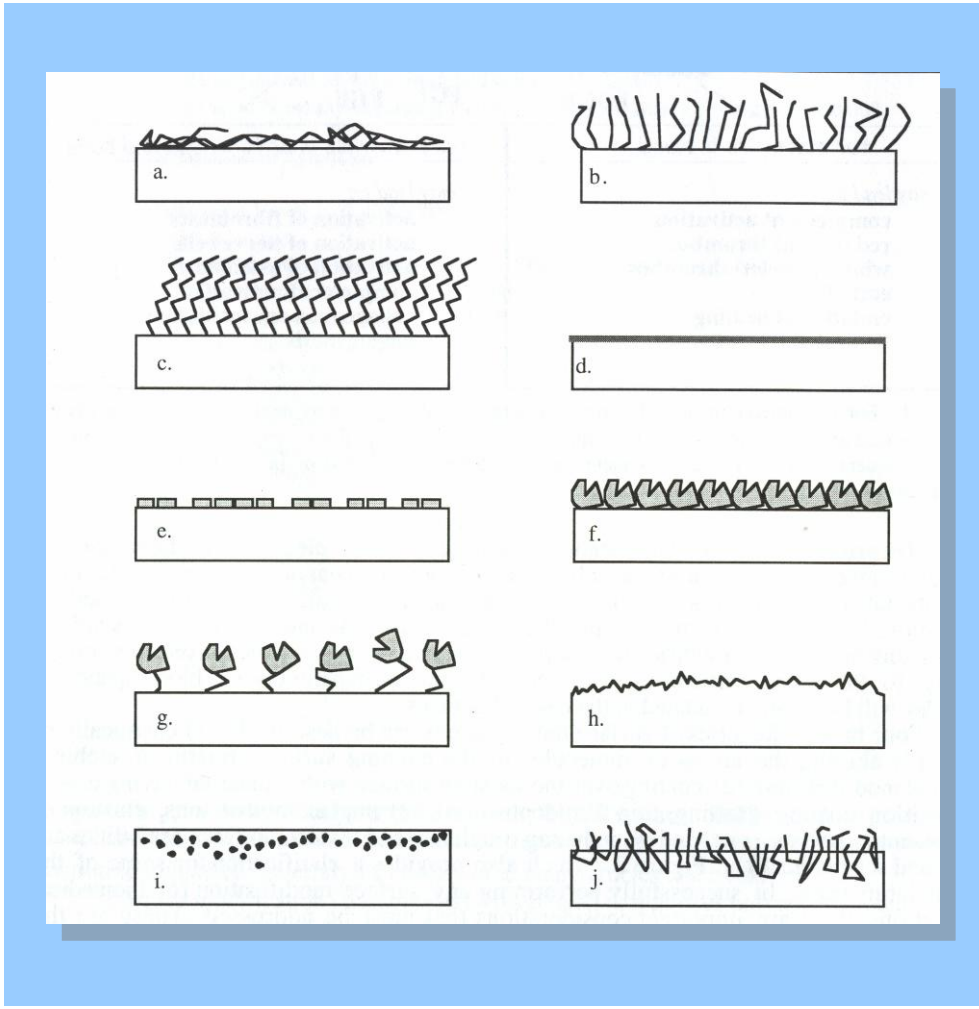


ÅNon covalent surfaces are effective for many applications; however, passive adsorption fails in many cases.
ÅCovalent immobilization is often necessary for binding of molecules that do not adsorb, adsorb very weakly, or adsorb with improper orientation and conformation to noncovalent surfaces.
ÅCovalent immobilization may result in better biomolecule activity, reduced nonspecific adsorption, and greater stability.

Surface modification processes

Surfaces can be modified physico-chemically by:
Åcoating
Åmodifying existing surface

Surfaces can be modified biologically by:
Ågrafting bioactive molecules to surface (i.e. lipids, proteins)



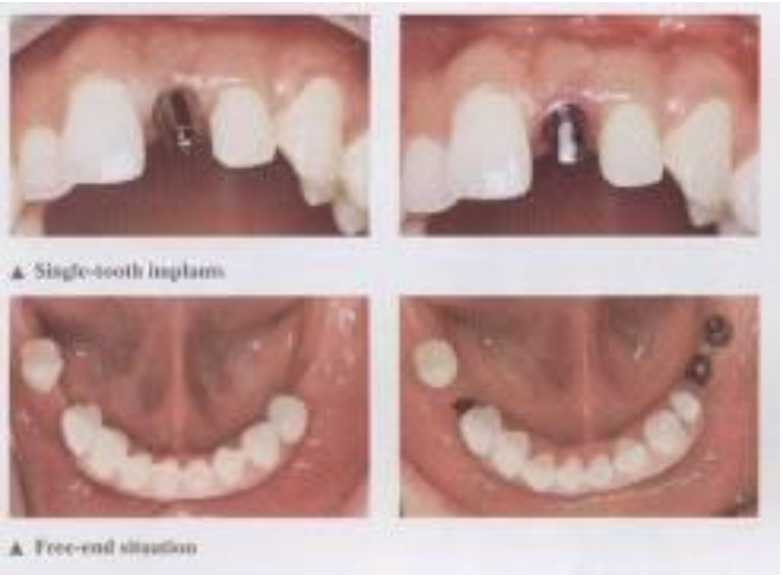
From B.D.Ratner et al., 1997, Surface Modification of Polymeric Biomaterials, Plenum Press

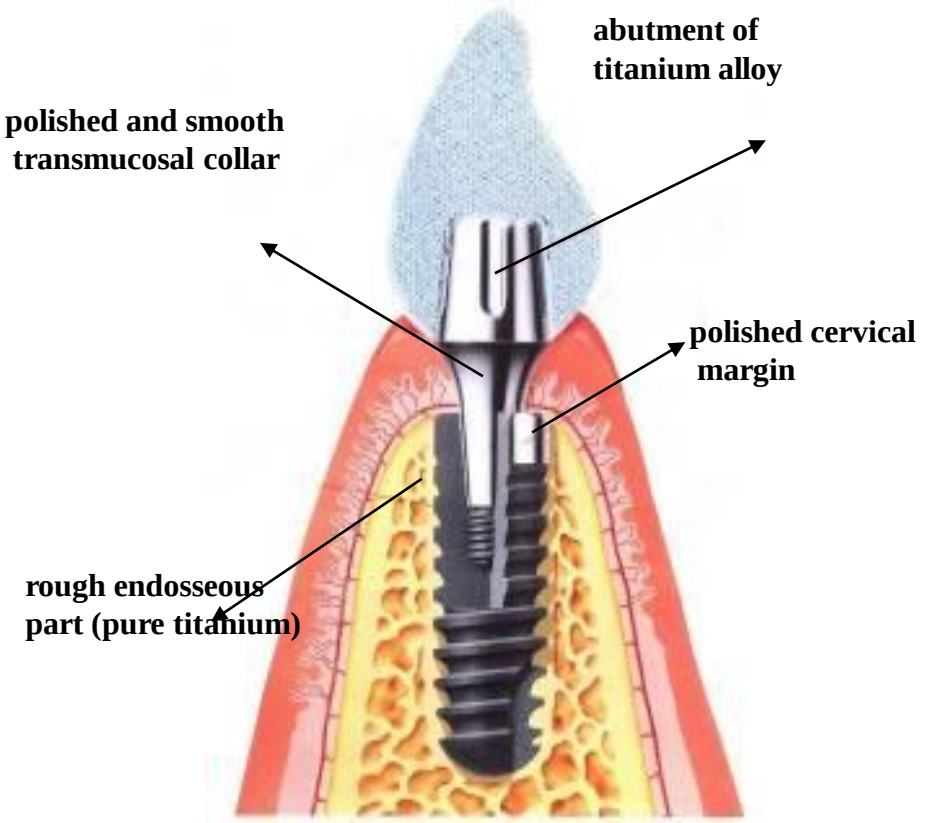
surface functionalisation and surface analysis

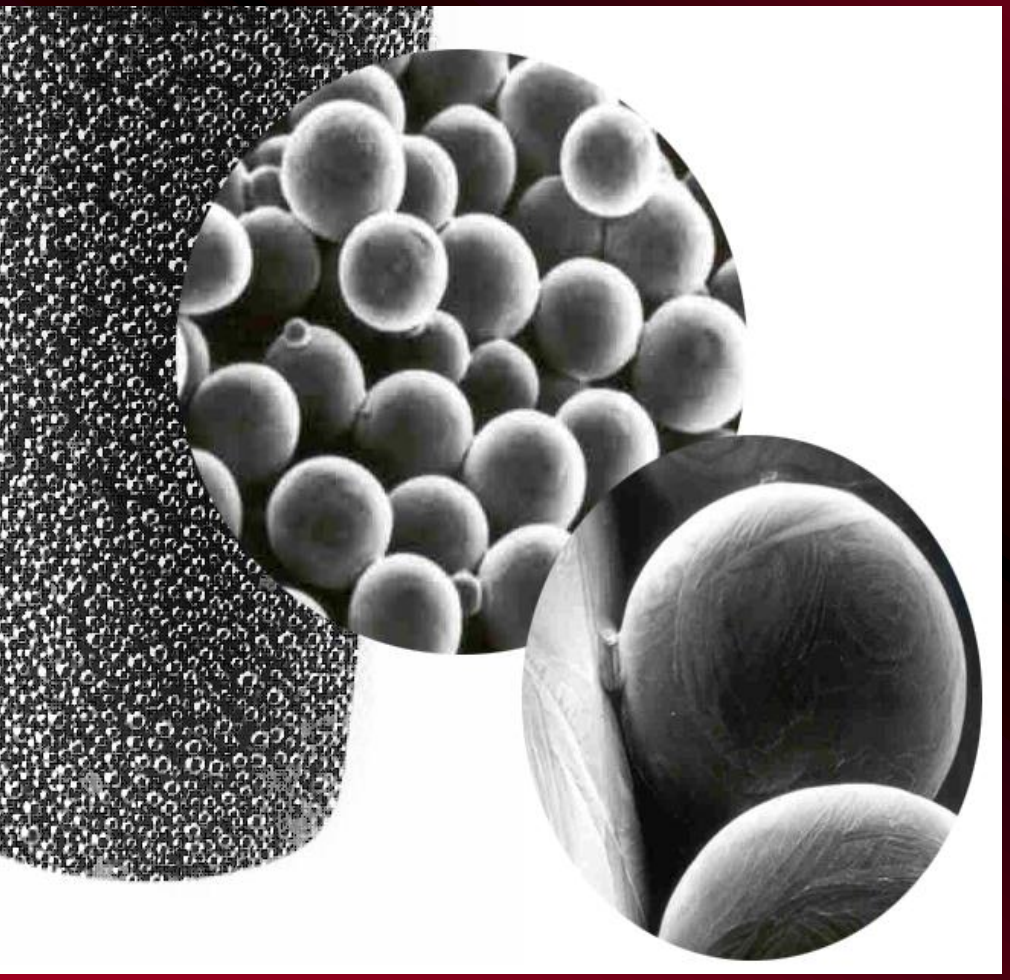
High surface sensitive techniques are needed to study the physics and chemistry of materials surfaces

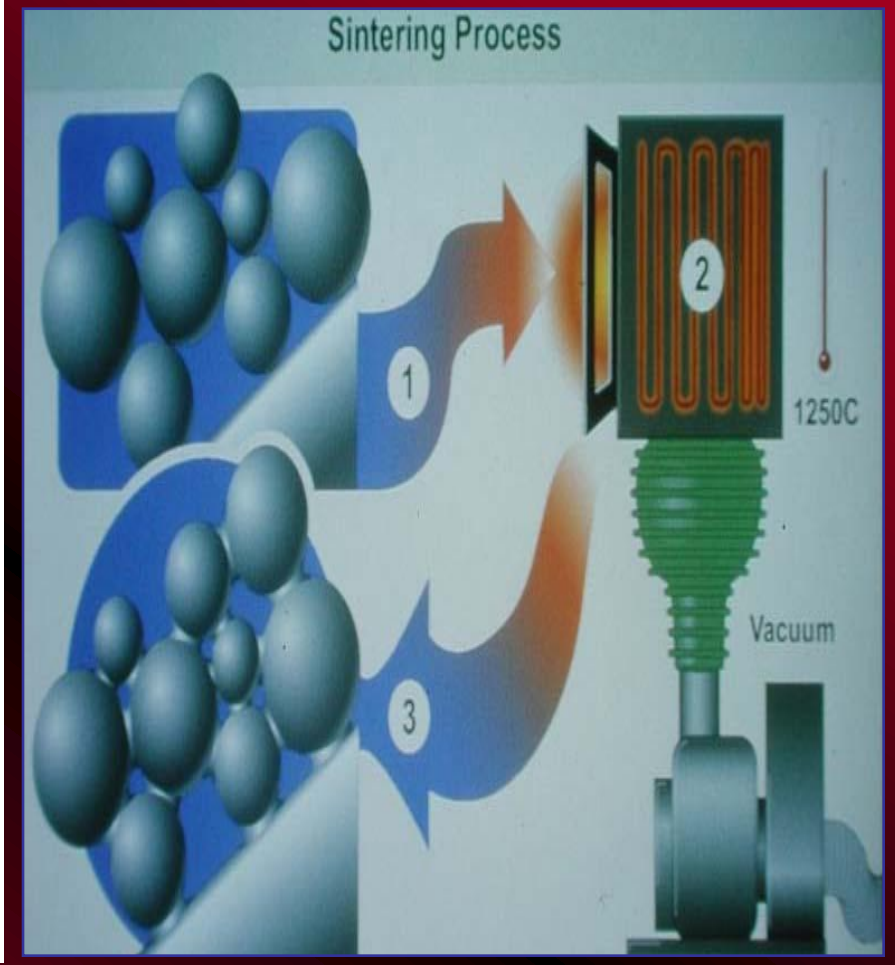
To be measured	Method
Surface Chemistry	XPS, SIMS, FTIR, mR
Texture/Roughness	SEM, AFM, SNOM
Modulus	AFM
Surface Energy	Contact angle

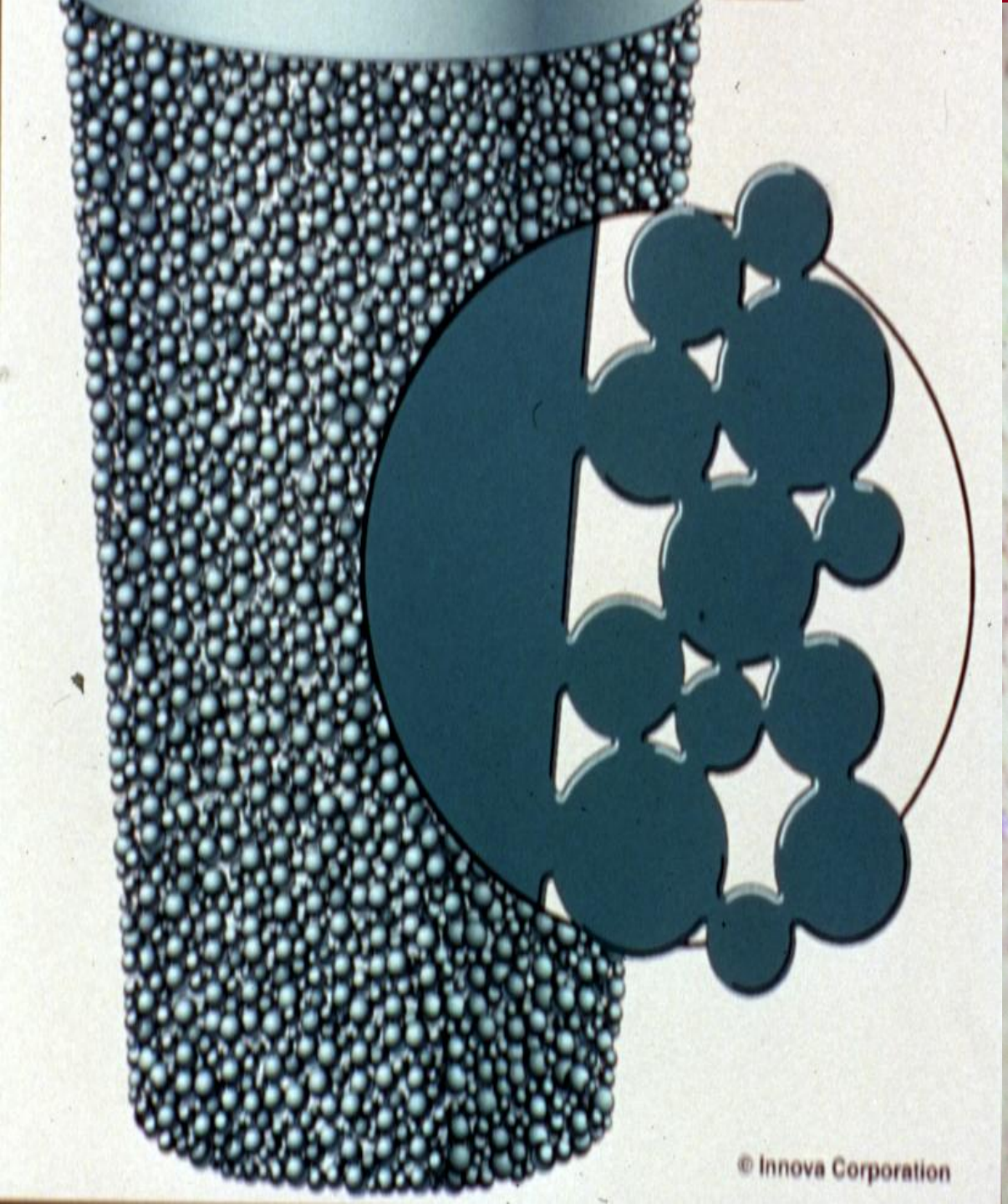
Titanium Dental Implants

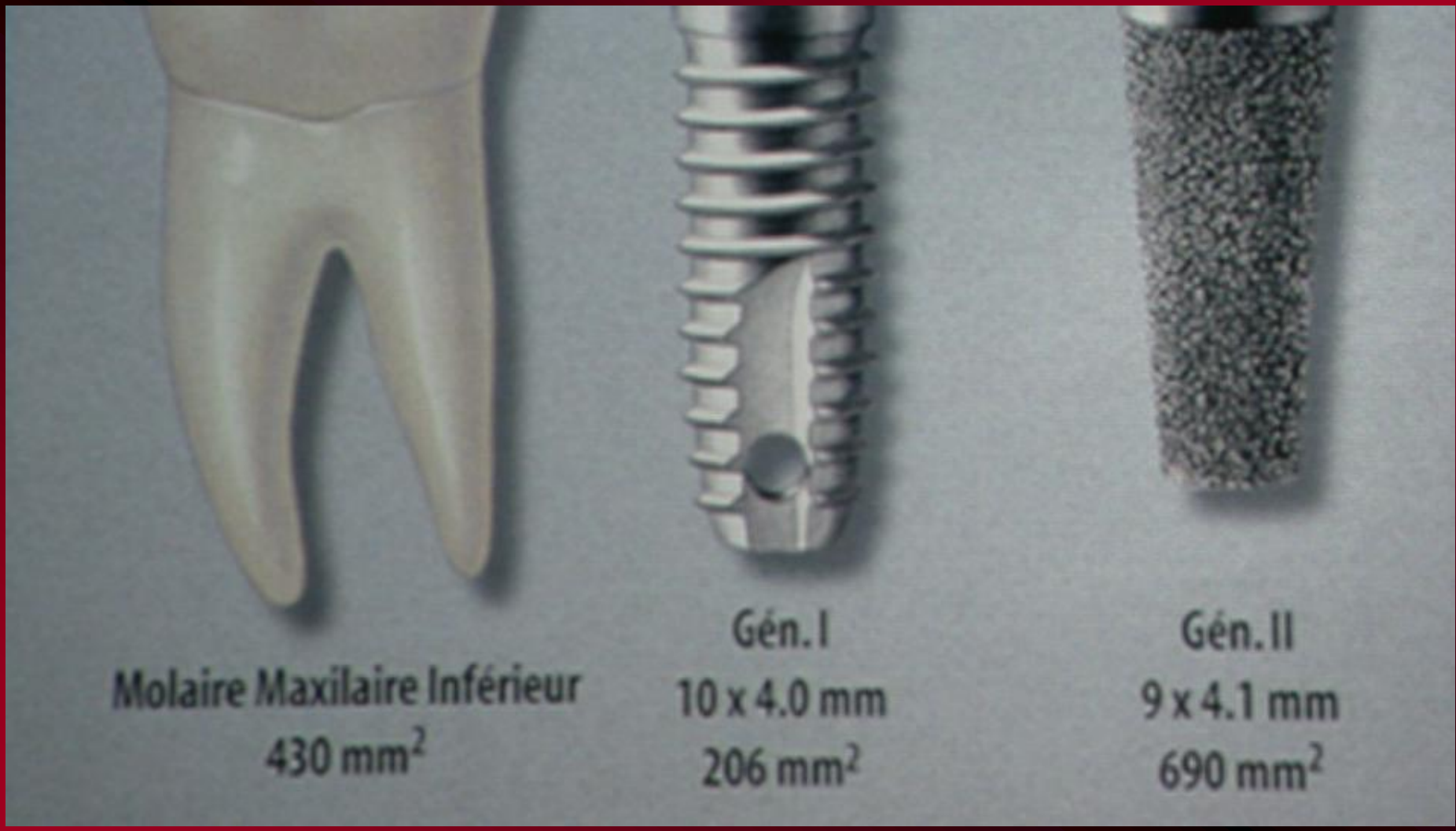




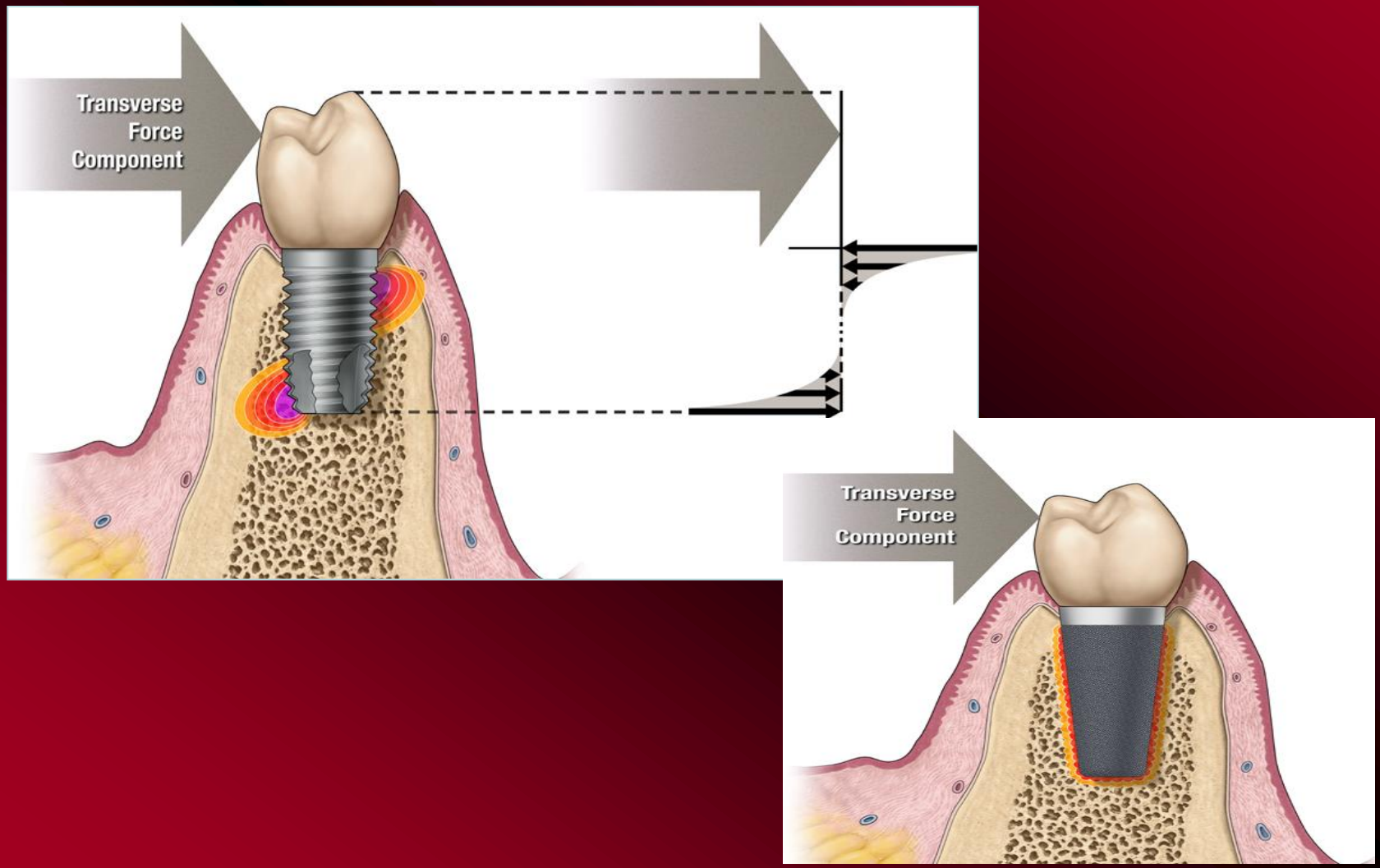



Sintering Process
1
2
3
1250C
Vacuum

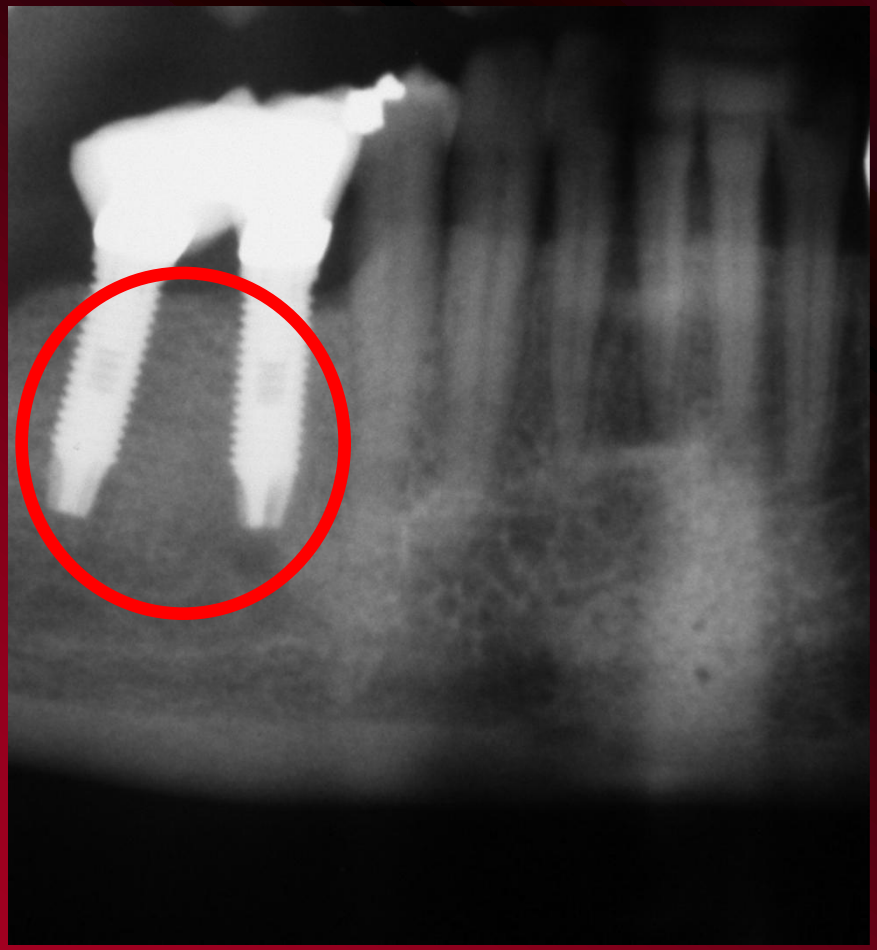

© Innova Corporation

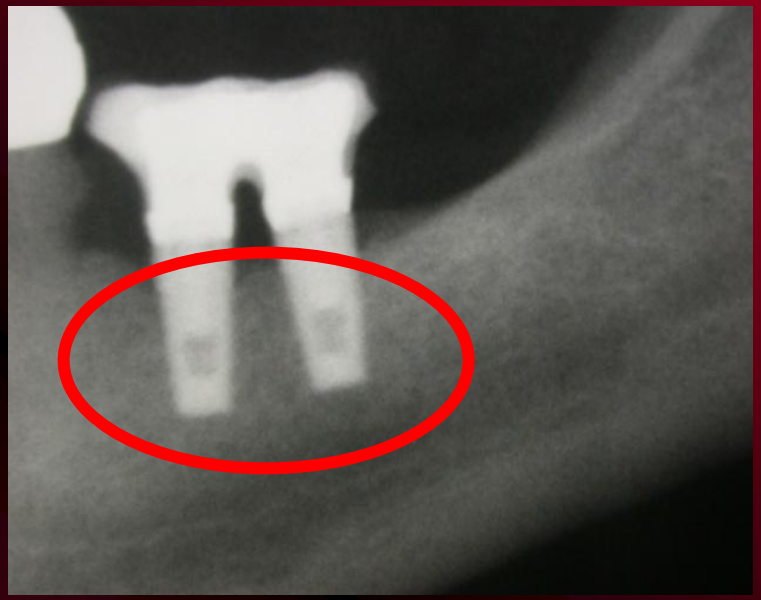

Molaire Maxilaire Inférieur
430 mm²
Gén. I
10 x 4.0 mm
206 mm²
Gén. II
9 x 4.1 mm
690 mm²

Resistance to transverse force component



Shorter to avoide neurological problems

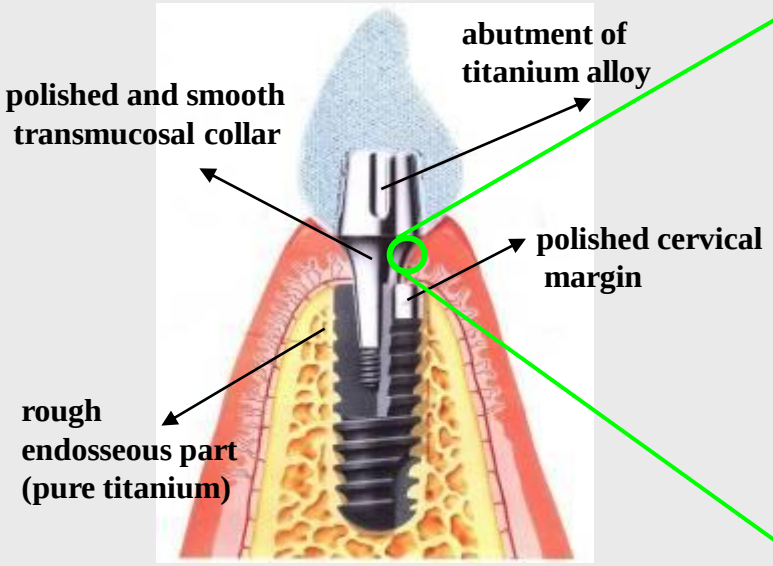


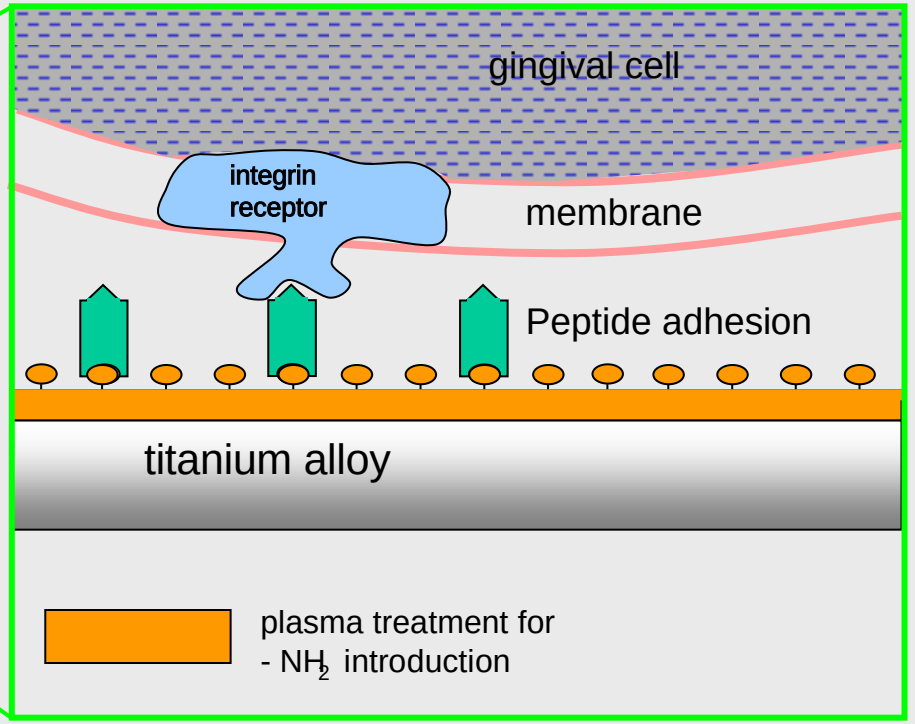


Deporter, D.A., Todescan, R. et al.

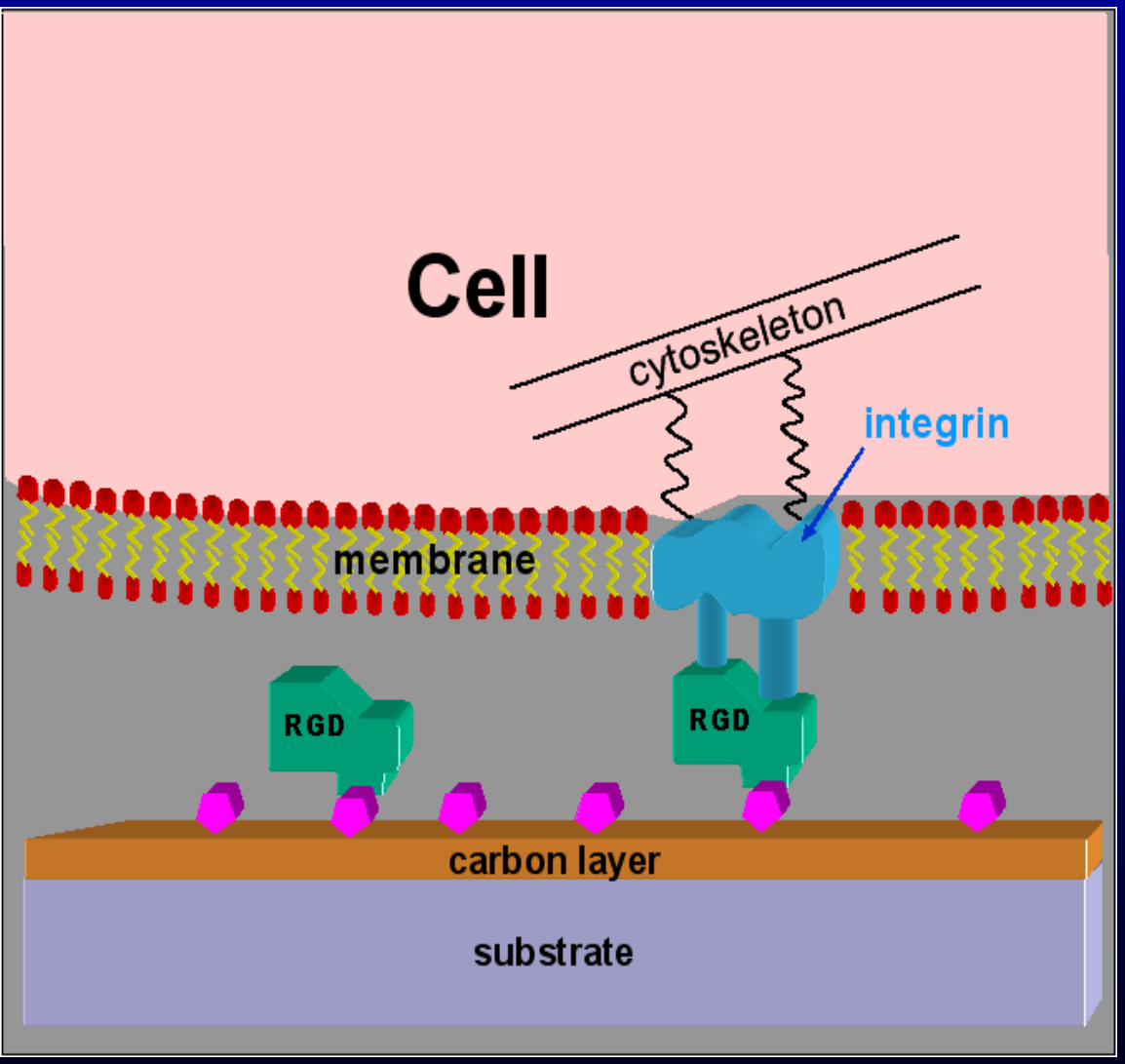
Length (mm)	# Used	# Failure	% Failure
7	44	1	2.3
9	89	4	4,5
12	16	2	12,5

Overall 5 year failure rate = 4,6%


abutment of
titanium alloy
polished and smooth
transmucosal collar
polished cervical
margin
rough
endosseous part
(pure titanium)


gingival cell
integrin
receptor
membrane
Peptide adhesion
titanium alloy
plasma treatment for
- NH_2 introduction

A Biological Functionalization to Stimulate the Soft Tissue Adhesion



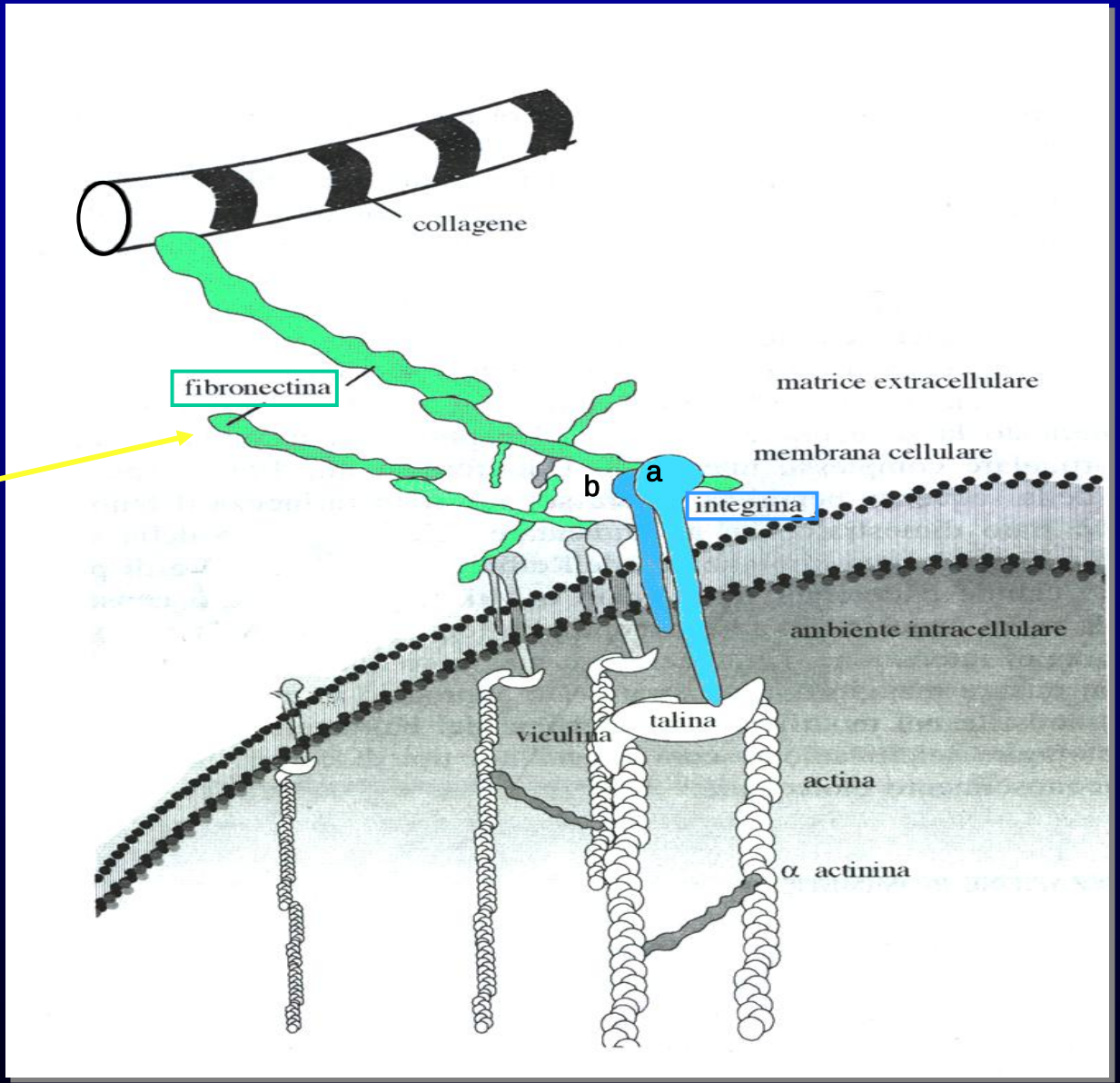
- Titanium alloy surface coating using a plasma assisted chemical vapor deposition process (PACVD) to reduce ion release from titanium and provide an amine-containing layer with adequate stability;
- PEG molecules immobilization creating a protein-resistant (non-fouling) surface;
- Cell adhesive, RGD-containing peptides immobilization stimulating the formation of the biological seal between the soft tissue and the implant.

ECM proteins and integrin receptors

Fibronectin

GLY-ARG-GLY-ASP-
-SER-TYR-CYS

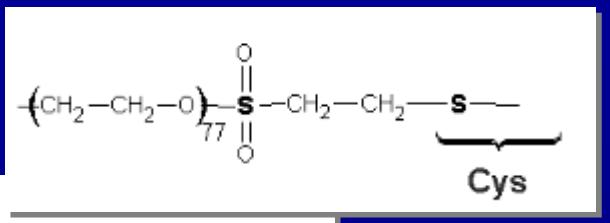
RGD
Adhesion
Peptide

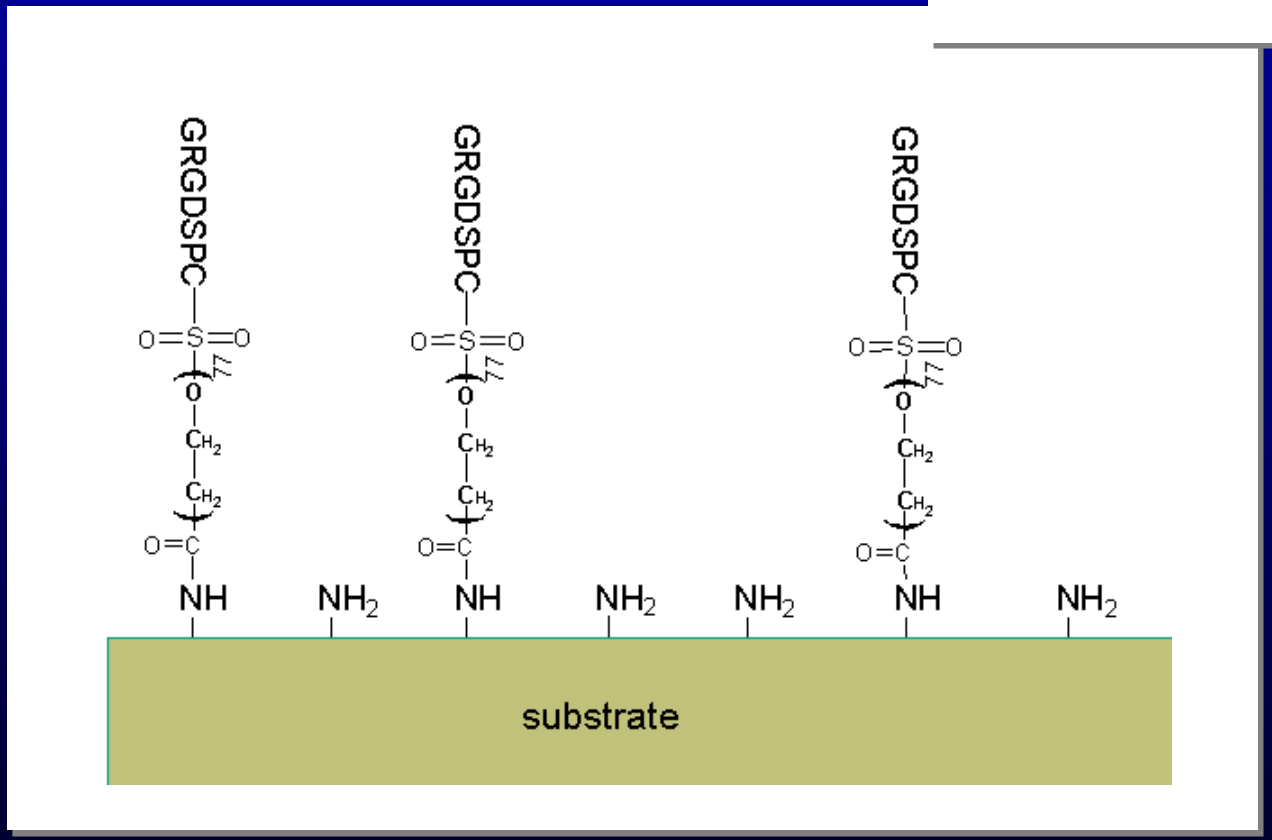


Titanium alloy functionalization: Overview

Step 1: amide bond through the N-hydroxysuccinimide ester *(NHS)*

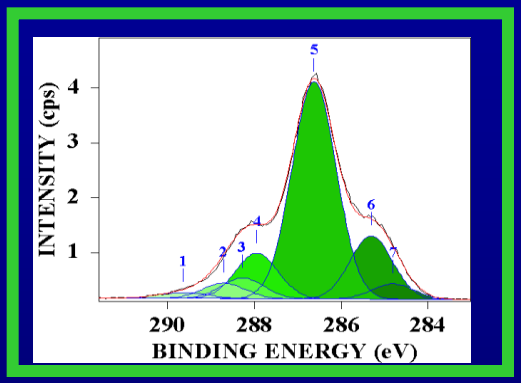
Step 2: thiol chemistry *(Vinylsulfone)*

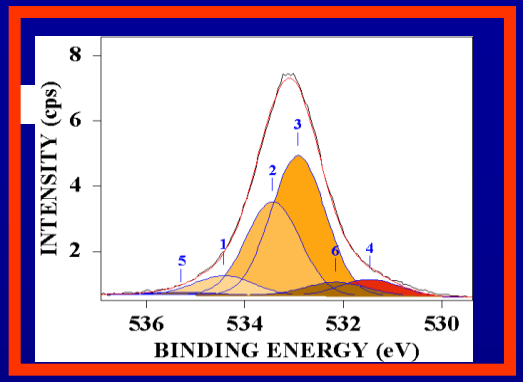


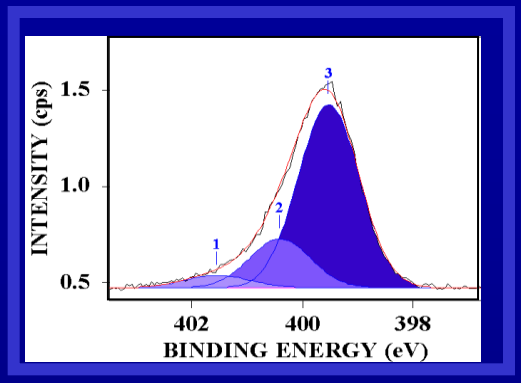


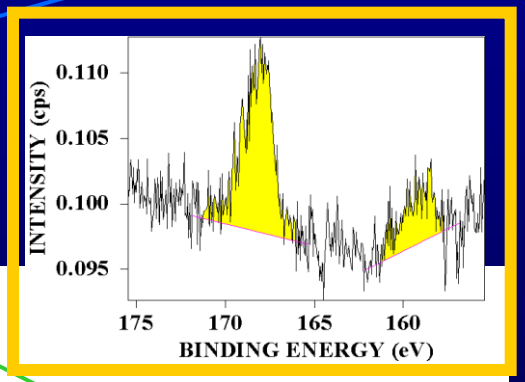
Fluorescent derivative PEG: $5.5 \cdot 10^{13}$ molecules/cm^2

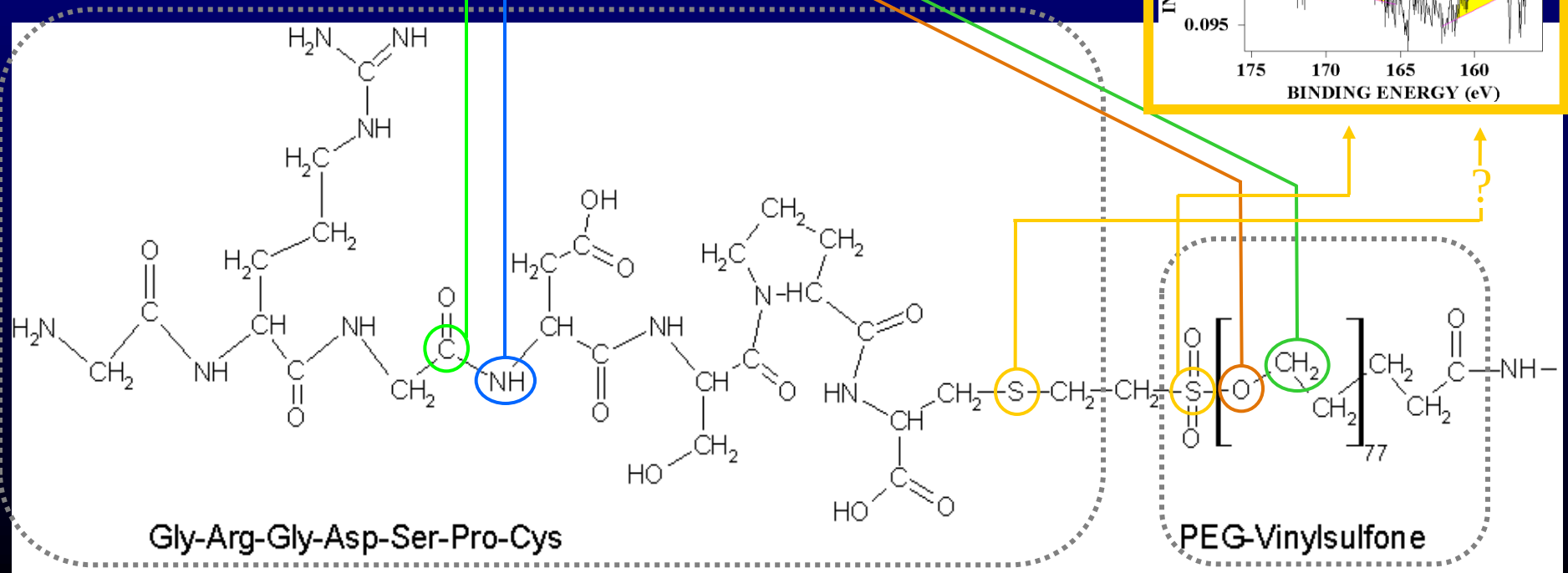
Titanium alloy functionalization: XPS analysis





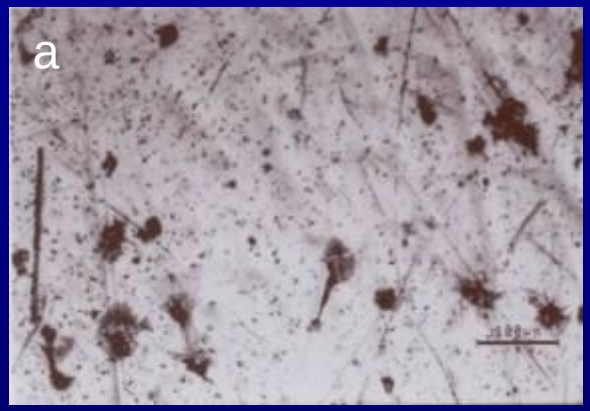


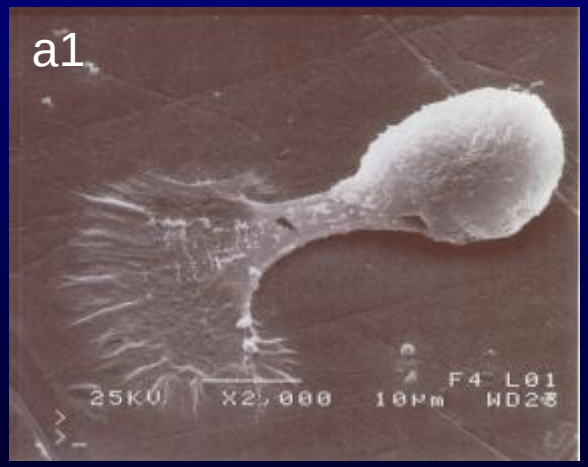




Titanium alloy functionalization: Human gingival cells (HGF-1) adhesion

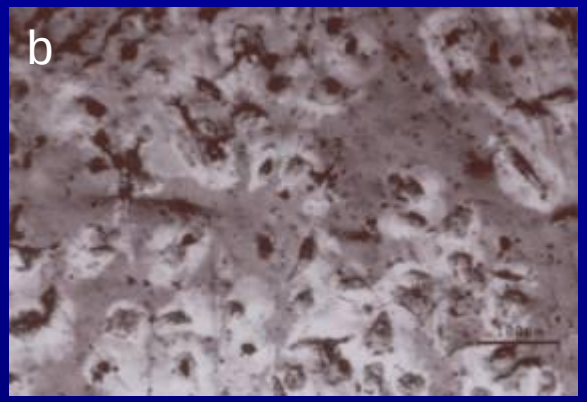
titanium alloy

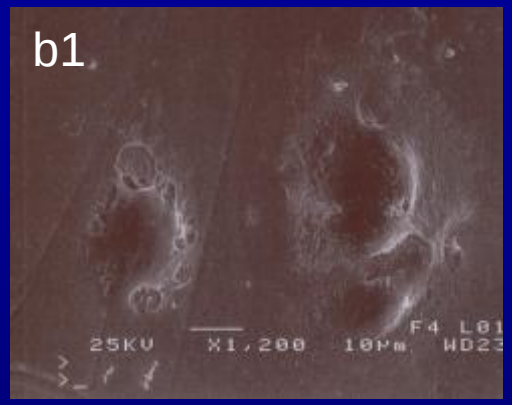


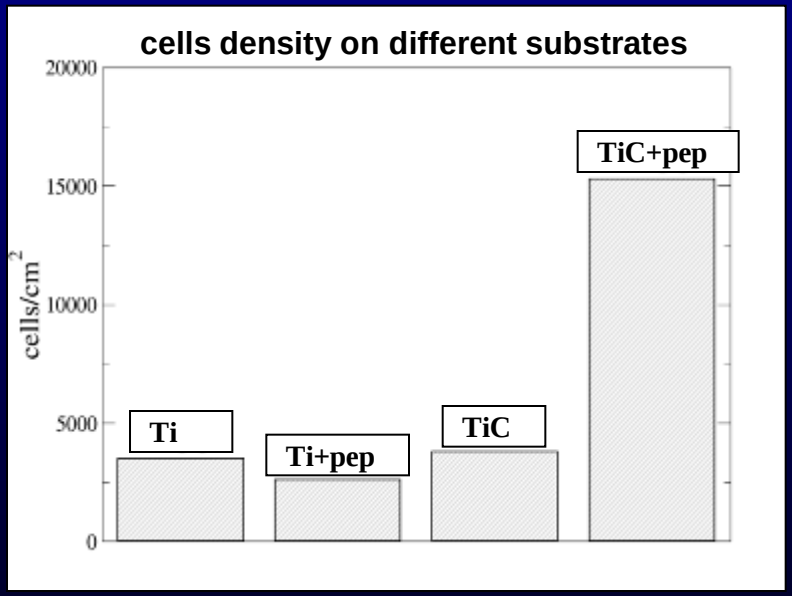


Cell images obtained with a laser scan microscope (a and b) and with a scanning electron microscope (a1 and b1)

RGD modified titanium alloy



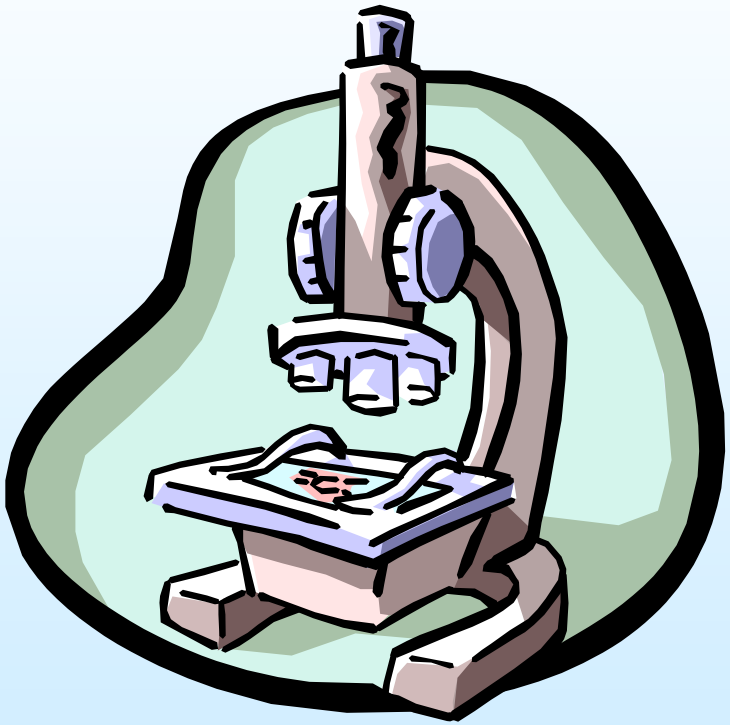




17.500 cell/cm^2
Incubation 24 h in serum free medium plus cycloheximide (25 ug/ml)

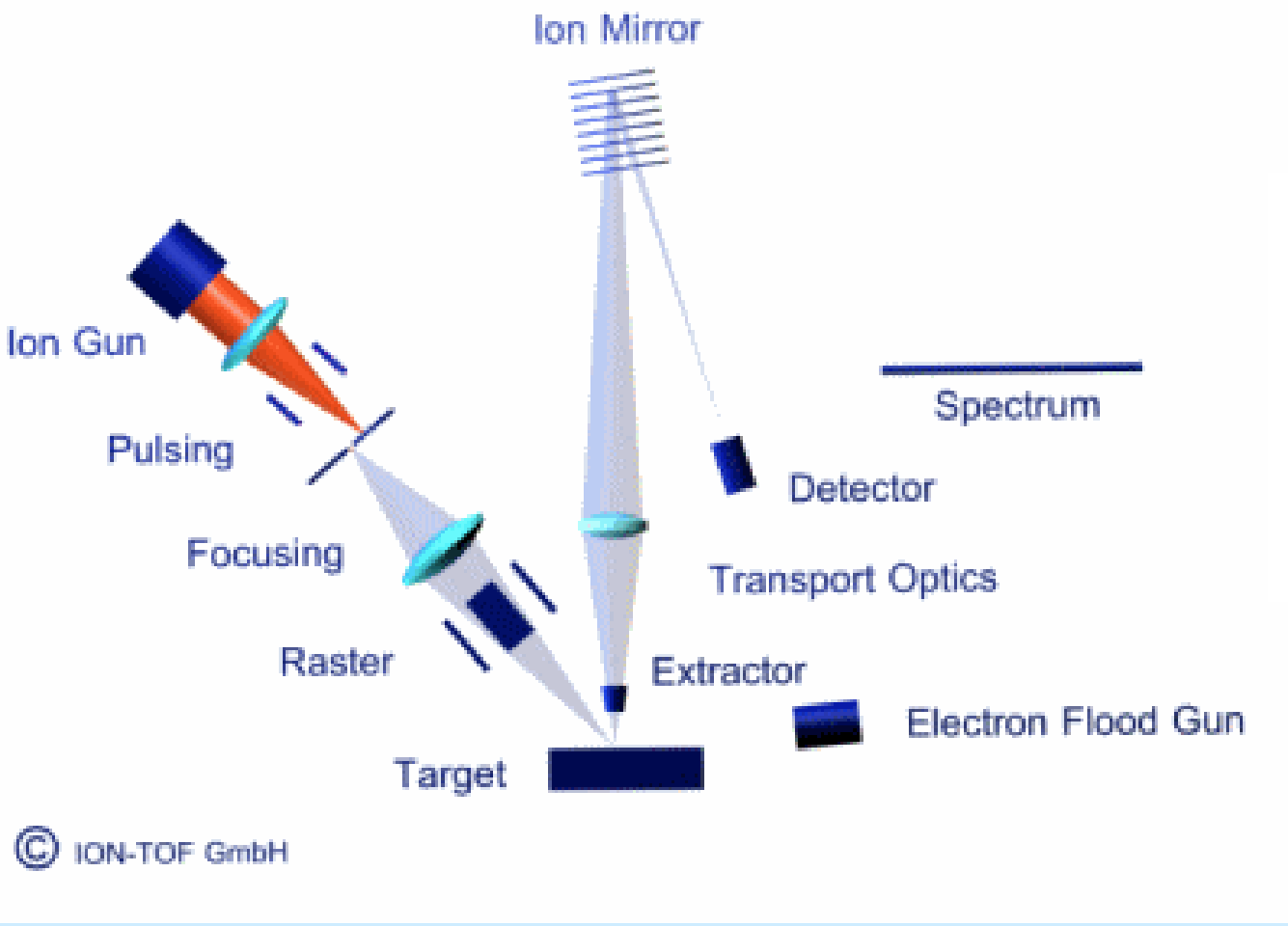
Biomaterials

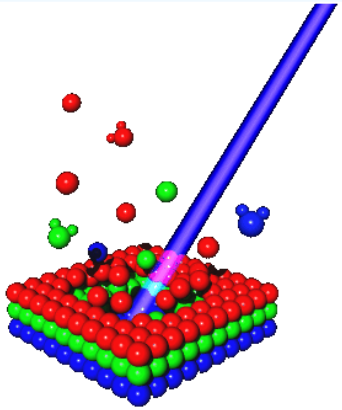
T E S T O N ò C L I N I C A L G R
POLYMERS



- Uncontrolled presence of contaminants and additives
- Surface roughness

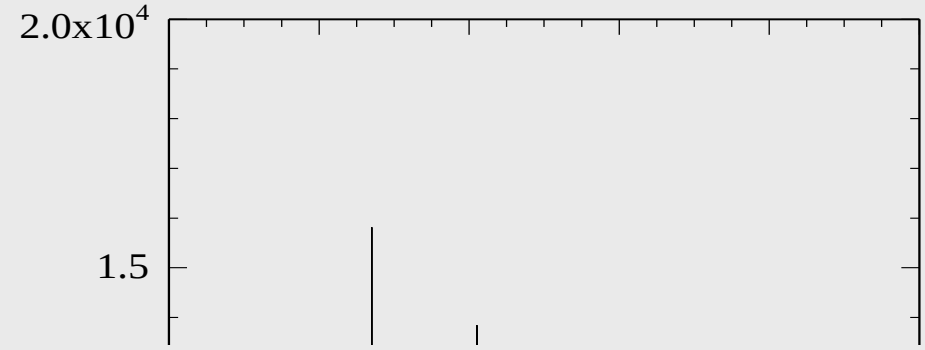
TOF-SIMS: Static Sims

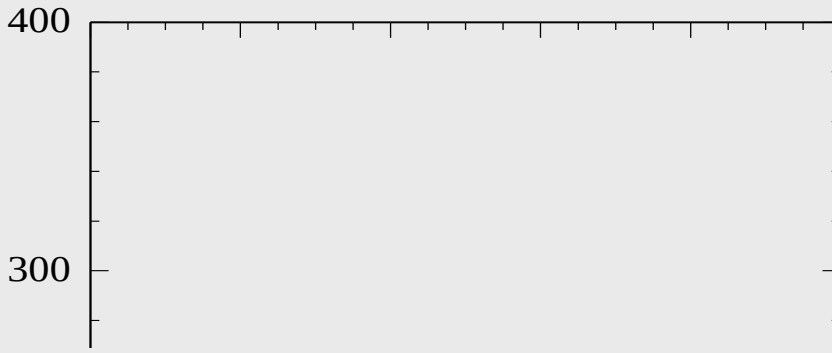




Sampling depth: 5-15 A

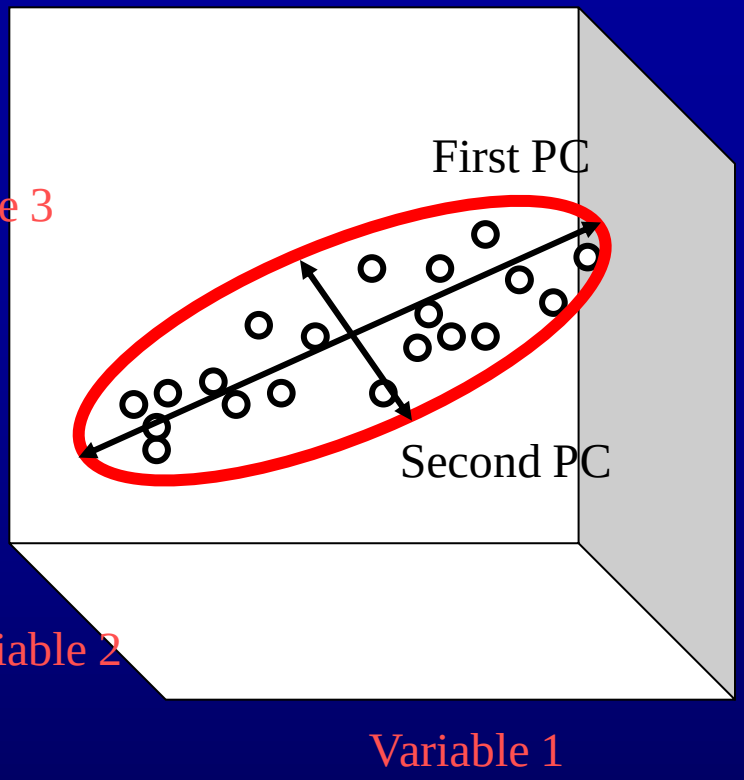
PMMA: Tof Positive Ion Spectra

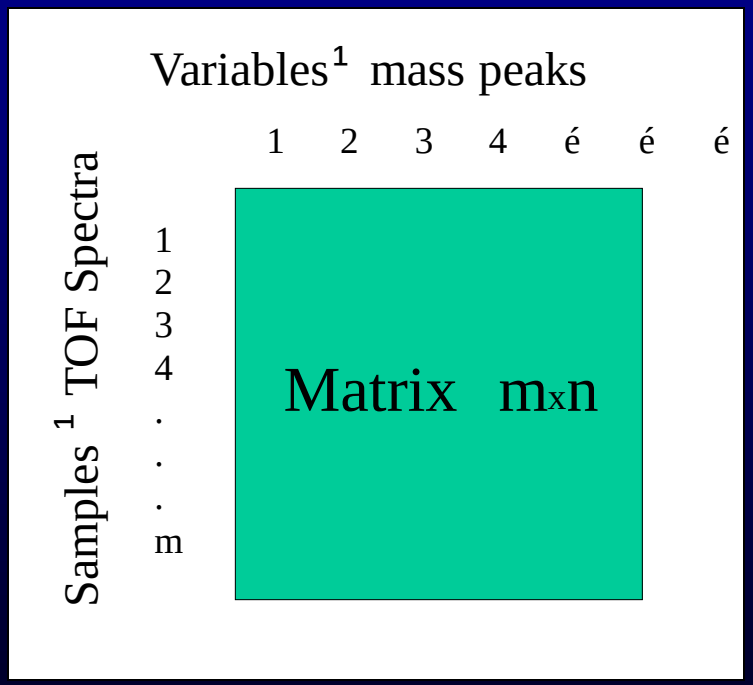




PCA

(Principal Components Analysis)





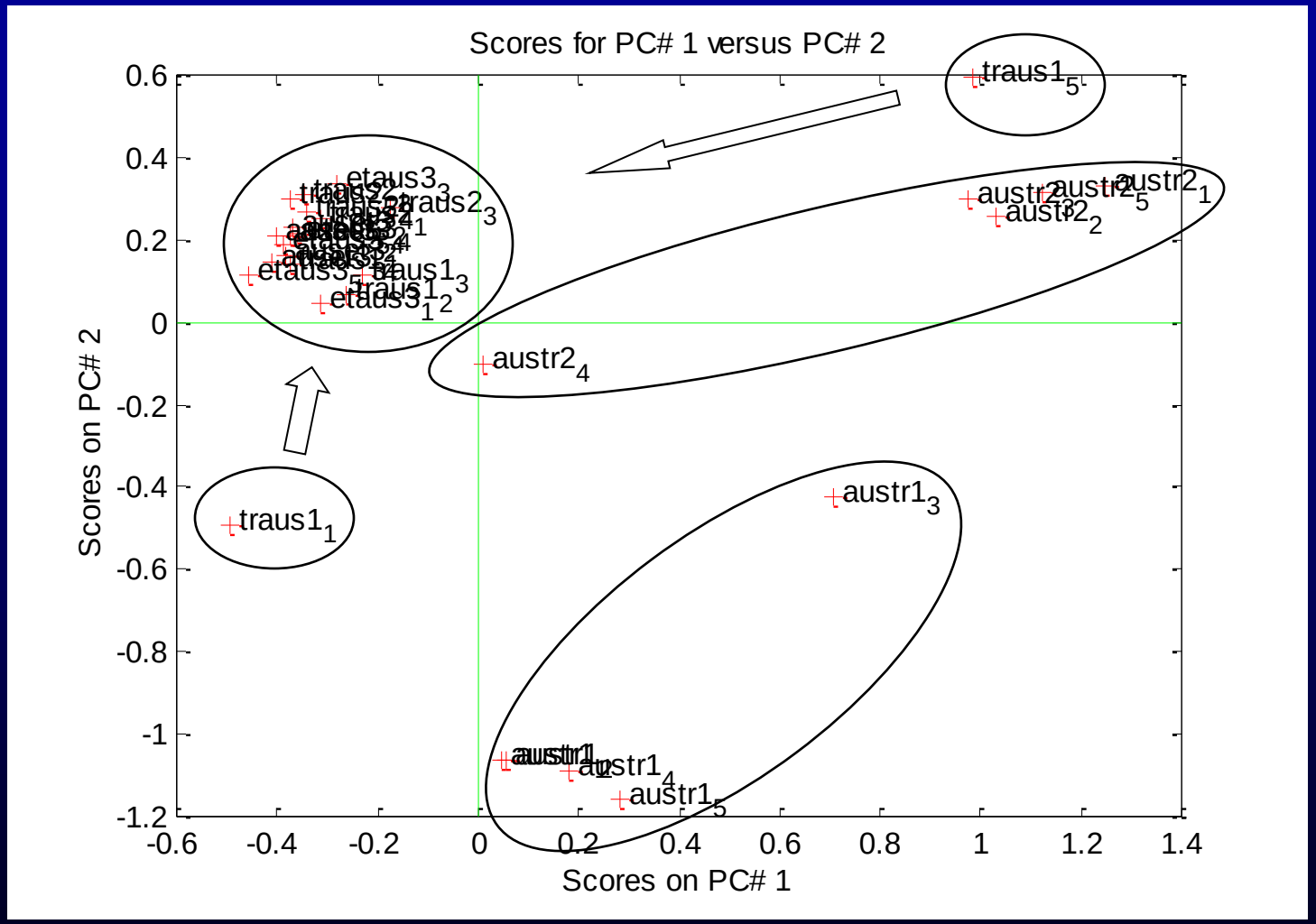
$$X = t_1 p_1^T + t_2 p_2^T + \ldots + t_k p_k^T + E$$

$k<\min(m,n)$

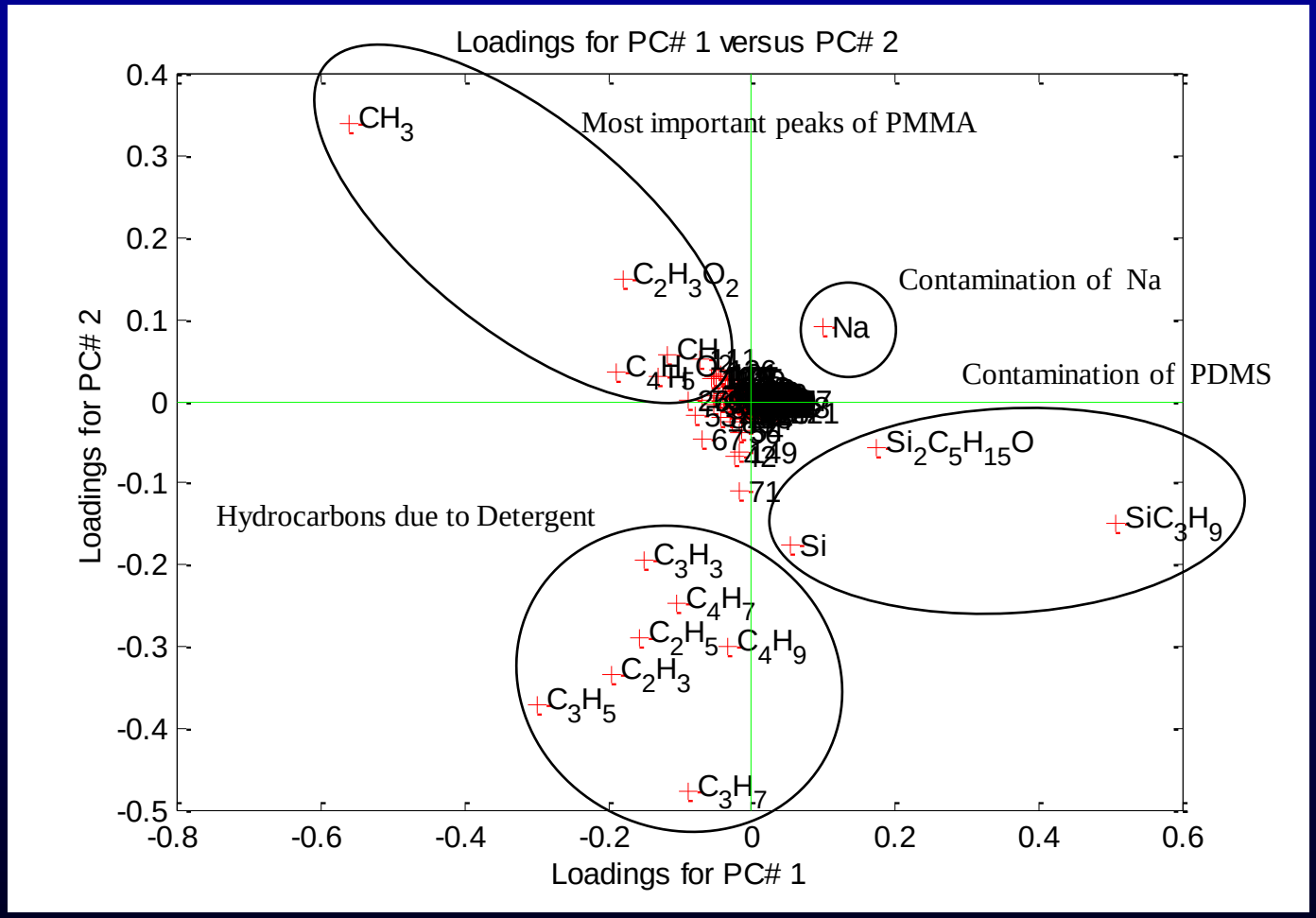
t_i - *scores* (sample info)

p_i - *loadings* (variable info)

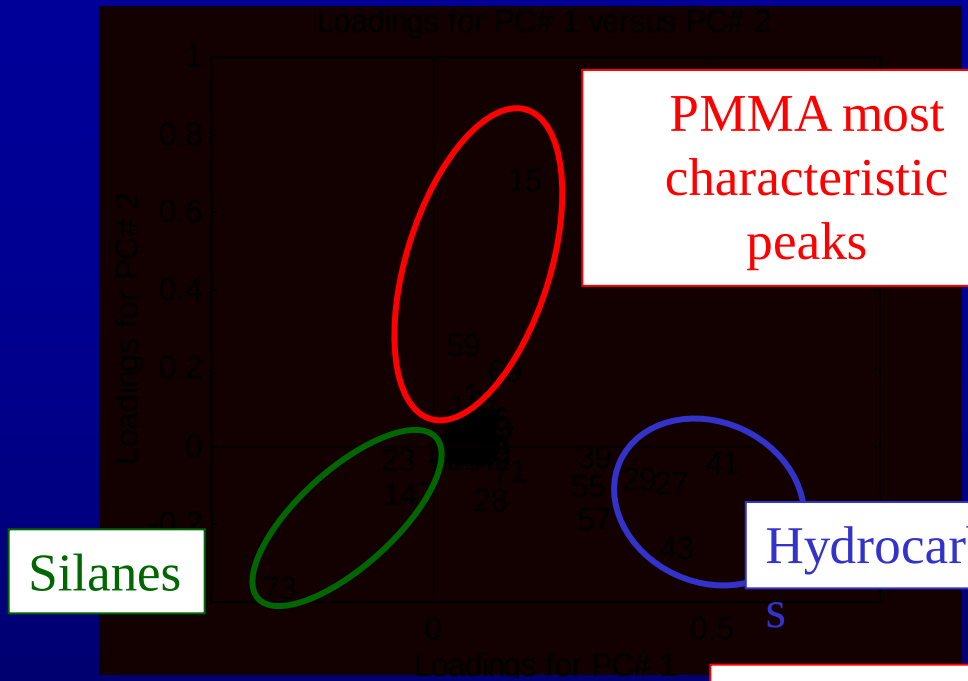
PMMA Positive spectra analysis

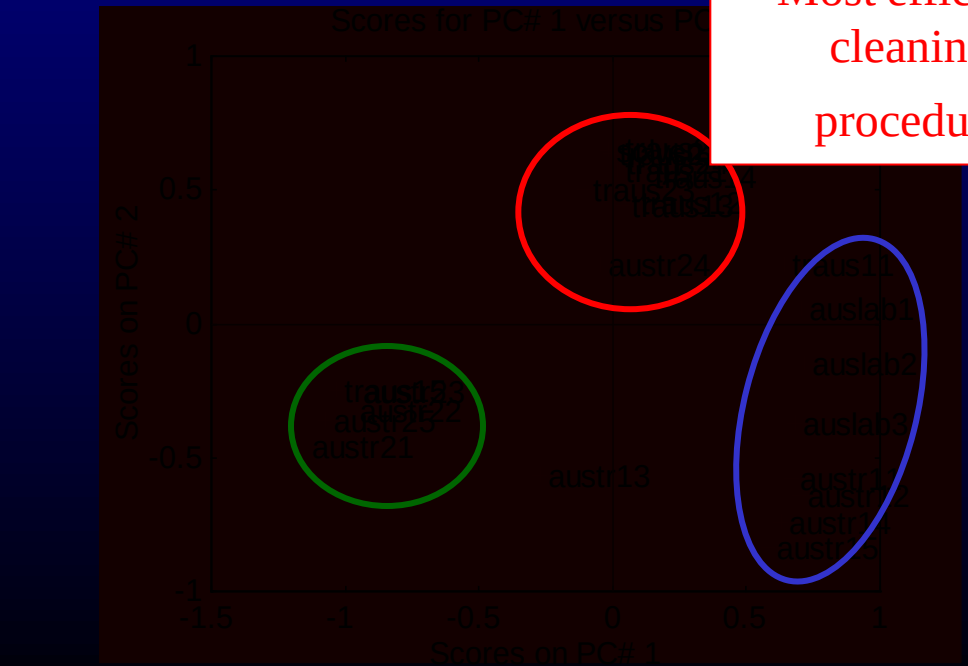


PMMA Positive spectra analysis



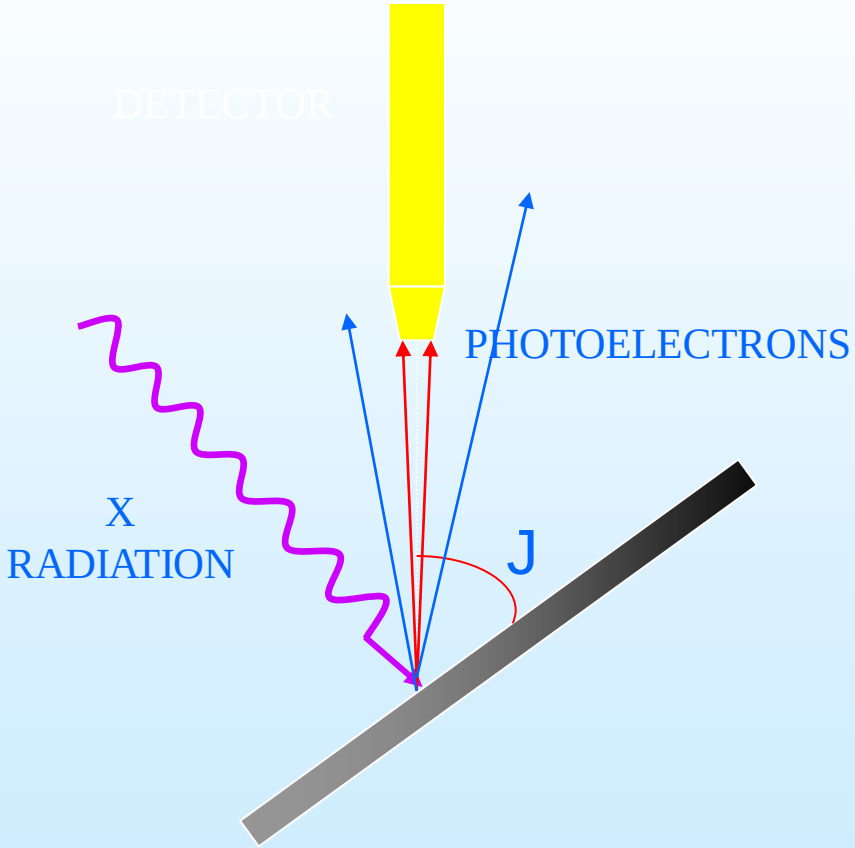
PCA application to PMMA samples treated with different cleaning processes





XPS (X-ray Photoelectron Spectroscopy)

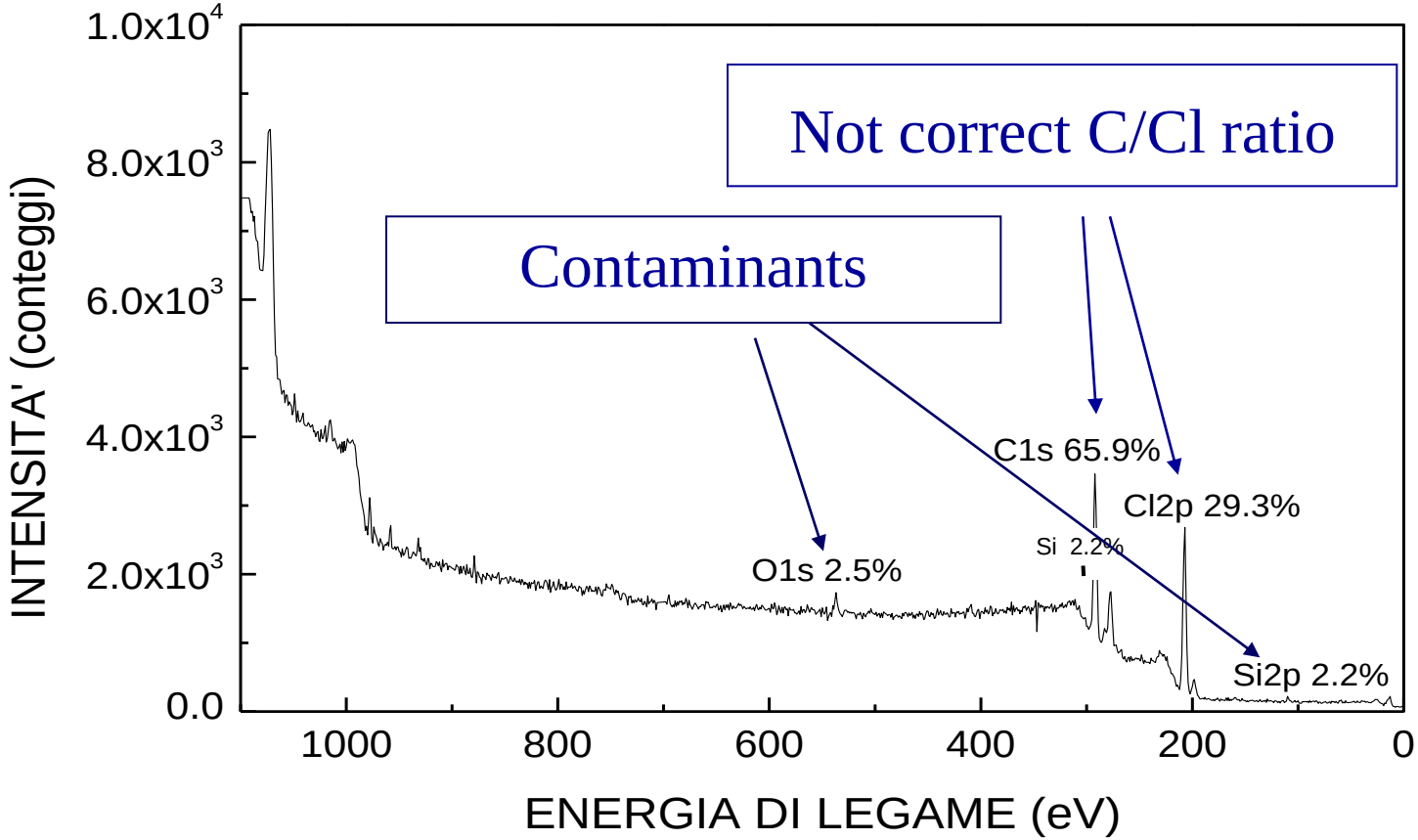
- surface chemical composition
- chemical bonds



$$E_K = h\nu - E_B - f$$

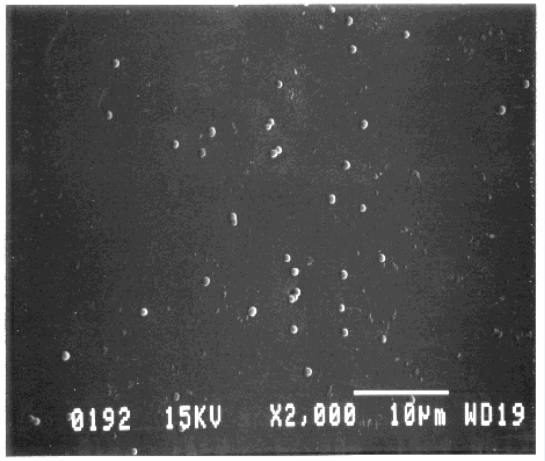
J = 90° - d = 5-6nm
J = 165° - d = 2 - 3nm

Biomaterials: Polymers



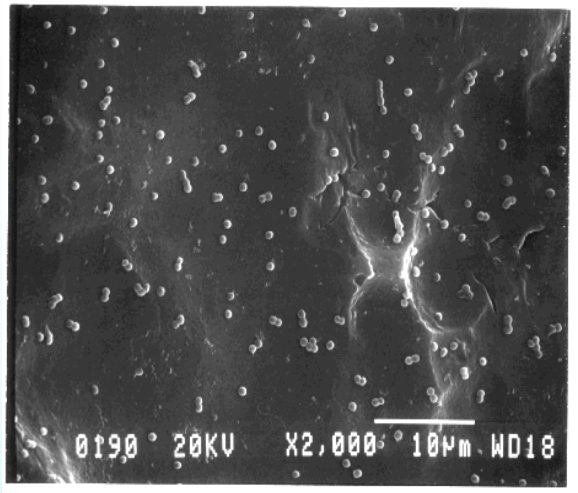
PVC

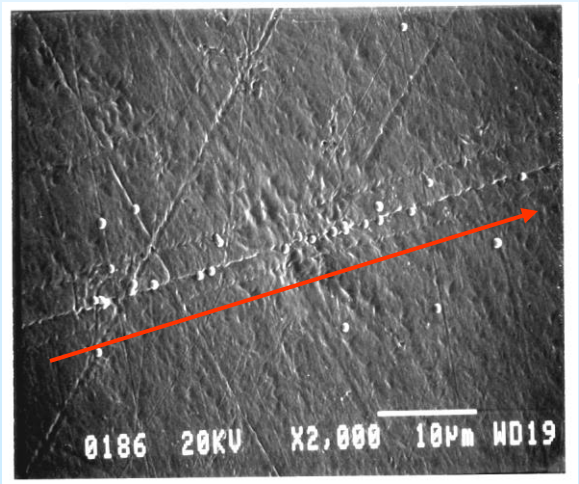
Biomaterials: Bacteria on Polymers



Staphylococcus epidermidis on PMMA 2000X

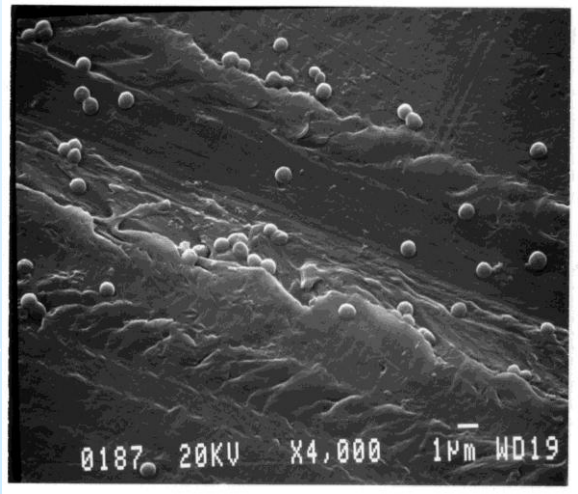
Staphylococcus epidermidis on PP 2000X



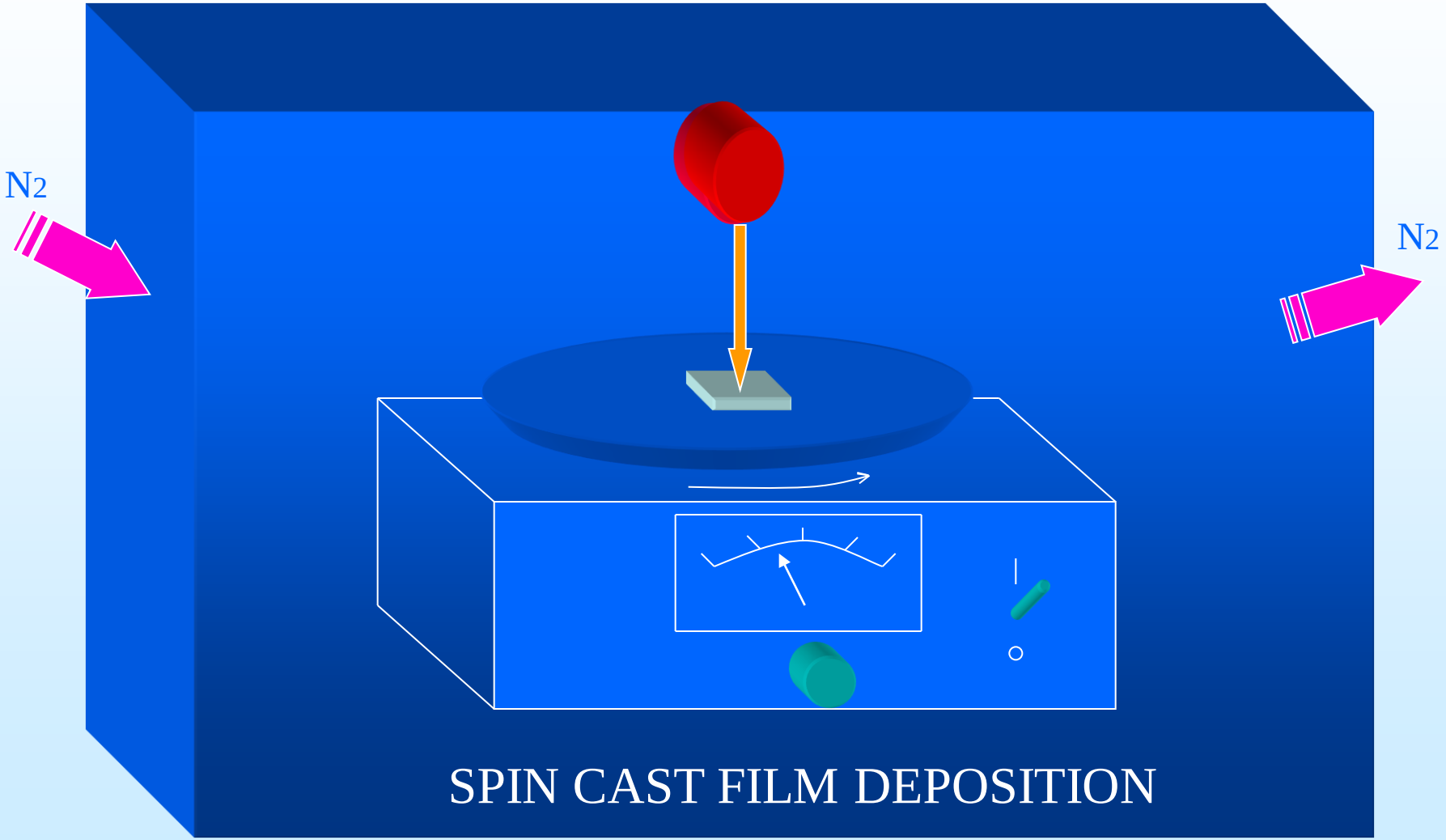


Staphylococcus epidermidis on PVDF 2000X

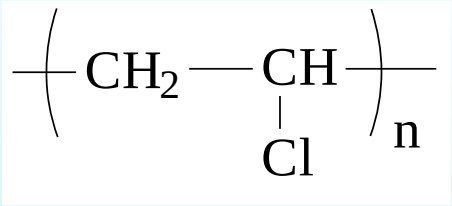
Staphylococcus epidermidis on PVDF 4000X



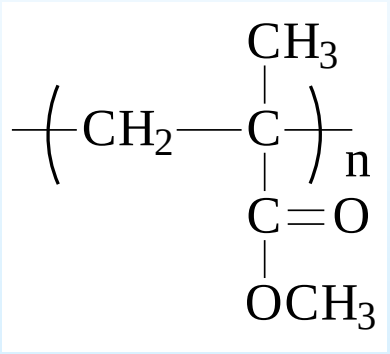
Roughness


N2
N2
SPIN CAST FILM DEPOSITION

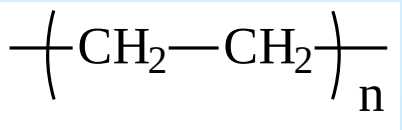
PVC
(mainly acid)



PMMA
(mainly basic)



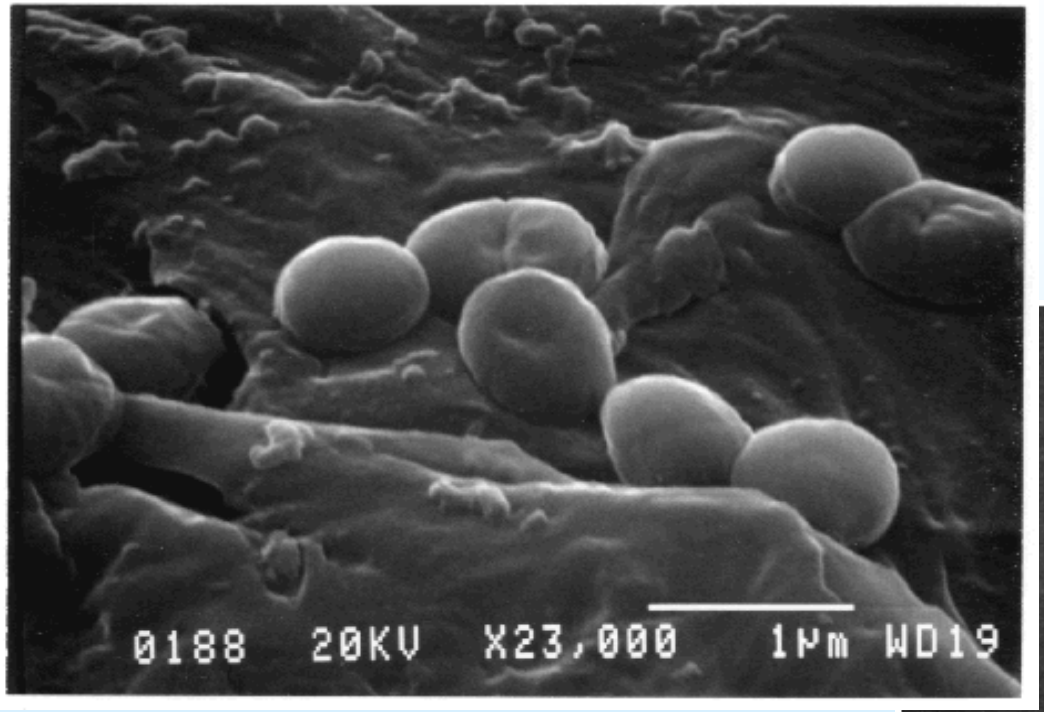
LDPE
(dispersive)



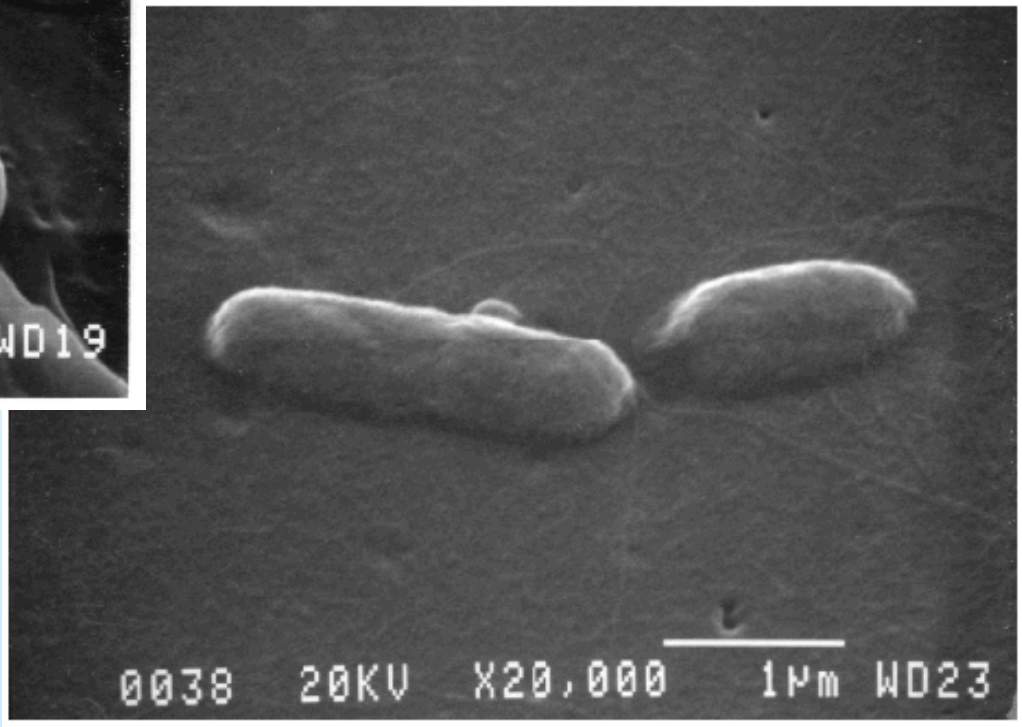
XPS Analysis	Nominal	Experimental
PVC	**C = 66.7%** **Cl = 33.3%**	**C = 66.3%** **Cl = 33.2%** **O = 0.5%**
LDPE	**C = 100%**	**C = 100%**
PMMA	**C = 71.4%** **O = 28.6%**	**C = 72%** **O = 28%**

Biomaterials: Bacteria on Polymers

Staphylococcus epidermidis

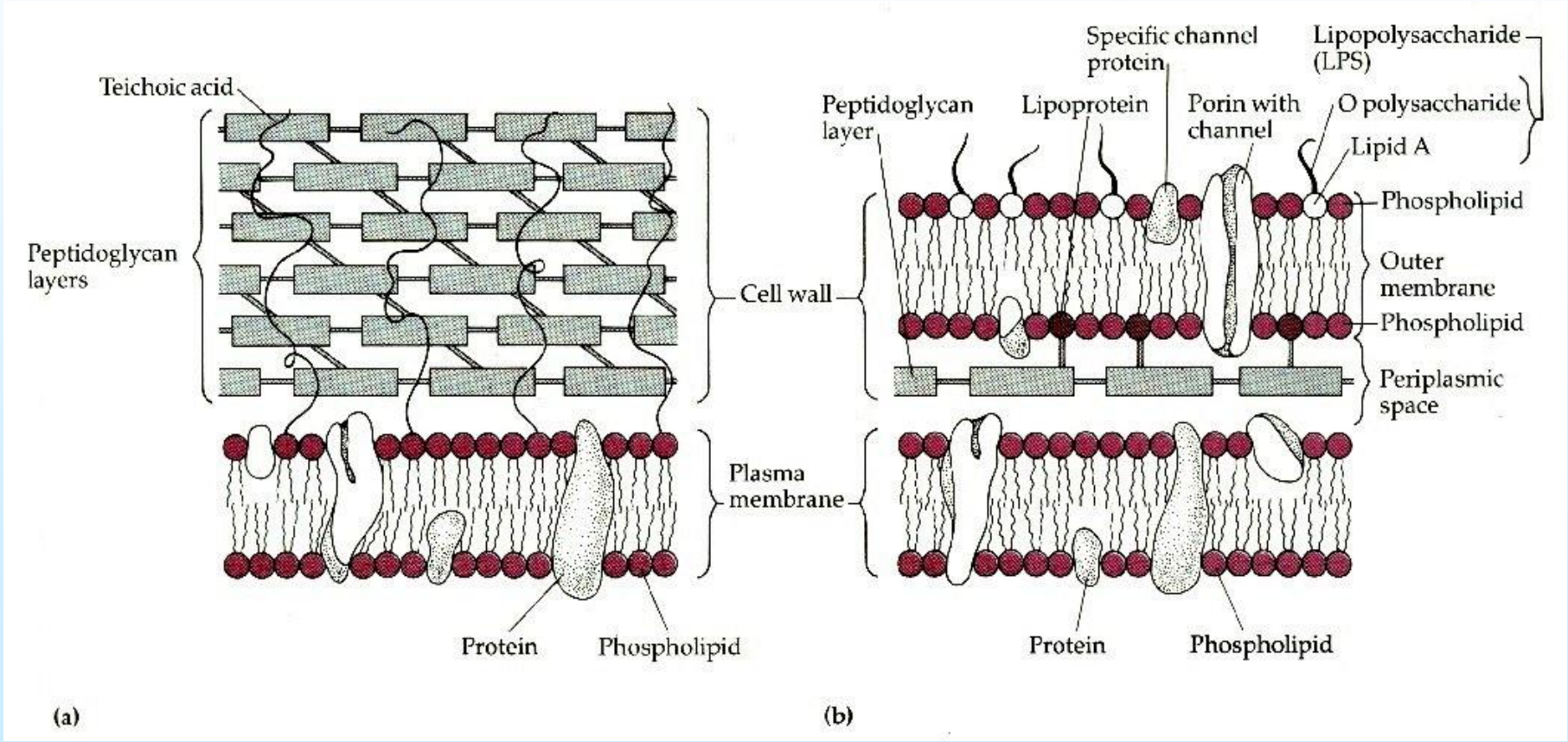


Escherichia coli



Biomaterials: Bacteria on Polymers

Two different bacterial strains: the one gram+ (a) and the other gram- (b) are caracterized by a different bacterial wall



Biomaterials: Bacteria on Polymers

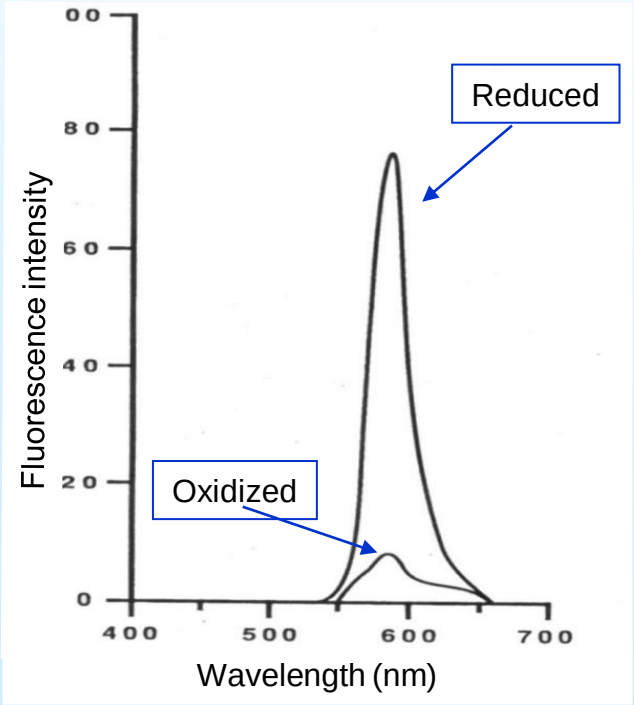
THE BIOLOGICAL EXPERIMENT

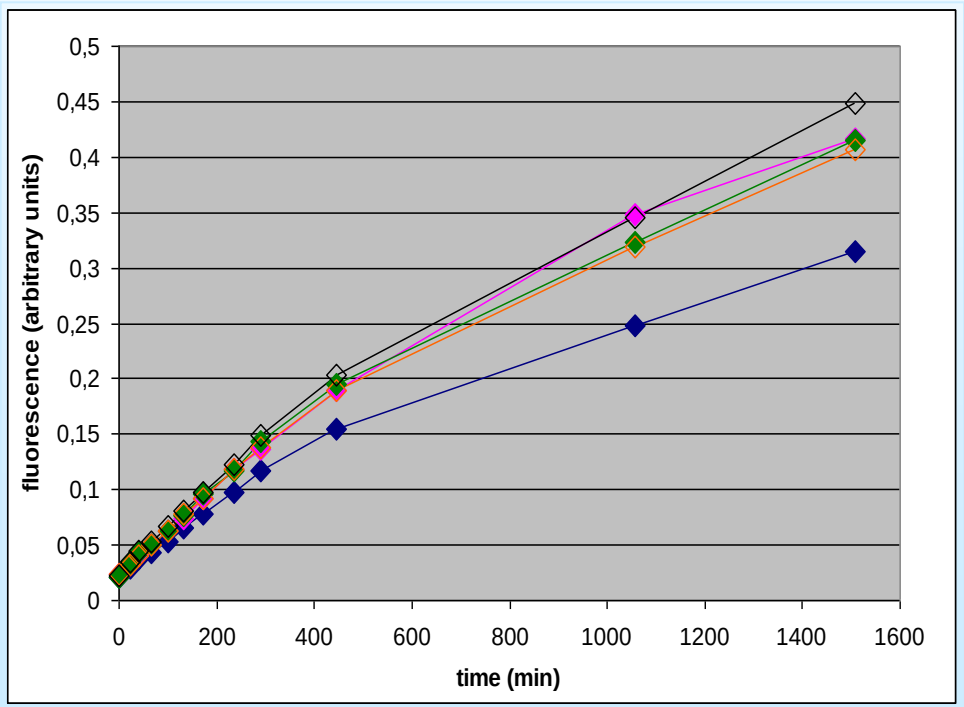
- Selection of bacteria from an unique colony;
- Growth of bacteria in a physiological solution;
- Bacteria are suspended in a bacteriostatic solution containing the polymeric surface;
- after 2 hours:
  - One of the polymeric surfaces is prepared for the SEM analysis;
  - The other polymeric surfaces are used to determine the bacterial density using a fluorimetric marker.

Biomaterials: Bacteria on Polymers

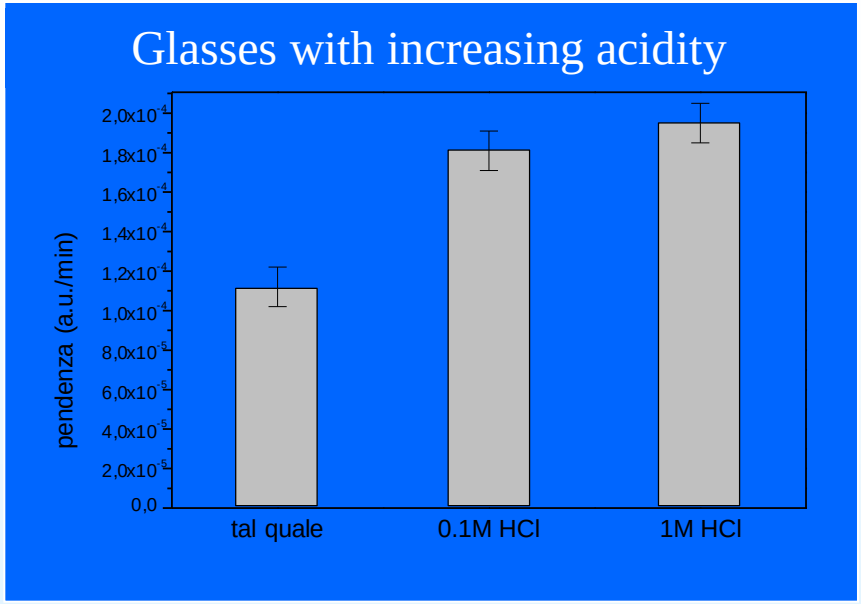
Detection of the metabolic activity of the bacteria via a fluorometric / colorimetric growth indicator (Alamar Blue)

Indirect measurement of the bacterial density on the polymeric surface.



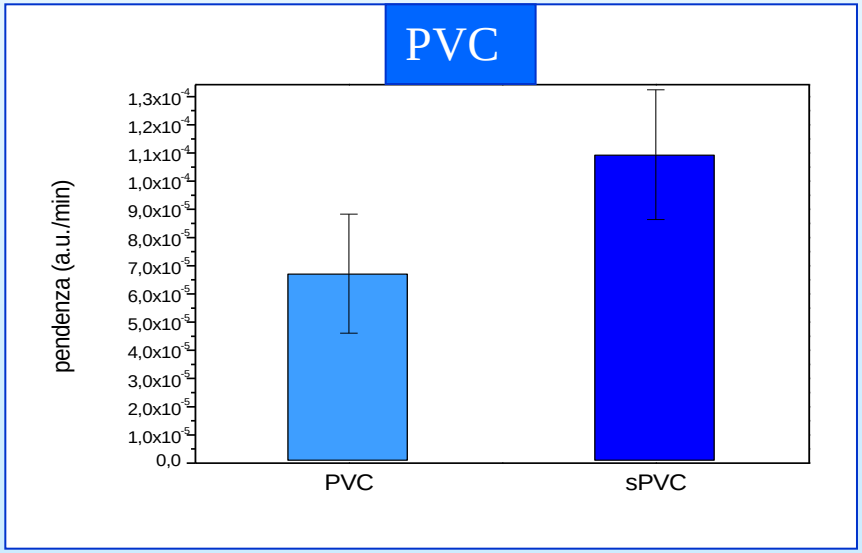


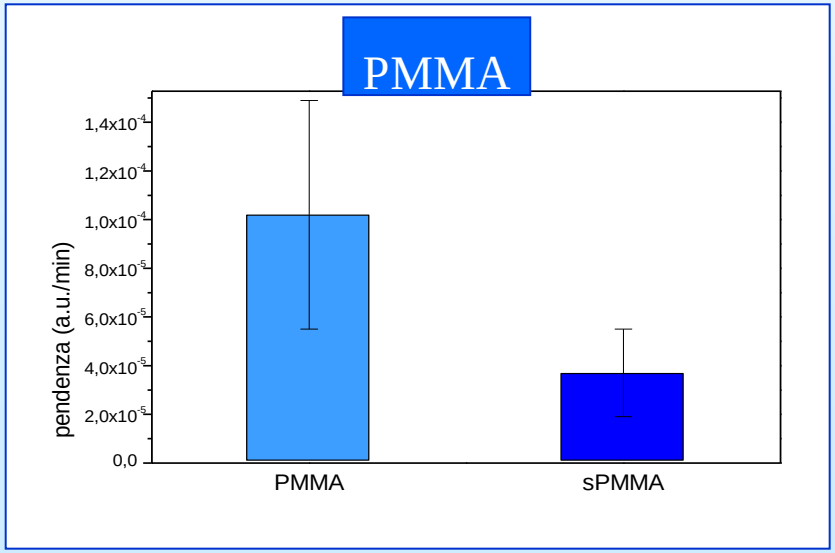
Biomaterials: Bacteria on Polymers



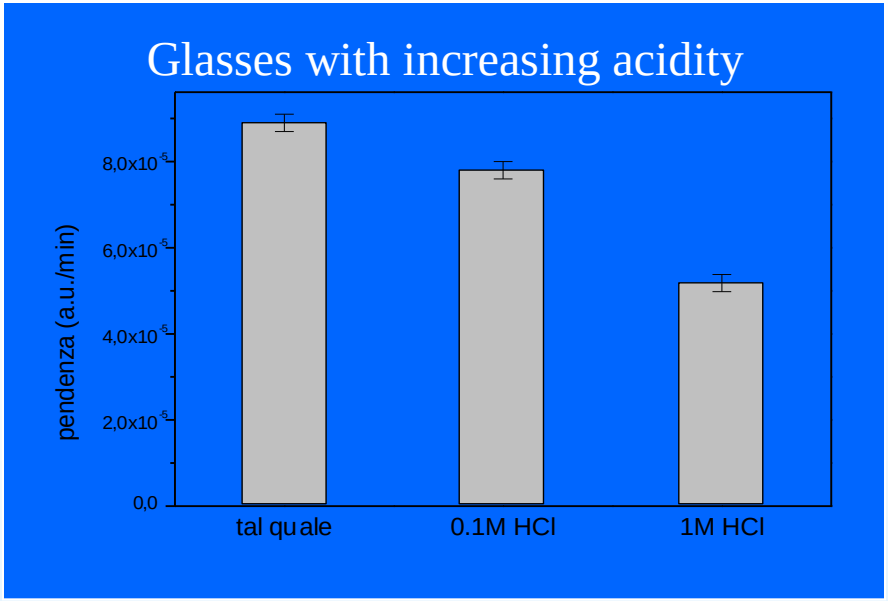
Escherichia coli

Gram -



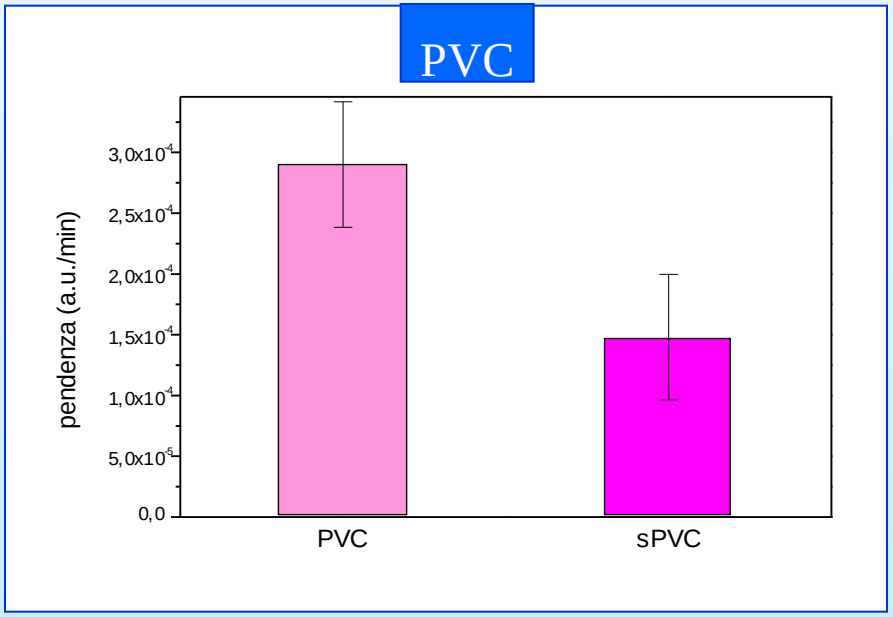


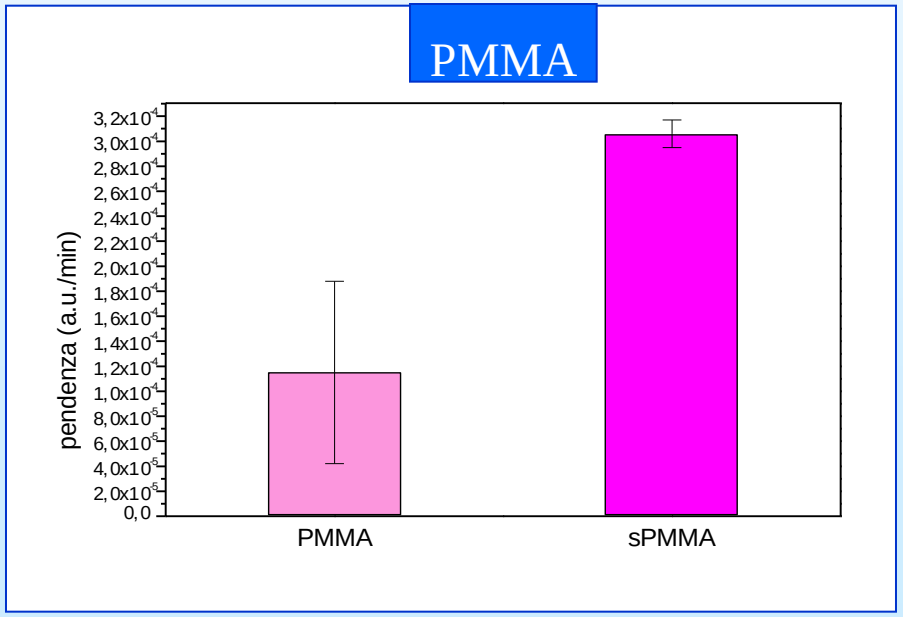
Biomaterials: Bacteria on Polymers



Staphylococcus epidermidis

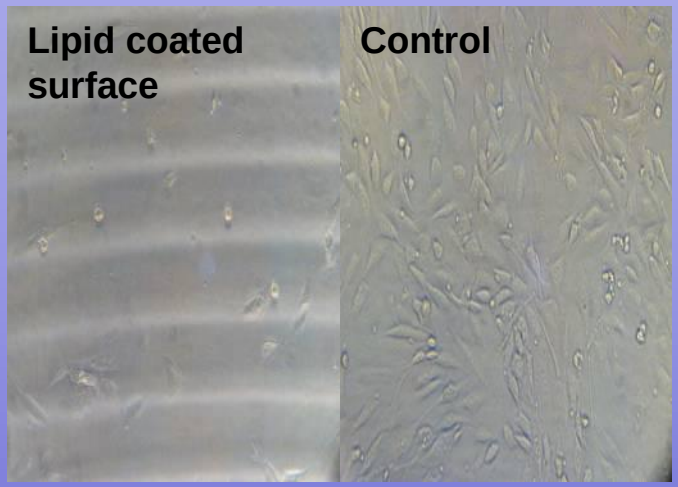
Gram +





Lipid-based coating

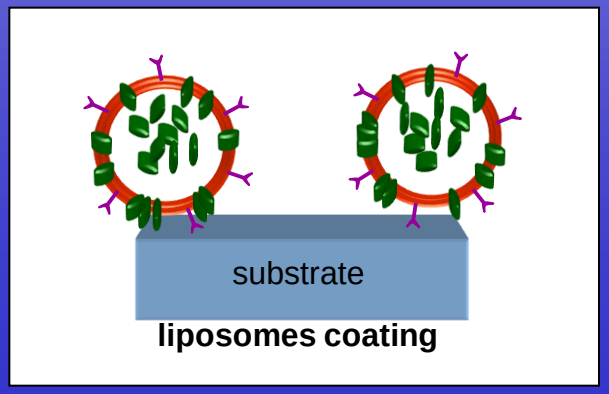
Ånon-fouling surface for controlling aspecific protein adhesion



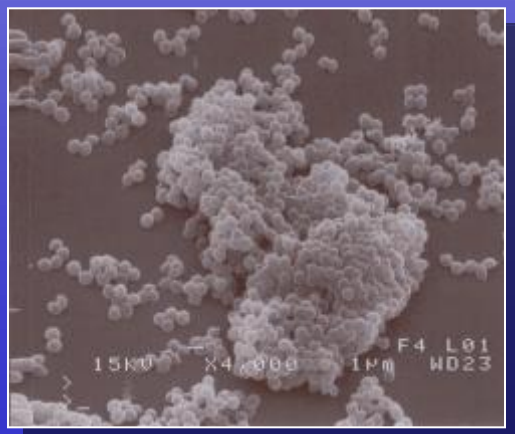
Contrast phase microscope image of mouse fibroblasts (NIH-3T3) adhesion

Ålocal drug delivery system for applying antimicrobial molecules to the surface of artificial implants

Prevention of bacterial adhesion and colonisation of polymeric surfaces by *deposition of lipid-based films loaded with an antibiotic (rifampicin)*



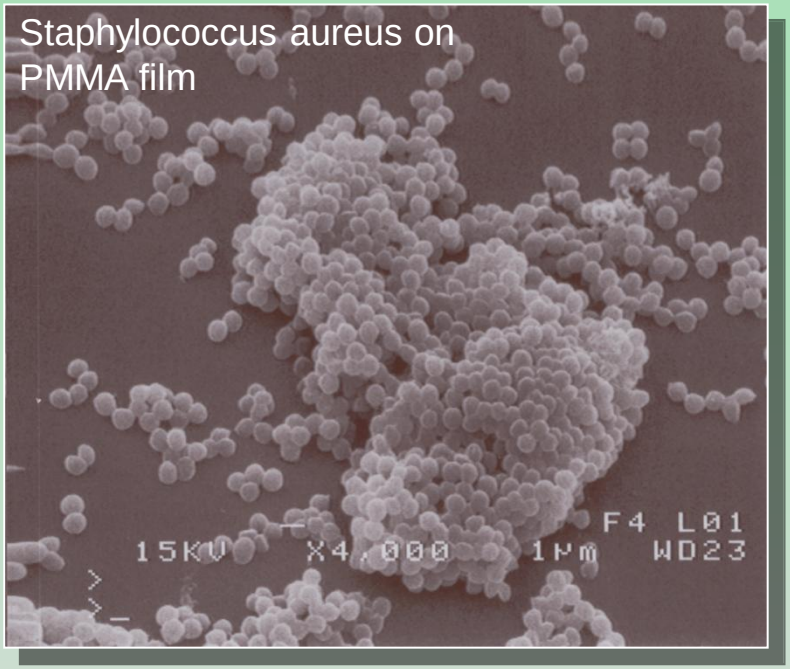
SEM image of *Staphylococcus aureus* on PMMA film



Bacterial infections on catheters

Most common pathogens isolated from hospital infections (%) [1]

Phatogens	1986-1989	1992-1999
Coagulase-negative staphylococci	27	37
Staphylococcus aureus	16	13
Enterococcus	8	13
Gram-negative rods	19	14
Escherichia coli	6	2
Enterobacter	5	5
Pseudomonas aeruginosa	4	4
Klebsiella pneumoniae	4	3
Candida spp.	8	8



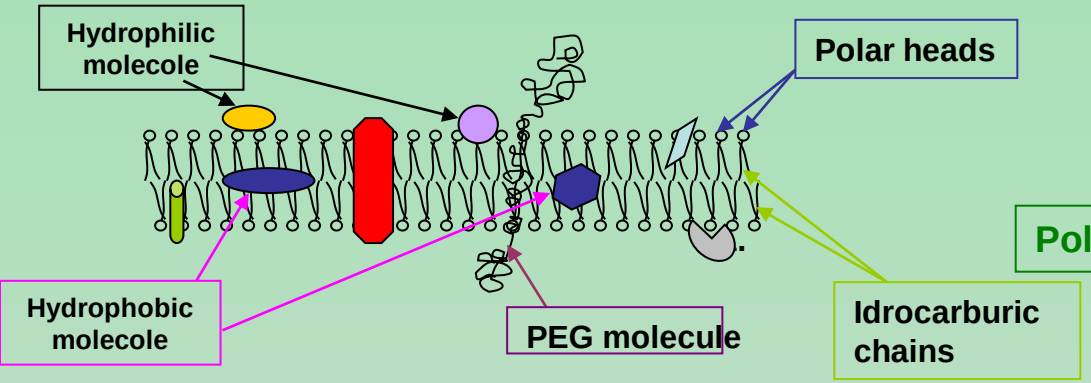
In the United States approximately 80,000 bloodstream infections occuring each year are associated with CVC [1]

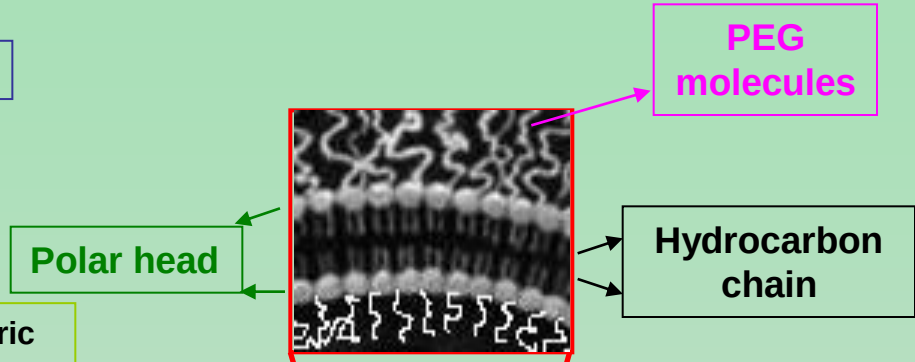
Annual cost of caring for patients estimated around $2.3 billion [1]

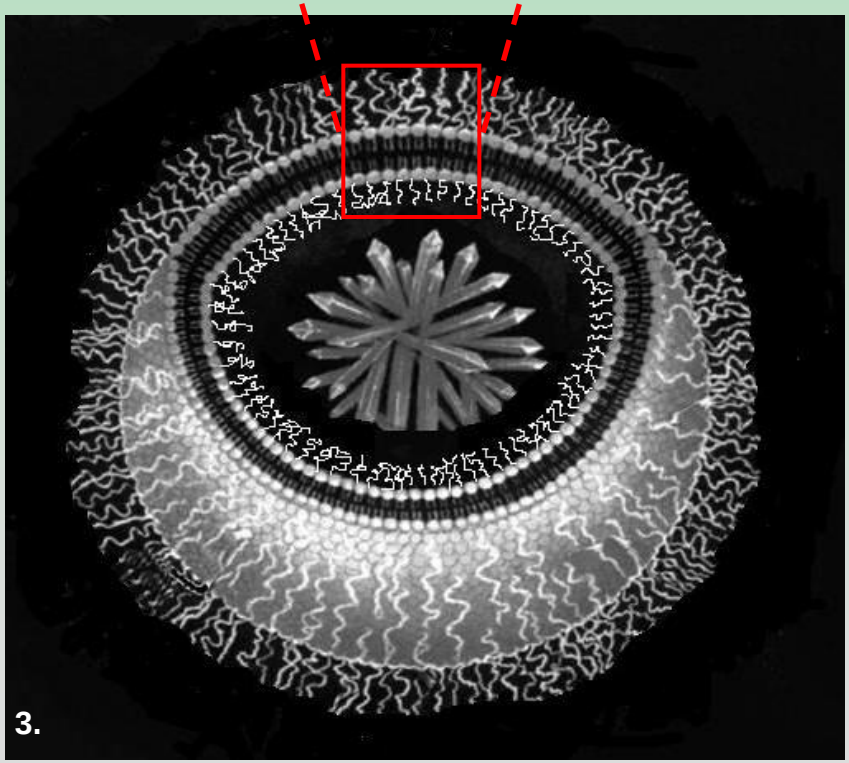
1. MMWR august 9, 2002 / Vol. 51 / No. RR-10

"Role of chemical interactions in bacterial adhesion to polymer surfaces" *Biomaterials 25 (2004) 2029–2037*

Liposomes as drug delivery systems







Spherical particles made of lipid molecules (phospholipids, cholesterol)

These vesicles can be used as drug delivery system carrying active agents such as antibiotics

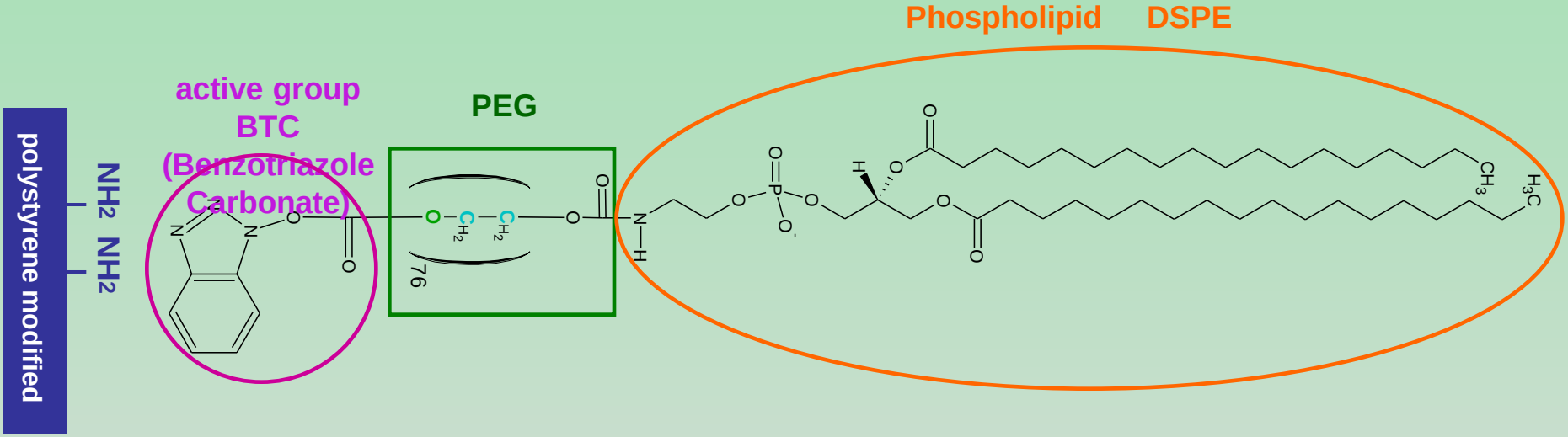
The release of the agent can be actively controlled for instance by pH, temperature variations, antibodies and fusion processes

2. From D.D.Lasic: from Physics to Applications, Elsevier, Amsterdam, The Nederlands, 1993

3. Adapted from: hp://www.science.mcmaster.ca/biochem/faculty/andrews/lab/projects/niosomes/picture1.jpg

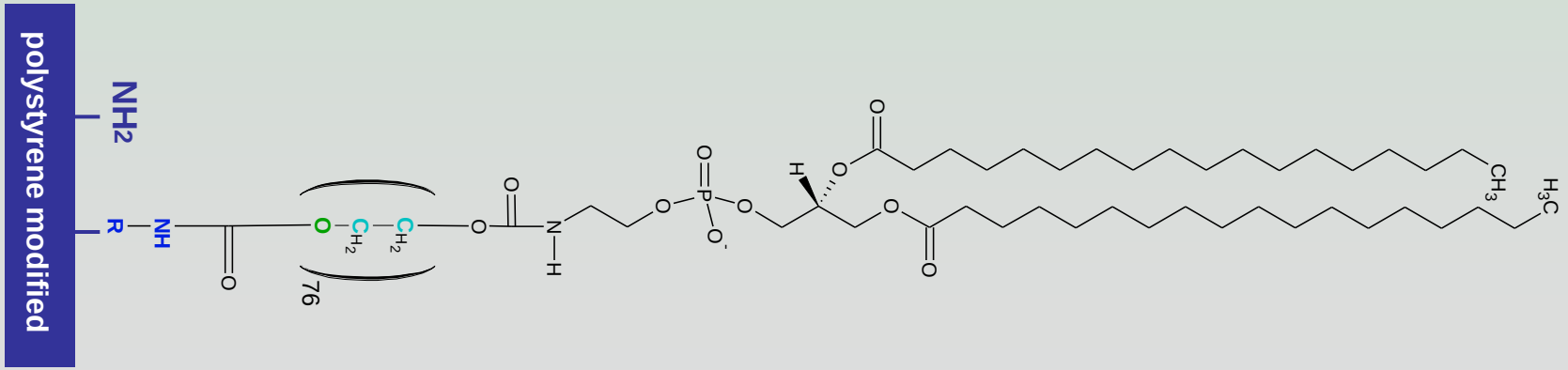
Liposomes linkage at amine surface

A carbamate linkage was obtained between amino groups on polystyrene surface and the active group at the end of DSPE-PEG inserted in liposome membrane



After incubation on surface

(30 mns, room temperature, carbonate buffer 8pH)

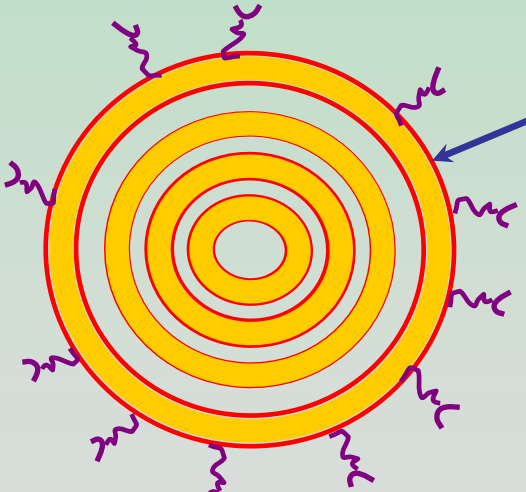


Biomembrane preparation and surface attachment

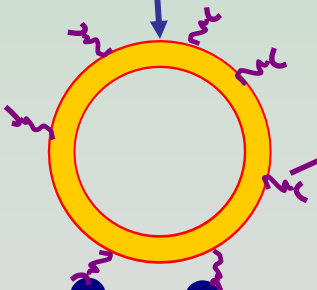
Experiments are performed with liposomes made of the same lipid formulation but different in number of lamella and in size.

Membrane composition (% molar)		
Phosphatidylcholine **(PC)**	Disteroyl-phosphatidylethanolamine-polyethyleneglycol-benzotriazole carbonate **(DSPE-PEG-BTC)**	Cholesterol **(CH)**
65	**5**	**30**

MULTILAMELLAR VESICLES (MLV) diameter 300-400 nm



LARGE UNILAMELLAR VESICLES (LUV) diameter 100 nm



Activated PEG-BTC (Benzotriazole Carbonate)

NH_2 groups

modified polystyrene

The selected polymeric model surface

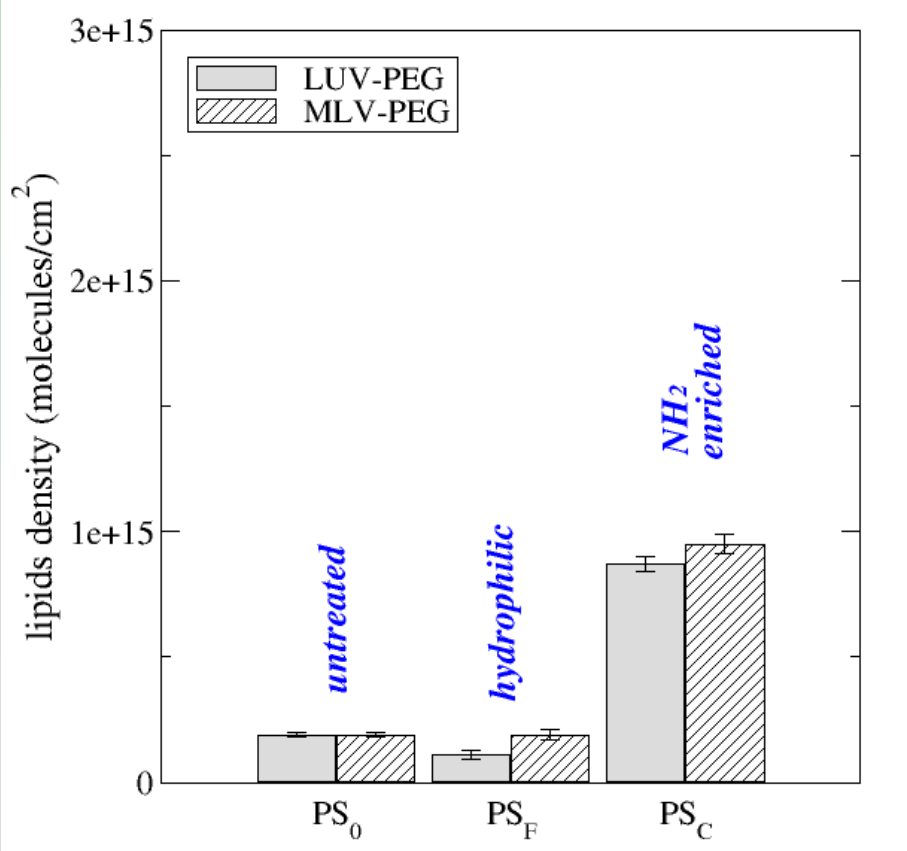
We chose polystyrene as a model surface because it is commercially available in various versions and inexpensive

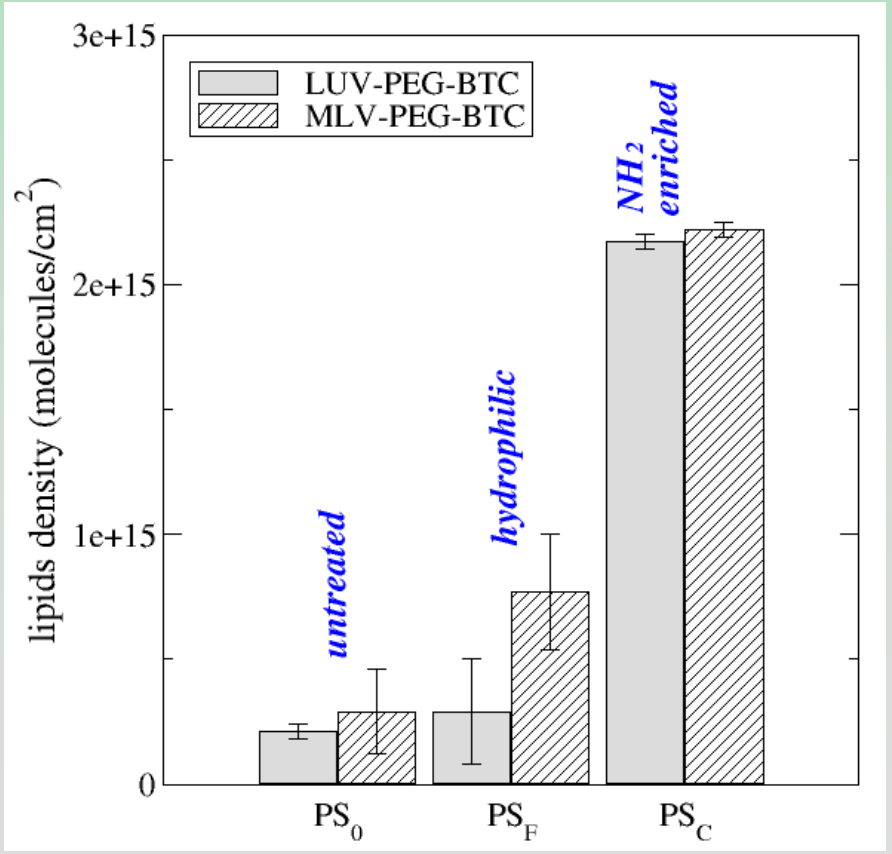
SAMPLE	BOTTOM AREA (cm^2)	SOURCE
PS_0 - untreated	0.44	Biomat s.n.c., Italy
PS_F ï hydrophilic surface	0.32	BD Biosciences, USA
PS_C ï Costar 2388 NH_2 enriched ($2*10^{13}$ amine/cm^2)	0.32	Corning Incorporated, USA

Quantification of liposome coatings

The number of lipid molecules bonded to the surface was quantified using a fluorescent lipid inserted into the liposome membrane: 1% phosphatidylethanolamine- lissamine rhodamine B (PE-Rhod) relative to the total lipid weight/liposomes. The adherent film was removed by detergent and measured in solution by microplate fluorescence reader.

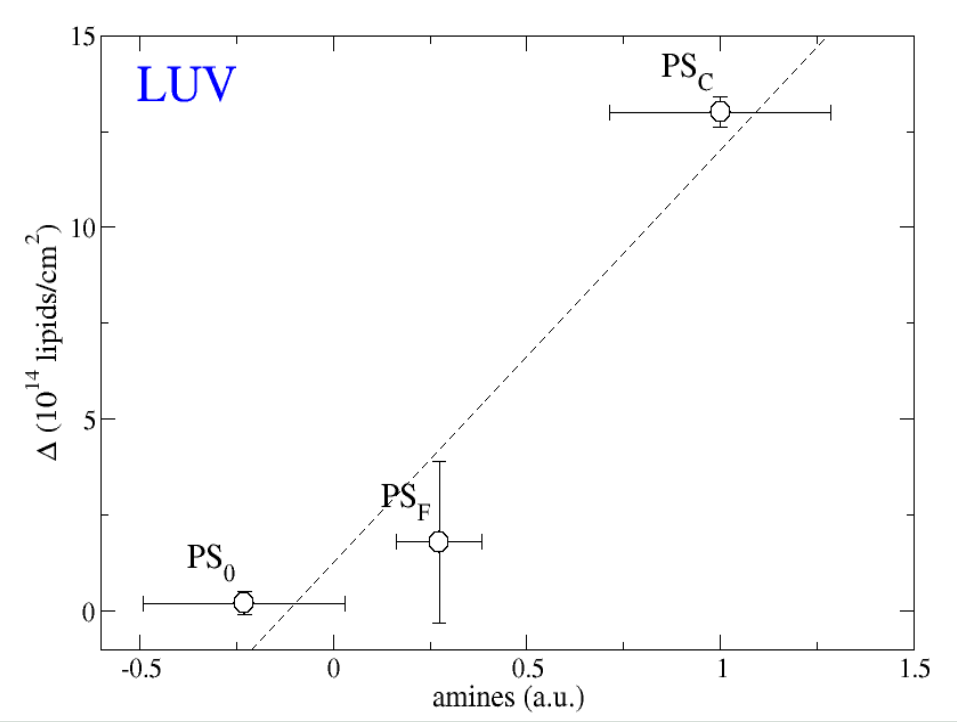
The comparison of liposomes with BTC-active and inactive PEG shows the contribution of covalently bonded molecules

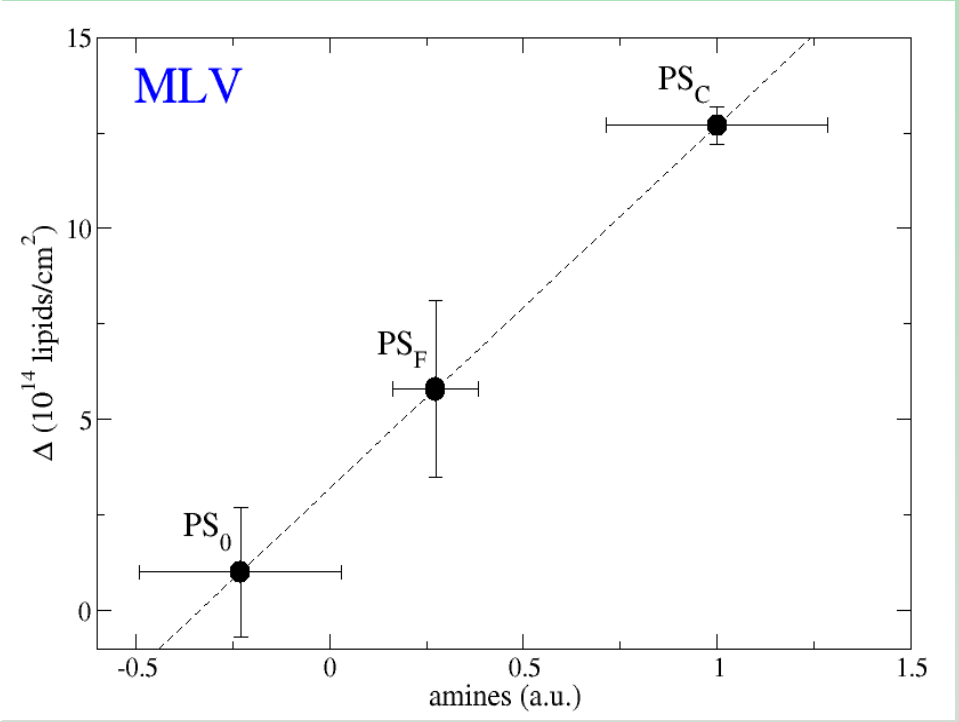




Specific lipid adhesion D on polystyrene surface vs amine density

The specific adhesion D is the difference between the liposomes with PEG alone and liposomes with PEG-BTC.

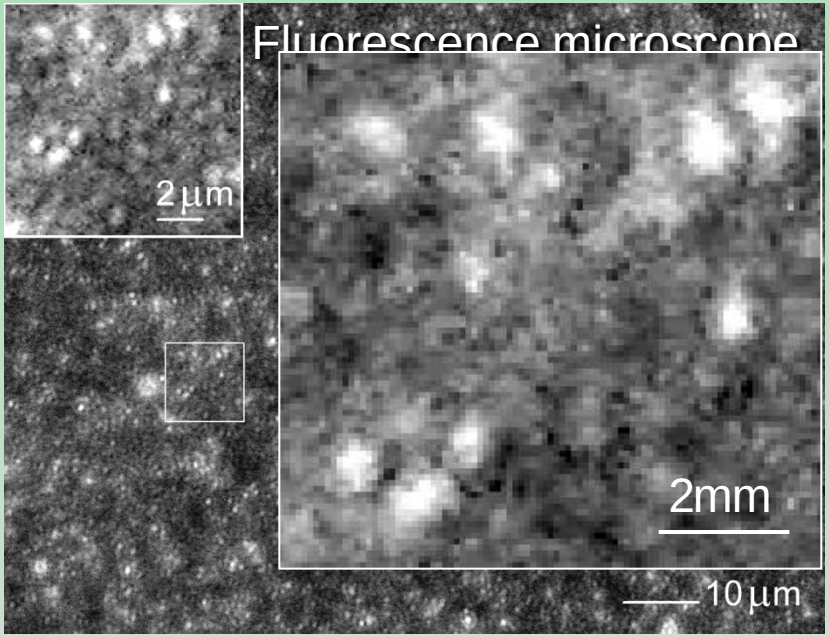


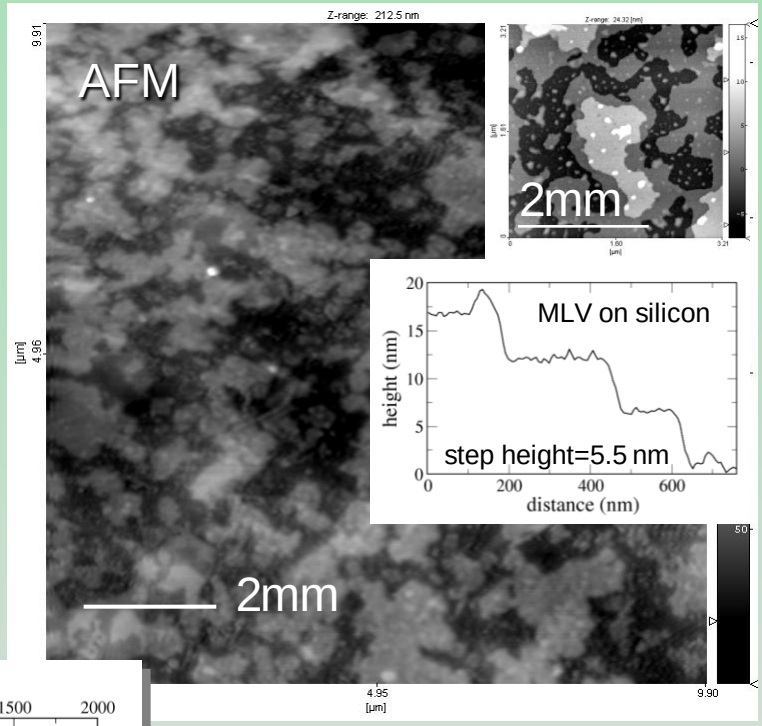


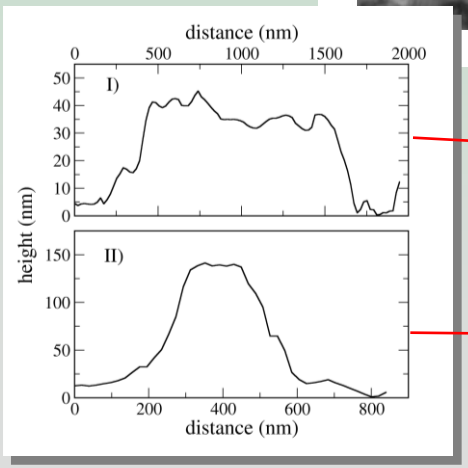
The amine quantification on substrate was performed using fluorescamine, a marker that becomes fluorescent (fluorophore) after the bond with amino groups of polystyrene.

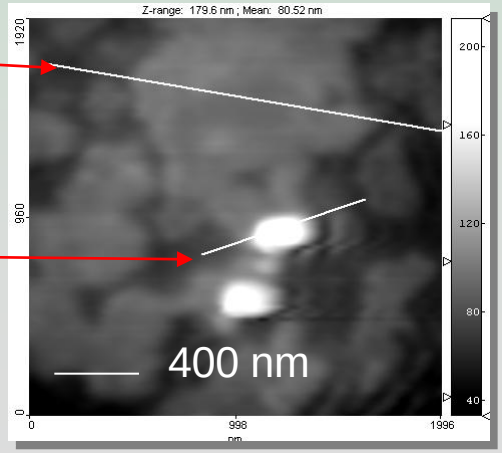
The value of amine quantification was normalized to the NH_2 enriched polystyrene .

Multilamellar liposome distribution and morphology on NH_2-riched polystyrene surfaces





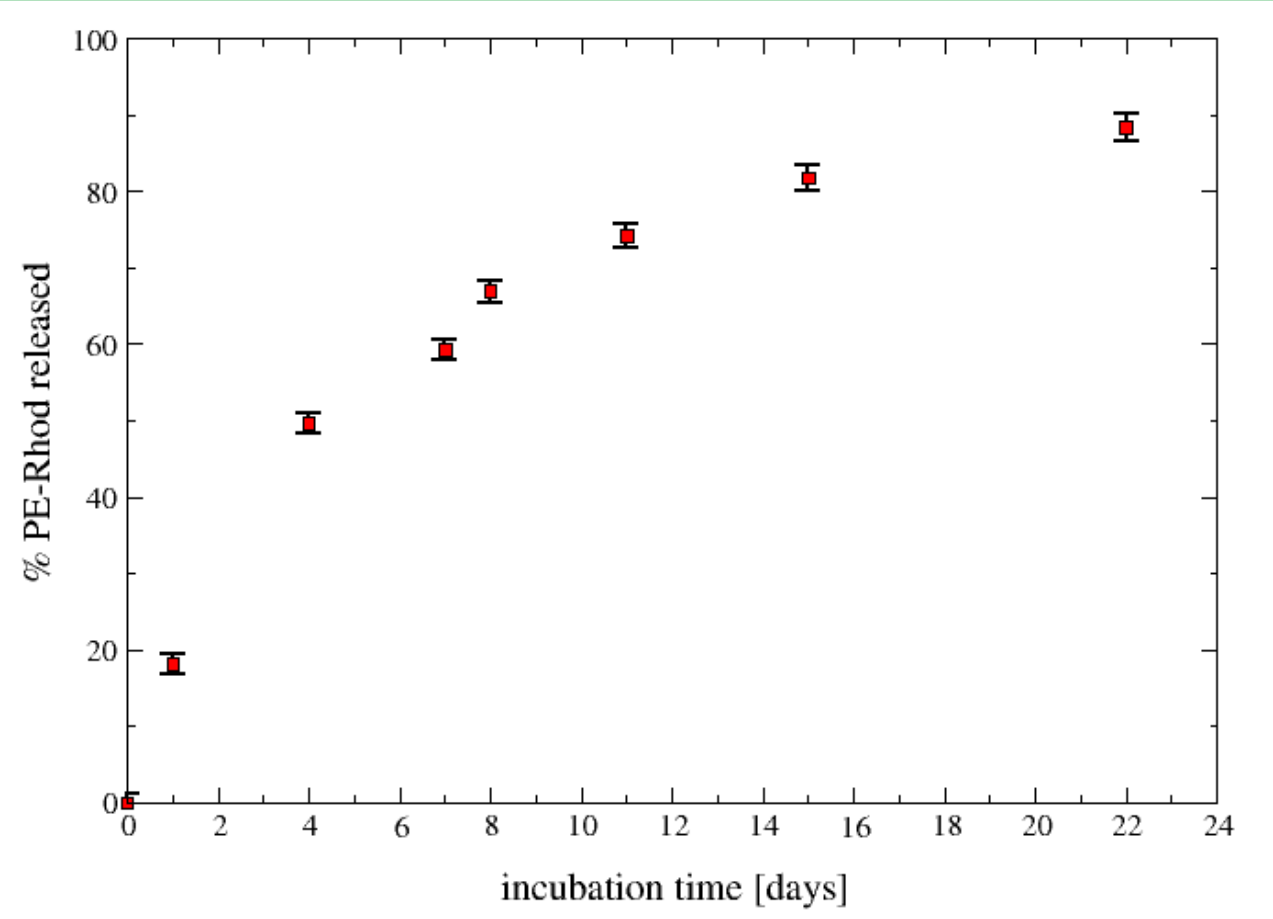




- Fluorescence microscopy: quite uniform lipid distribution
- AFM: multilayer lipid structures (~ 8 bilayers) on the surface
- Liposome density quantification is consistent with AFM and fluorescence microscopy analyses

Multilamellar film stability on NH_2-rich polystyrene surfaces

The multilamellar stability on surface was performed in phosphate buffer at pH 7.4 at 37 °C for several days using the Rhod-PE fluorescent marker in the liposomes

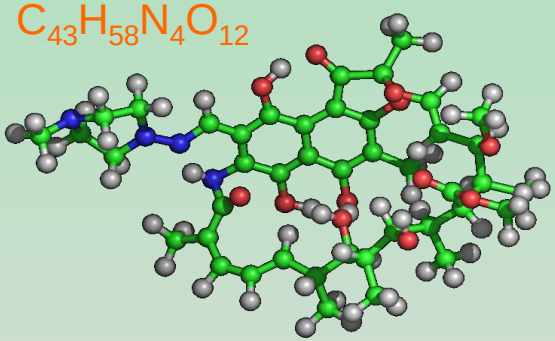


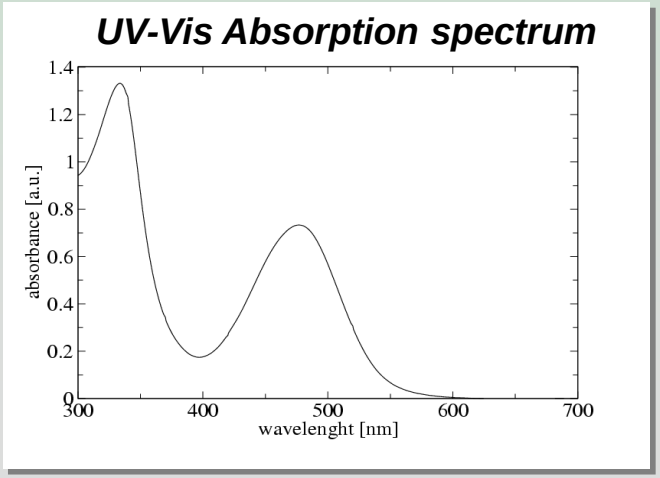
The selected antibiotic and its encapsulation

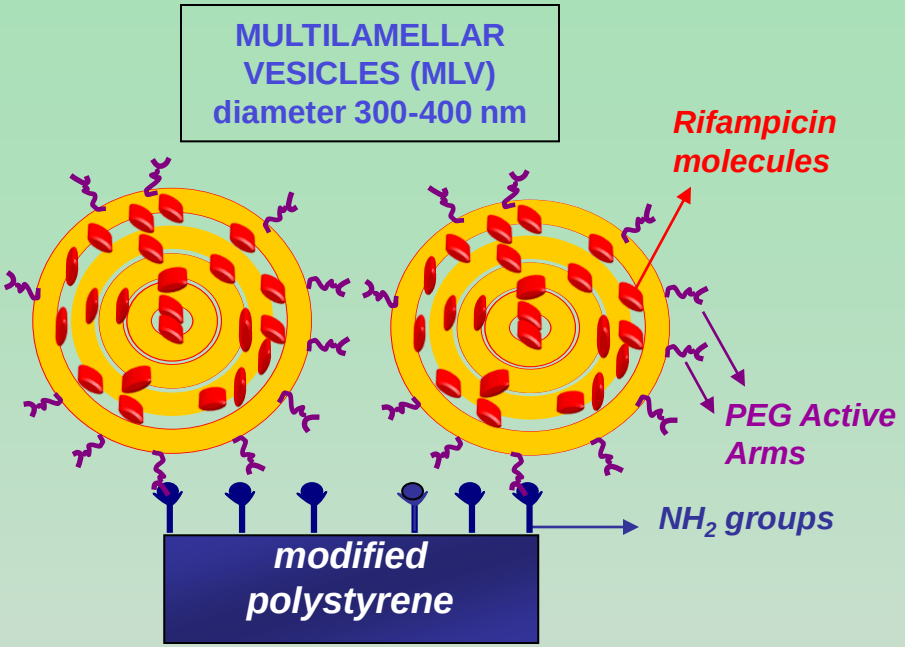
Rifampicin is a specific inhibitor of bacterial deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase. It is a broad-spectrum antibiotic especially active against Gram+ bacteria

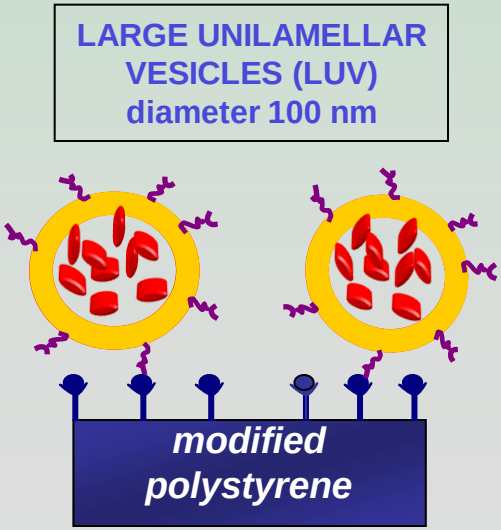
Rifampicin molecule

$C_{43}H_{58}N_4O_{12}$





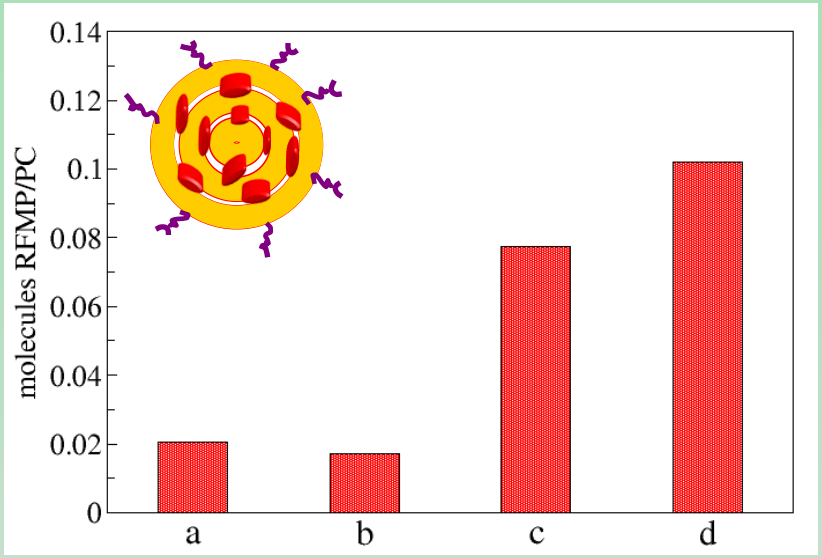


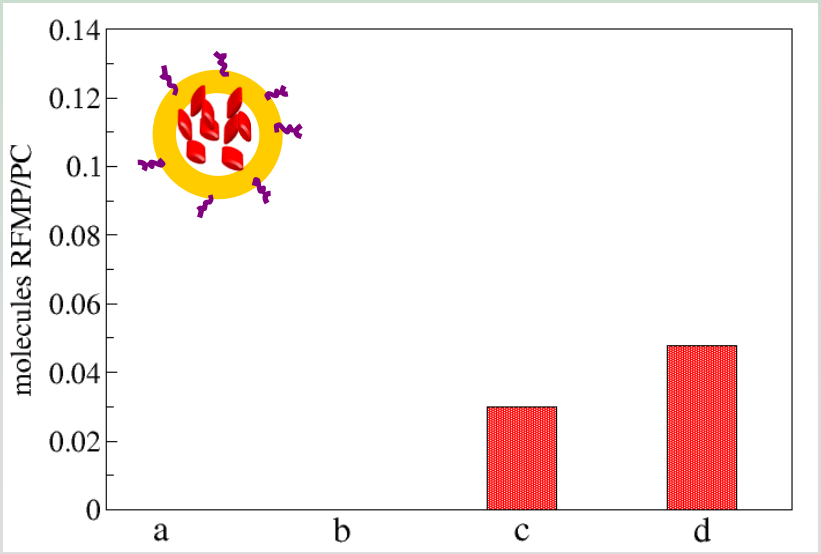


Rifampicin encapsulation depends on lipid formulation

Different membrane composition of MLV and LUV (% molar)

a)	**PC** *55*	**Chol** *30*	**DSPE-PEG** *5*	**DSTAP** *10*
b)	**PC** *50*	**Chol** *30*	**DSPE-PEG** *5*	**DSTAP** *15*
c)	**PC** *50*	**Chol** *30*	**DSPE-PEG** *5*	**DOTAP** *15*
d)	**PC** *50*	**Chol** *26*	**DSPE-PEG** *4*	**DOTAP** *20*





PC: phosphatidylcholine

Chol: cholesterol

DSPE-PEG: disteroylphosphatidylethanolamine (polyethyleneglycol)

POSITIVELY CHARGED LIPIDS:

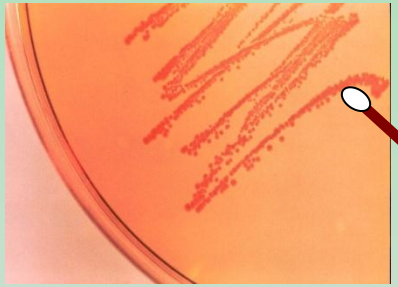
DSTAP: Distearoyl-3-Trimethylammonium-Propane (Chloride Salt)

DOTAP: (Dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride

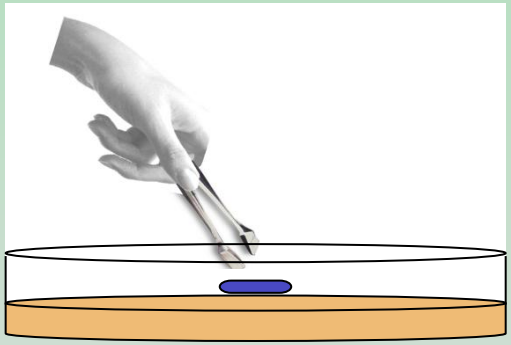
Antimicrobial activity of rifampicin-loaded d-liposomes

Kirby-Bauer modified test using Staphylococcus epidermidis for the determination of the zone inhibition diameter after 24 hours at 37°C

A Staphylococcus epidermidis was spread on agar plate (a culture solid nutrient)



B The NH_2 polystyrene surface, covered with rifampicin loaded d-liposomes, has been put on to bacteria agar culture



C

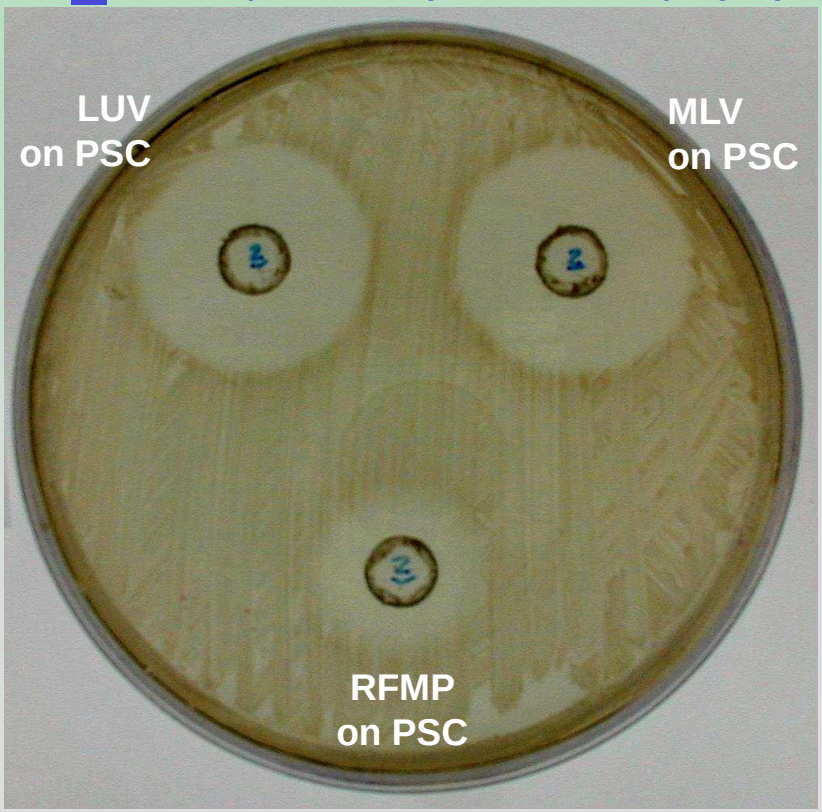
After 24 hours at 37°C

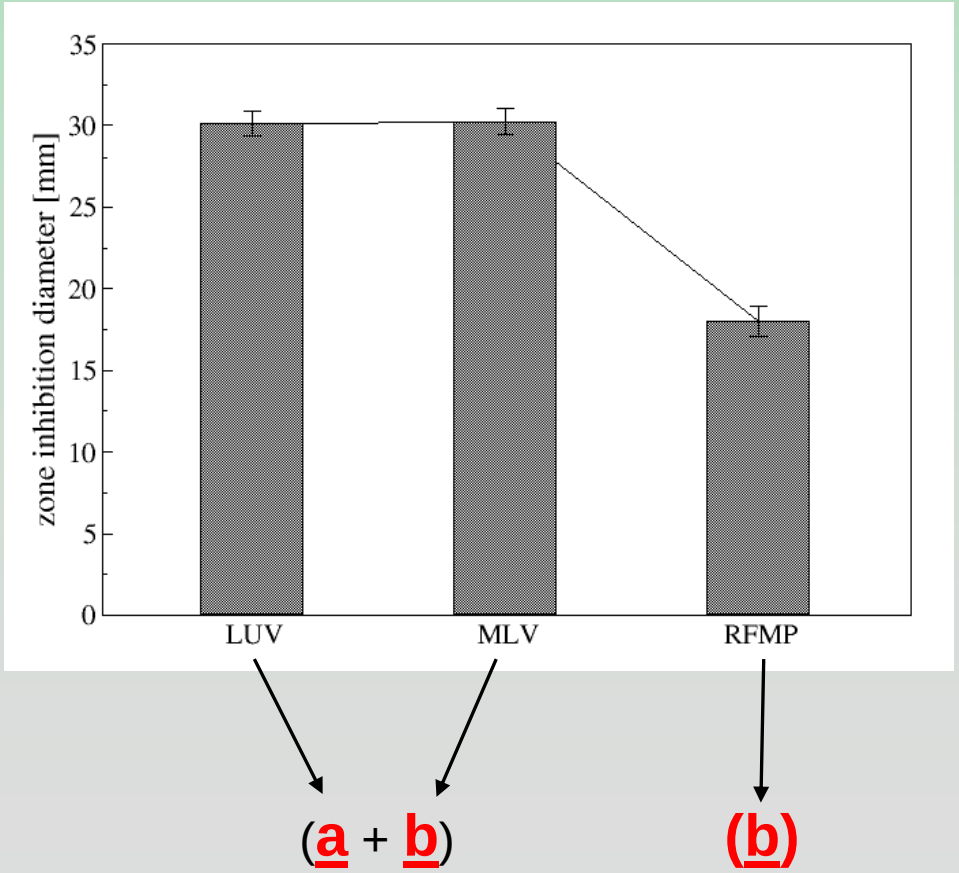
Antimicrobial activity of polystyrene surface coated with rifampicin-encapsulated liposomes and free rifampicin

Two contributions since liposomes are not rinsed:

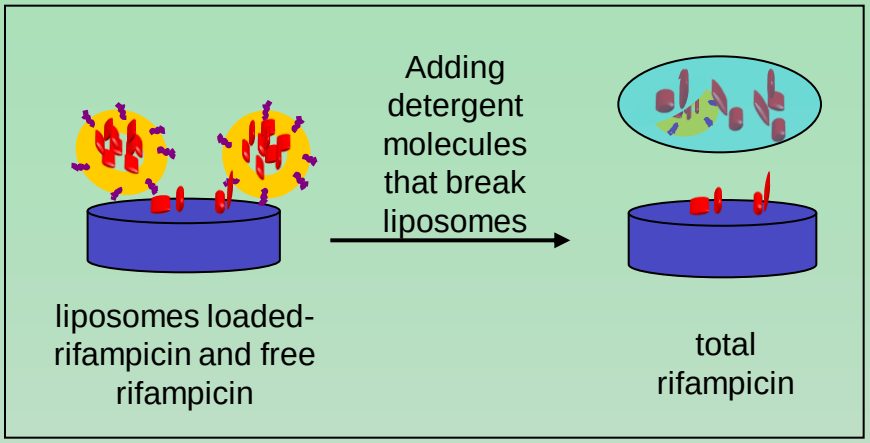
a. Rifampicin encapsulated within liposomes (LUV and MLV formulations)

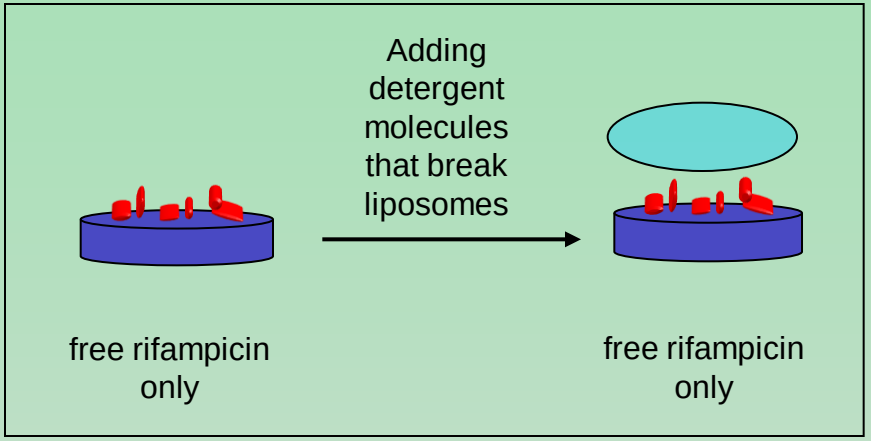
b. Rifampicin freely bonded to polystyrene surface





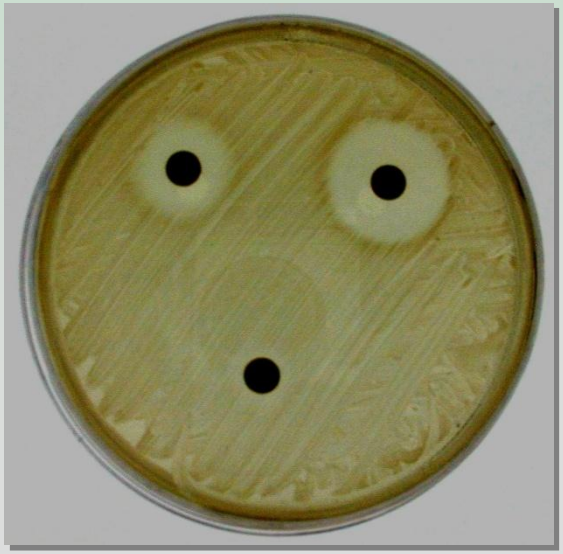
Determining contribution from rifampicin inside liposomes

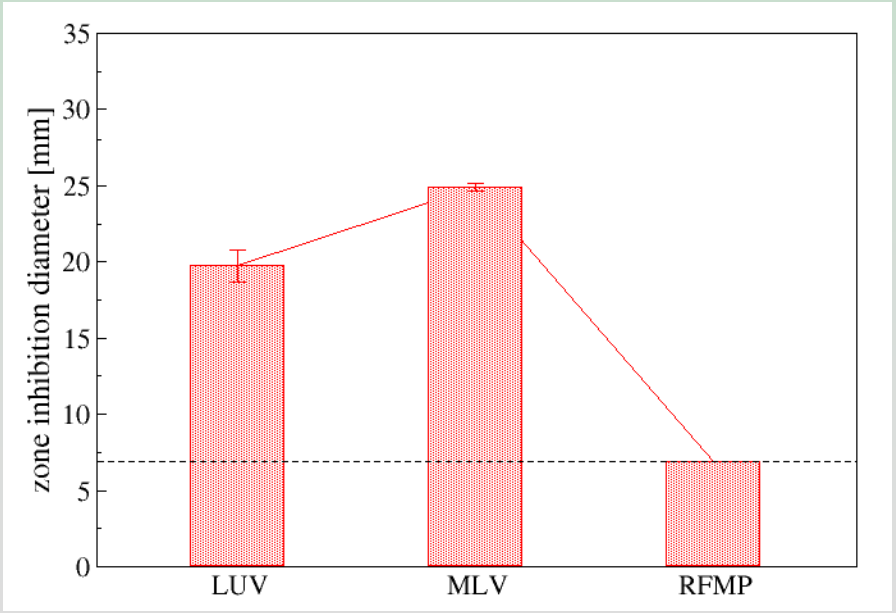




The detergent molecules do not remove free rifampicin from sample.

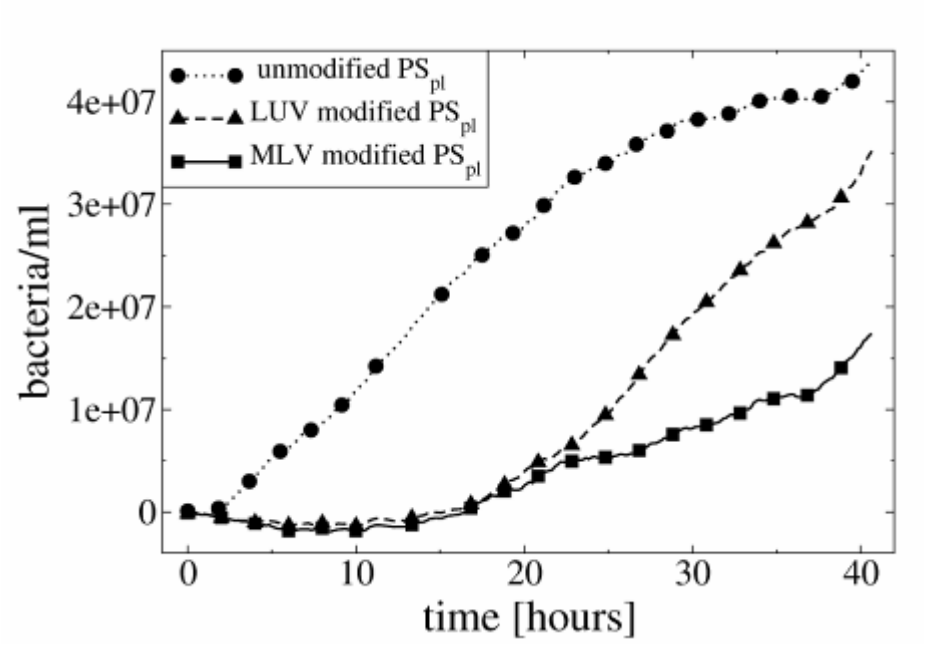
The removed solution is transfered to filter paper and then stamped onto the bacteria agar plate.





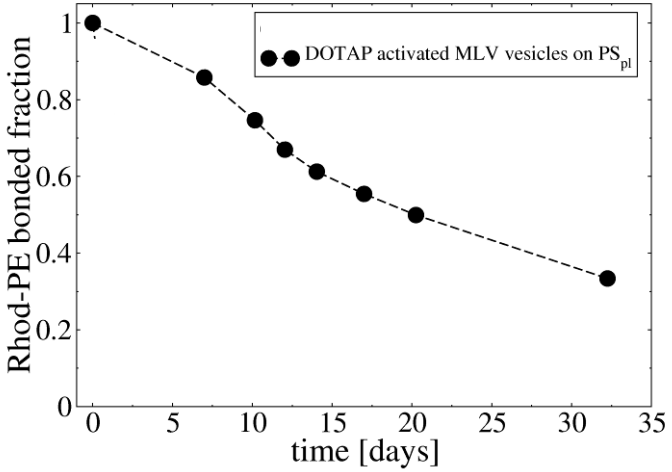
Anti-microbial activity of the rifampicin-coated polymeric surfaces

***Staphylococcus epidermidis* growing cultures on unmodified substrate and modified with drug-loaded LUV and MLV liposomes**



A reduced bacterial proliferation was detected on amine modified polystyrene (PSpl) using both LUVs and MLVs (10% and 2% respectively of the bacteria initially inoculated)

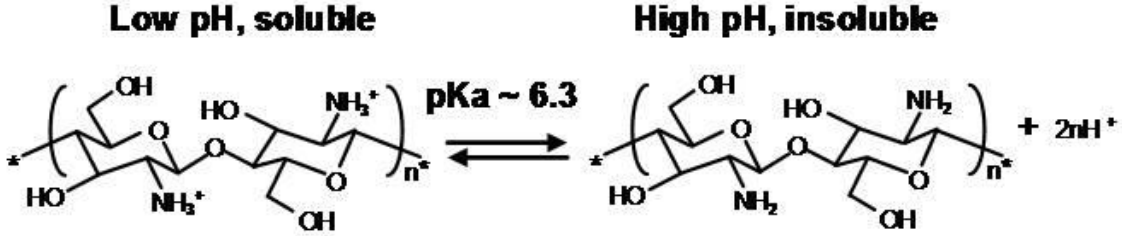
Time-dependent stability of the deposited lipid structures

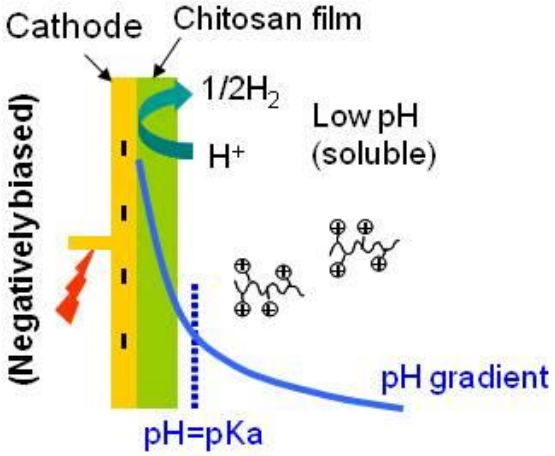


The stability of deposited lipid films was determined at 37 ̄C, obtaining a half-time of ~20 days.

1. Possible solution for bacterial infections: drug delivery with liposomes
2. Two kinds of liposomes bond to modified polystyrene with the same lipids density (2.2×10^{15} lipids molecules/cm^2)
3. AFM and fluorescence microscopy analyses are consistent with lipids density quantification
4. Preliminary test on Staphylococcus epidermidis: promising antimicrobial activity results

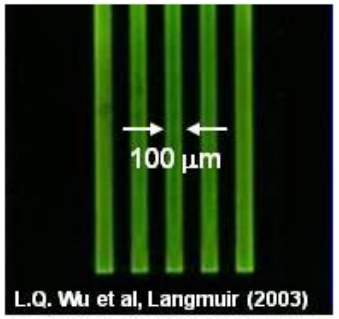
Chitosan Electrodeposition

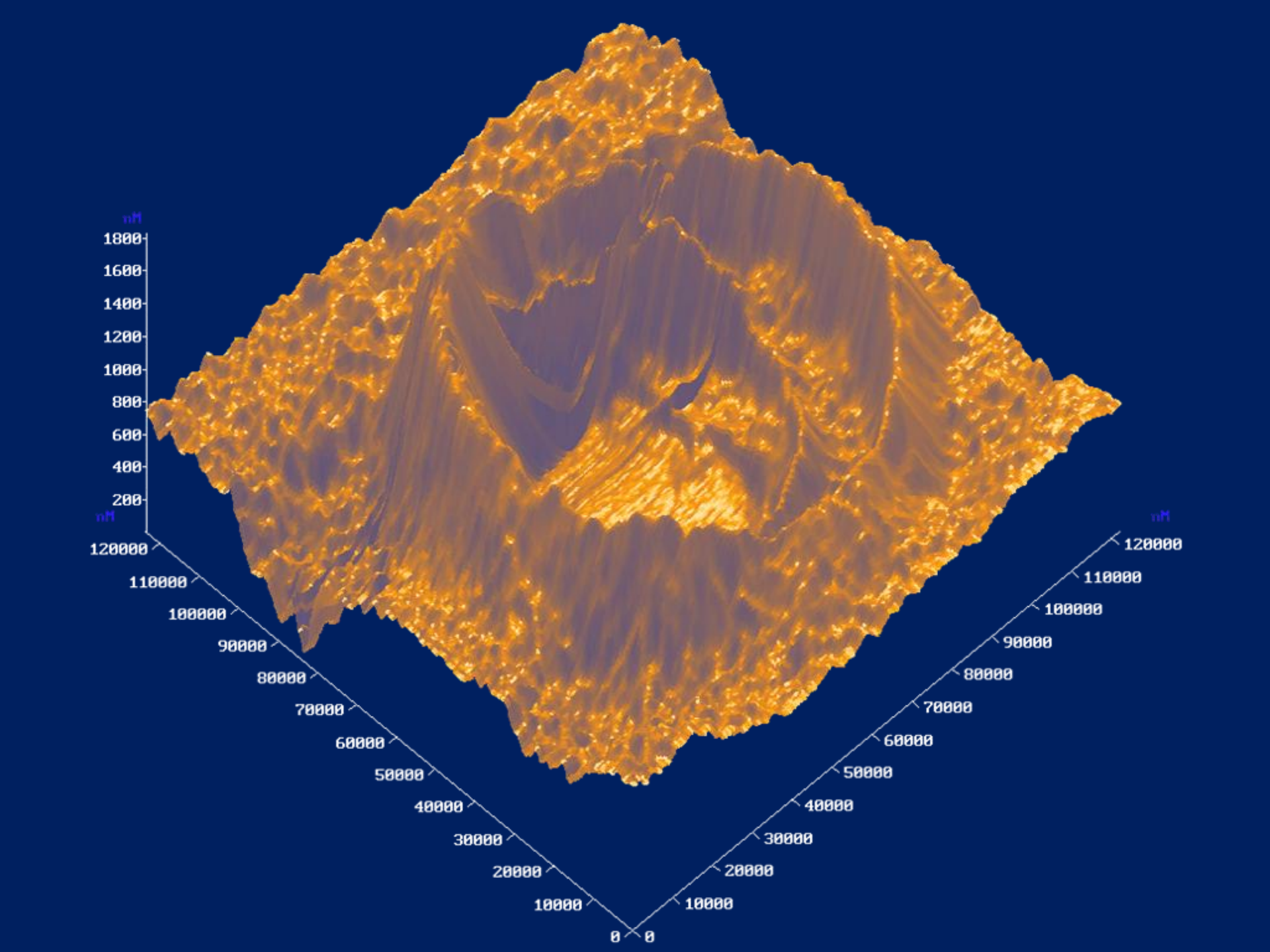




- High pH region at negative electrode due to hydrogen evolution
- Chitosan molecules deprotonated, immobilized at electrode surface

- Addressable signal guided deposition
- Temporally and spatially programmable (1-2 µm or better)




nM
1800
1600
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10000
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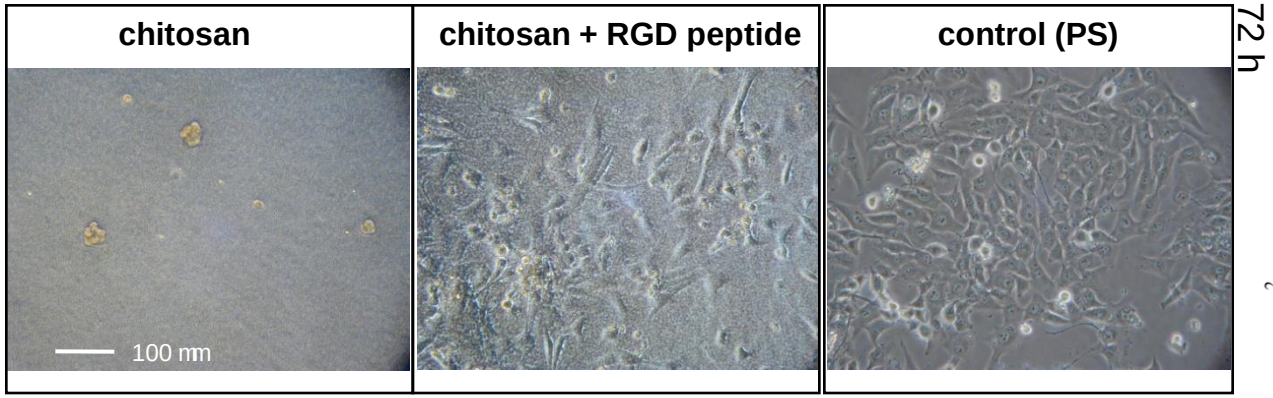
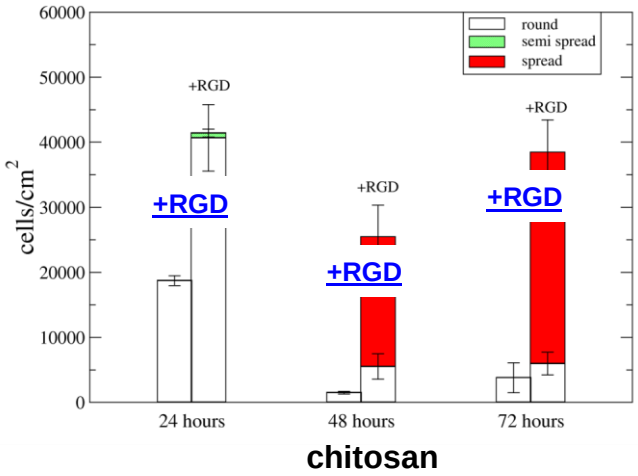
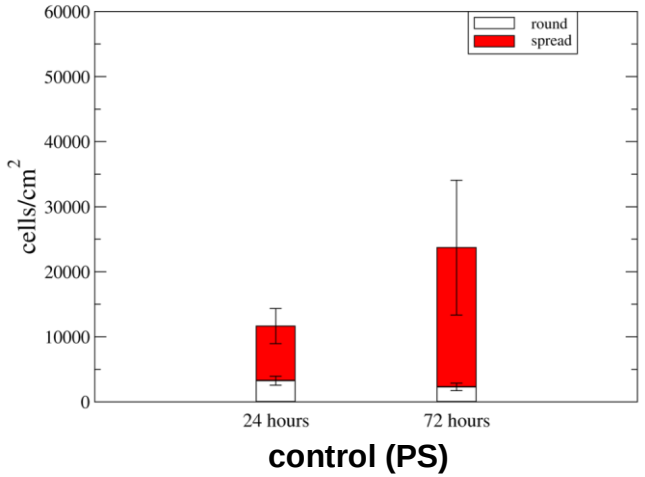
80000
90000
100000
110000
120000
nM

Human osteoblast-like cells (MG-63) adhesion on deposited chitosan films

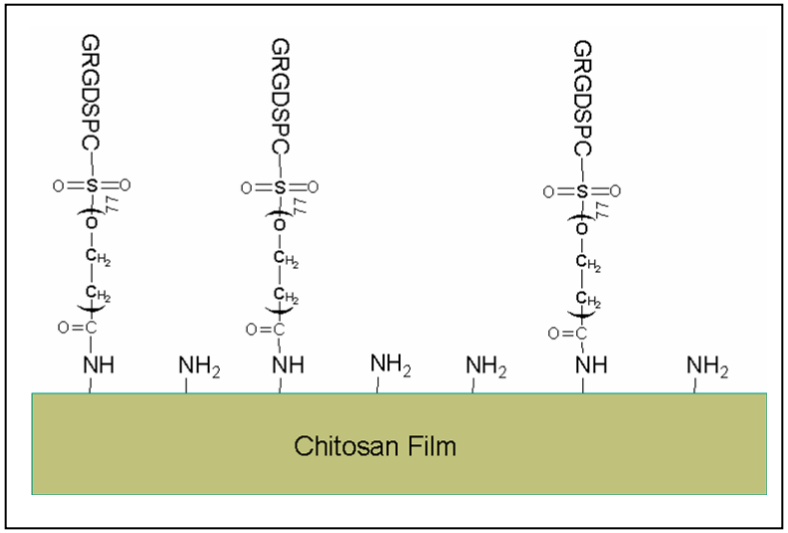


Extent of cells adhesion and proliferation





Surface functionalization process

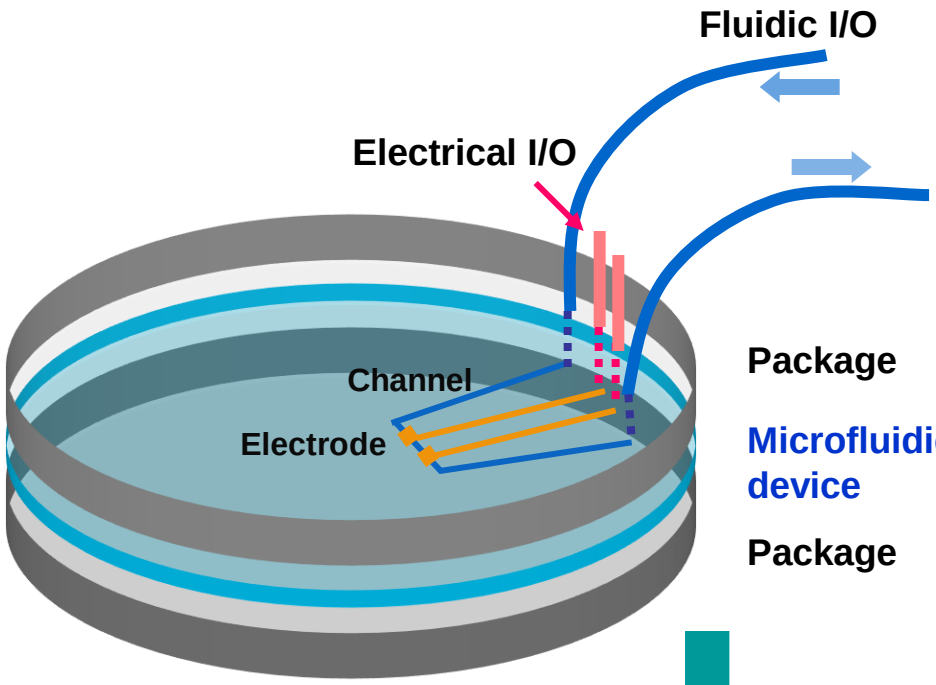


A two step chemical reaction procedure was developed in order to attach a RGD adhesion factors to an activated surface:
Step 1: amide bond through the N-hydroxysuccinimide ester (NHS)
Step 2: thiol chemistry (Vinylsulfone)

But at the mscale ?

mscale

Chitosan electrodeposition

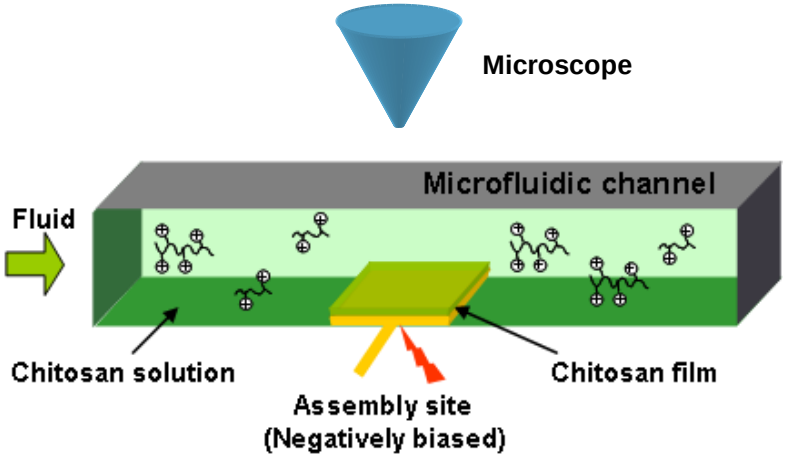


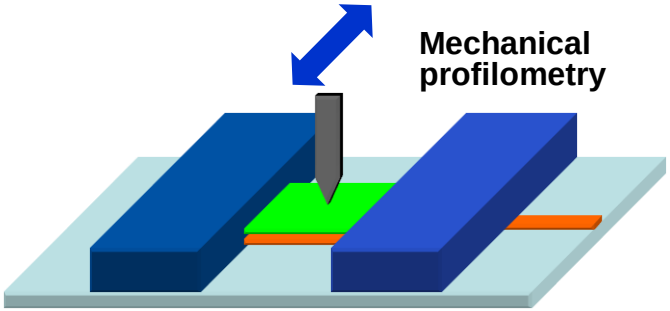
Microfluidic device and package

Leak tight fluidic system

Non-permanent sealing

Post fabrication biomolecule assembly

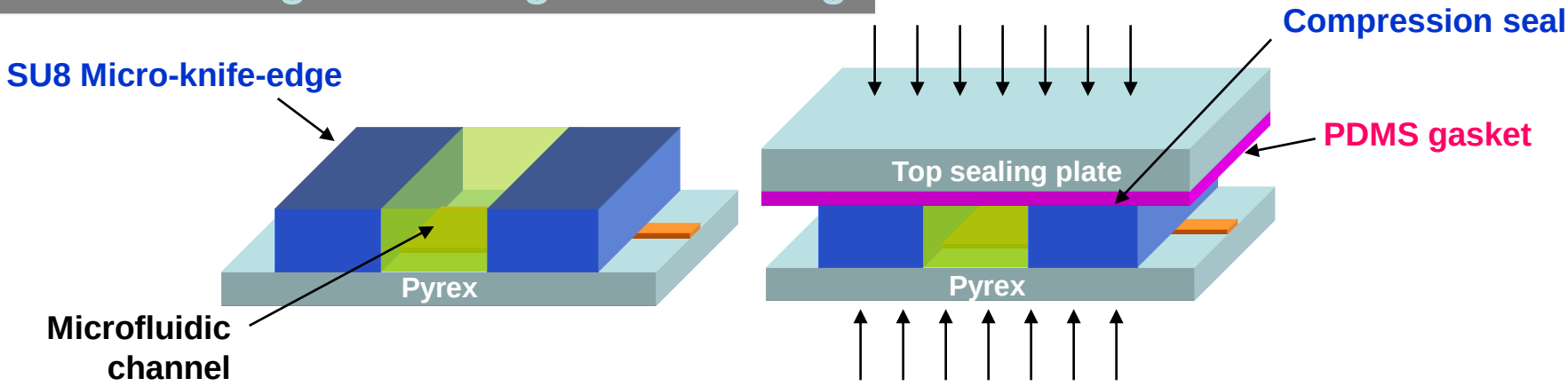


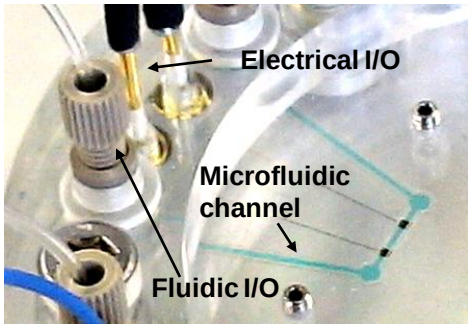


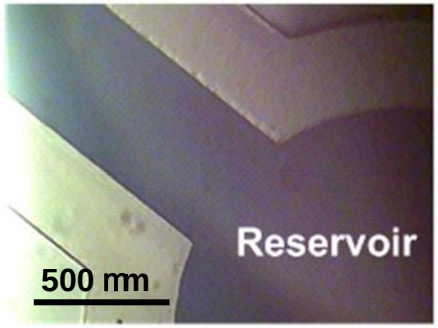
Ex-situ, post process analysis

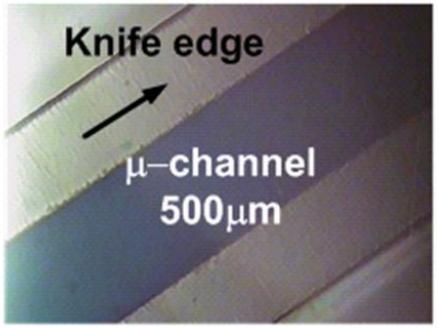
Compression Sealing

SU8 knife edge / PDMS gasket sealing







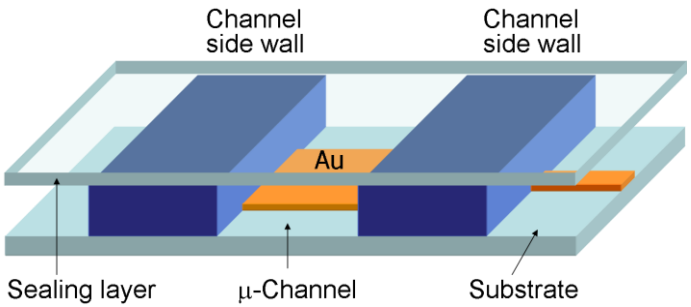


Testing flow condition

Flow rate : ~ 50 ㎖/min

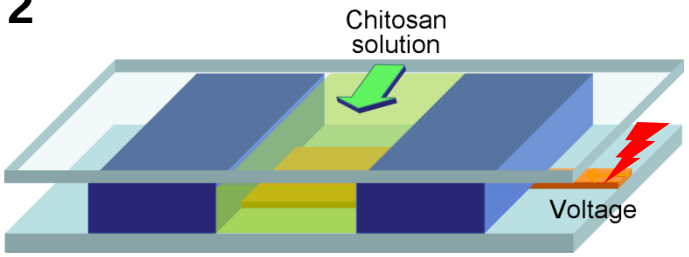
Chitosan Platform for Biomolecule Assembly in Microfluidics

1



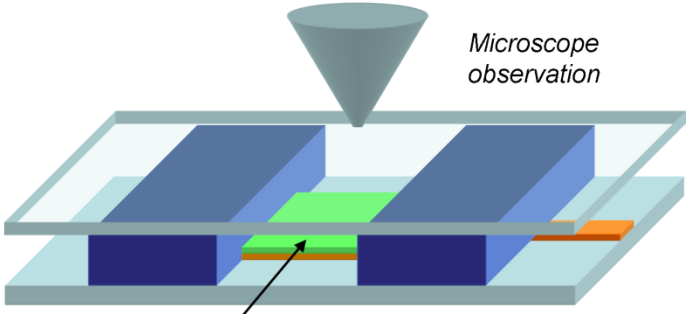
Microfabrication

2



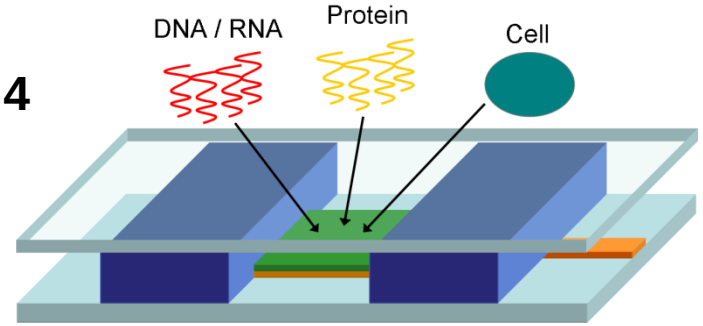
Chitosan solution and electrodeposition

3



Chitosan film

4

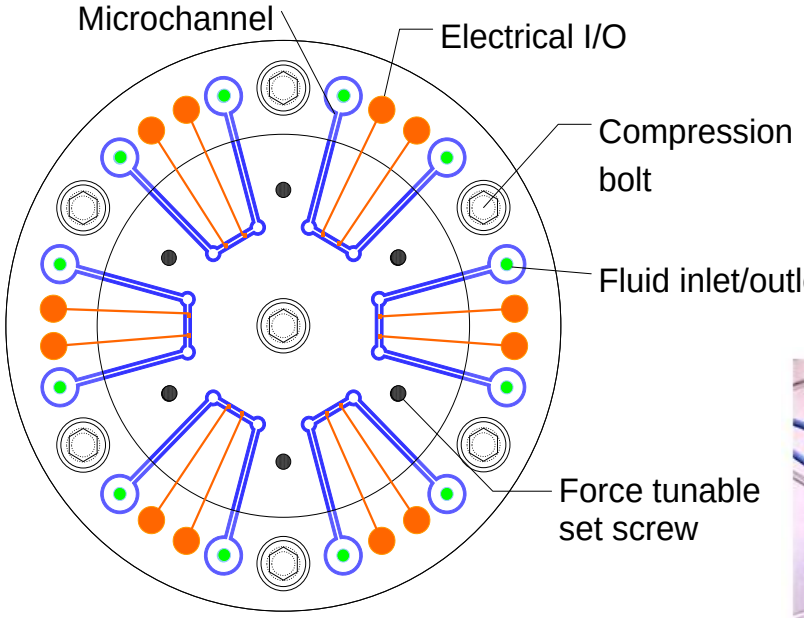


Biomolecule assembly

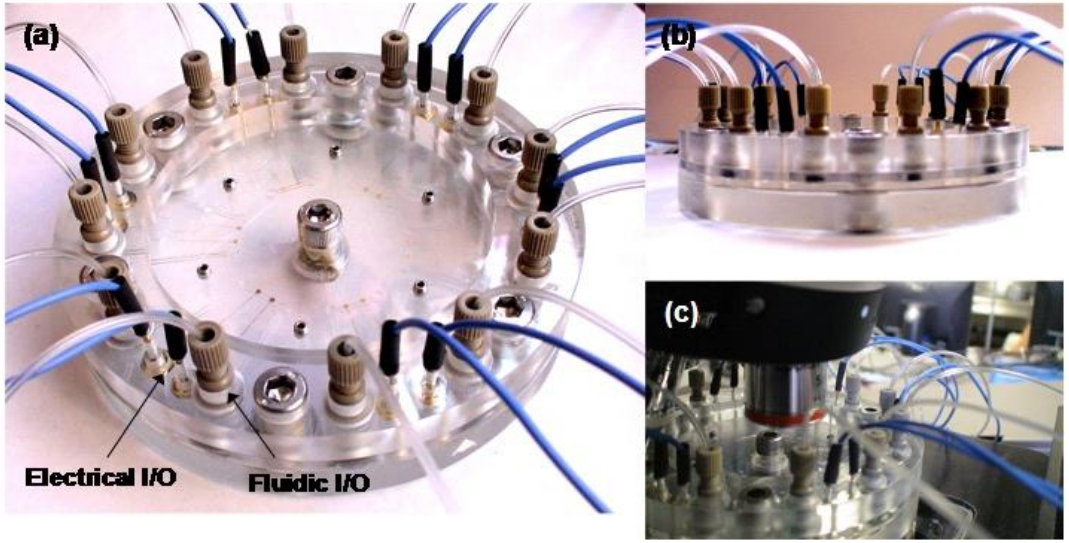
- **Transportation and reactions in microfluidic channel**
- **Biologically friendly environment for biomolecules interaction**
- **Optical observation, ex-situ analysis**

Microfluidic Package

Top view



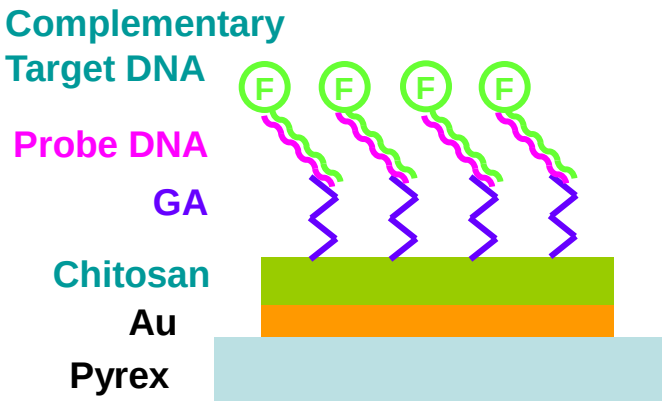
Optical images



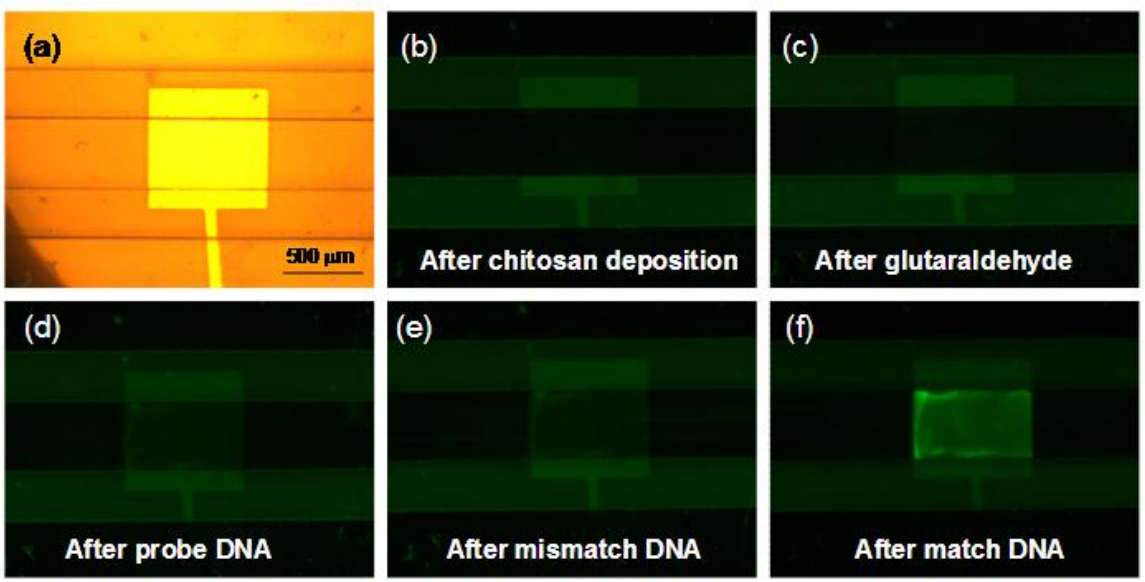
- **Plexiglas packaging system**
- **Clear access for *in-situ* optical microscopy**

RESULT: Chitosan-mediated DNA Assembly in Microfluidics

The idea: Chitosan film deposition and probe DNA coupling hybridization of target DNA



Fluorescence micrograph



- Signal-directed sequential assembly of DNAs onto addressable sites
- Mismatch target DNA didnôt react wit
- Probe DNA on the electrode retained its hybridization capacity

HAEMOCOMPATIBILITY

Why ?

Pyrolytic carbon (PyC) shows good anti-trombogenic properties, as it induces low levels of protein adsorption and platelet adhesion and activation.

For this reason it is currently used in artificial heart valves.

However, life-long treatment with anti coagulation drugs is compulsory.

Which goal ?

Find a surface coating that allows to avoid drug treatment

How ?

1) Developing suitable surface functionalization

- non toxic

- long lasting (> 30 years !!!)

2) Identifying the role of surface features and properties such as:

- carbon atoms hybridisation and surface termination
- surface and sub-surface structure
- é .

through

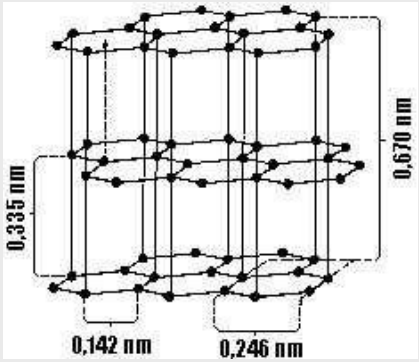
a thorough comparative investigation of the haemocompatibility of other carbon coatings (NCG, HOPG, NCD...)

so that

it becomes possible to engineer the surface

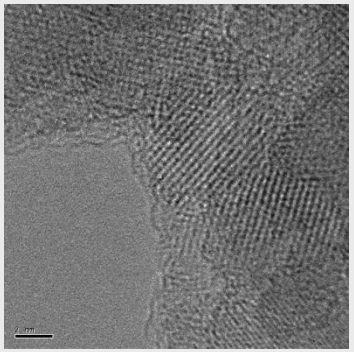
CARBON MATERIALS

Pyrolytic carbon:
Wide range of graphitic microstructures
Variable content of impurities

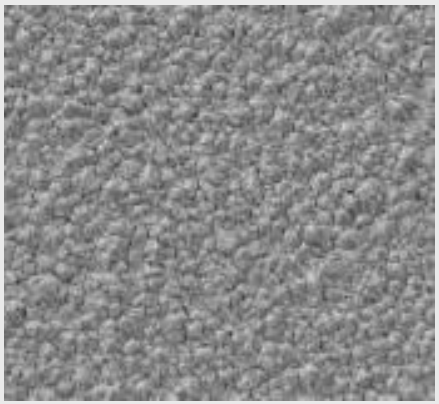


HOPG
pyrolitic graphite with a high degree of preferred crystallographic orientation

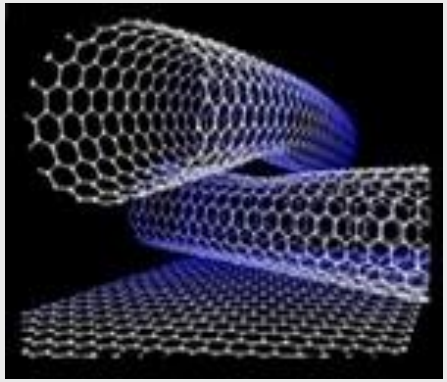
Nanographite
CVD grown nanostructured graphite



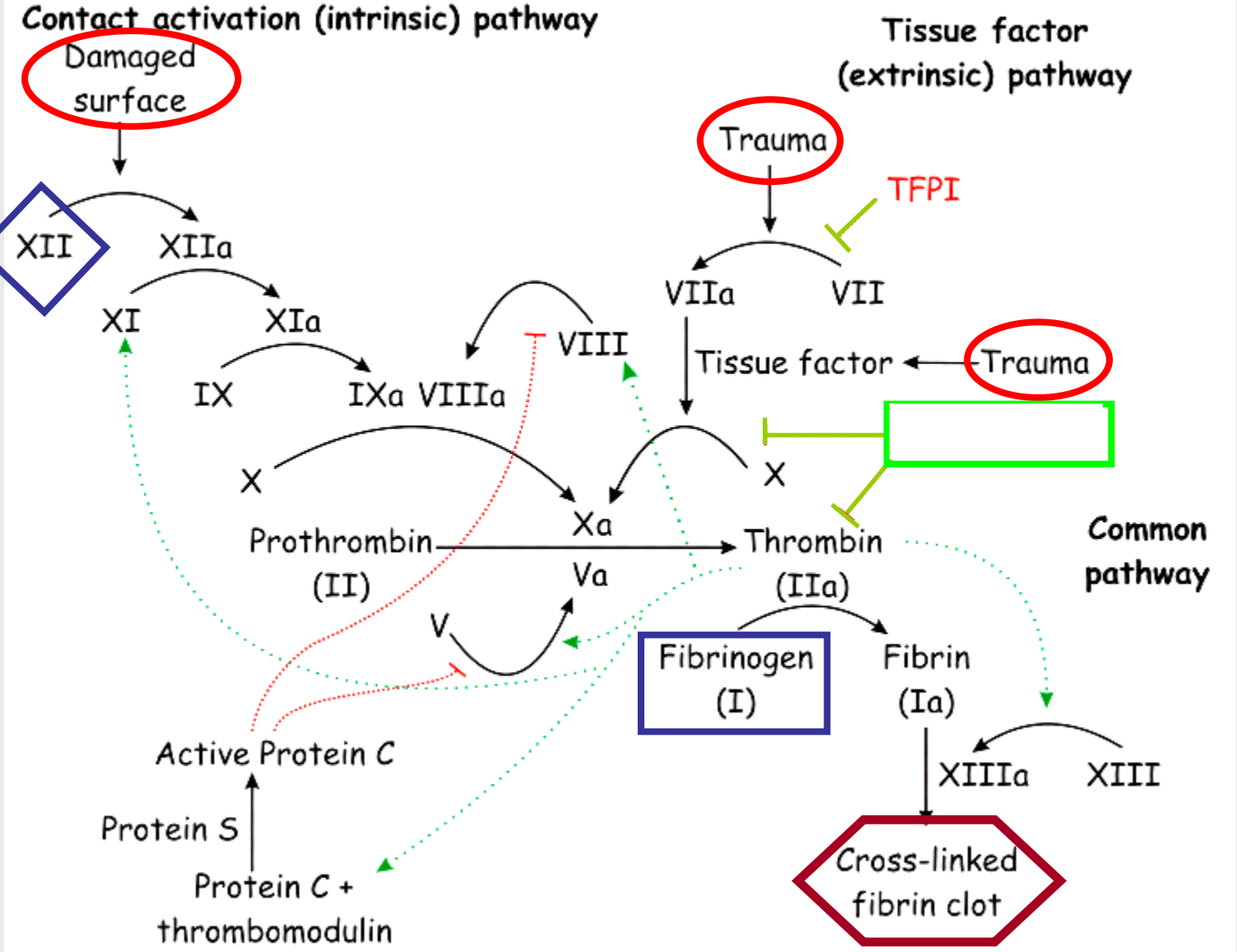
Nanocrystalline Diamond
Diamond nanocrystallites covered by sp^2 material



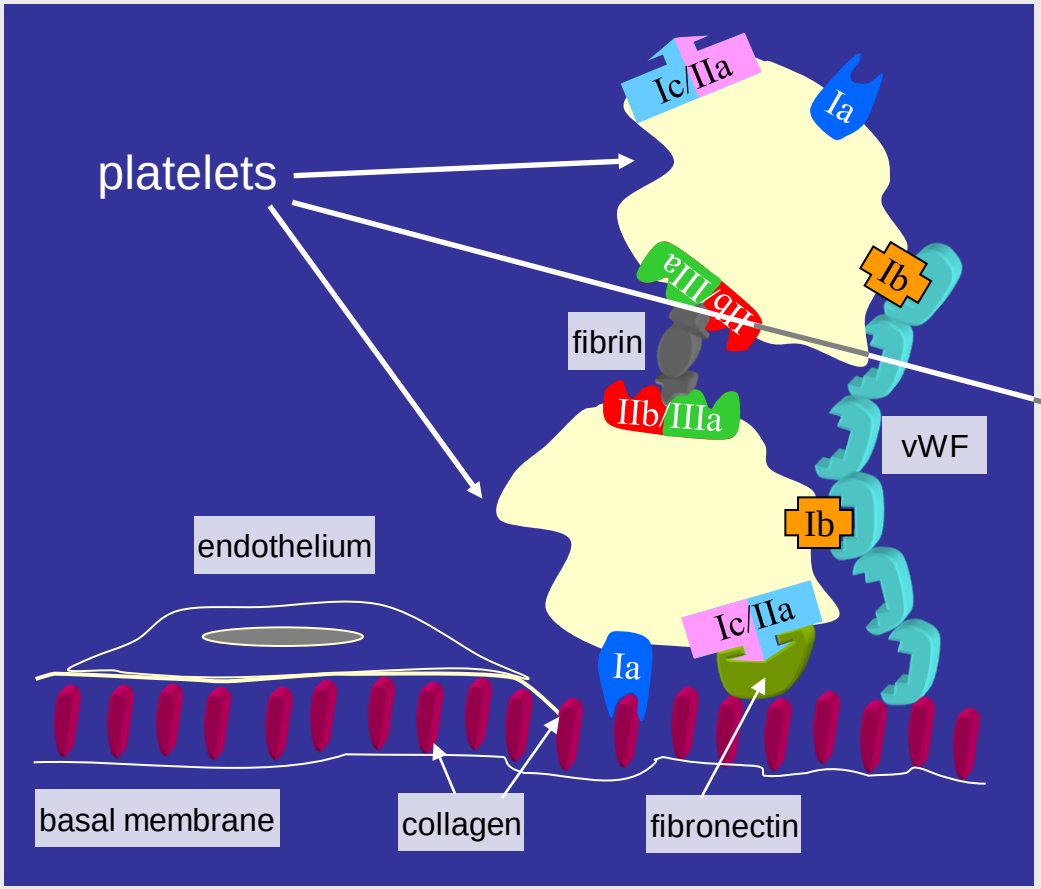
Carbon Nanotubes
Graphene sheets rolled up in tubular shape

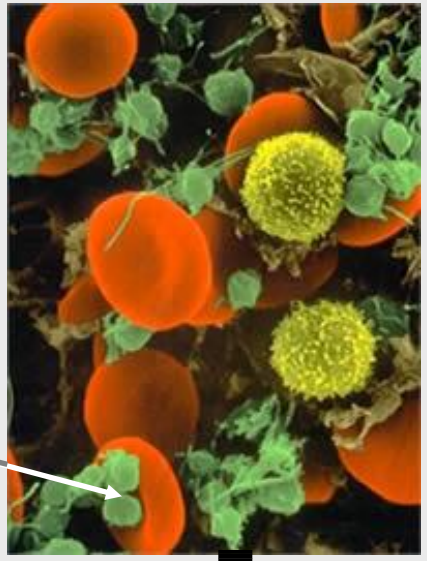


COAGULATION CASCADE



PLATELETS ADHESION & ACTIVATION

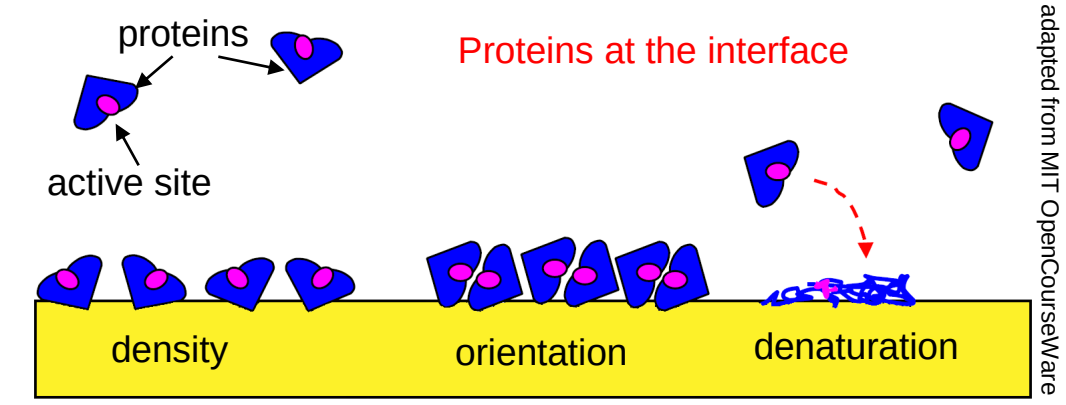




Fibrin bridges activated platelets to develop the thrombus

ANALYSIS

TECHNIQUE	Micro-BCA protein assay (on PPP)
INFO	Plasma protein surface density
MOTIVATION	Surface coverage by protein is needed for platelet adhesion
TECHNIQUE	Immunofluorescence analysis (on PPP)
INFO	Factor XII and Fibrinogen surface adsorption and distribution
MOTIVATION	Surface coverage is needed for platelet adhesion



ANALYSIS

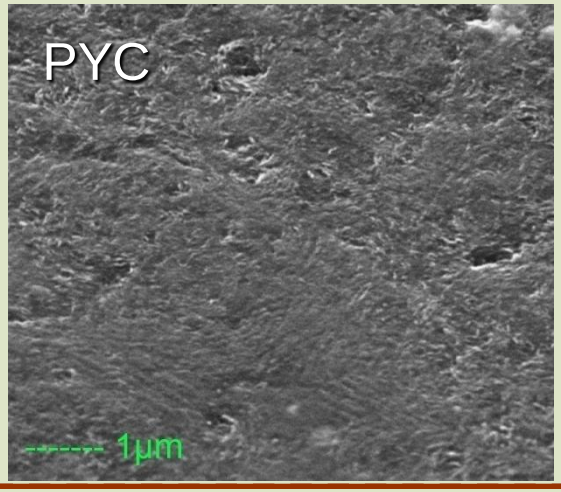
TECHNIQUE	SEM (on PRP incubated surfaces)
INFO	Platelets adhesion and activation
MOTIVATION	Platelet presence and activation are crucial to thrombus formation

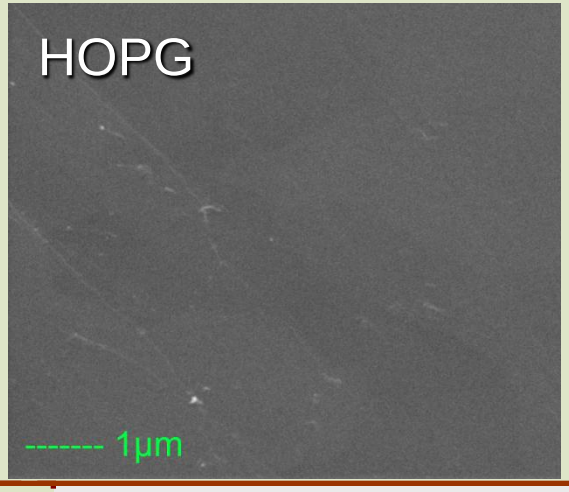
TECHNIQUE	Calcein Fluorescence Analysis (on PRP)
INFO	Platelets vitality test
MOTIVATION	Platelets vitality must not be affected

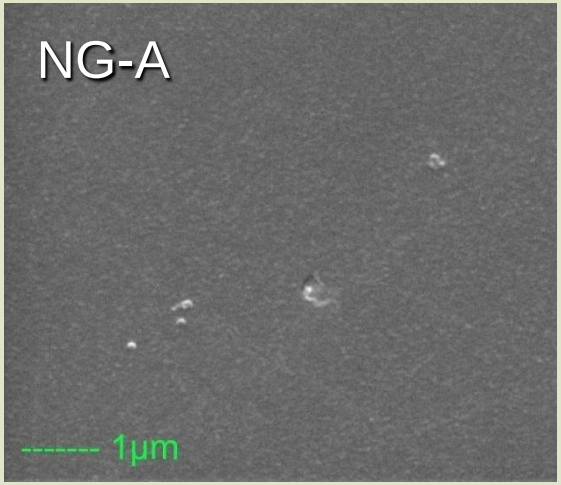
SURFACE CHARACTERIZATIONS

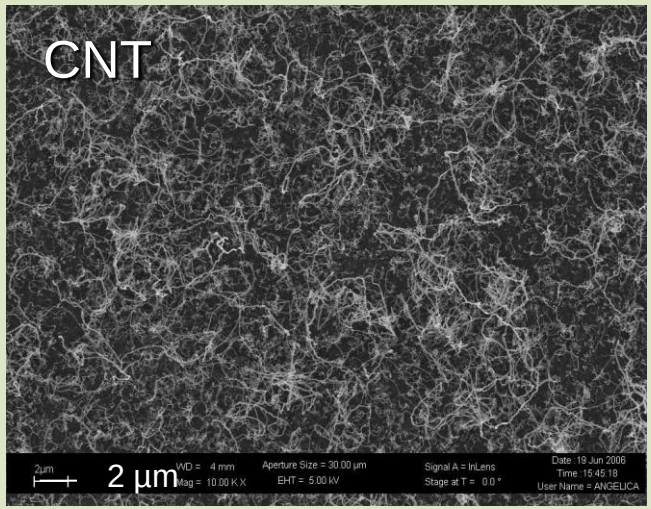
TECHNIQUE	AFM & SEM	INFO	Morphology
TECHNIQUE	XPS	INFO	Hybridisation & contamination

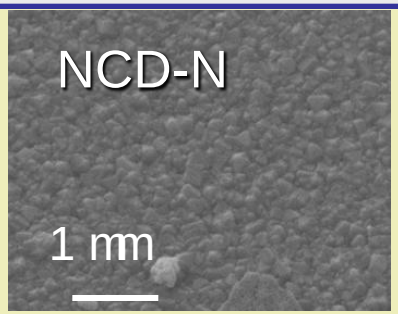
CARBON MATERIALS SURFACES

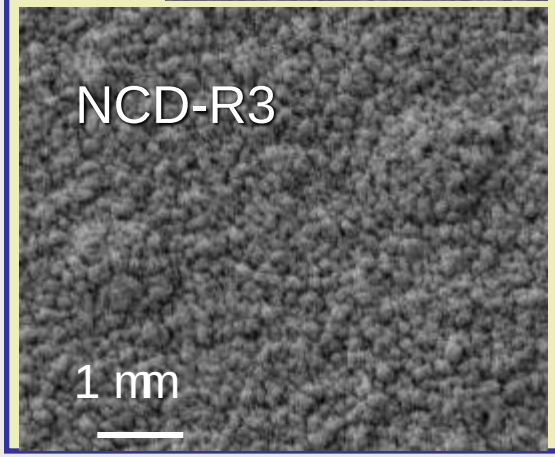


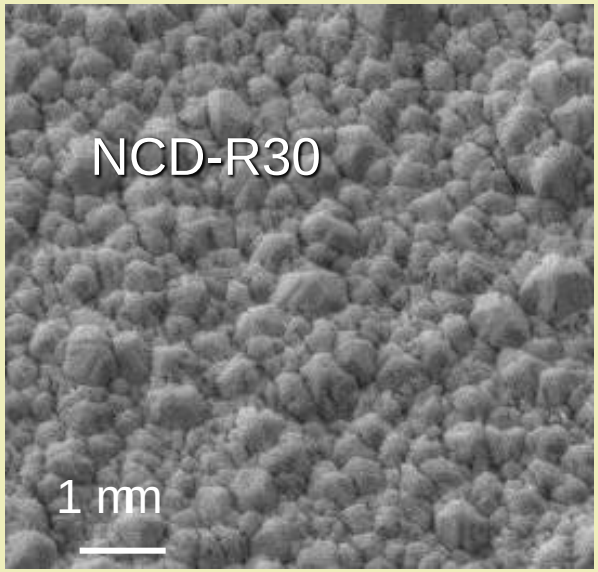






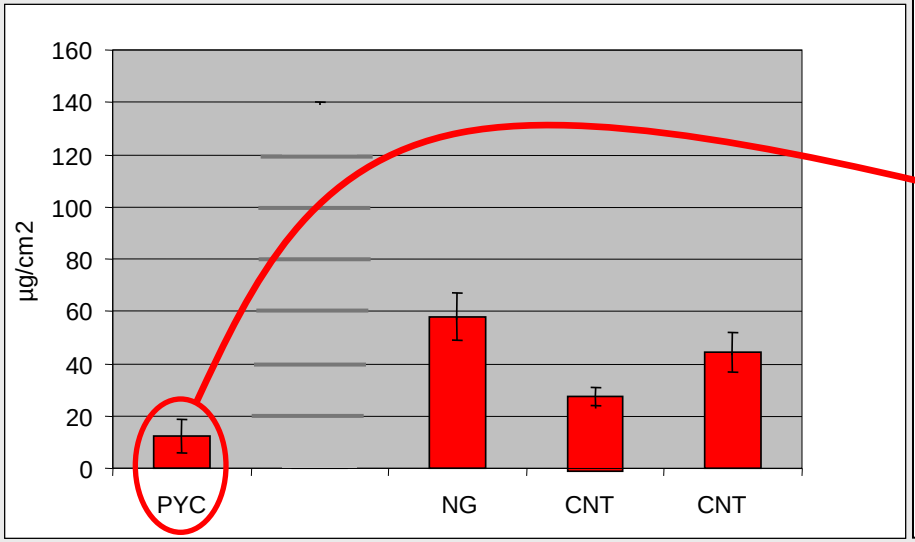


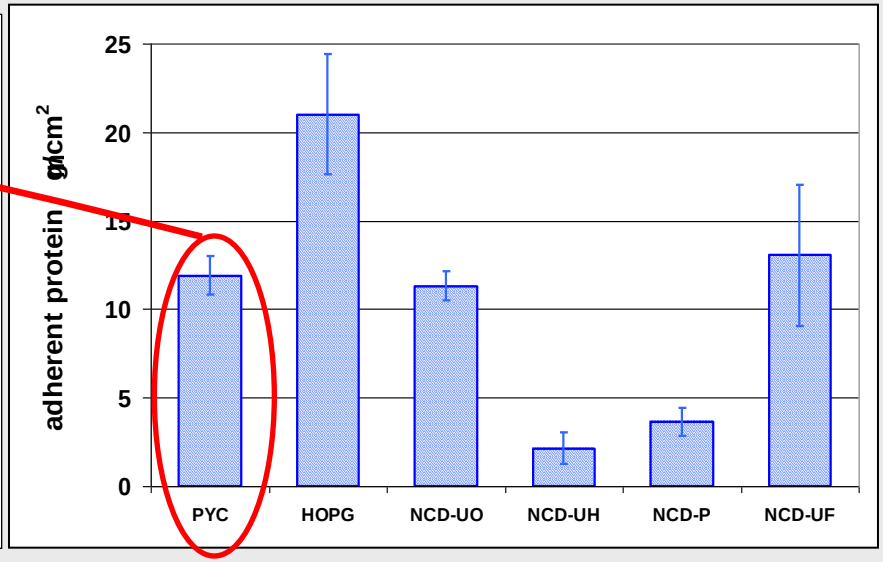




Haemocompatibility

PROTEIN SURFACE DENSITY



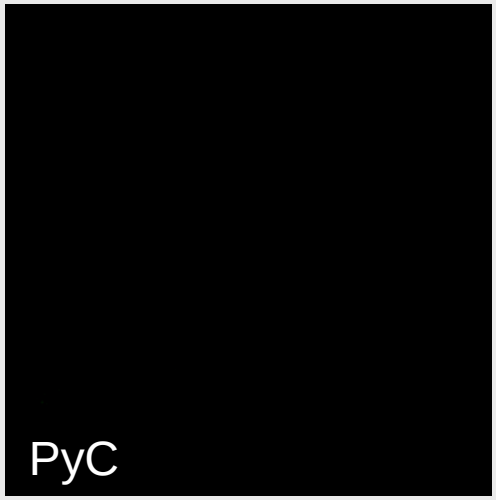


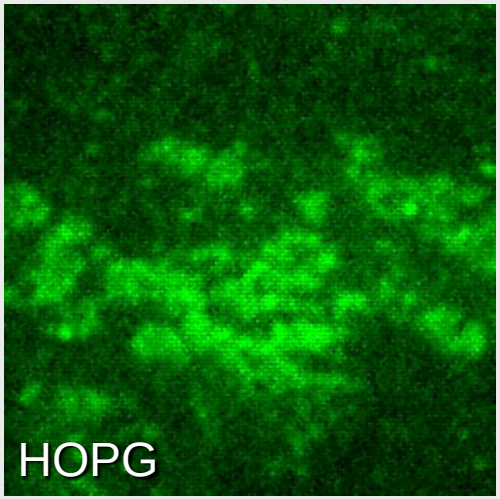
NCD has the lowest extent of protein adsorption

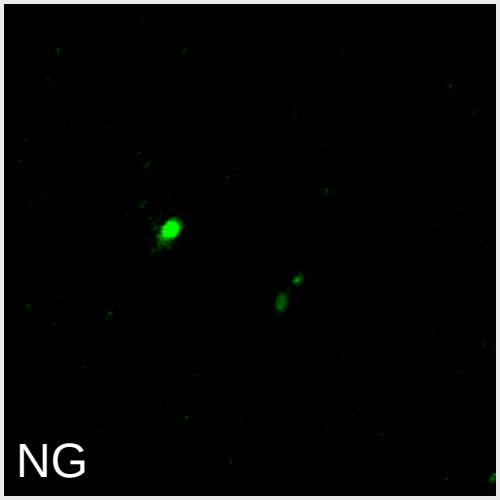
NG has the highest protein adsorption

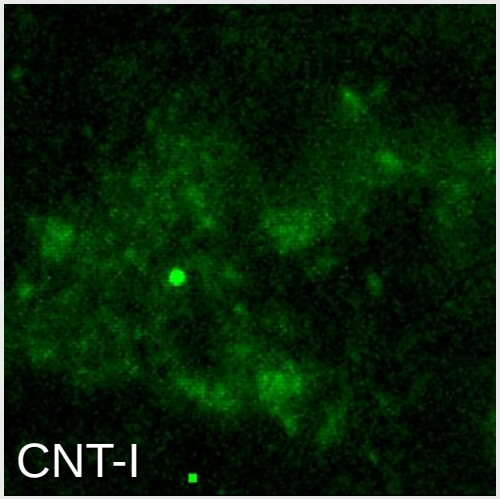
CNTs	difficult quantification due to incertitude in the exposed surface
PyC	almost all adsorbed proteins are eluted after 2 h incubation with SDS
HOPG	an additional overnight incubation is needed

FACTOR XII SURFACE DISTRIBUTION

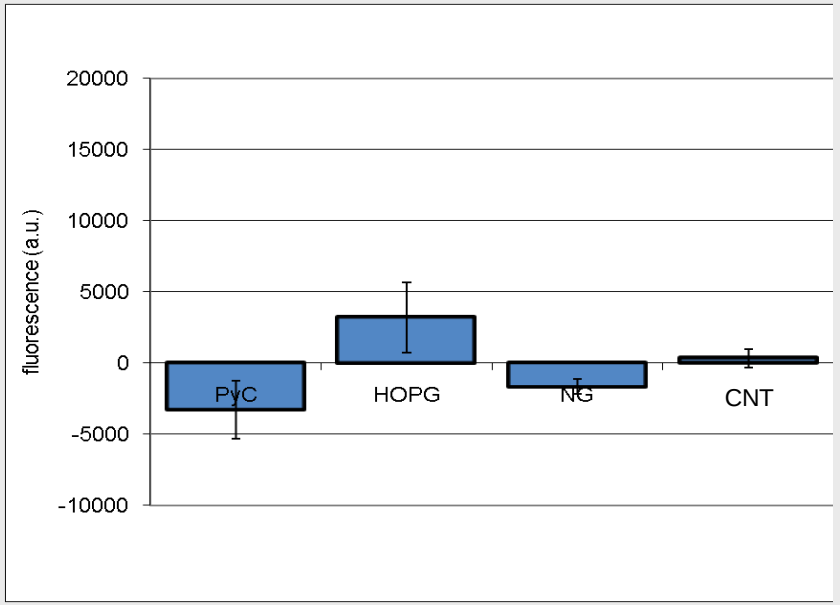






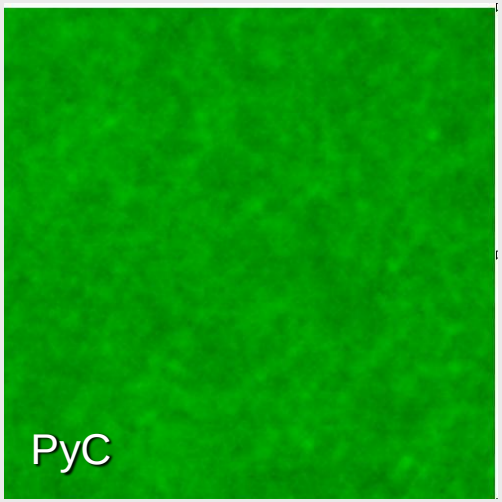


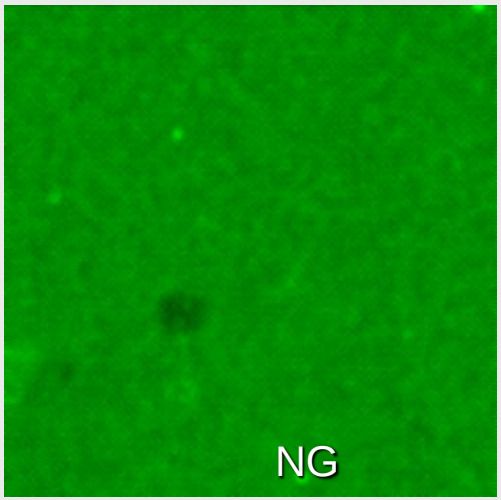
Only **HOPG** adsorbs **Factor XII**

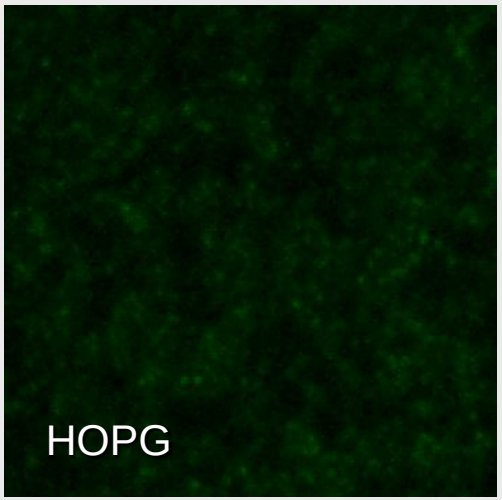


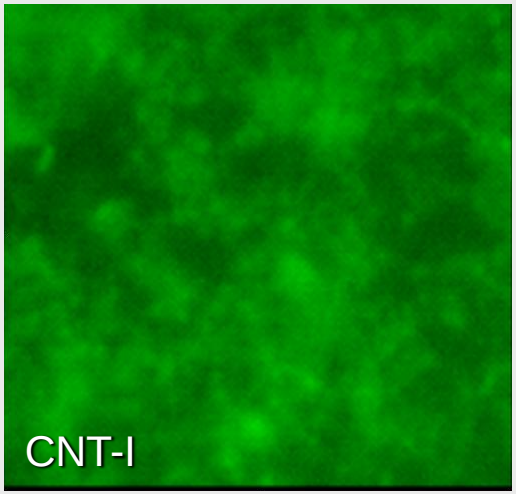
Factor XII *activates the intrinsic pathway of the coagulation cascade*

FIBRINOGEN SURFACE DISTRIBUTION

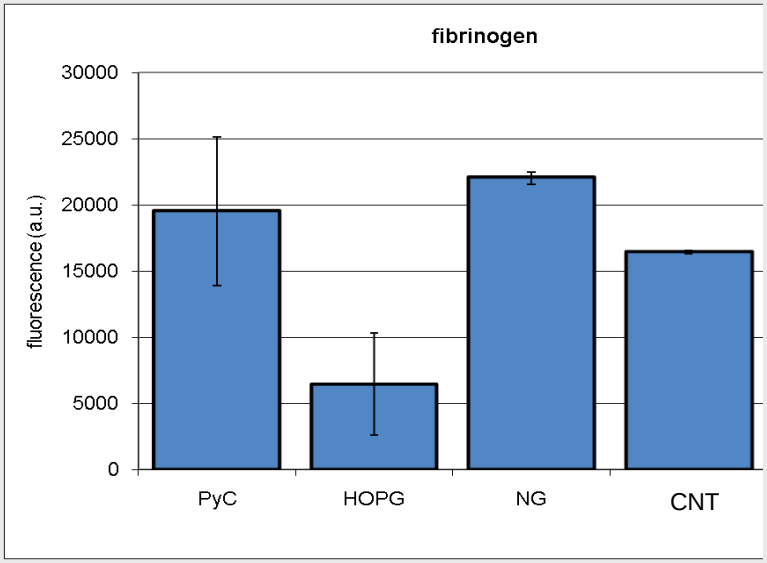




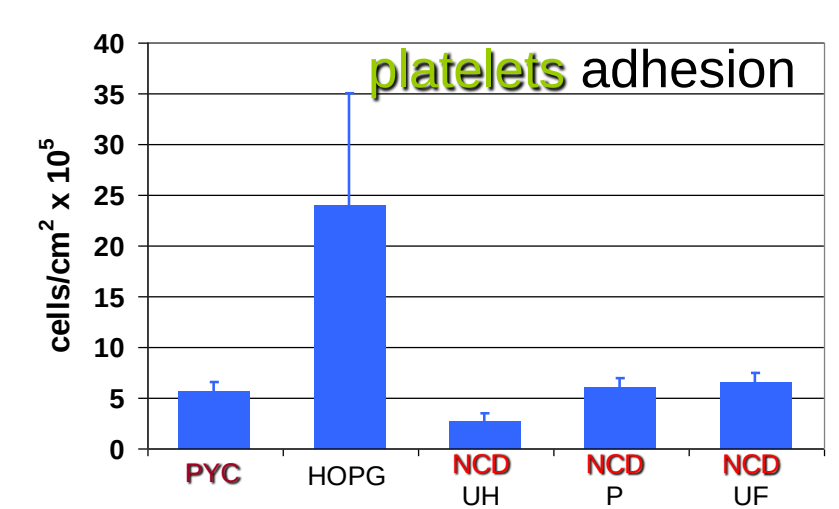


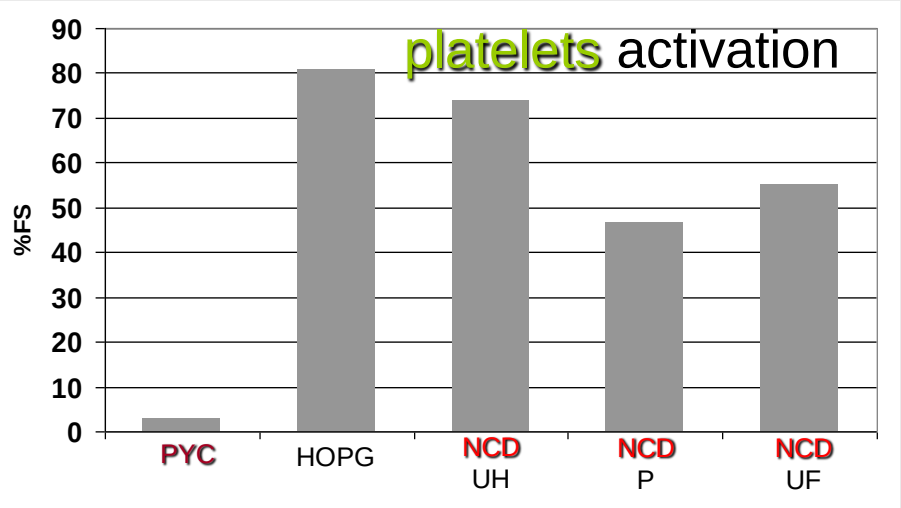


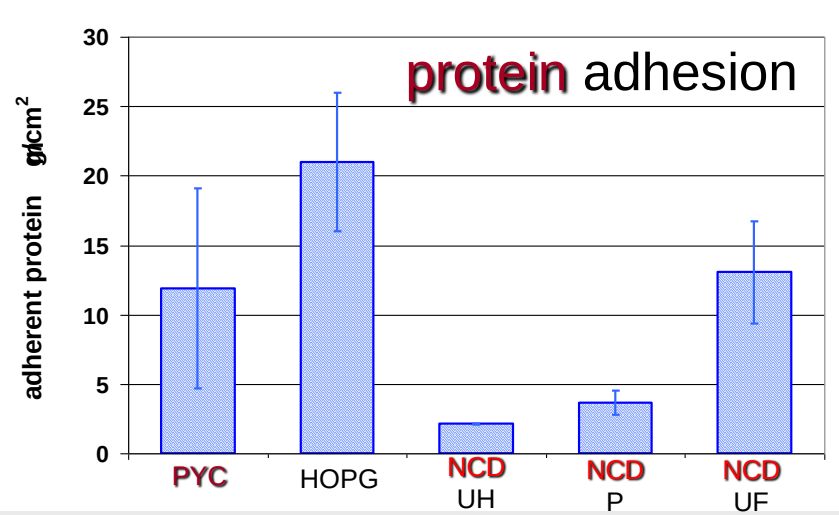
PYC & **NG** : Homogeneous Surface Distribution



PLATELETS & PROTEINS





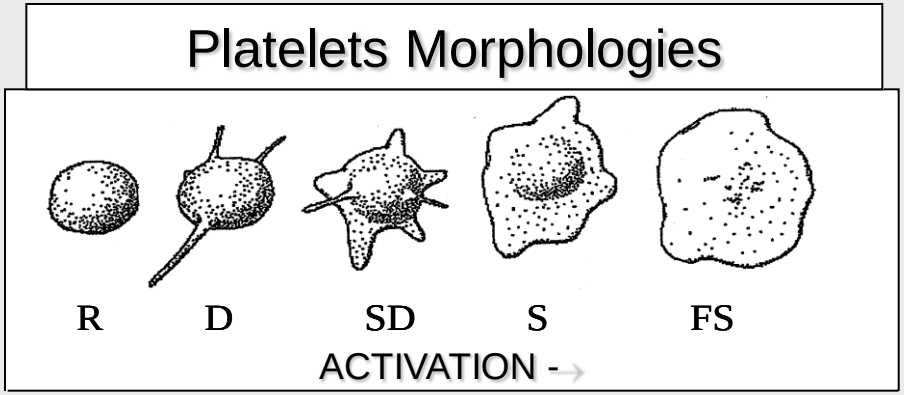


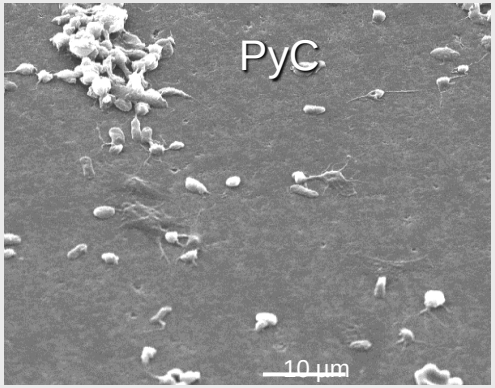
PyC shows low protein adhesion and weak platelets activation

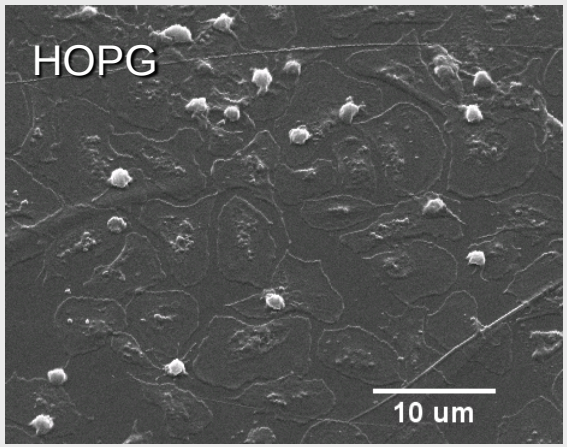
HOPG show a large amount of adhering proteins and activated (FS) platelets

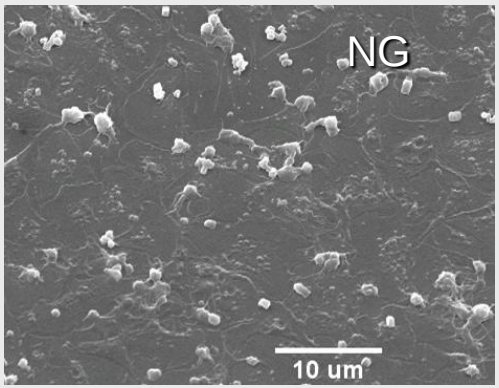
NCDs have low protein and platelets adhesion but high fraction of activated platelets

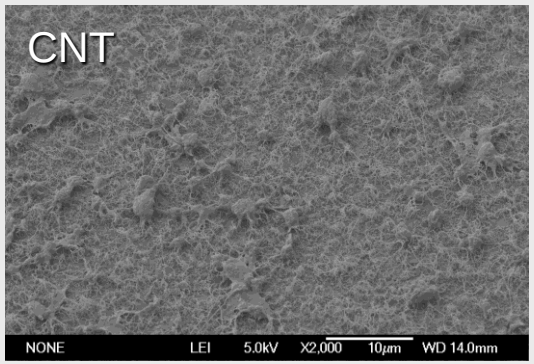
PLATELETS ADHESION & ACTIVATION

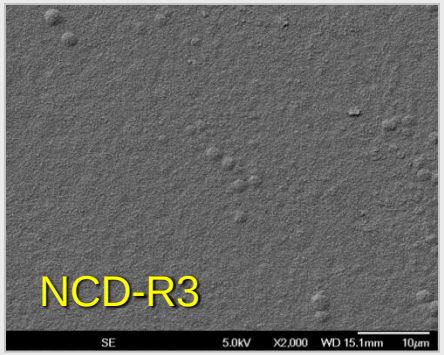


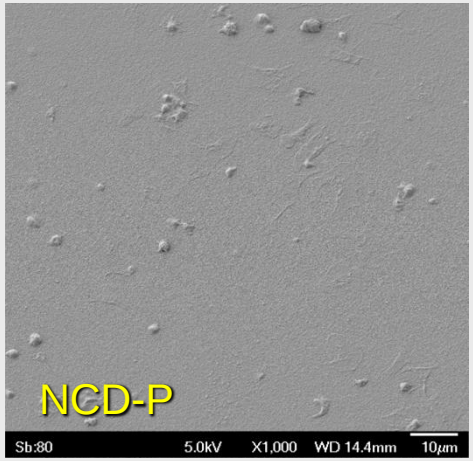






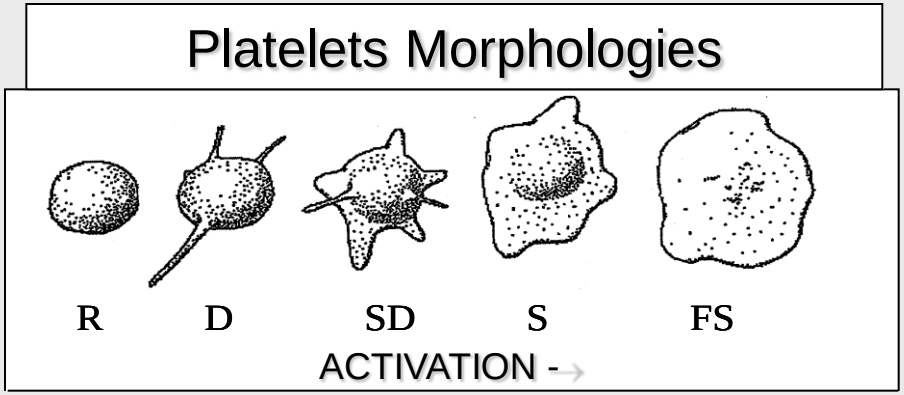


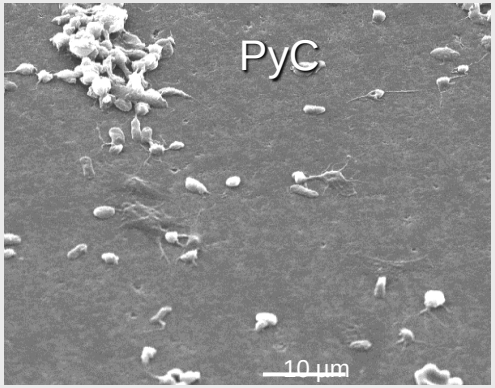


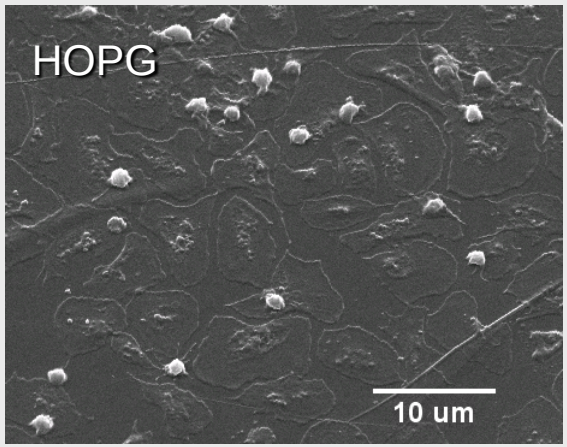


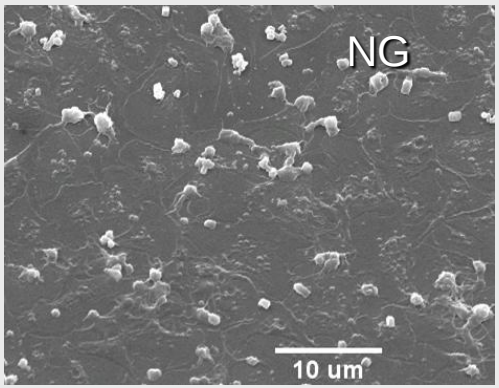
Haemocompatibility

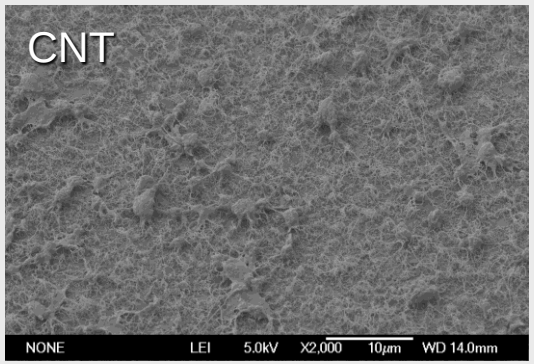
PLATELETS ADHESION & ACTIVATION

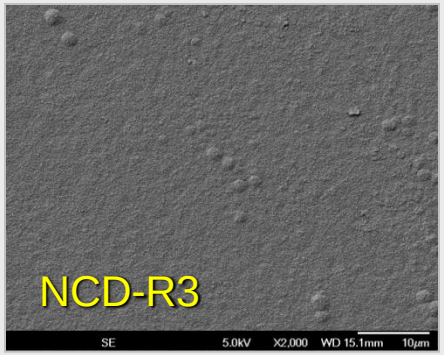


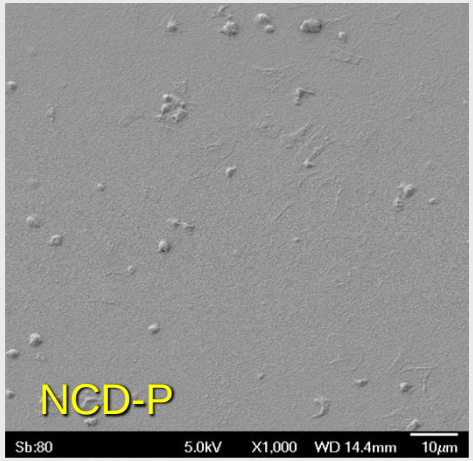






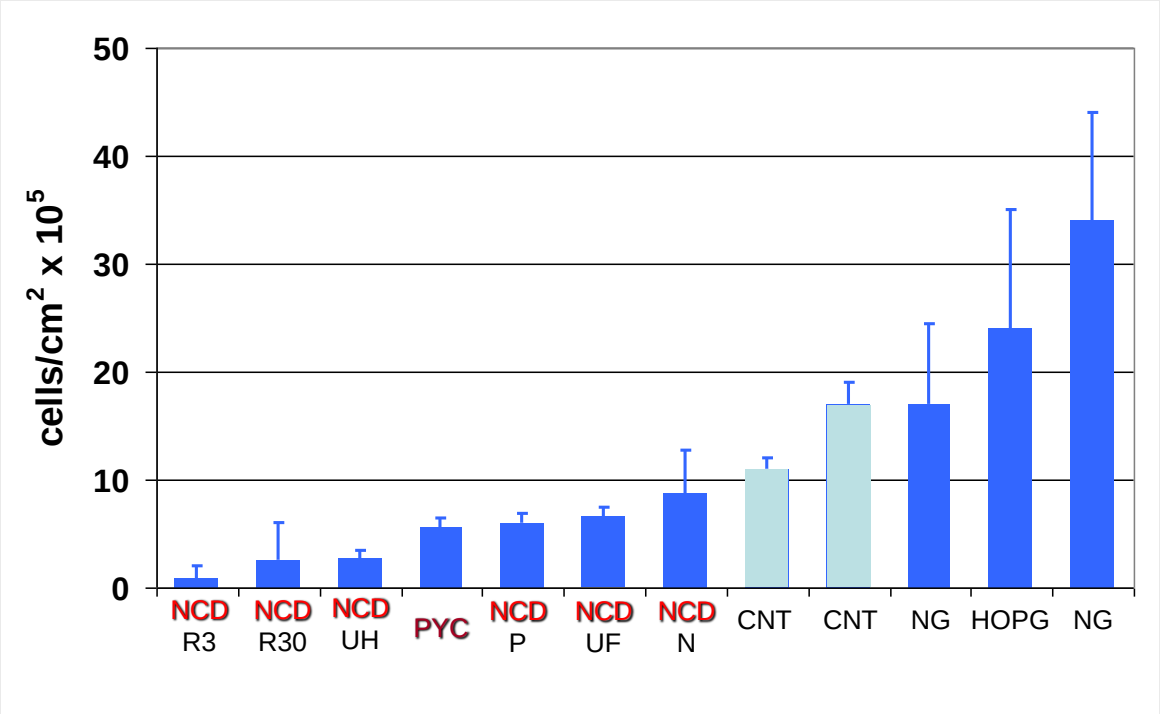






PLATELETS ADHESION

Randomly picked areas
(4-6 regions/sample)
Field size 100 x 100 µm²

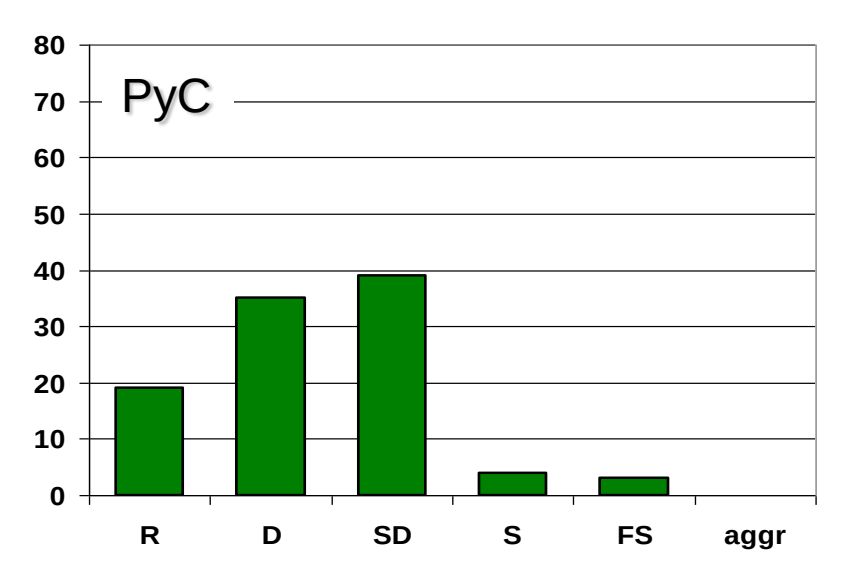


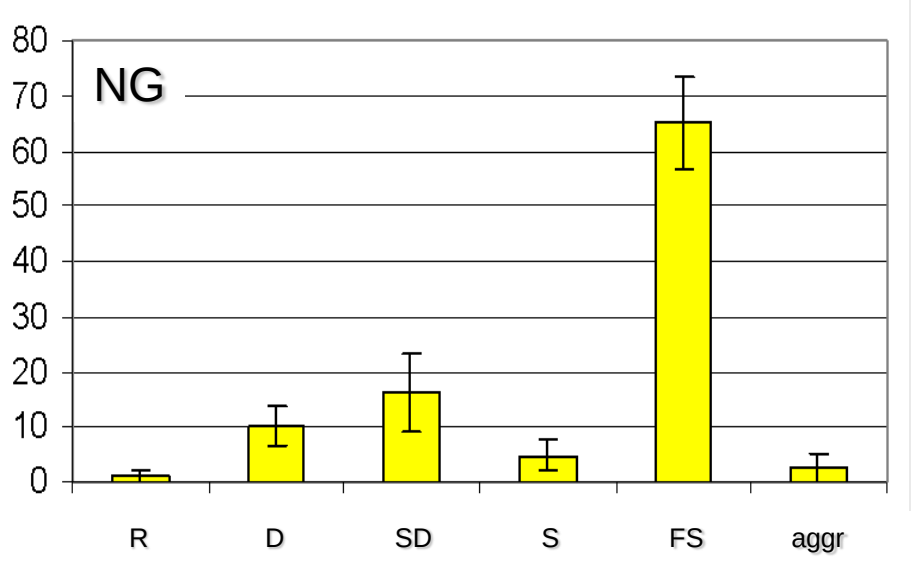
NCDs shows low platelet adhesion (sometimes lower than PyC)

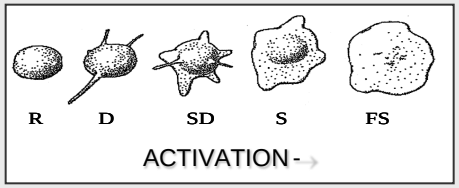
HOPG & NG show large amount of adhering platelets

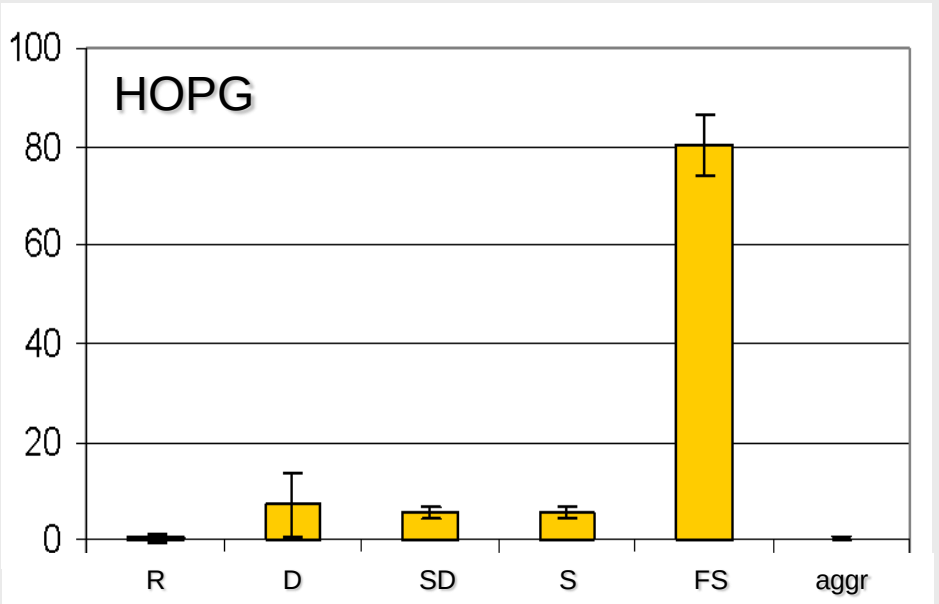
CNTs surface value is approximate

PLATELETS ACTIVATION







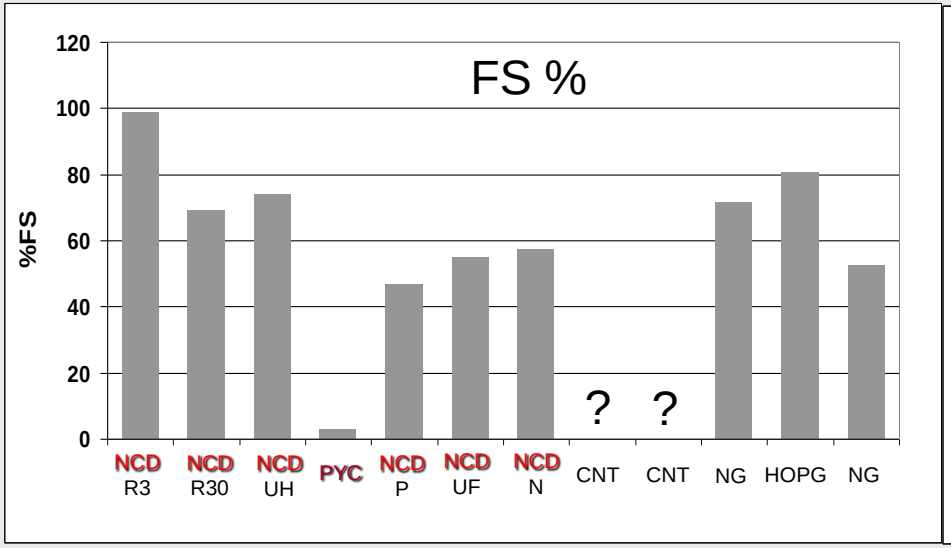


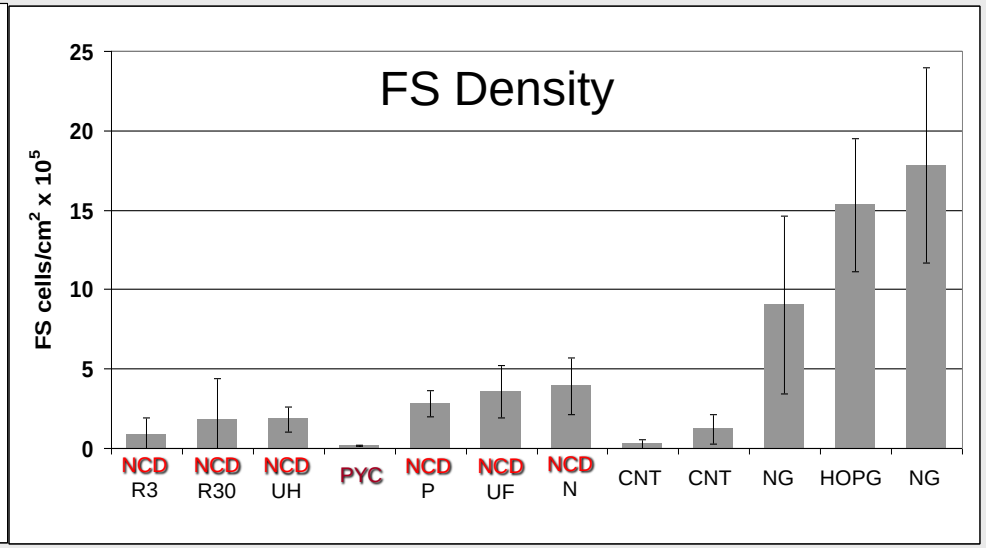
PyC shows low platelet activation

HOPG & **NG** show a large amount of activated cell (FS)

CNTs cut adhering platelets

PLATELETS ADHESION & ACTIVATION

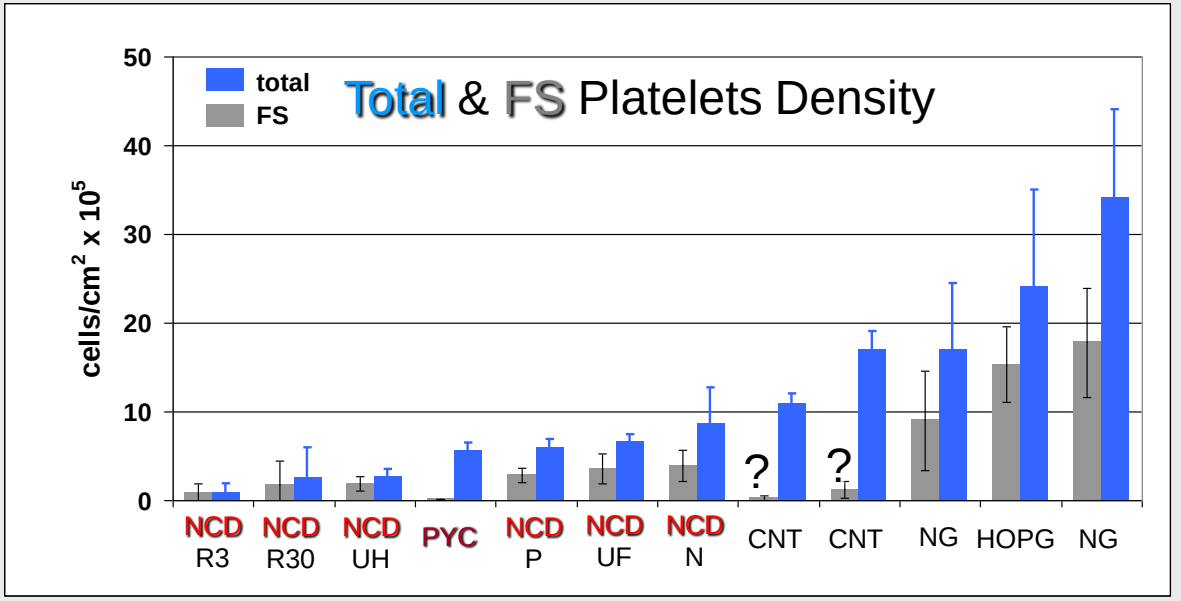




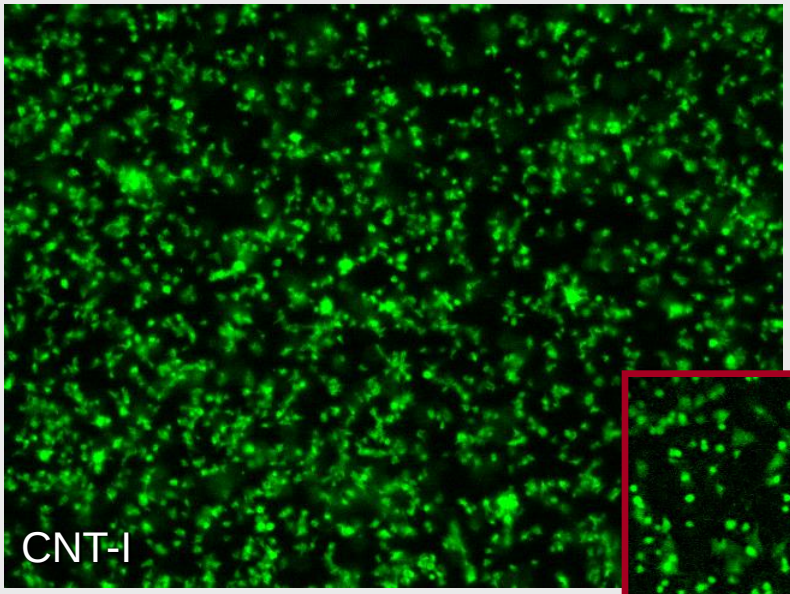
PyC has a very low amount of activated platelets

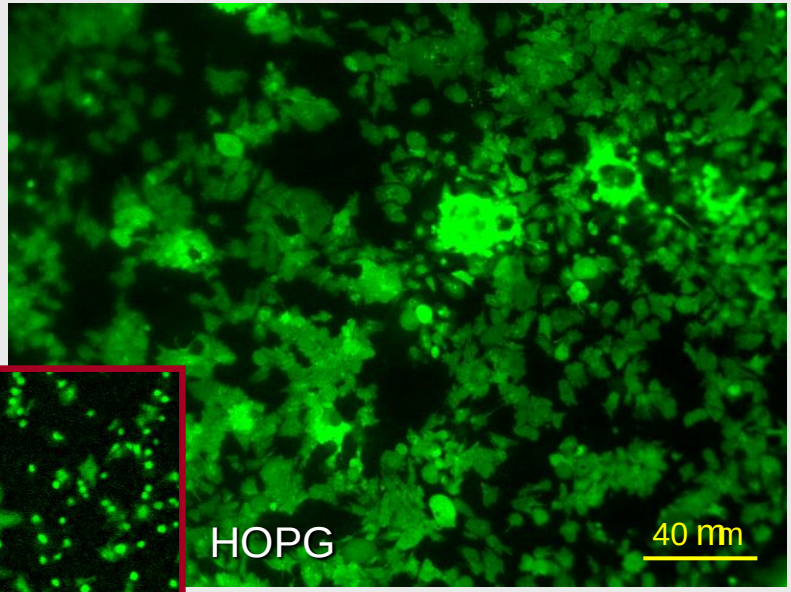
HOPG & NG have a large amount of (mostly activated) platelets

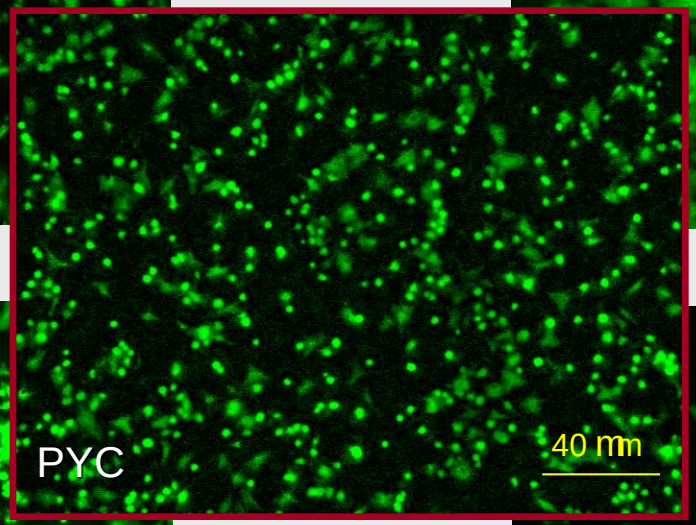
NCDs have low amount of (mostly activated) platelets

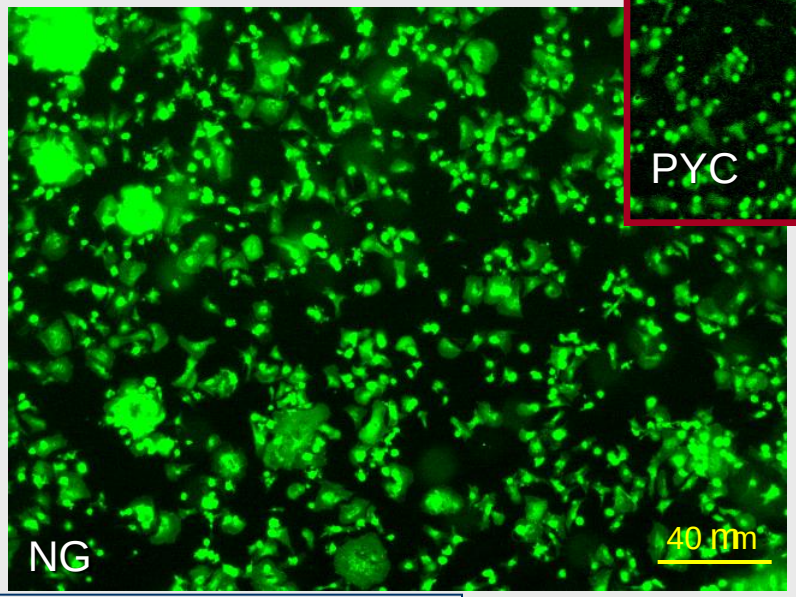


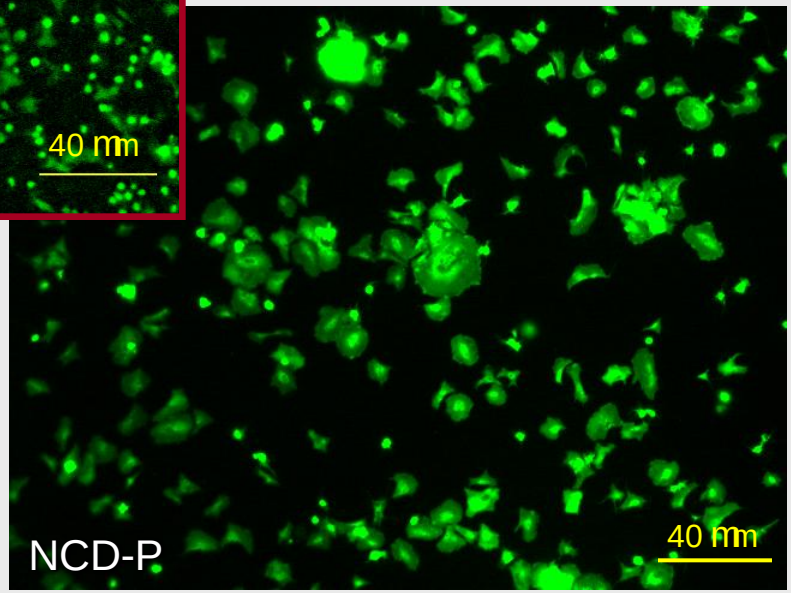
PLATELETS VITALITY TEST





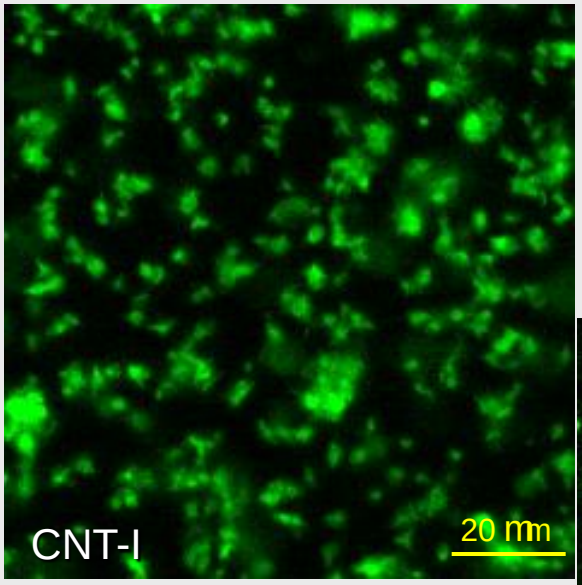


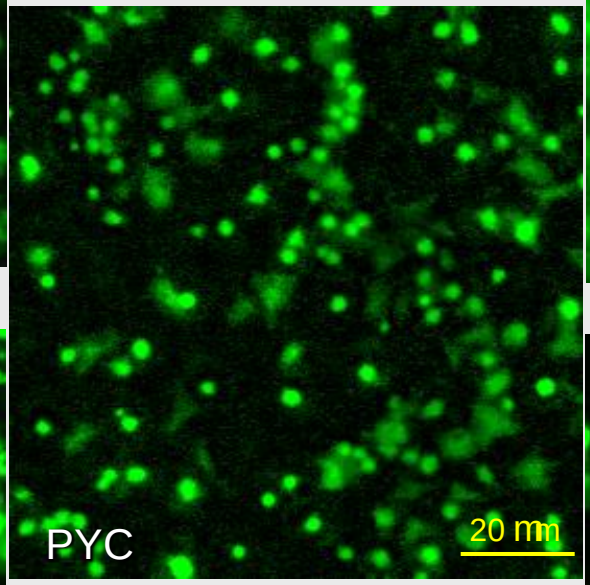


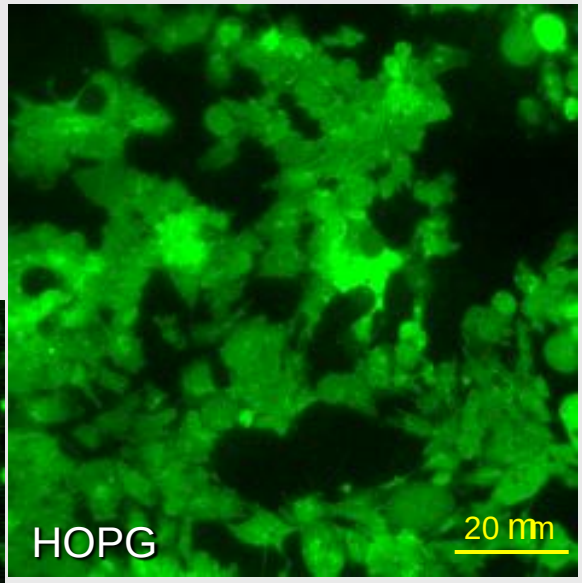


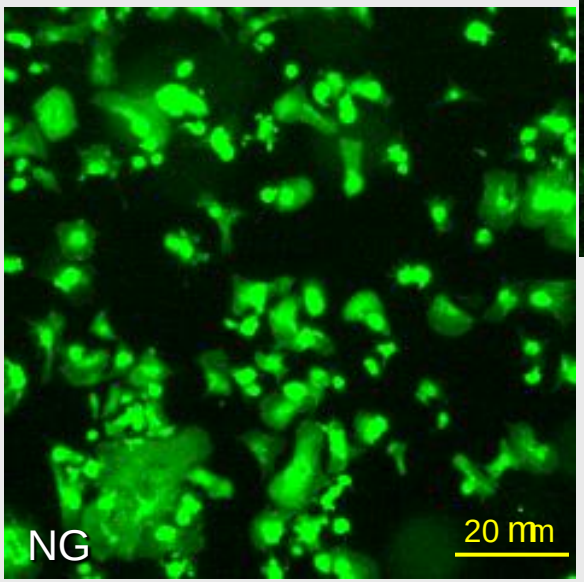
PLATELETS VITALITY TEST

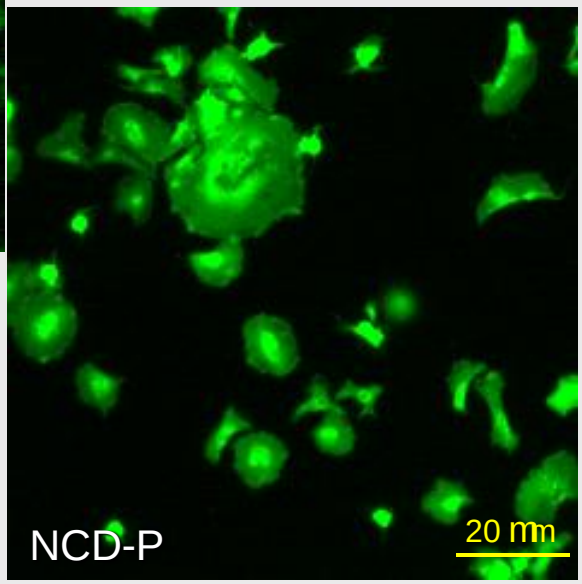
Field size
100 x 100 μm²



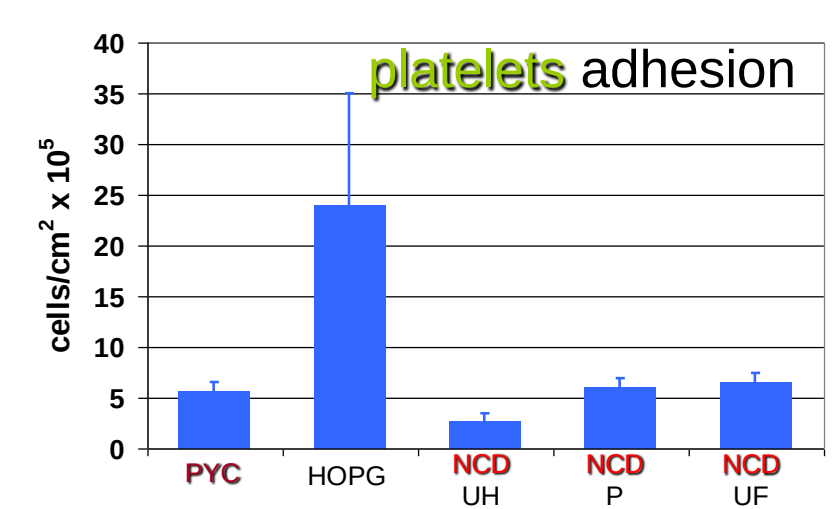


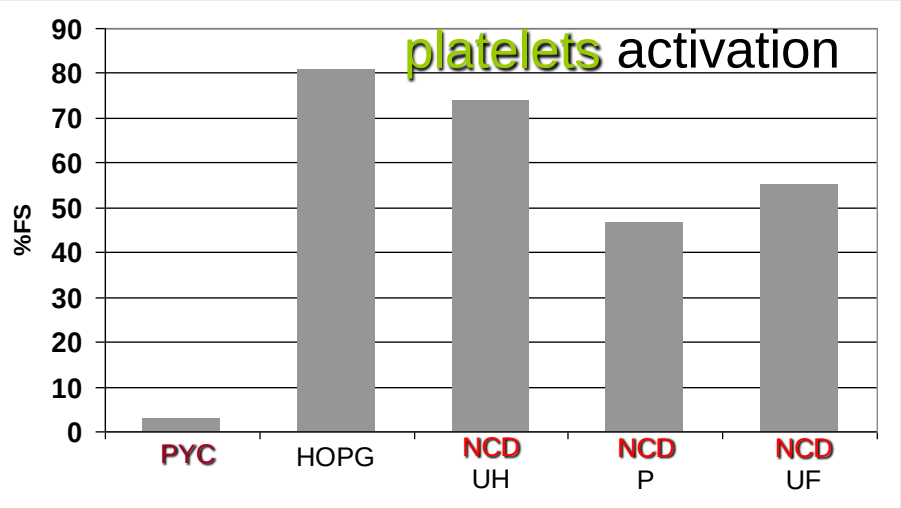


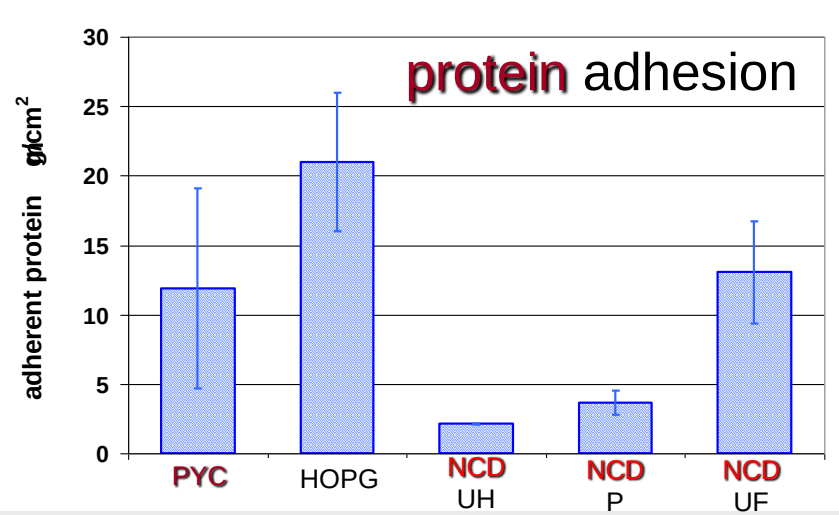




PLATELETS & PROTEINS





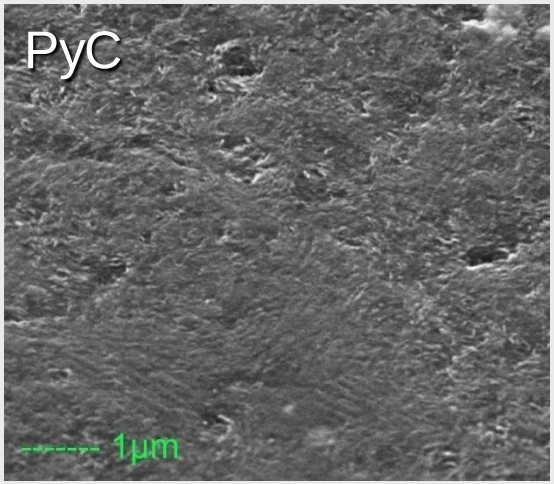


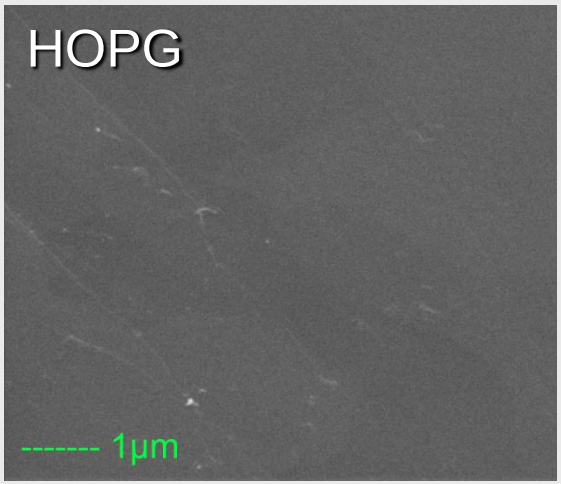
PyC shows low protein adhesion and weak platelets activation

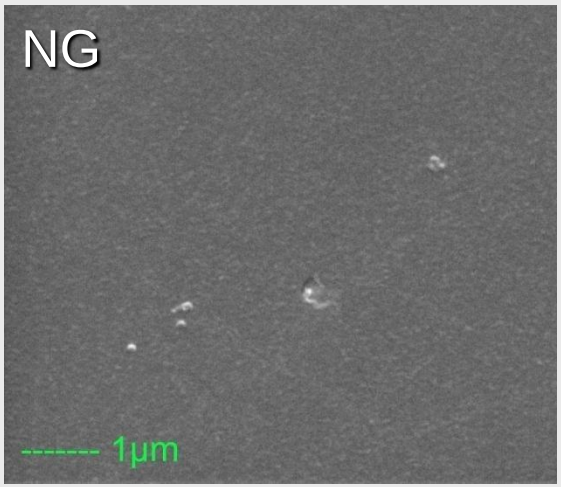
HOPG show a large amount of adhering proteins and activated (FS) platelets

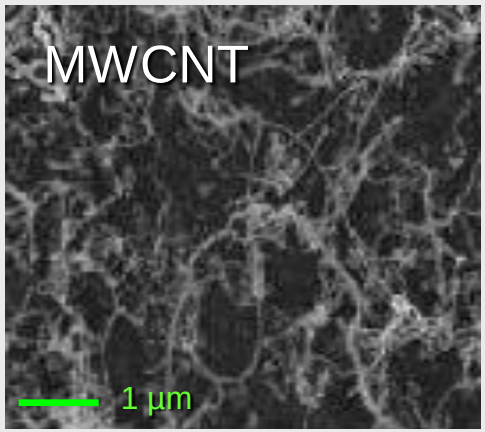
NCDs have low protein and platelets adhesion but high fraction of activated platelets

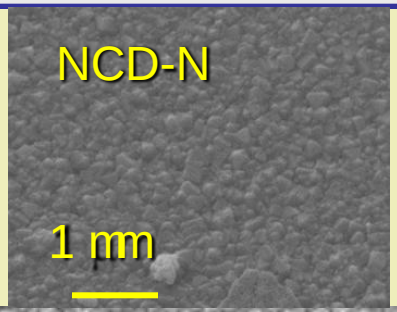
SURFACE MORPHOLOGY

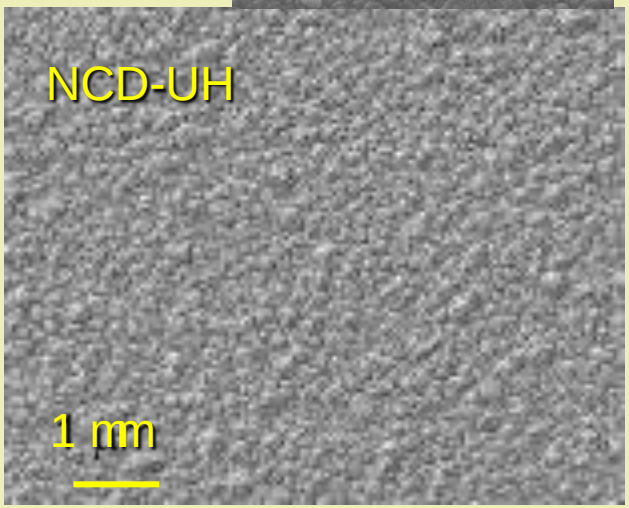


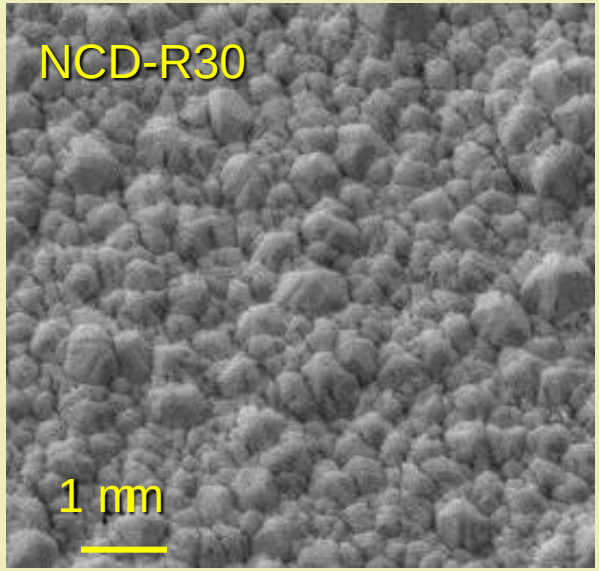




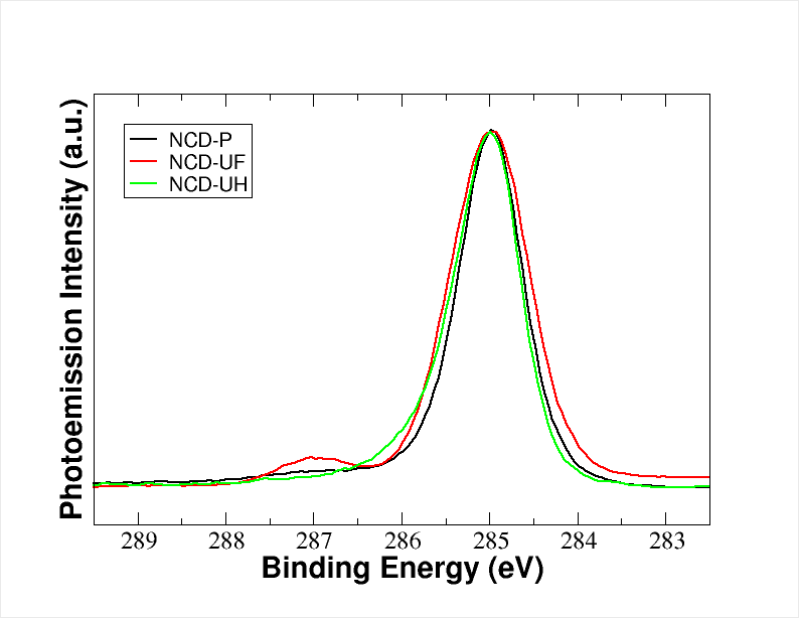


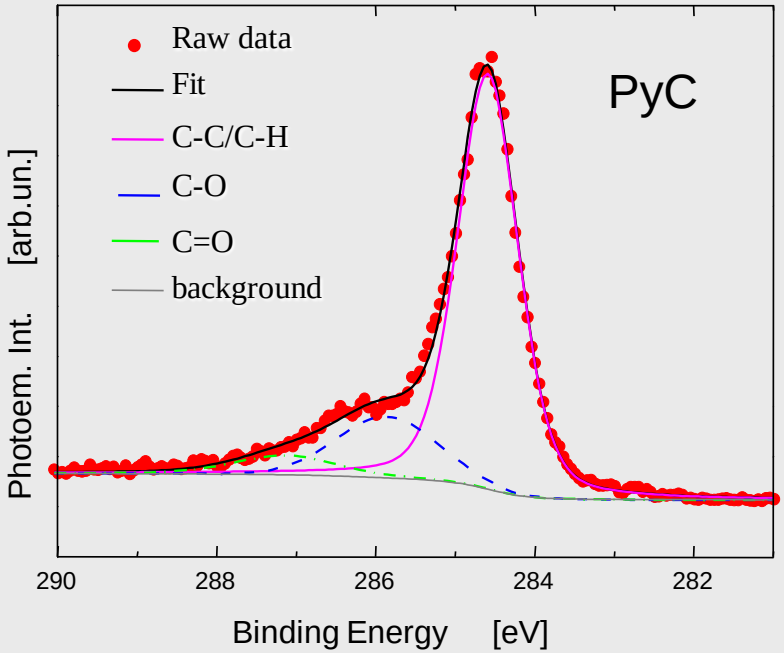






SUBSURFACE COMPOSITION





	C	O	F	Si	W	Mo
PyC	86.5	10.5	-	3	-	
NCD-P	90.2	7.9	-	-	-	2
NCD-UO	90.8	8.6	0.6	-	-	-
NCD-UH	82.2	10.6	-	-	7.2	-
NCD-UF	90.9	1.1	8	-	-	-

HOW TO IMPROVE NCD HAEMOCOMPATIBILITY ?

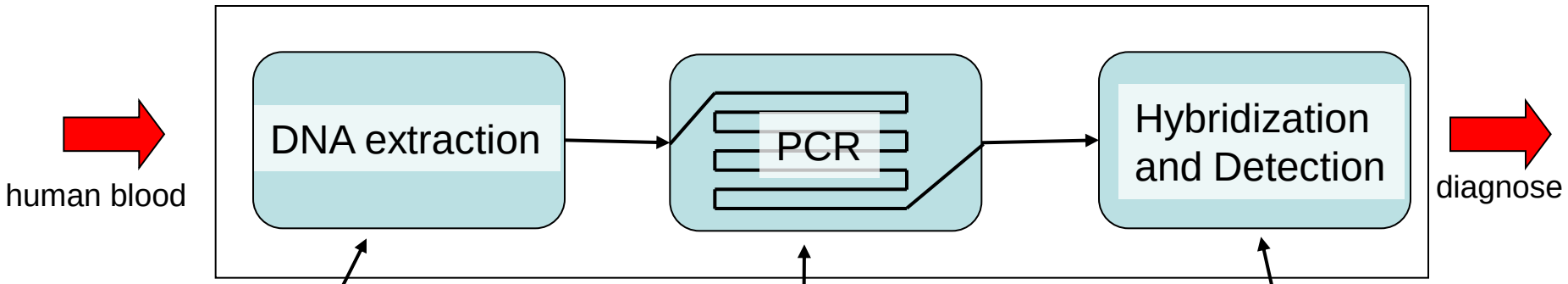
- Si addition ?
- Controlled surface texturing ?

Development of a *Lab-on-chip* for genomic analysis

ÅGenomic DNA extraction

ÅAmplification of target genes by PCR

ÅPCR products hybridization
ÅOptical (fluorescence) detection



ÅSurface modified to attach and release DNA (in opportune working conditions)

1

ÅSurface modified to maintain enzyme (Taq) activity
ÅSurface modified to increase hydrophilic properties

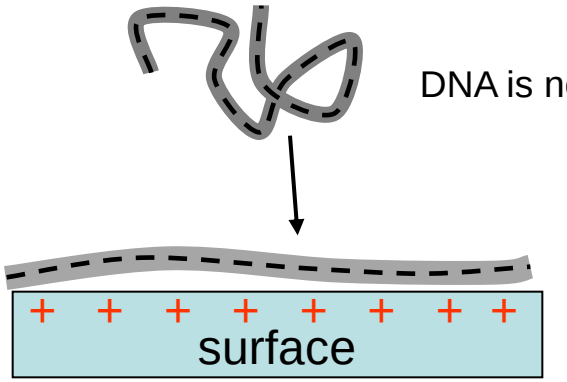
2

ÅSurface modified to avoid aspecific DNA adhesion

3

Surface challenges

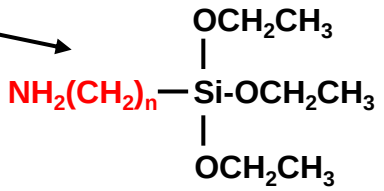
Silicon functionalization for DNA extraction-elution (electrostatic interaction)



DNA is negatively charged

Introduction of positively charged groups on the surface (Si oxide):

- Aminosilanes (APTES)
- Lipids
- Chitosan

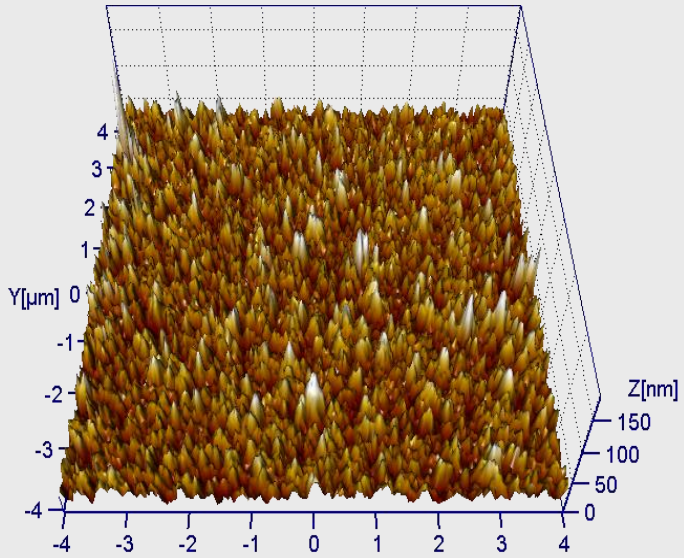


Silanization procedures using different silicon substrates

1. Introduction of a first surface layer → hydrogenated layer
   - oxide layer → thermally grown
   - oxide layer → PECVD
2. Reaction with silane molecules (APTES) → **WET conditions**
   - **DRY conditions** → **different pressures**

Silanized PECVD silicon oxide: AFM and XPS analysis

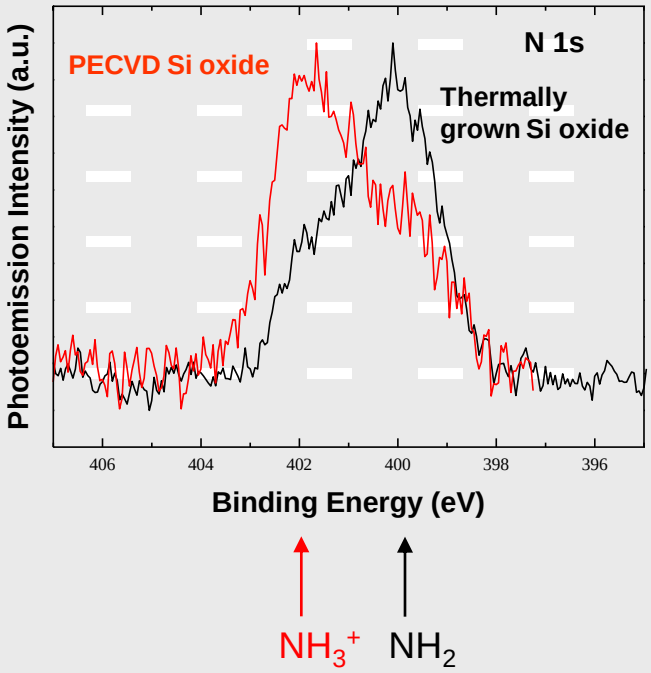
PECVD Si oxide + APTES WET, Sa=7.6 nm



Among the different tested silicon substrates, the silanized PECVD silicon oxide was selected for the DNA extraction and elution

<u>Normalized N1s core line</u>:

- Thermally grown Si oxide + APTES: higher density of NH_2 groups
- PECVD Si oxide + APTES: higher density of NH_3^+ groups

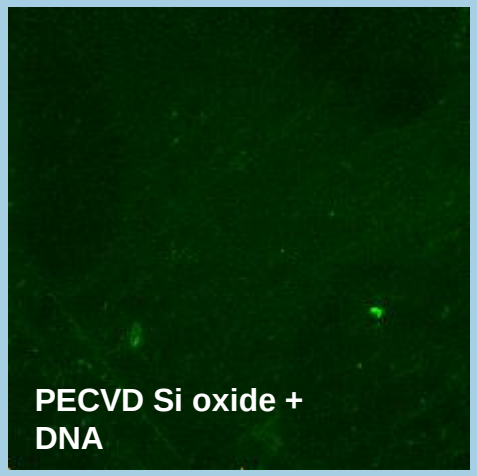


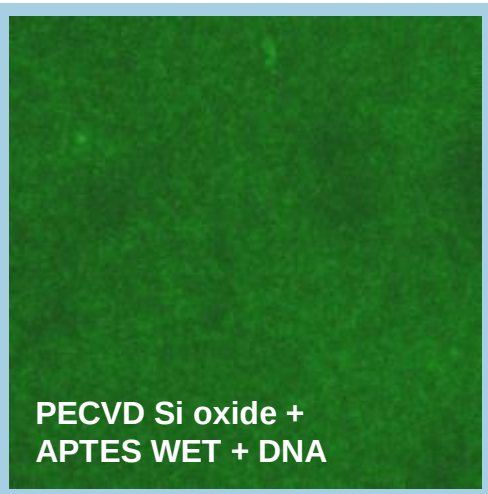
Nitrogen content= 3.3%

DNA adhesion and elution on silanized PECVD silicon oxide

DNA adhesion: pH=7.5

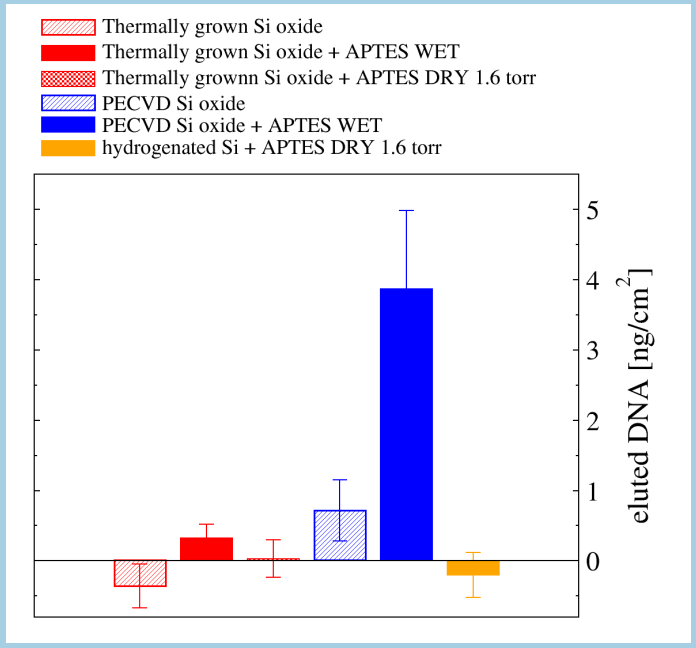
Fluorescence microscopy images of DNA adherent on surfaces (100x100 mm^2). PicoGreen staining.



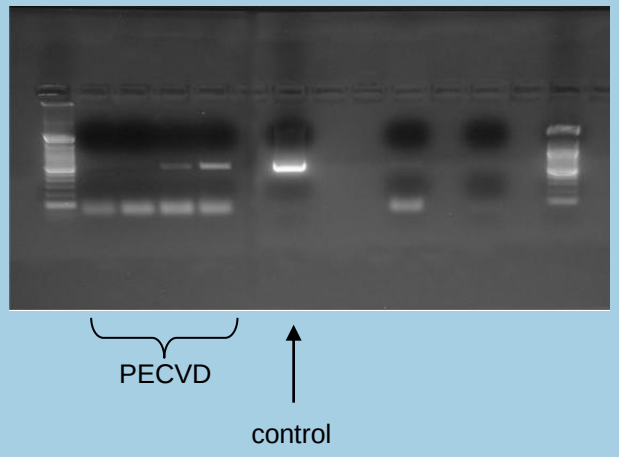


DNA elution: pH=10.5

Quantification of eluted DNA by fluorescence on different silicon substrates.
PicoGreen staining.

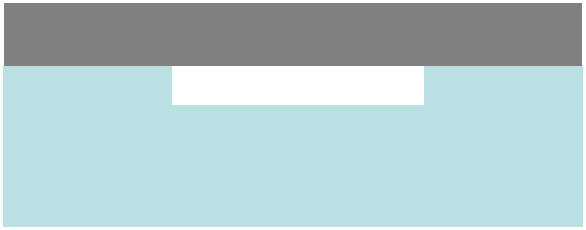


Electrophoresis after PCR of eluted DNA



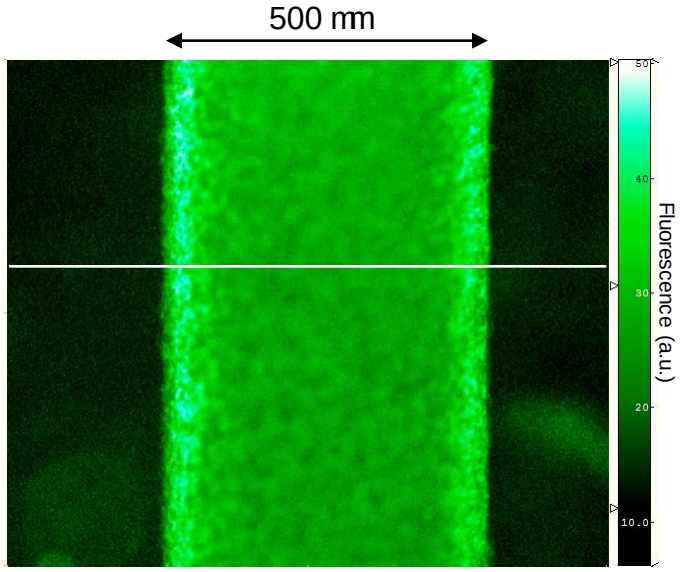
PEG passivation of microchannel device

ÅSurface modified to maintain the PCR enzyme (Taq) activity
ÅSurface modified to increase hydrophilic properties

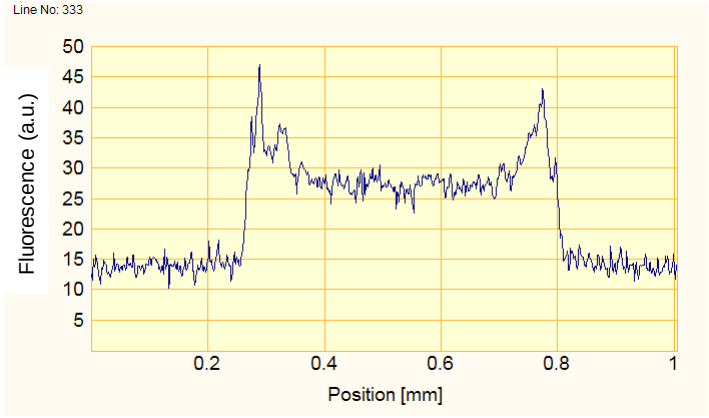


Thermally grown Si oxide + APTES DRY + Fluorescein-PEG-NHS

Pyrex + APTES DRY + Fluorescein-PEG-NHS



Fluorescence microscopy image of functionalized channel



Fluorescence intensity profile

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