Soft pulsed laser transfer of biomaterials

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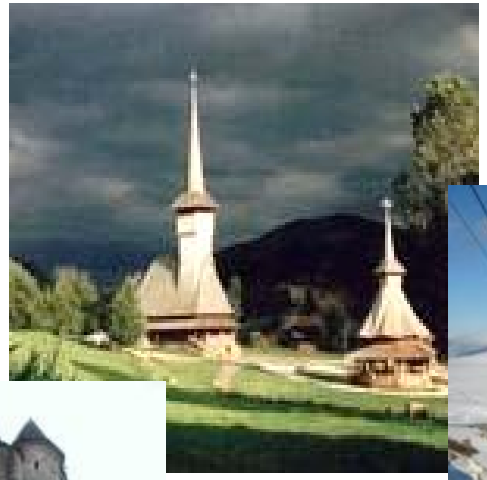
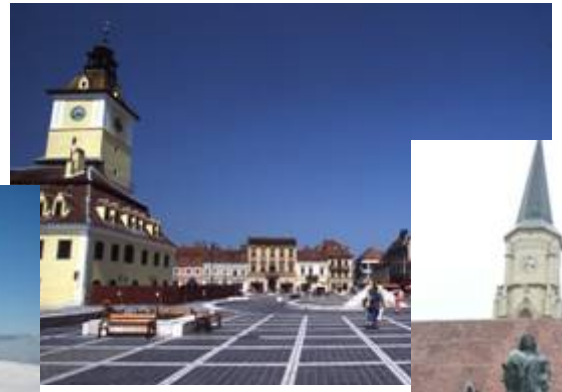










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CURICULUM VITAE

1. Surname, name: *MIHAILESCU Ion N.*
2. Date and place of birth: *30 mai 1947, Slatina, Olt*
3. Nationality: *Romanian*
4. Education:
- July 1982: Ph.D. from the Central Institute of Physics, Bucharest; Ph.D. thesis: "Interaction of 1.06 mm laser radiation of ms duration with metallic targets in vacuum"
-July 1969: Graduated from the University of Bucharest Faculty of Physics after majoring in Nuclear Physics and Optics, Spectroscopy, and Plasma Physics; graduation thesis: "Statistical fluctuations in nuclear reactions"
5. Career/Employment:
- 1990 to date: Senior research scientist 1st degree, National Institute for Lasers, Plasma, and Radiation Physics, "Laser-Surface-Plasma Interactions" Laboratory - http://lspi.inflpr.ro
- 1990 to date: Professor, Faculty of Physics, University of Bucharest
- 1975 to date: Head of "Laser-Surface- Plasma Interactions" Laboratory
- 1975-1989: Research scientist, Institute of Physics and Technology of Radiation Devices, Central Institute of Physics, Bucharest
- 1969–1975: Physicist, Lasers Department, Institute of Atomic Physics (IFA), Bucharest
6. Prizes:
2012 *– IFA Honorary Prize and Medal for contributions in the field of laser – matter interactions*
1994 *- "Galileo Galilei" Award of the International Commission for Optics (ICO) "for outstanding contributions to the field of Optics under unfavorable circumstances"*
1975 *- "Constantin Miculescu" Prize of the Romanian Academy for contributions to Laser Interactions Physics and Applications*

ISI Articles: 471; Books: 4; Book chapters: 20; Citations : 2500 j + 266 b; h-index = 26

Biomaterials

Biomaterial (as defined by National Institutes of Health from USA): *a substance or a combination of synthetic or natural substances, different from a drug, which can be used for long time as a whole or for a part of a vivid tissue, replacing the entire tissue, organ or organism function.*

Key asset:

- Meet minimal biological requirements: biocompatibility combined with the absence of any adverse effect (non-toxic and non-allergic)

Other requests:

- resistance to physiological fluids;
- non-interference with the body's natural immune system;
- lifelong resistance to mechanical stress;
- easy manufacturability in any desired shape.

Classifications

1. *Biologically inactive, nearly inert*
2. *Porous:* facilitate tissue in-growth into the pores
3. *Bioactive:* firmly bind to tissues
4. *Resorbable:* are gradually replaced by tissue over time

A. *Inorganic:* Metals, metals alloys, aluminates, silicates, phosphates, carbonates, …

B. *Organic:* Biopolymers, proteins, enzymes, …

C. *Hybrid inorganic - organic*

i. *first generation*: bioinert

ii. *second generation*: bioactive and biodegradable

iii. *third generation*: intelligent design to stimulate specific responses at the molecular level

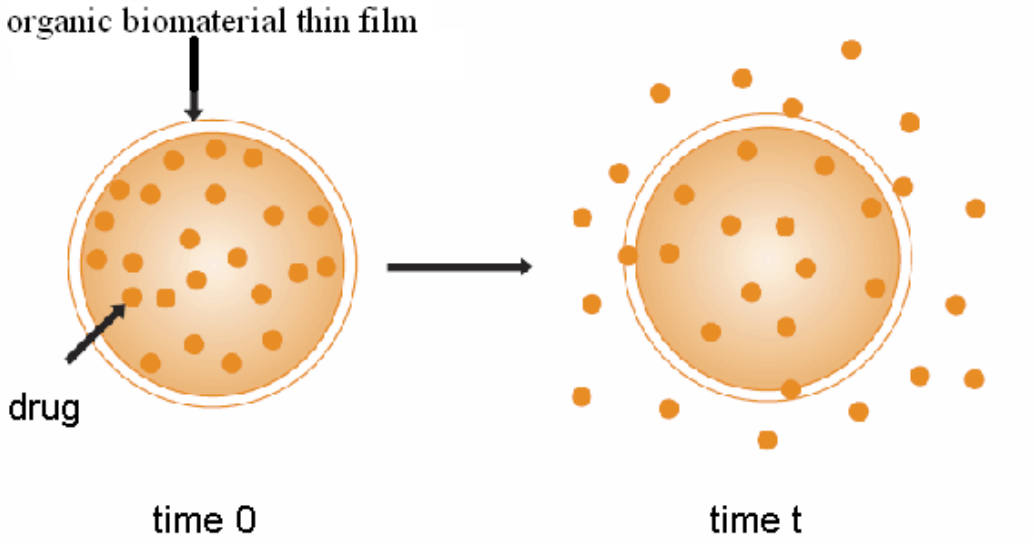
Why organic biomaterial thin films?

Organic biomaterials are generally: - rather expensive
- have modest/poor mechanical and wear properties

Important note: only the outer layer of biomaterials enter in contact with the biological/vivid (tissue) → possible solution: application as thin films

Potential utilization: - coating of active substances for drug delivery
- biosensors
- coating of biomimetic, generally metallic implants

Thin films in drug delivery

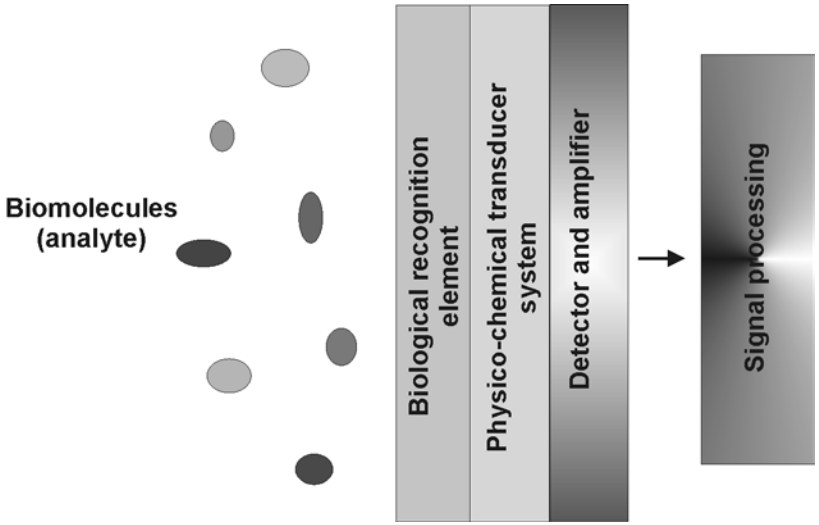


Schematic representation of reservoir diffusion controlled drug delivery device

Thin films may be applied in order to:

- slow the rate of release of an active component;
- improve the dispersion / flow properties; or
- increase the absorption into the systemic circulation.

Biosensors



Biosensor:

1. biological receptor (the recognition site for interaction with the analyte to be detected)

2. transduction system, and

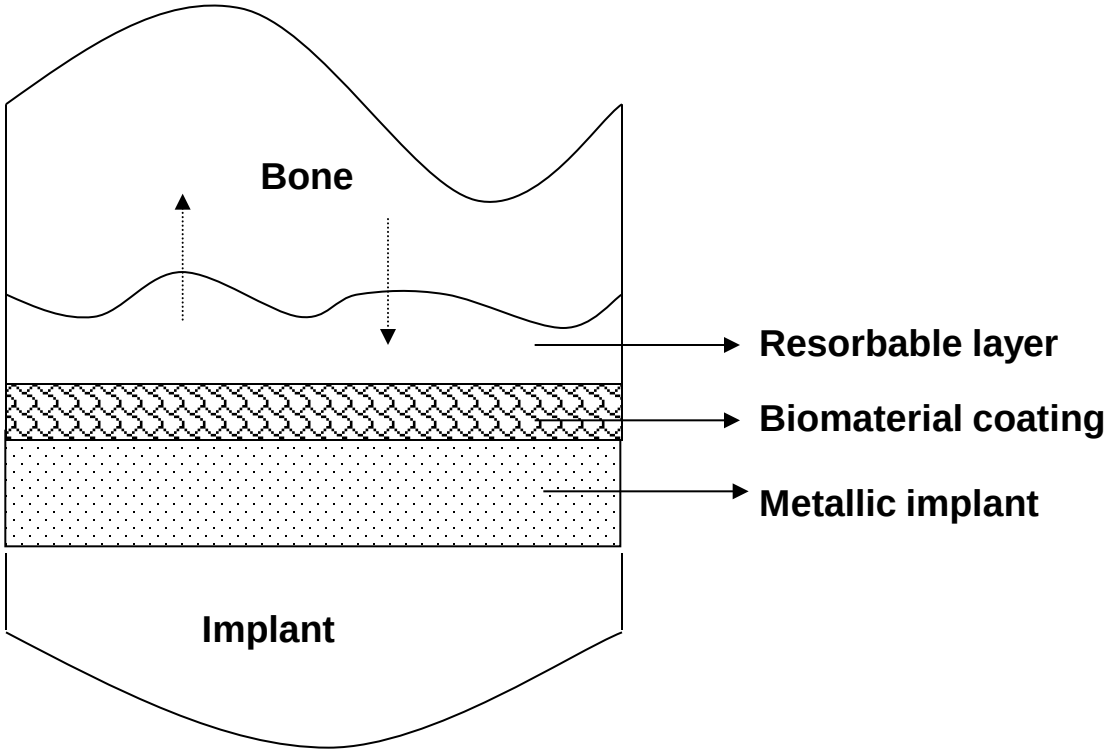
3. signal output

The **biosensing mechanism** is based upon the interaction between the **biologically active recognition element** and the **analytes**.

Biomimetic coatings for metallic or other implants

Main drawback of organic biomaterials: generally fragile in bulk

Alternative solution



How to deposit organic biomaterial thin films?

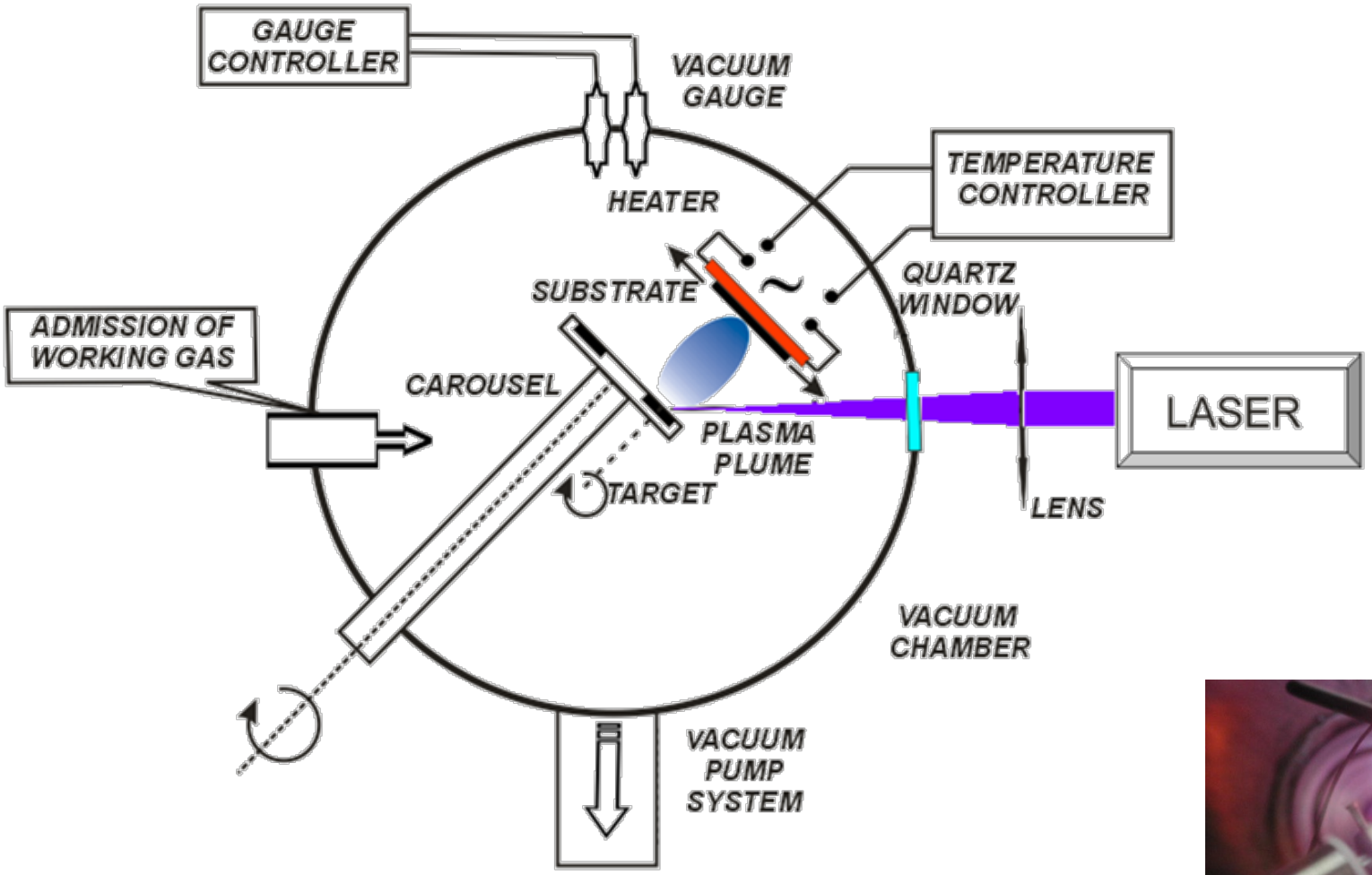
- Plasma methods (plasma spraying, MS, ion and/or electron bombardment) are inappropriate for deposition of organic biomaterial thin films. The organic biomaterials suffer of irreversibly damage during evaporation and transfer.

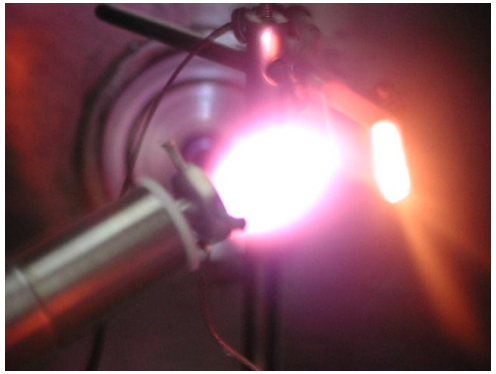
- The same generally applies to physical-chemical (spin-coating and Langmuir Blodgett) or laser “classical” methods: laser cladding, PLD.

- New laser methods were developed and extended for the transfer of organic biomaterials in form of thin films on different substrates: MAPLE (after 1999), laser direct write methods (since 1969).

Organic biomaterial thin films must resemble the biological ones in composition, structure, morphology, and functionality!

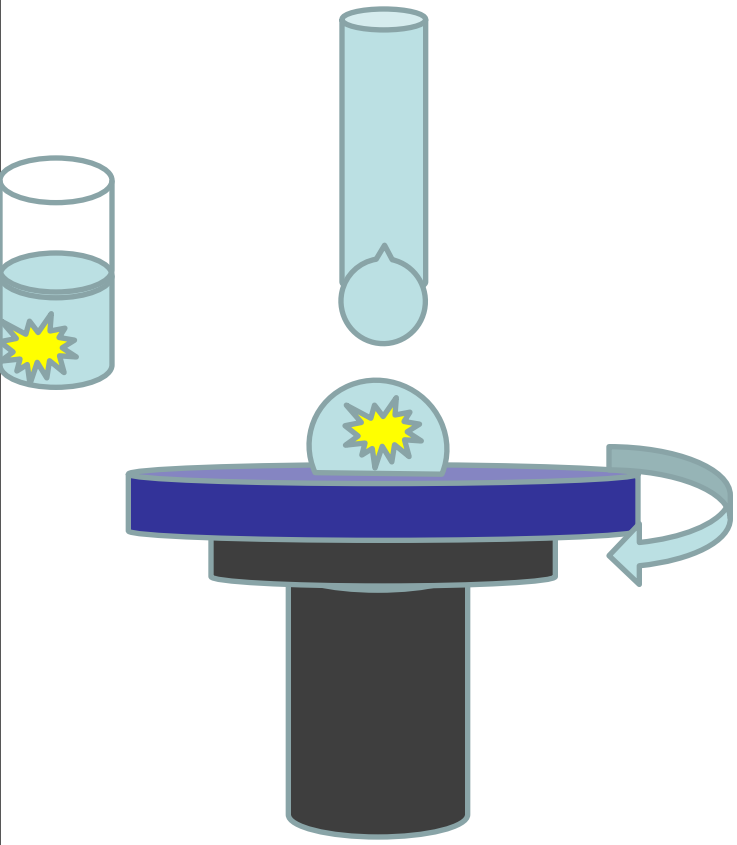
Experimental PLD setup



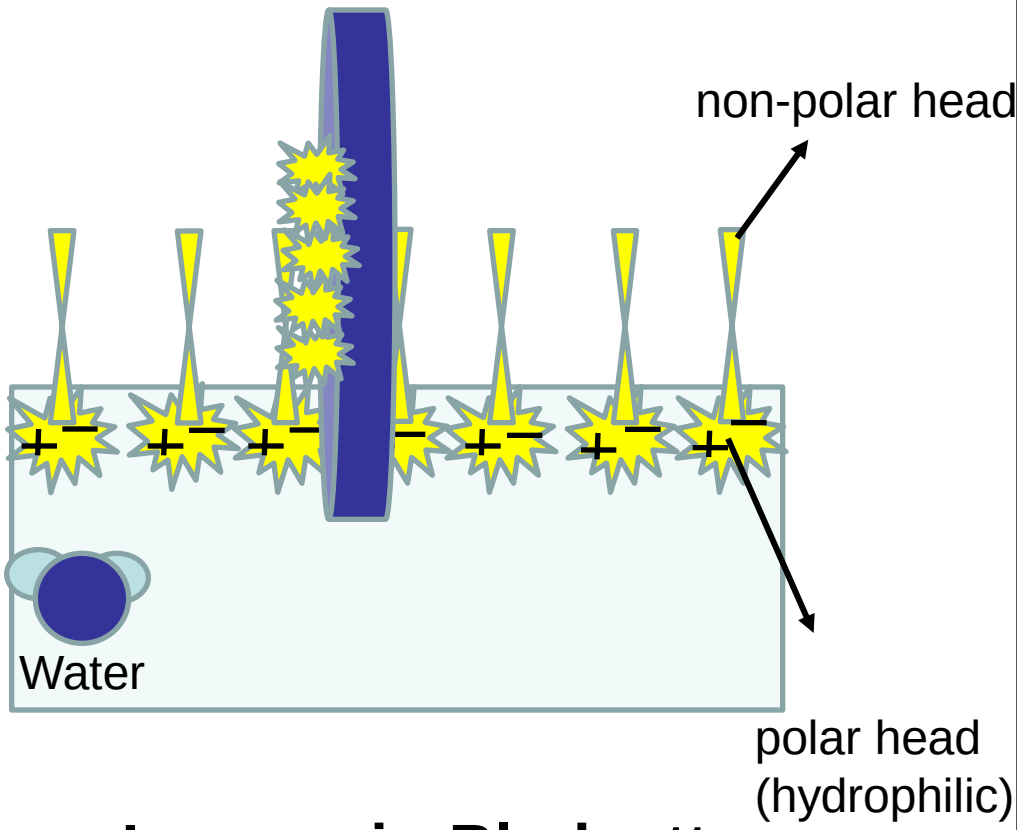


Set-ups in LSPI lab

Techniques to deposit thin organic coatings



Spin coating

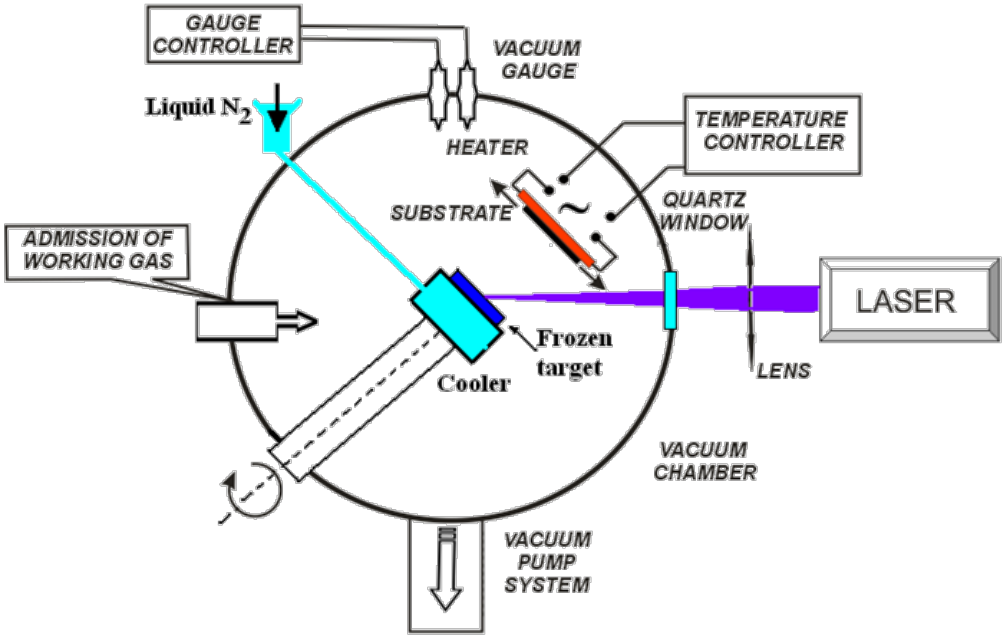


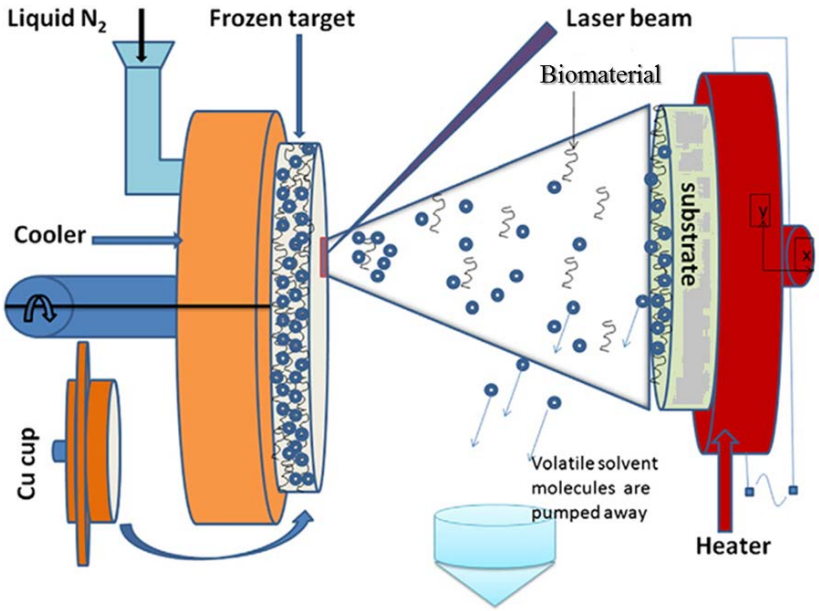
Langmuir–Blodgett

Issue in biotechnologies and biomimetism:
difficulties to prepare multistructures and multilayers.

Matrix-Assisted Pulsed Laser Evaporation (MAPLE)

Main differences PLD vs MAPLE: target preparation and interaction mechanisms





- the composite target is obtained by mixing a solvent with an organic/biologic material
- the mixture is frozen
- during the deposition the target is kept at low temperature using a *cooler*

MAPLE

Photographs of cooler (left) and MAPLE targets before the laser strikes the target (center), and after deposition (right), showing the eroded area on the target corresponding to the region where the laser hit.
**protein in deionized water, 248 nm*

General requirements:

- laser fluence must have proper values, lower than in PLD
- incident laser energy must be majoritary absorbed by solvent molecules and not by organic molecules of the base material (0.5 - 10) %
- frozen solvent should be characterized by a high absorption at working laser wavelength
- solvent has to be selected so that the solute presents a good solubility
- solvent has to present a high freezing point
- solvent must not produce chemical reaction under laser radiation exposure

Biomaterials for drug delivery

Triacetate-pullulan polysaccharide

- a linear homopolysaccharide of glucose, often described as a linked polymer of maltotriose subunits (1, 6)
- has many potential food, pharmaceutical, and industrial applications

Hydroxyapatite

- bioactive, bioresorbable, can promote osteoconductivity
- main constituent of inorganic part of bone (≅ 65% of volume); most insoluble Ca phosphate

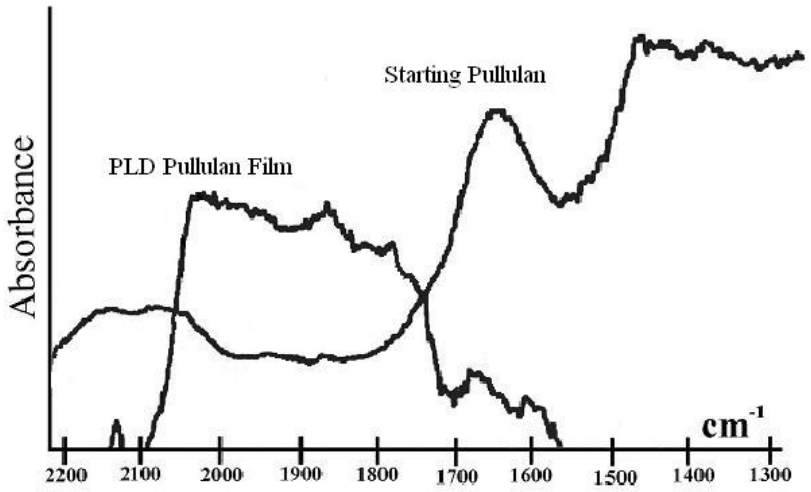
Alendronate

- Bisphosphonates (BPs) are widely used for the management of specific disorders of bone metabolism, such as Paget bone disease, osteoporosis, fibrous dysplasia, myeloma and bone metastases

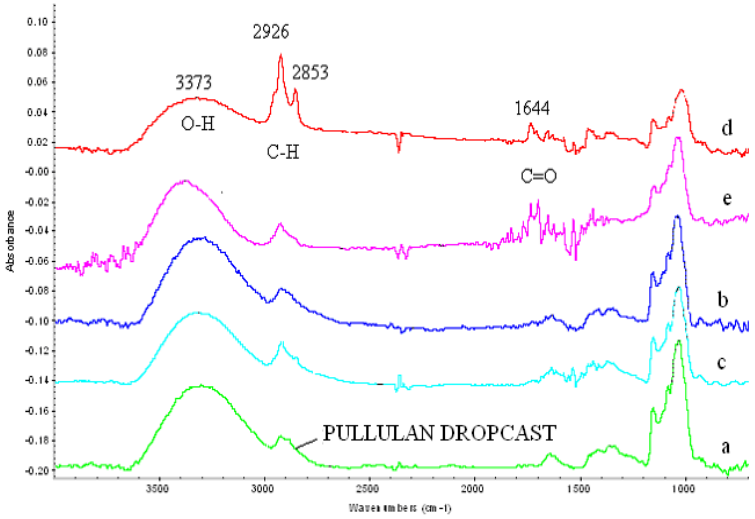
Levan

- homopolysaccharide of d-fructofuranosyl residues joined by β-2,6 with multiple branches by β-2,1 linkages;
- great potential as a functional biopolymer in foods, feeds, cosmetics, pharmaceutical and chemical industries

Triacetate-pullulan polysaccharide



Major differences between starting material and the PLD (248 nm, 390 mJ/cm^2) film which demonstrate the **<u>degradation</u>** of the structure during PLD.

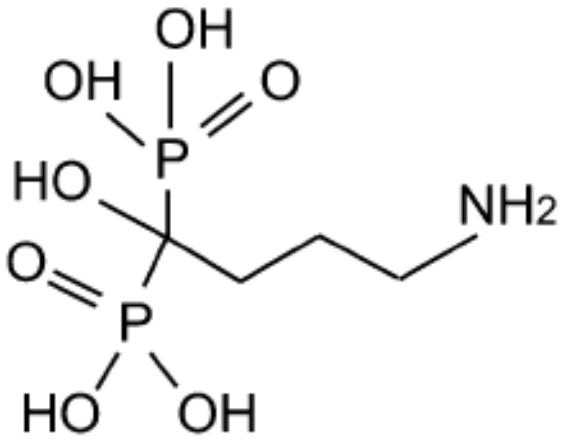


a. Pullulan dropcast
b. 2% pullulan in deionized water
c. 2% pullulan in 3-butanol
d. filtered 2% pullulan in 3-butanol
e. filtered 2% pullulan in DMSO

Laser fluence 240 mJ/cm^2

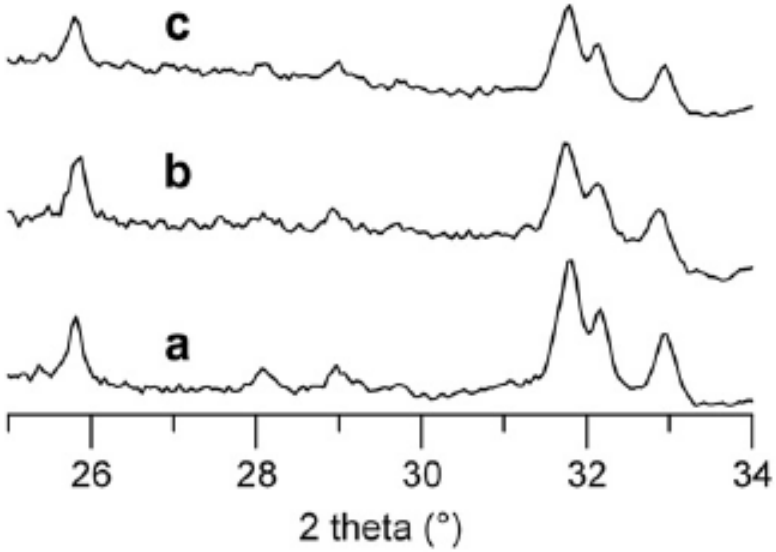
<u>No decomposition</u> by MAPLE of 2% solutions in deionized water and 3-butanol!

HA –AL– XRD investigations



Alendronate

HA: $Ca_{10}(PO_4)_6(OH)_2$

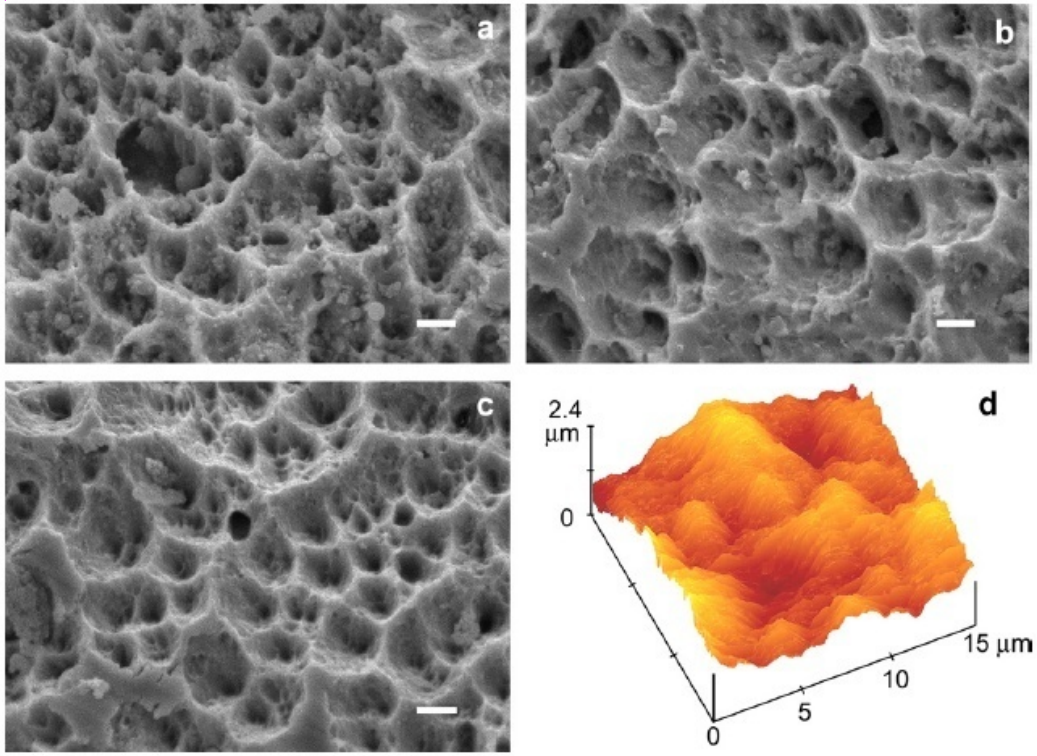


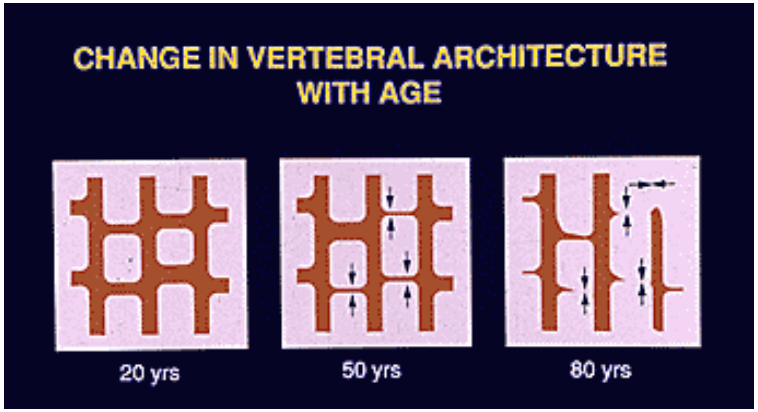
Powder X-ray diffraction patterns of the thin films deposited from: (a) HA, (b) HA-AL7(4%), (c) HA-AL28(7%)

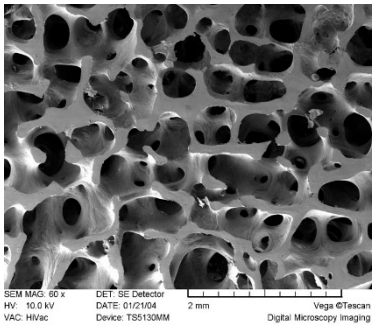
The slight increase of the broadening of the diffraction peaks when increasing alendronate concentration is indicative for a modest decrease of the length of the crystalline domains as the alendronate content in the apatite nanocrystals increases up to 7.1%.

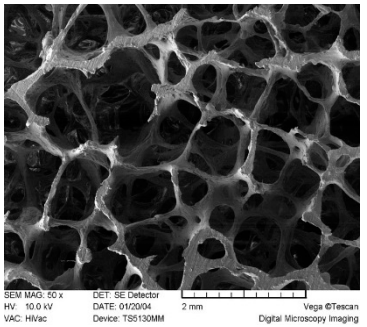
** 3.9 or 7% AL with HA; 0.25 g of HA-AL powder suspended in 5 ml deionized water, 248 nm, 750 mJ/cm^2*

SEM and AFM studies





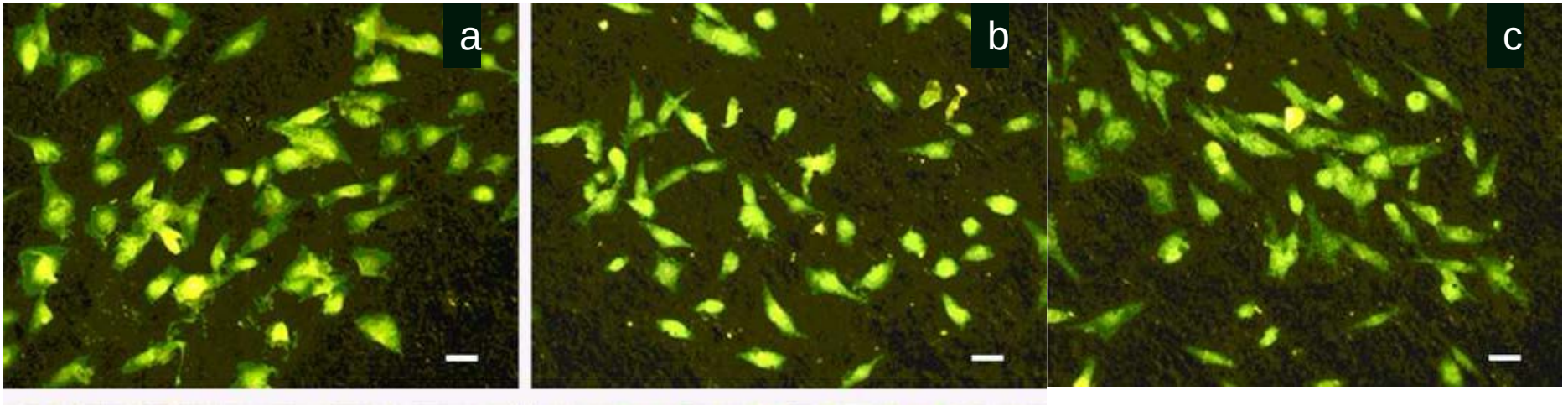




SEM micrographs of thin films deposited from: (a) HA, (b) HA-AL7, (c) HA-AL28. Bars ¼ 2 mm. (d) AFM image of the surface of a thin film deposited from HA.

The films exhibit a **porous-like** structure (similar to human bone), with pores dimension of 2–4 µm, while only few grains are visible.
AFM analyses are quite similar for the different coatings.

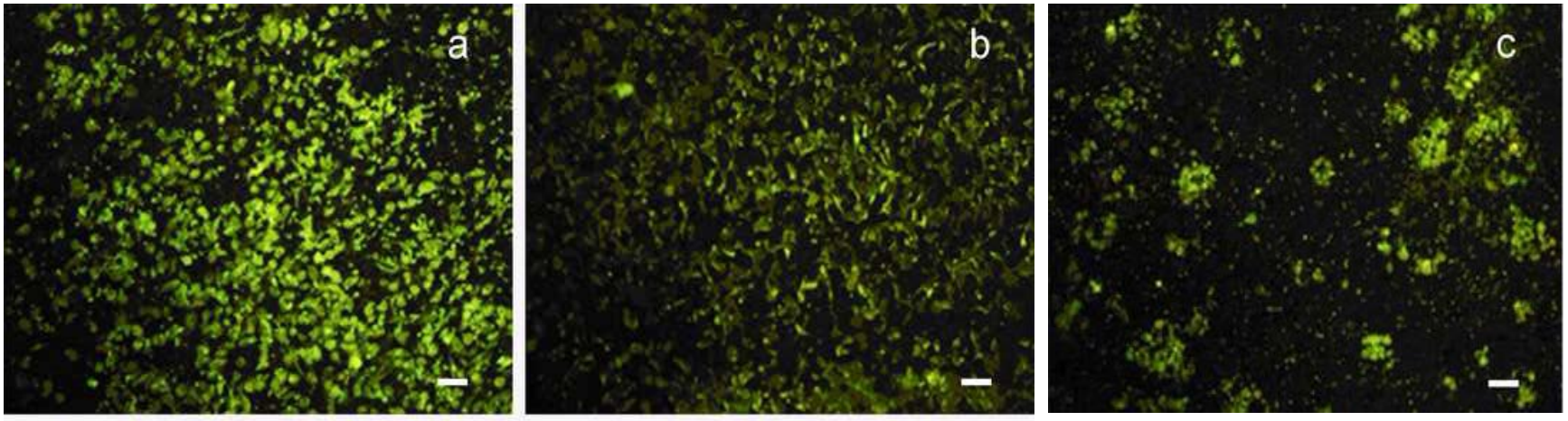
Florescence microscopy images of hOB on alendronate-HA coatings



Phallodin staining of culture after 24 hour from seeding: (a) Ti, (b) HA, (c) HA-AL7. Bars = 20 µm.

Presence of alendronate in HA thin films enhances osteointegration and bone regeneration!

Proliferation of osteoclast (hOC) culture on alendronate-HA coatings: 21 days



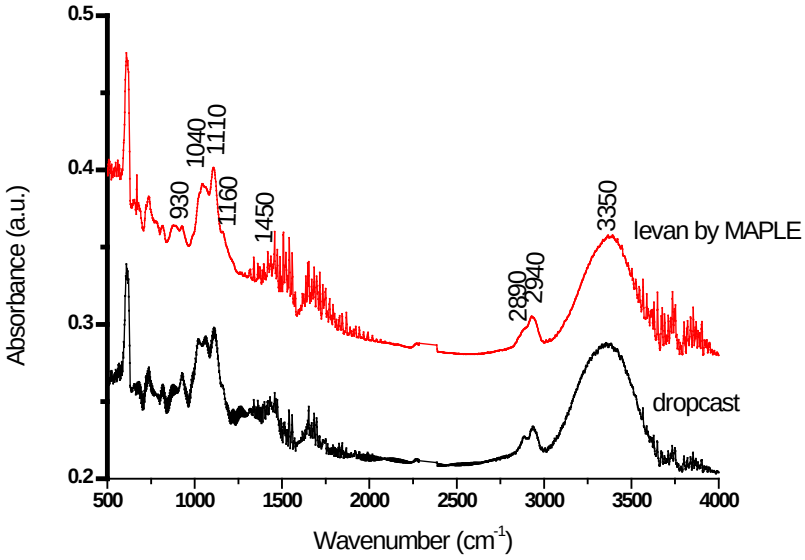
Phallodin staining of culture after 21 days from seeding: (a) Ti, (b) HA, (c) HA-AL7. Bars = 20 μm.

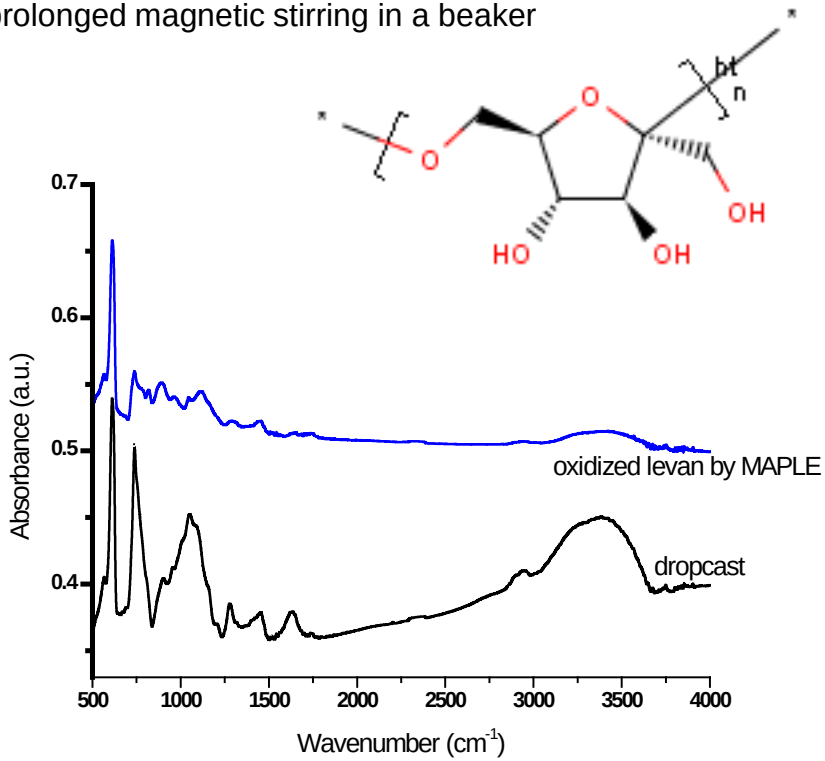
Presence of alendronate prevents the undesirable bone resorption!

LEVAN

Sample	Fluence (J/cm^2)	Solvent	Concentration (g/l)	Optimal parameters (substrate temperature, pressure, target-substrate separation distance)
Levan (L)	0.28	DMSO	5	100 °C, 5 Pa, 3.5 cm, 20000
Oxidized Levan (OL)*	0.35	DMSO	5	100 °C, 5 Pa, 3.5 cm, 20000

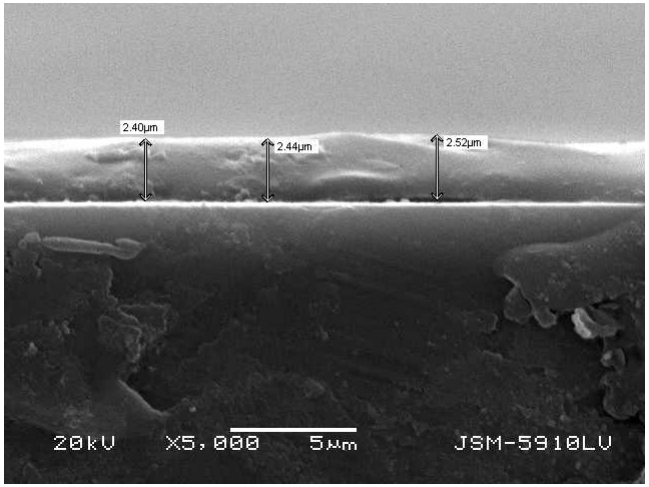
*Pure levan samples subjected to periodate oxidation by prolonged magnetic stirring in a beaker



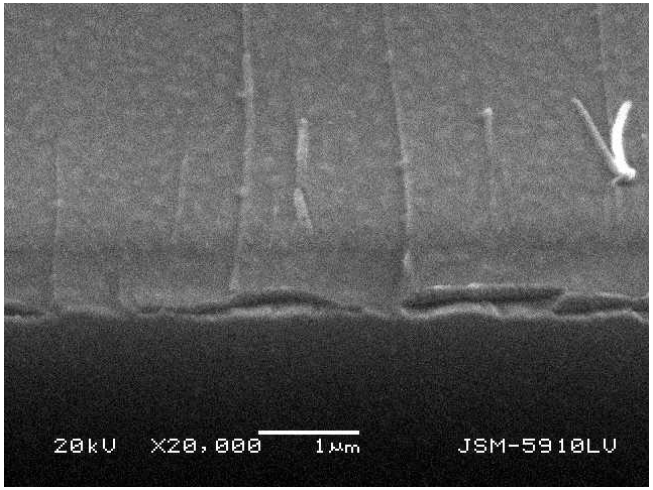


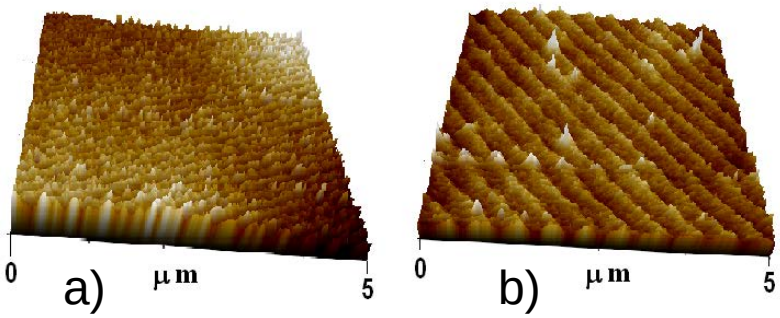
FTIR absorption spectra of L and OL dropcast and L and OL thin films, respectively, deposited by MAPLE

LEVAN



XSEM of L thin films on glass by MAPLE



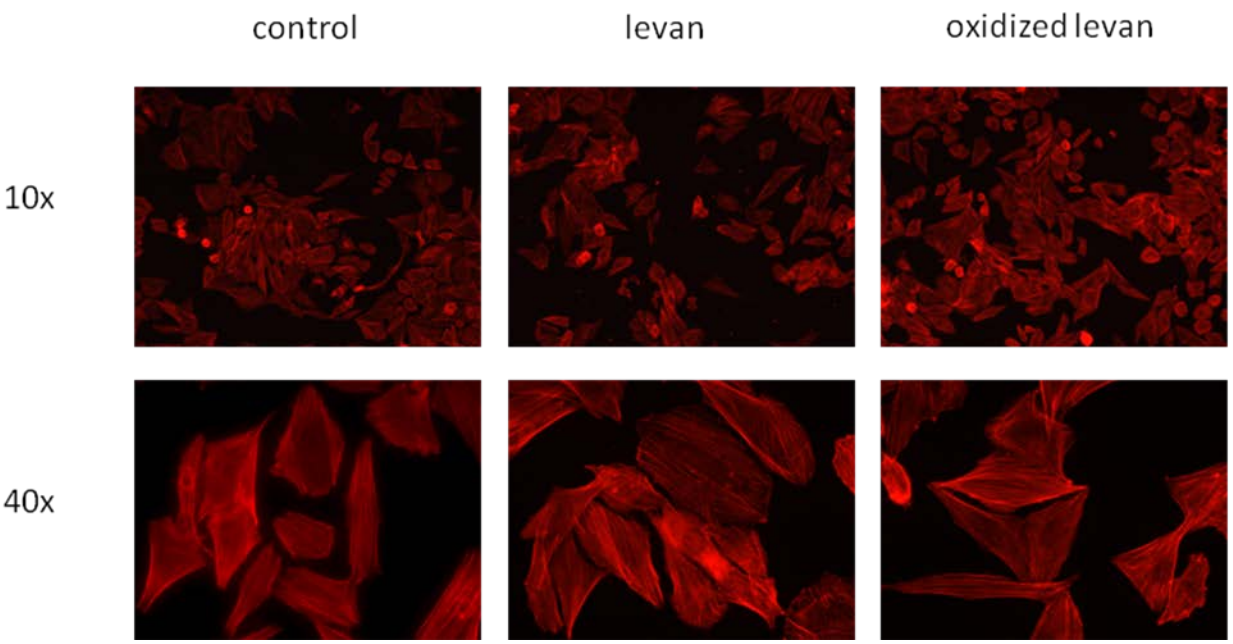


Typical AFM images of sample surfaces for a) L and b) OL coatings on Si

Possible formation mechanims: nanostructured assemebling!

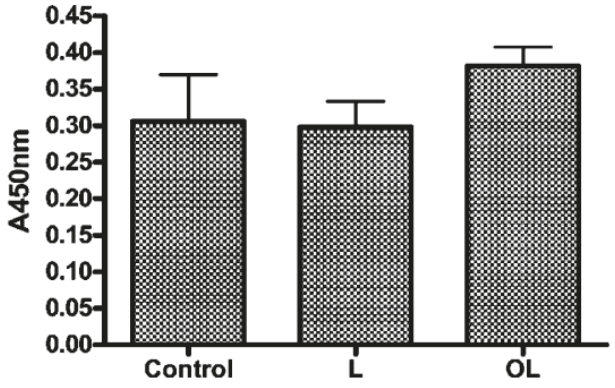
Sample	Rms (nm)	Ra (nm)	Rz (nm)
L coating on Si	0.972	0.762	4.158
OL coating on Si	1.203	0.962	4.818

LEVAN



- similar coverage of the control and tested surfaces
- no change in the filament organization pattern. Actin is uniformly spread throughout cell cytosol in parallel filaments sustaining cell shape and motility.

SaOs2 cells adhesion on control, L and OL coatings on glass by immunofluorescence microscopy



- L and OL coatings have no detrimental function over cells. Cells proliferation was similar on L and control samples.
- OL induces an increase in the ability of cells to divide and give rise to daughter cells. This result is supported by CA measurements where **hydrophilic** surfaces were revealed in the case of OL.

Biomaterials for biosensors

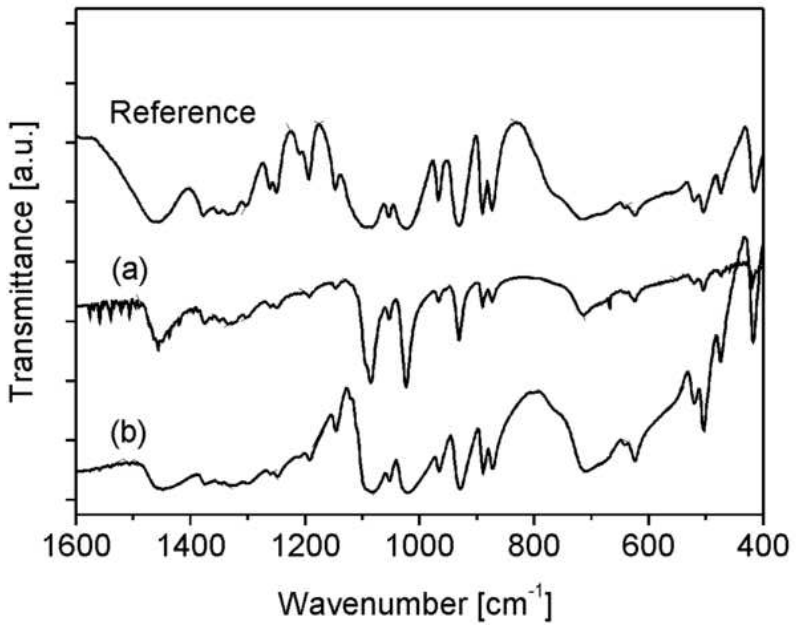
Urease

- an enzyme that catalyzes the hydrolysis of urea into carbon dioxide and ammonia;
- nitrogen concentration of the human serum in urea is a measure of kidney function

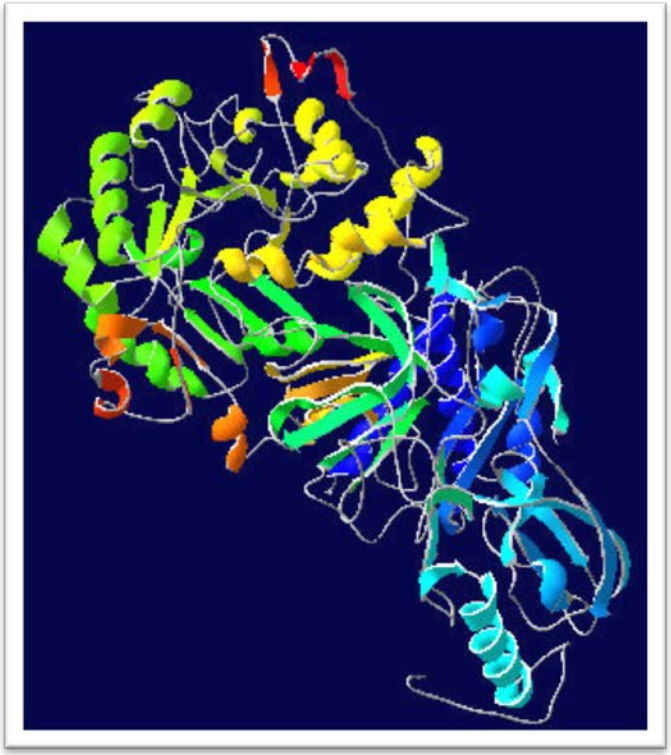
Mesotetraphenylporphyrin (TPP):

- a promising material for:
  - -detecting volatile organic compounds: alkanes (hexane), aldehydes (propanal), alcohols (methanol, ethanol), ketone (acetone) and amine (triethylamine);
  - -pH or oxygen sensing in solution
  - - tracer in photodynamic therapy

Urease: 1 or 10% in deionized water; 248 nm

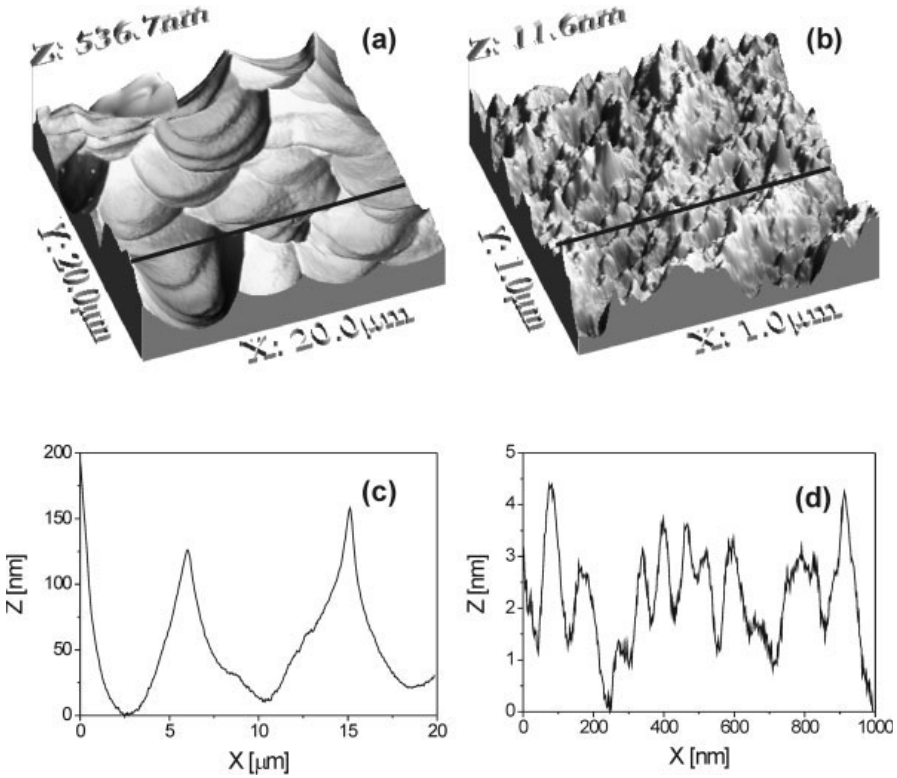


FTIR spectra of urease base material used for the preparation of the composite targets (Reference) and urease thin film deposited at 0.4 J/cm^2 laser fluence from the (a) 1 and (b) 10 wt.% urease concentration



Chemical composition and structure are preserved after the thin films growth process (in particular for 10% urease concentration)!

Urease thin film morphology



Tilted (a), detailed view (b) AFM micrographs, and corresponding surface profiles (c,d) of urease thin film deposited at 400 mJ/cm^2 laser fluence.

Interconnected island-like morphology

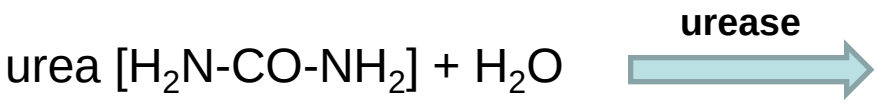
Minimum root-mean square surface roughness ~100 nm.

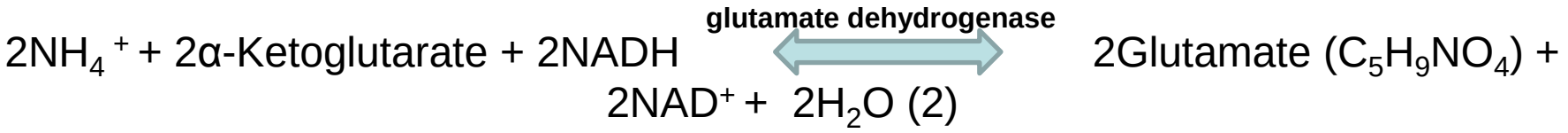
Morphology can be considered a real advantage, because it provides a considerably broader active area as recognition element in biosensor devices.

Urease enzymatic activity evaluation

➢The hydrolysis of urea was measured by coupling ammonia to NADH (nicotinamide adenine dinucleotide) oxidation reaction

➢ **Urease enzymatic activity and kinetics => determined by Worthington assay method**





The NADH molecule (reduced NAD) is oxidized to NAD+. NADH absorbs UV light at 340 nm but NAD+ does not.

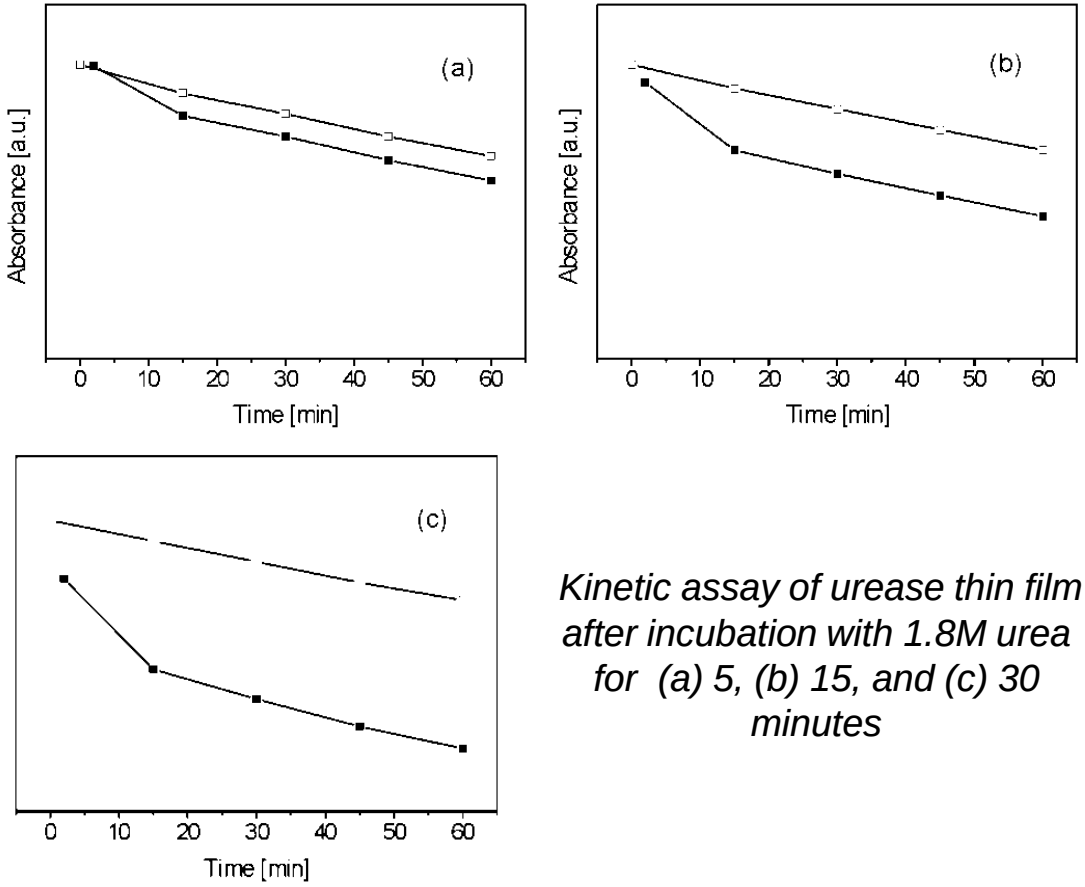
If the number of NADH molecules drops, the absorbance at 340 nm decreases – possible application to urea monitoring

Urease enzymatic activity evaluation

1. Thin films were immersed in the solutions and mixed with 1.8 M urea in 0.1 M potassium phosphate buffer.

2. Kinetic analyses were performed by measuring the absorbance of the obtained solution at 340 nm over one hour.

3. We compared the kinetic assay slope of the catalized reaction with the blank solution slope.

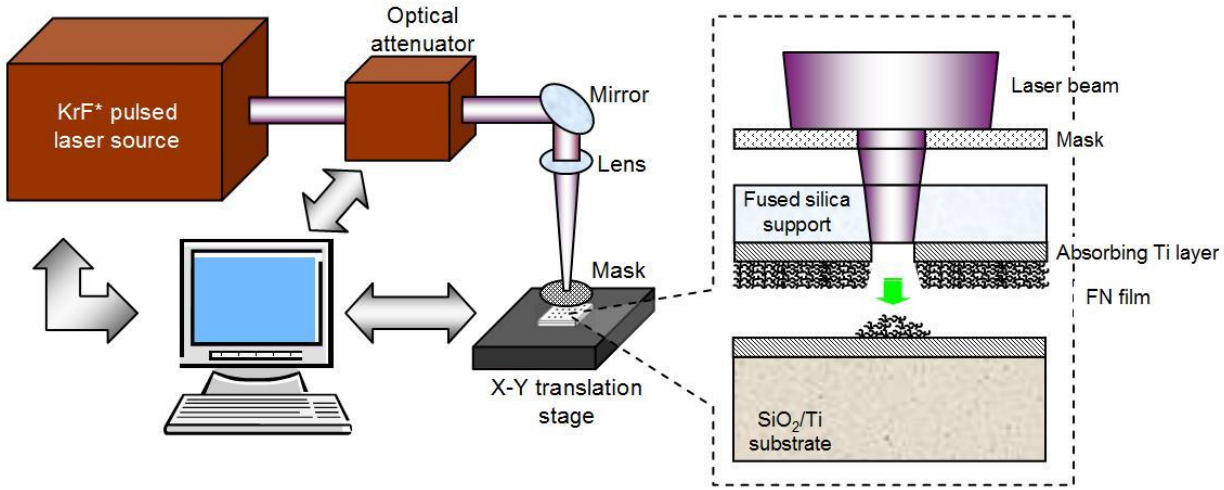


Kinetic assay of urease thin film after incubation with 1.8M urea for (a) 5, (b) 15, and (c) 30 minutes

Decrease of absorbance with incubation time: laser immobilised enzyme active in breaking down / diagnostic of urea!

Blister-based Laser Direct Write

Blister-based Laser Direct Write (BB-LDW): as a promising tool for clean, cold and liquid-free local transfer of various organic substances.



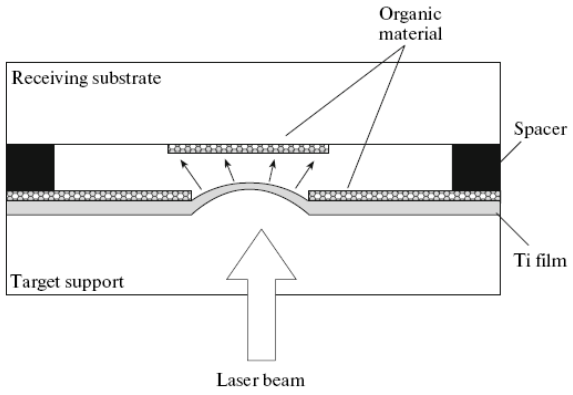
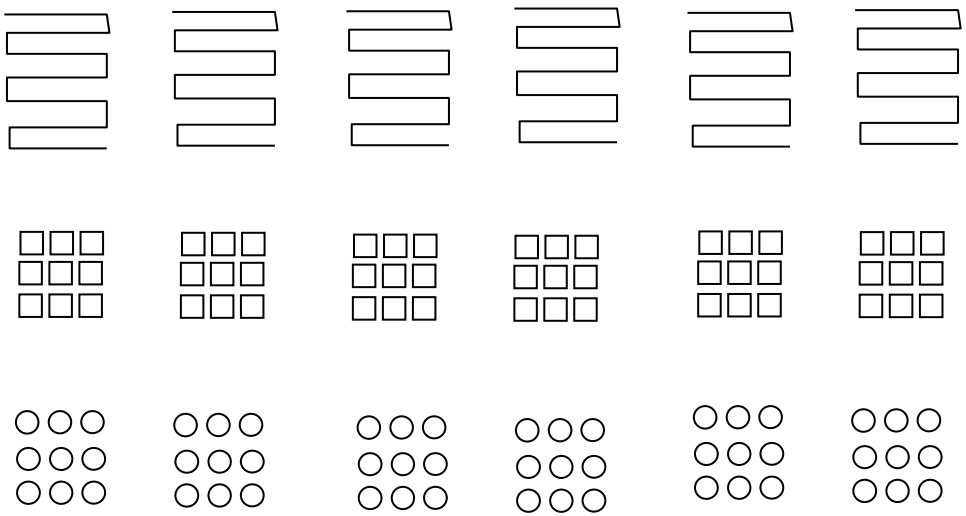


Diagram of blister-based LDW

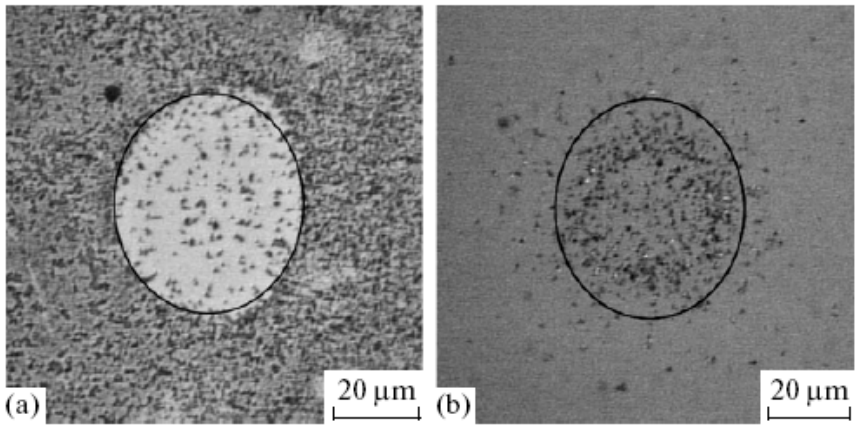
The main feature of the technique is non-destructive local deformation of an absorbing metal film on a transparent support avoiding the metal sputtering.

Microstructures of mesotetraphenylporphyrin (TPP)

- ribbon: a Langmuir film of Mesotetraphenylporphyrin (TPP) and stearic acid (SA) dissolved in chloroform (both 10^{-3} M); the ratio of TPP:SA = 1:1, deposited on (50 – 1500) nm Ti interlayer
- acceptor: a silica substrate placed at 10 μm from donor (ribbon)
- Nd:YAP laser, 5 ns @ 1078 nm; (240 – 900) mJ/cm^2

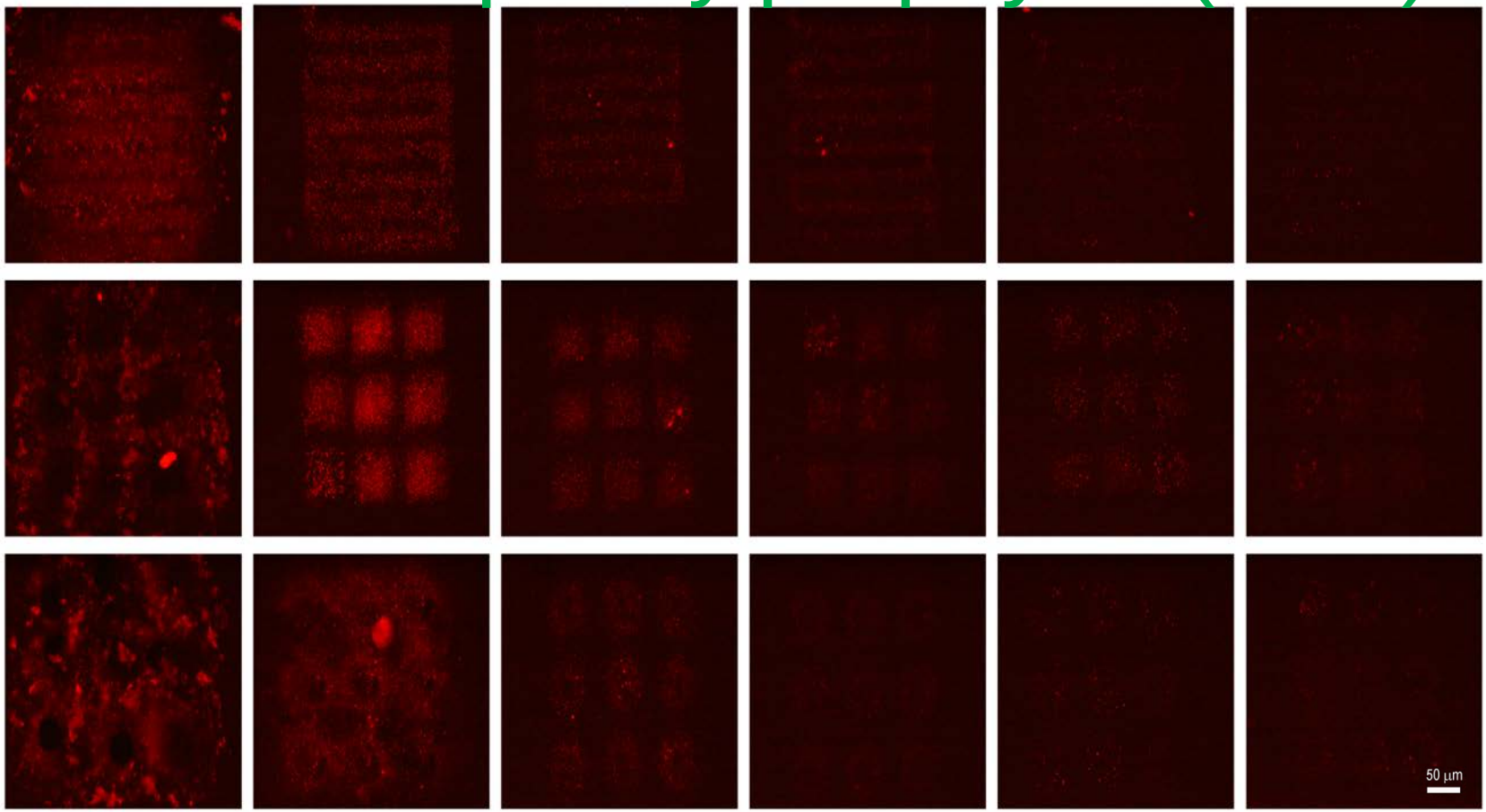


Fluence decreases



Clean laser transfer of TPP layer: (a) irradiated target surface; (b) receiving substrate with deposited TPP fragments. Ellipses drawn in both photos are identical.

Microstructures of mesotetraphenylporphyrin (TPP)



TPP exhibits natural fluorescence at 675nm or 734nm (red fluorescent channel of the epifluorescence microscope). Series of successfully transferred TPP corresponds to the 2nd and 3rd columns. The highest fluence generates splashing which seems to be aggregated. Lower fluences are optimal for obtaining discrete geometrical structures with a remarkable precision.

Biomaterials for coating of implants

Silver – antimicrobial properties, in concentration lower than 0.6%
Lignin (Lig) - complex, amorphous organic polymer found in plant tissues, usually bounded to cellulose; important source of natural antimicrobial compounds

Poly(methyl methacrylate) (PMMA) + Bioactive glasses (BG)

- Biocompatible, bioinert polymer, often used as a light or shatter-resistant alternative to glass
- increase the functionality and improve wear properties of the metallic implants

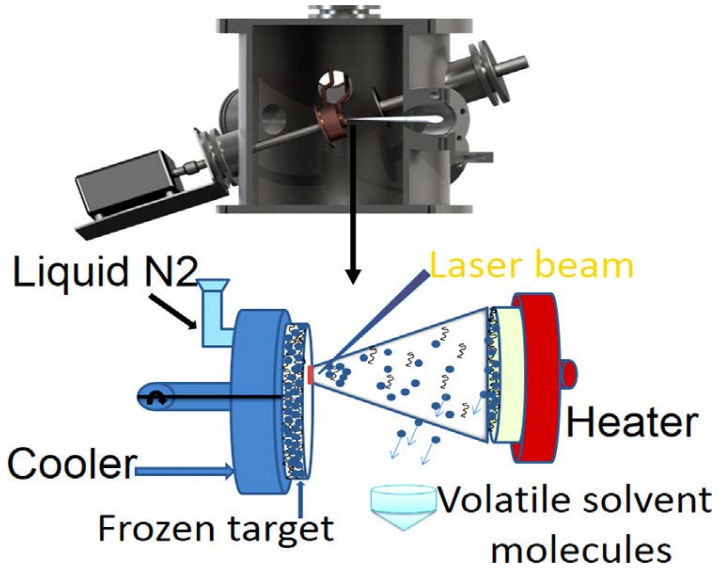
Extracellular matrix (ECM) proteins-HA

- HA is capable to induce mineralization, while ECM proteins (as e.g. fibronectin or vitronectin) are used for material bioactivation by adsorption and cell adhesion at the interface
- Fibronectin (FN) is an ECM glycoprotein capable of binding to integrin receptors, which mediate the attachment of cells to surrounding tissues
- Vitronectin (VN) is a glycoprotein; about one-third of the protein's molecular mass is composed of carbohydrates

HA-Lig and Ag:HA-Lig – Experimental details

Targets: MAPLE targets of HA-Lig (10% w/v) and Ag:HA-Lig (1% w/v) powders dissolved in distilled water

Substrates: Ti substrates,Ti modified with TiO_2 nanotubes, <111> single-crystalline Si wafers



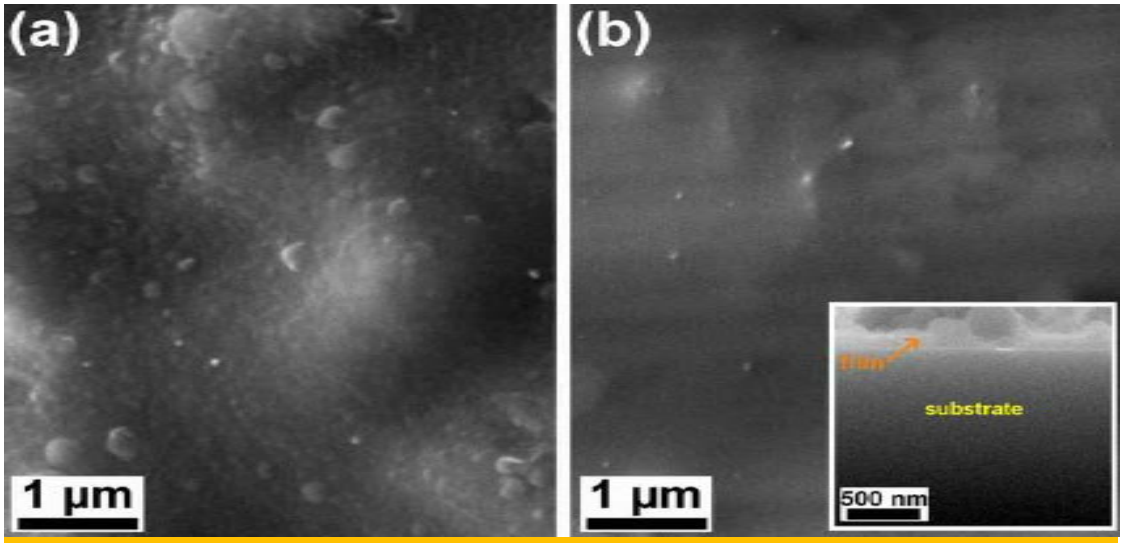
Code samples:

- ***HA-Lig*** (hydroxyapatite/lignin deposited on titanium substrate)
- ***Ag:HA-Lig*** (silver doped hydroxyapatite/lignin deposited on titanium substrate)

KrF* laser source: λ= 248 nm, pulse duration = 25 ns, 10 Hz laser repetition rate

- laser fluence 0.7 J/cm^2
- 6.5 Pa , 35 000 laser pulses, target-substrate separation distance 35 mm, spot size 25 mm^2, RT

HA-Lig and Ag:HA-Lig – SEM ANALYSES

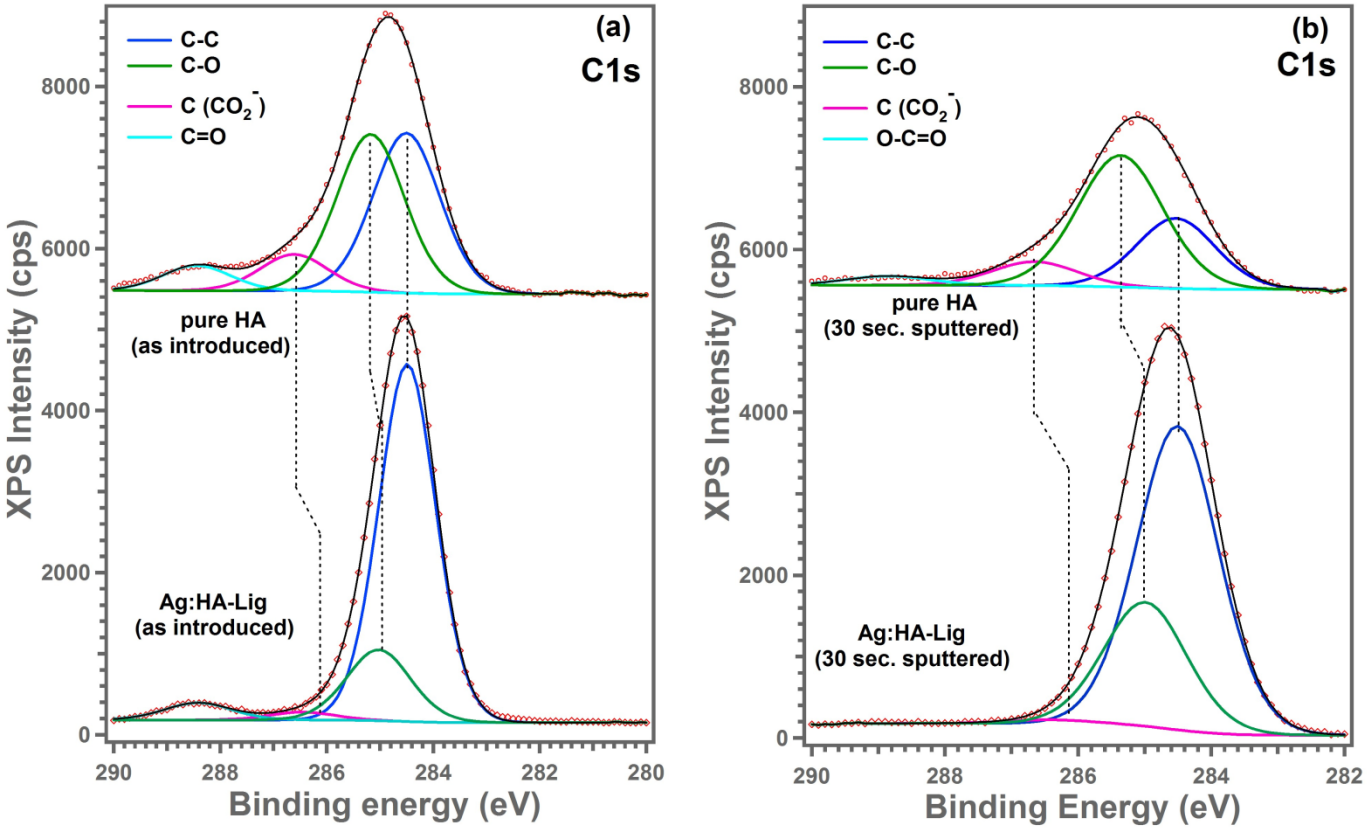


Top-view SEM micrographs of the HA-Lig (a) and Ag:HA-Lig (b) films deposited onto TiO_2/Ti substrata by MAPLE. Inset: cross-view SEM micrograph of Ag:HA-Lig film deposited onto silicon wafer

- MAPLE deposition conditions led to rather smooth films with a homogenous and pore-free microstructure, without particular morphological features.
- No morphological differenced have been evidenced between the HA-Lig/Ti and Ag:HA-Lig/Ti films.
  - ~180±10 nm nm film thickness

The quantitative EDS estimations indicated the synthesis of calcium deficient HA films, as the Ca/P ratios seem to have slightly altered during the ablation process down to a value of ~1.33 (lower than the 1.667 theoretical Ca/P ratio of stoichiometric HA).

HA-Lig and Ag:HA-Lig – XPS SPECTRA

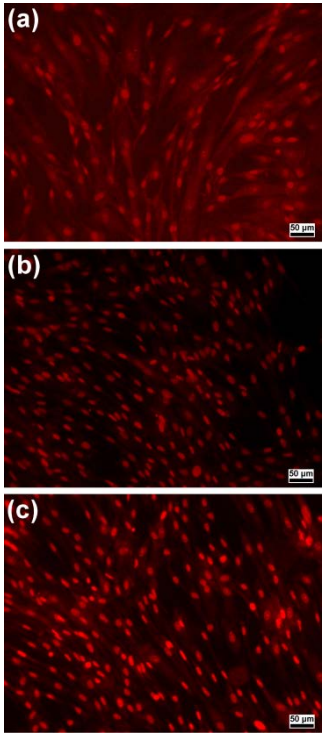


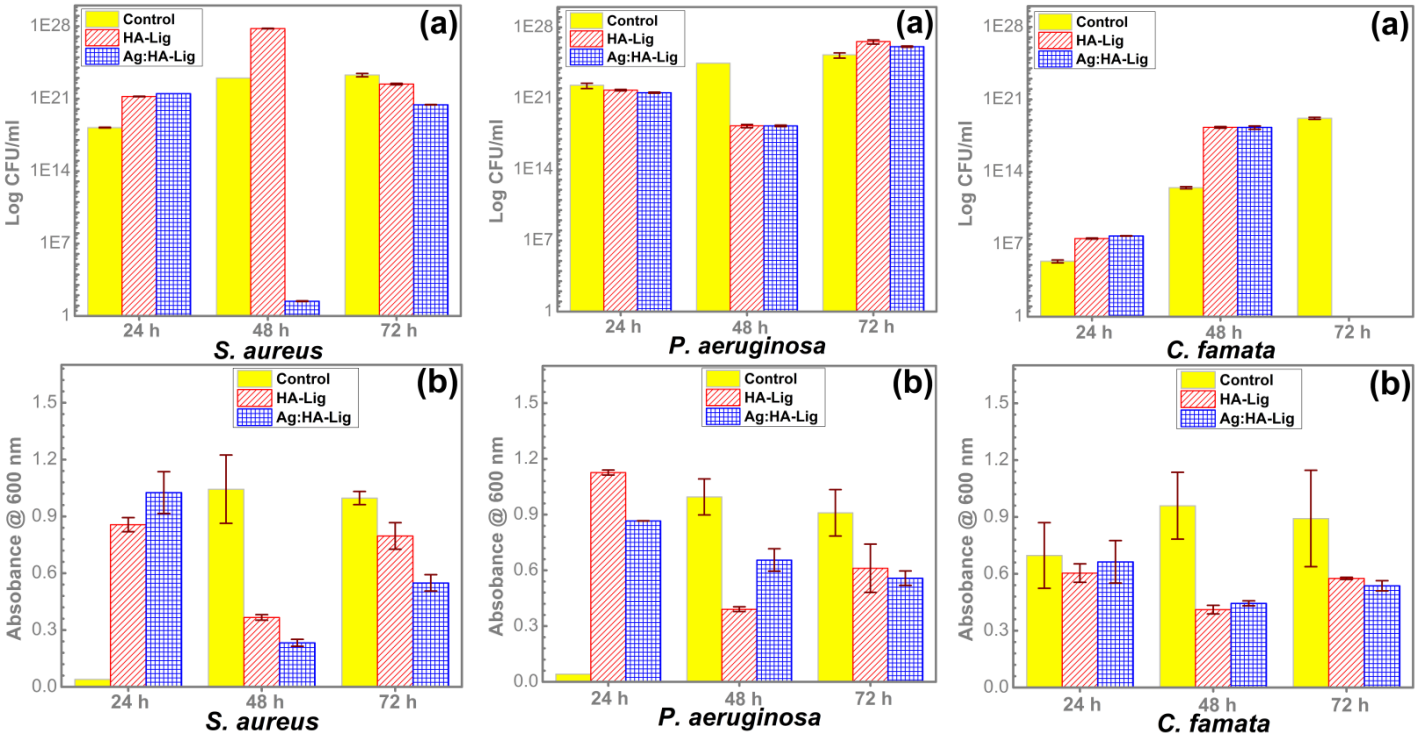
C 1s core level high resolution XPS spectra of pure HA (a) and Ag:HA-Lig (b) films

- The Lig signature was revealed to be dispersed in the HA matrix, as evidenced by a massive increase of the C-bonded carbon signature, accompanied by a slight increase of the component associated with oxygen-bonded C or oxygen-containing radicals

- Lig has been effectively transferred into the HA composite film

HA-Lig and Ag:HA-Lig – BIOLOGICAL ASSAYS





Fluorescence microscopy images of nuclei of WJ-MSCs grown on different substrata: pure HA (a); HA-Lig (b); and Ag:HA-Lig (c) films. Magnification: 200X

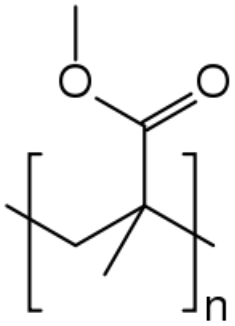
The biological assays demonstrated that the organic-inorganic lignin-hydroxyapatite composite coatings synthesized by MAPLE could provide an efficient protection against microbial biofilms, without inducing any cytotoxicity towards tested Wharton's Jelly-derived Mesenchymal Stromal Cells .

BG-PMMA

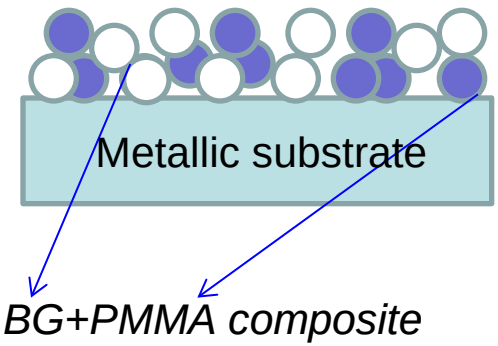
Metals and alloys used in implants are susceptible to corrosion in body fluids → release of ions that accumulate in vital organs

Most common corrosion types of metallic medical devices:
- pitting
- galvanic
- crevice

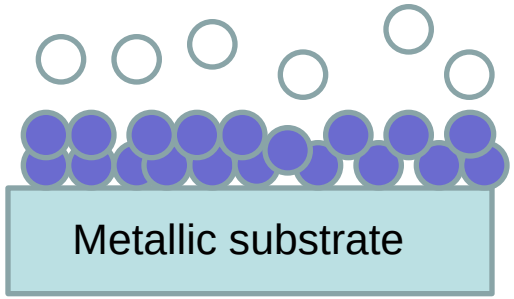
Proposed solution: apply protective BG-PMMA coating→ PMMA insulator and barrier against ions



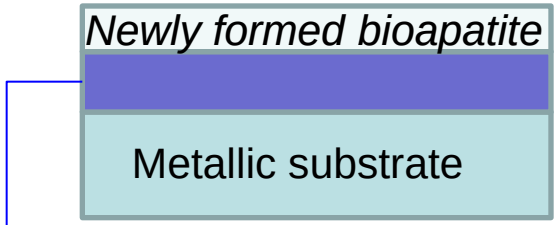
$(C_5O_2H_8)_n$



BG+PMMA composite



Immersion in body fluids



Continuous , compact layer of PMMA

Experimental details

- *Target preparation:*

0.6g PMMA +0.08g BG in 19.3 ml chloroform

- *Two types of BG :*

BG61: SiO_2 61,1%, Na_2O 10.3%, K_2O 2.8%, CaO 12.6%, MgO, 7.2%, P_2O_5 6%

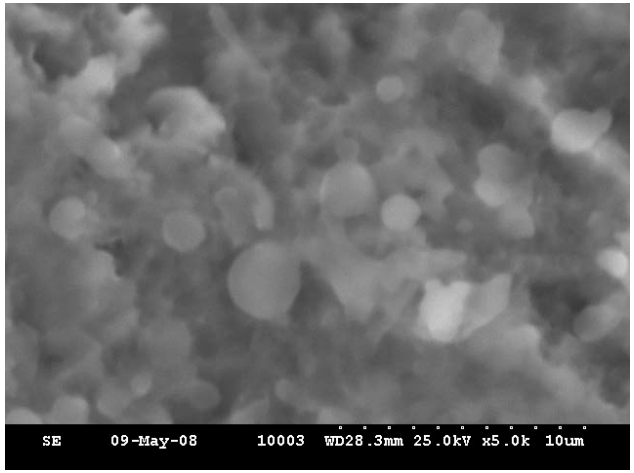
BG57: SiO_2 57%, Na_2O 11%, K_2O 3%, CaO 15%, MgO 8%, P_2O_5 6%

Deposition substrates: Ti gr.4 disks

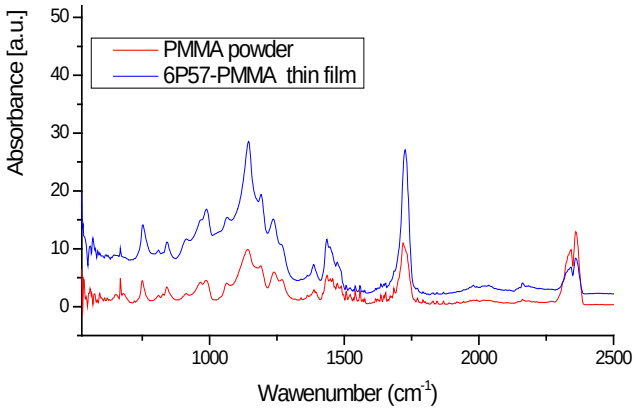
Laser parameters: λ=248 nm, τ= 25 ns, F=0.55J/cm^2

Immersion in SBF: 28 days (for BG57) or 42 days (for BG61)

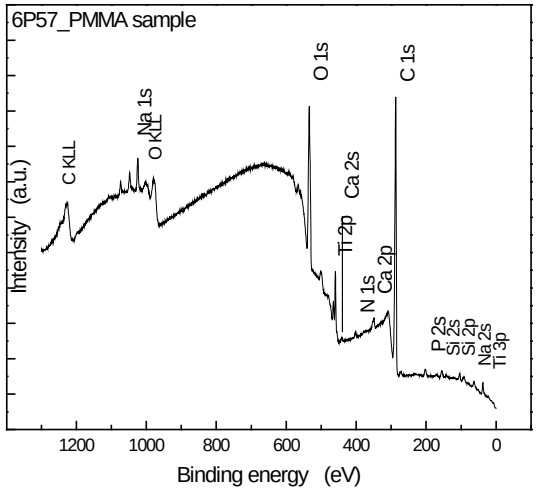
Results



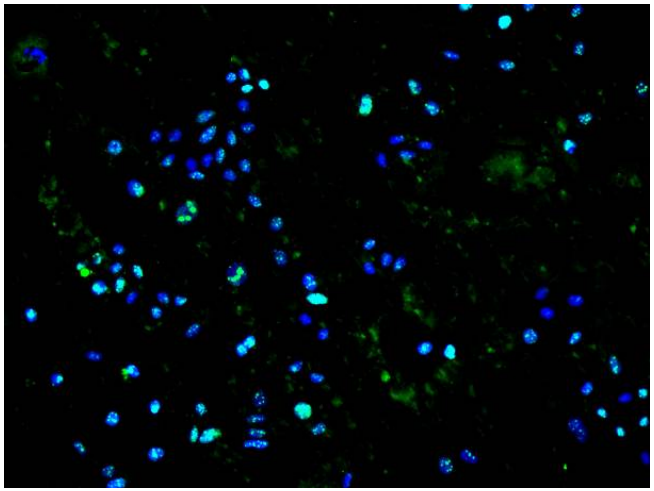
SEM micrograph showing a typical surface morphology of a BG57-PMMA coating



FT-IR spectra of PMMA powders and of a BG57-PMMA coating obtained by MAPLE



XPS survey spectrum of the BG57-PMMA coating

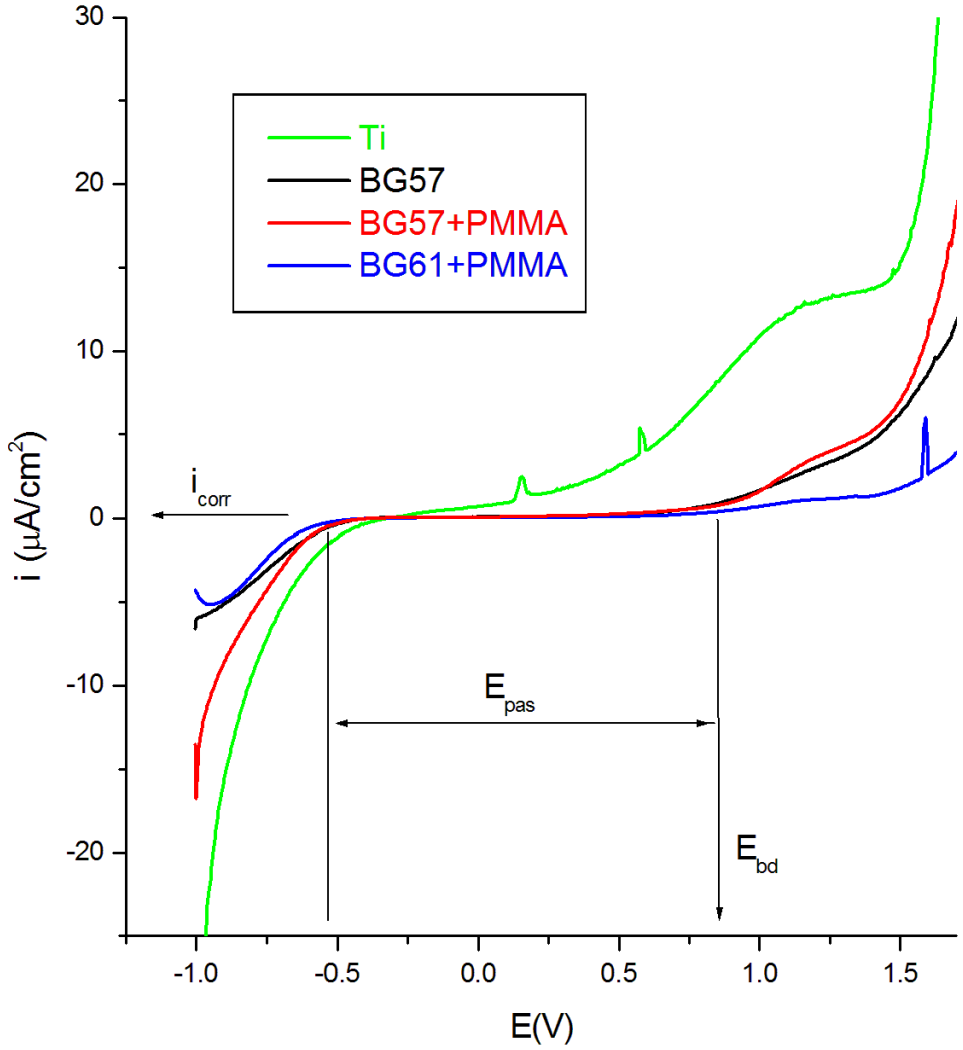


Fluorescence microscopy of cells cultured on MAPLE deposited structures BG57 – PMMA

The film contains PMMA and bioglass. Biological properties similar to simple BG57 and BG61 bioactive glass coatings

Higher proliferation of cells on BG57+PMMA!

Corrosion measurements



Polarisation curves in SBF

Corrosion parameters for the bioglass-polymer samples determined from the polarization curves

Material	E_{corr} (mV)	i_{corr} ($\mu A/cm^2$)	E_{bd} (mV)	E_{pas} (mV)
Ti	-357	1.320	141	541
BG57/Ti	-309.3	0.56	535.2	1037.4
BG57+PMMA/Ti	-251	0.053	1274	1192
BG61+PMMA/Ti	-224	0.022	1454	1512

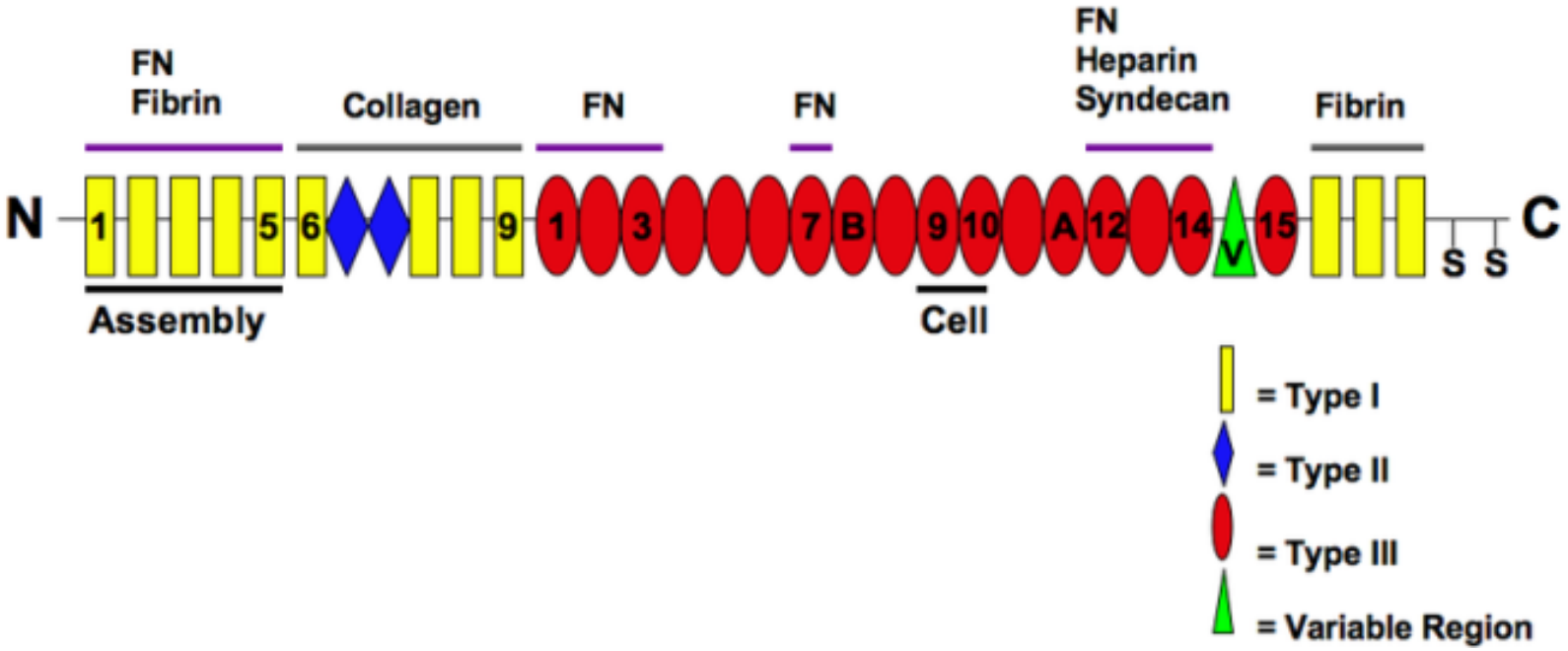
E_{corr} – corrosion potential; i_{corr} – corrosion current density ~ corrosion rate;
E_{bd} - breakdown potential;
E_{pas} – passivation potential ~ transpassive region dimension

The corrosion rate (given by i_{corr}) decreased 2.35 times after coating with BG57, to drop 25 times for BG57 + PMMA and 60 times in case of BG61+PMMA => the bioglass-polymer nanocomposite coatings protect very well the titanium implant against corrosion.

E_{bd} increases about 10 times after coating => significant increase of shielding effect

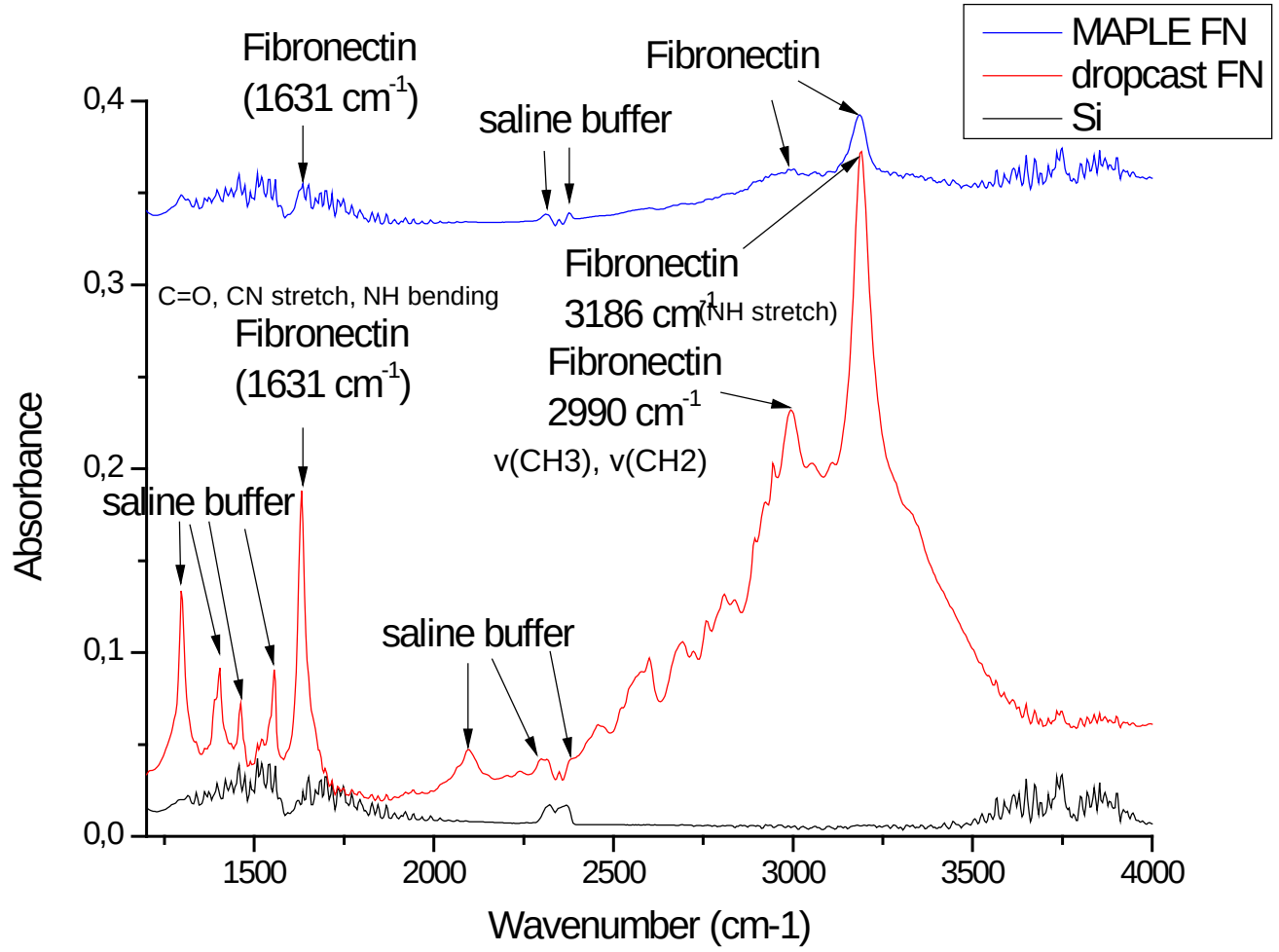
HA-ECM proteins

ECM proteins: Fibronectin, vitronectin



Fibronectin structure

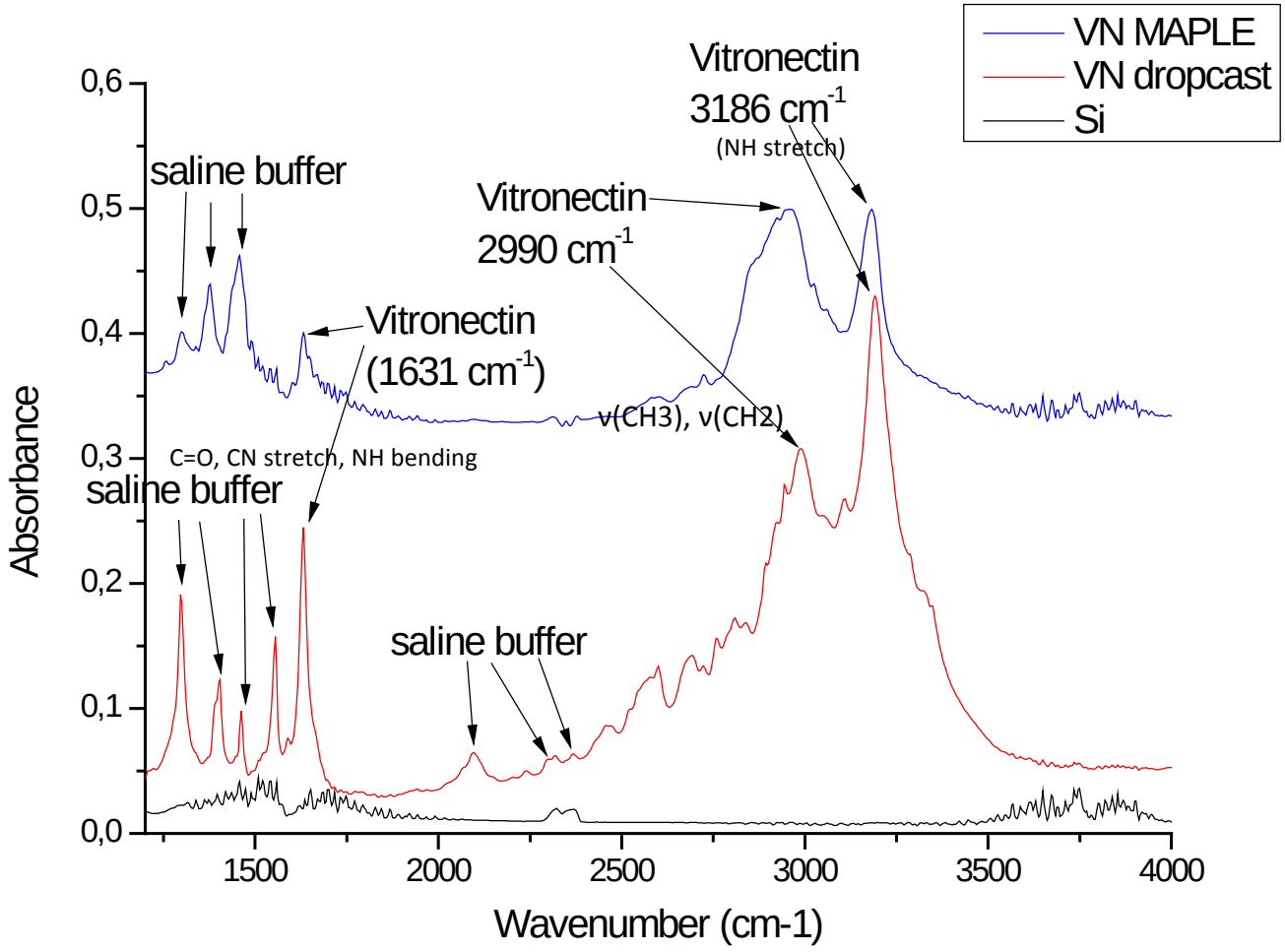
Protein investigations: FT-IR studies



IR absorption bands of FN structures obtained on silicon substrates

** 1.8 mg/ml in deionized water based saline buffer, 700 mJ/cm^2, 15000 subsequent laser pulses*

Protein investigations: FT-IR studies



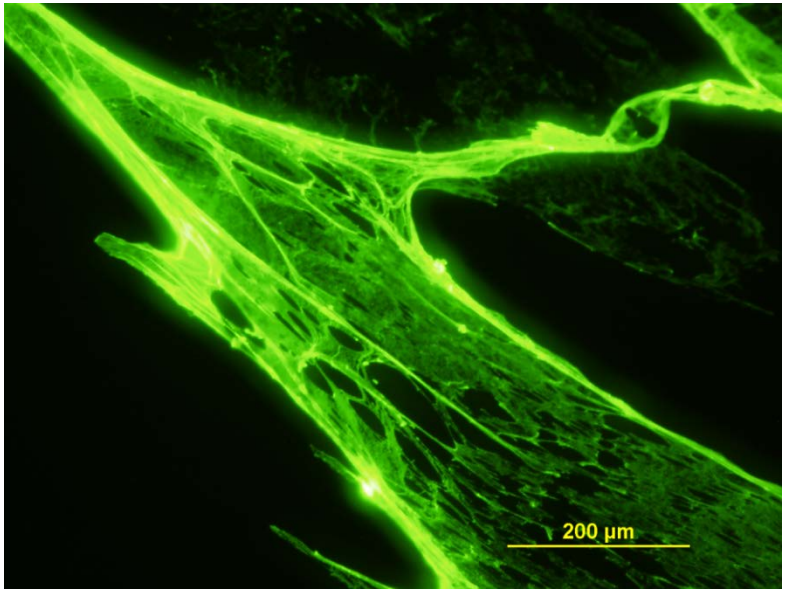
IR absorption bands of VN structures obtained on silicon substrates

The peaks of proteins deposited by MAPLE are matching very well the ones from dropcast!

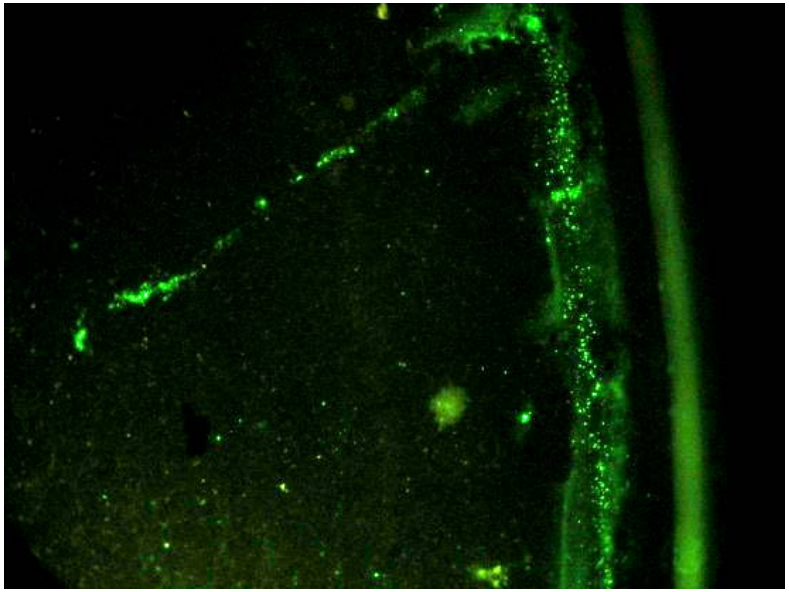
** 1.8 mg/ml in deionized water based saline buffer, 700 mJ/cm^2, 15000 subsequent laser pulses*

Antibody staining

Experiments with anti-human FN and anti-human VN rabbit polyclonal serum.
The secondary antibody for FN and VN was an FITC-conjugated anti-rabbit IgG.



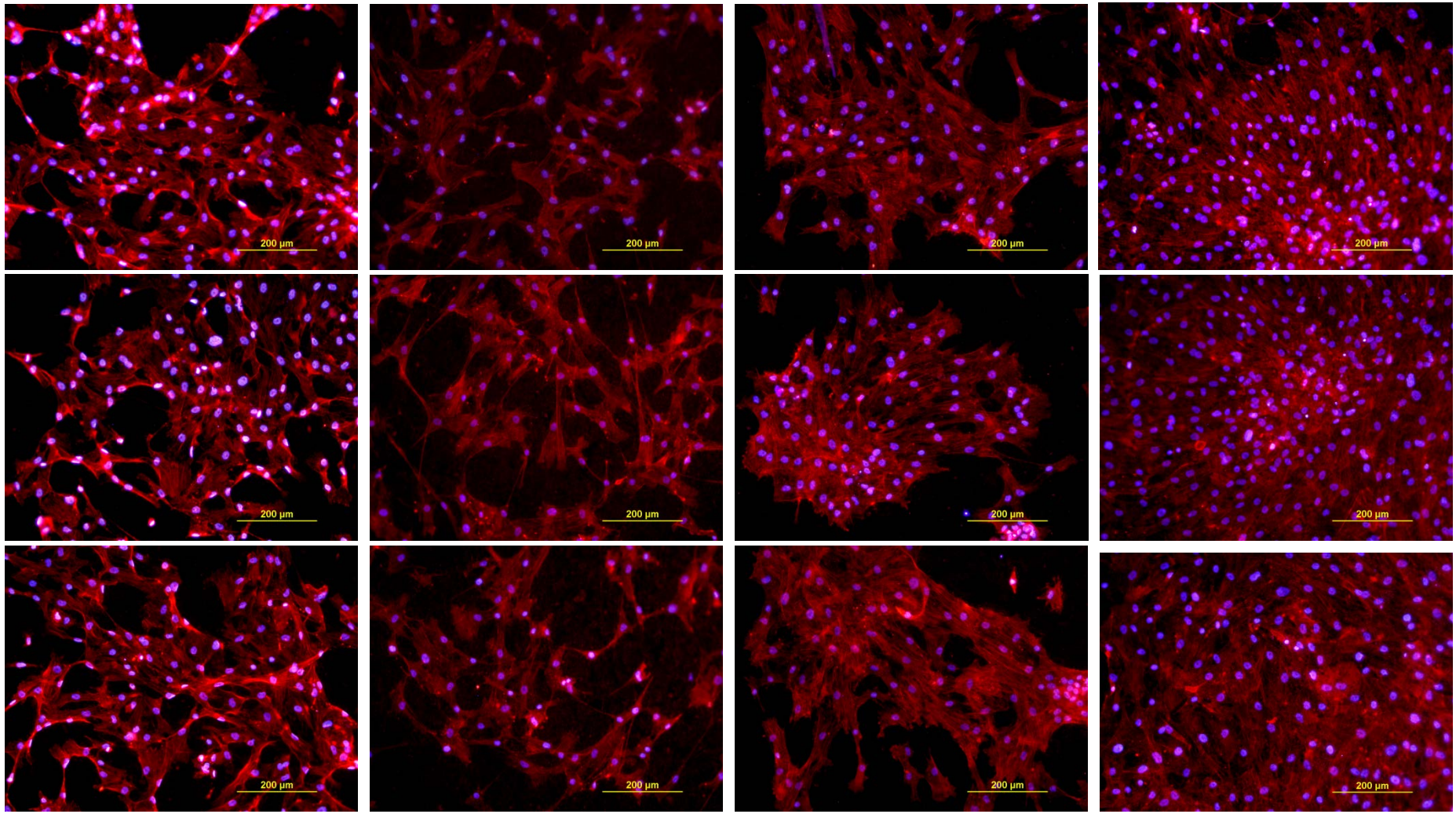
Immunofluorescence detection of fibronectin structures



Immunofluorescence detection of vitronectin structures

Fibrilar structure of FN and necklace-like confined structure in case of VN were evidenced: **the physiological conformation of transferred FN and VN after MAPLE is confirmed**!

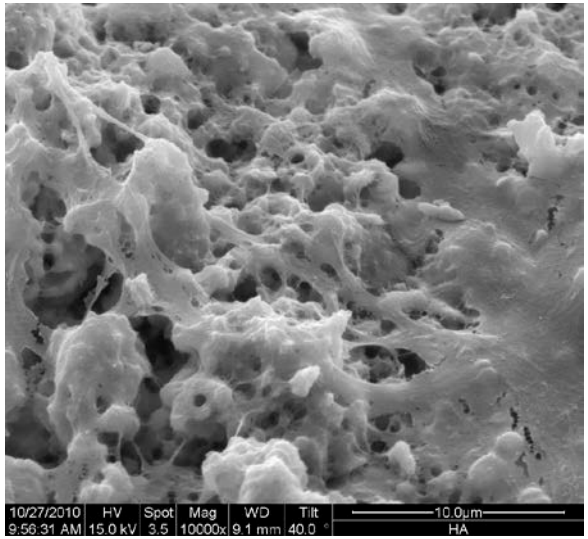
HOB precursor cells: Actin and nuclei staining 7 days



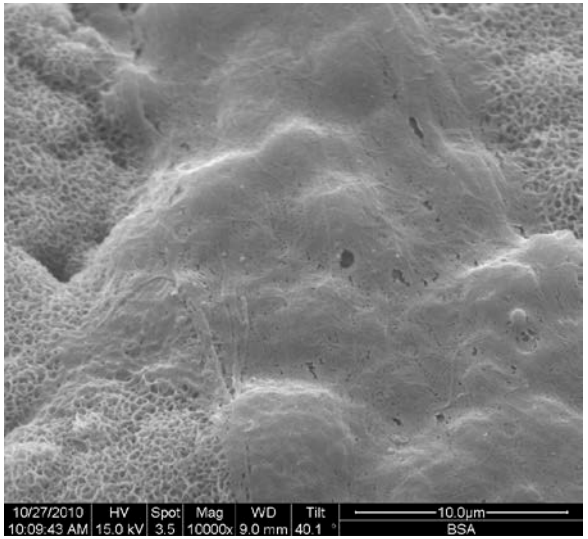
Higher potential for adhesion and spreading in the case of Ti/HA/FN and VN/HA/Ti structures as compared to HA/Ti and BSA/HA/Ti

Scanning electron microscopy studies 7 days

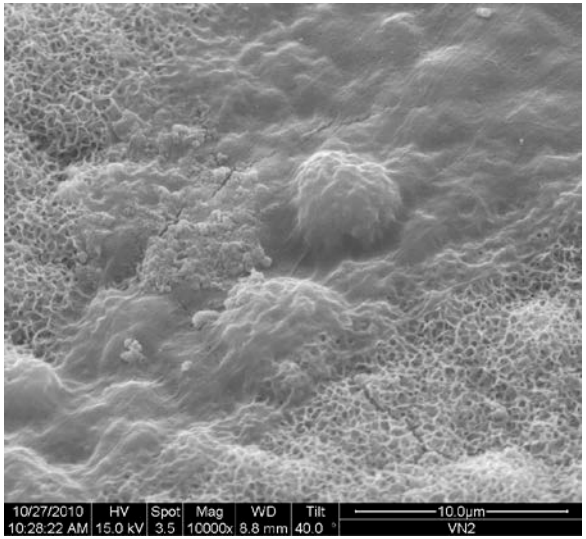
HA/Ti



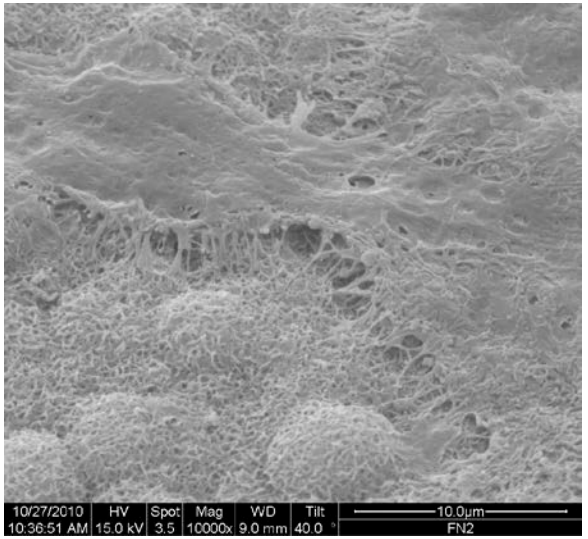
BSA/HA/Ti



VN/HA/Ti

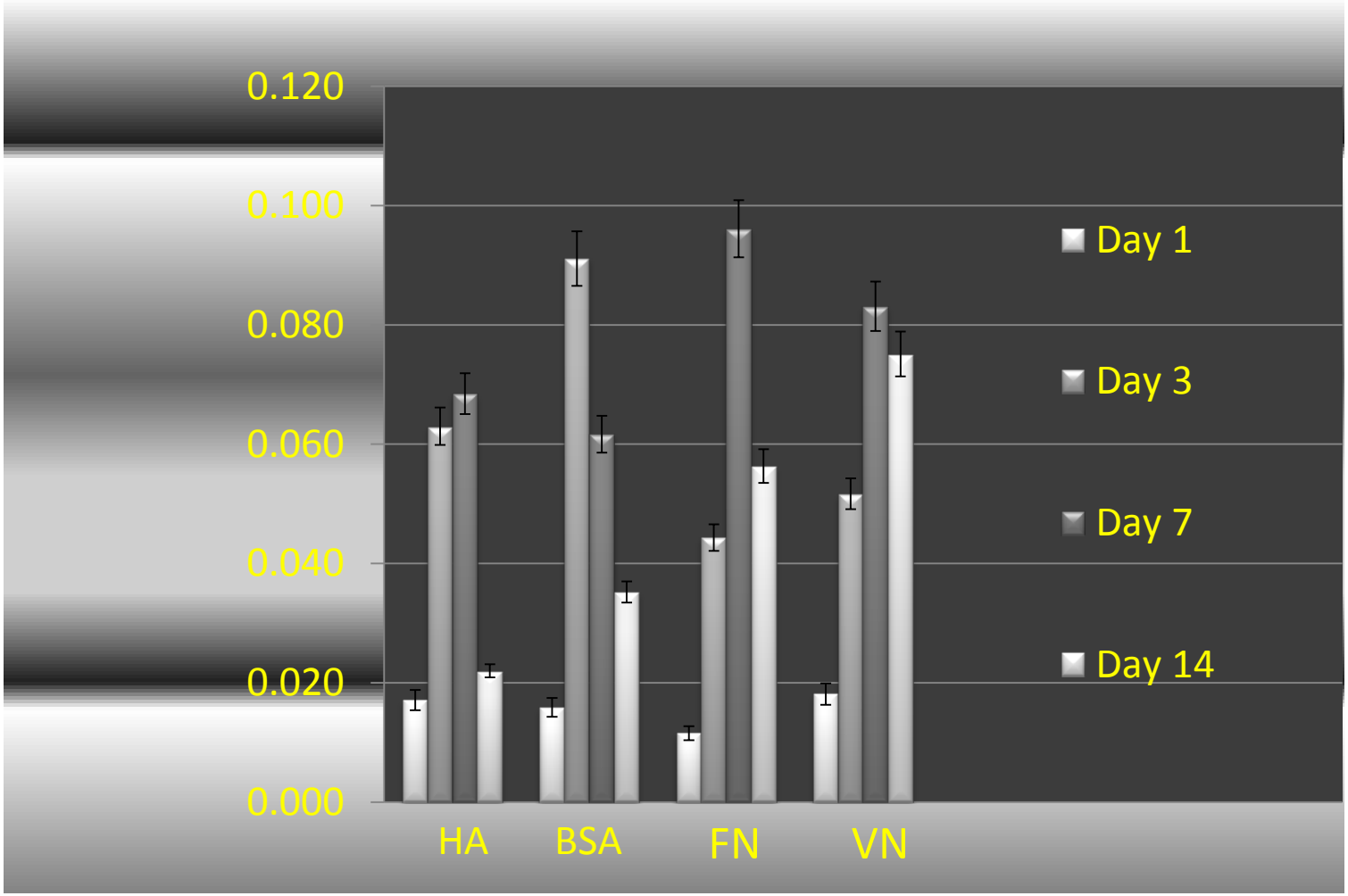


FN/HA/Ti



Flat cell morphology, intimate contacts and long filopodia were visualized for FN and VN covered structures as compared to HA/Ti and BSA/HA/Ti controls.

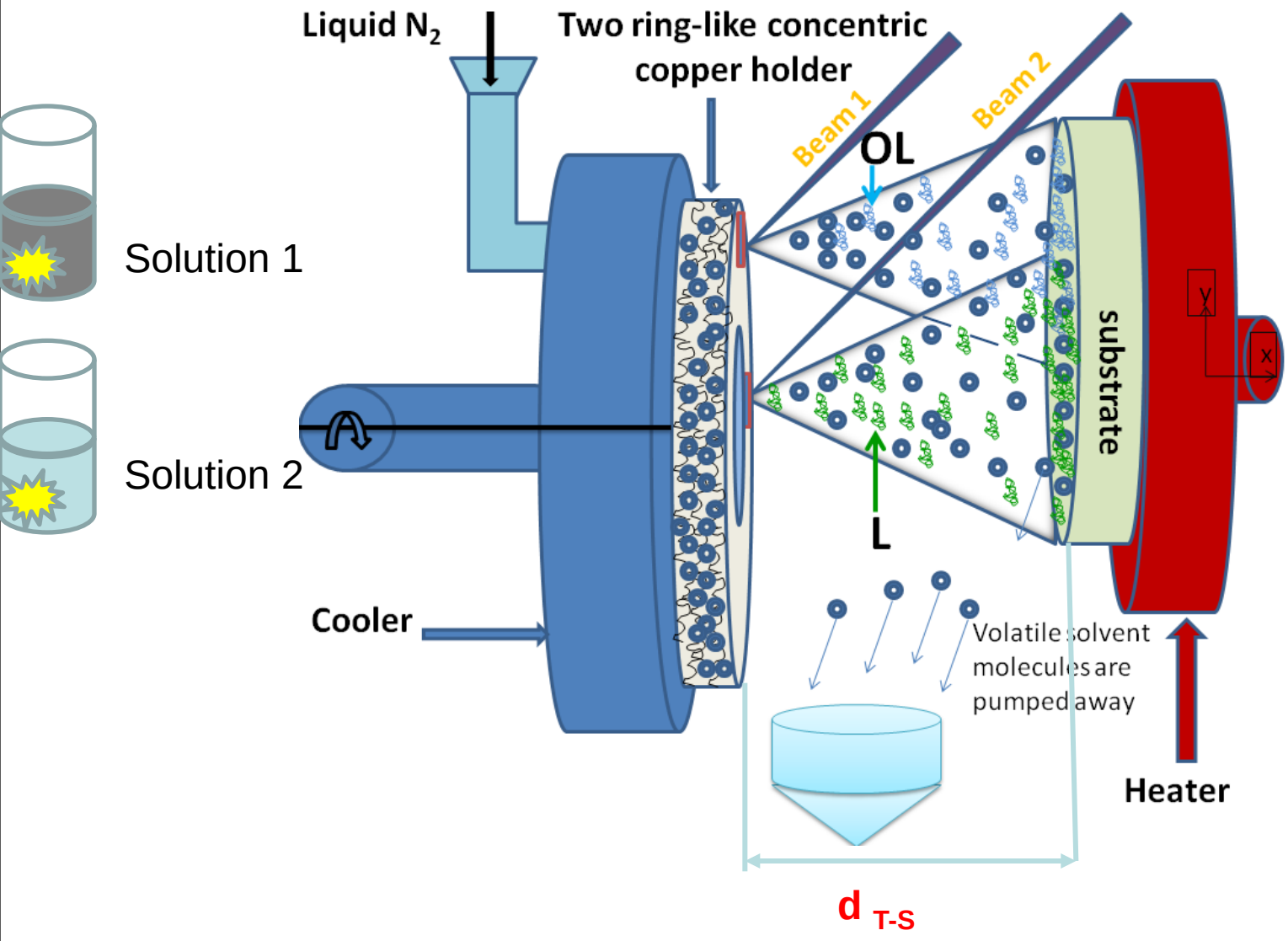
Cell culture – human osteoprogenitor cells: MTS assay



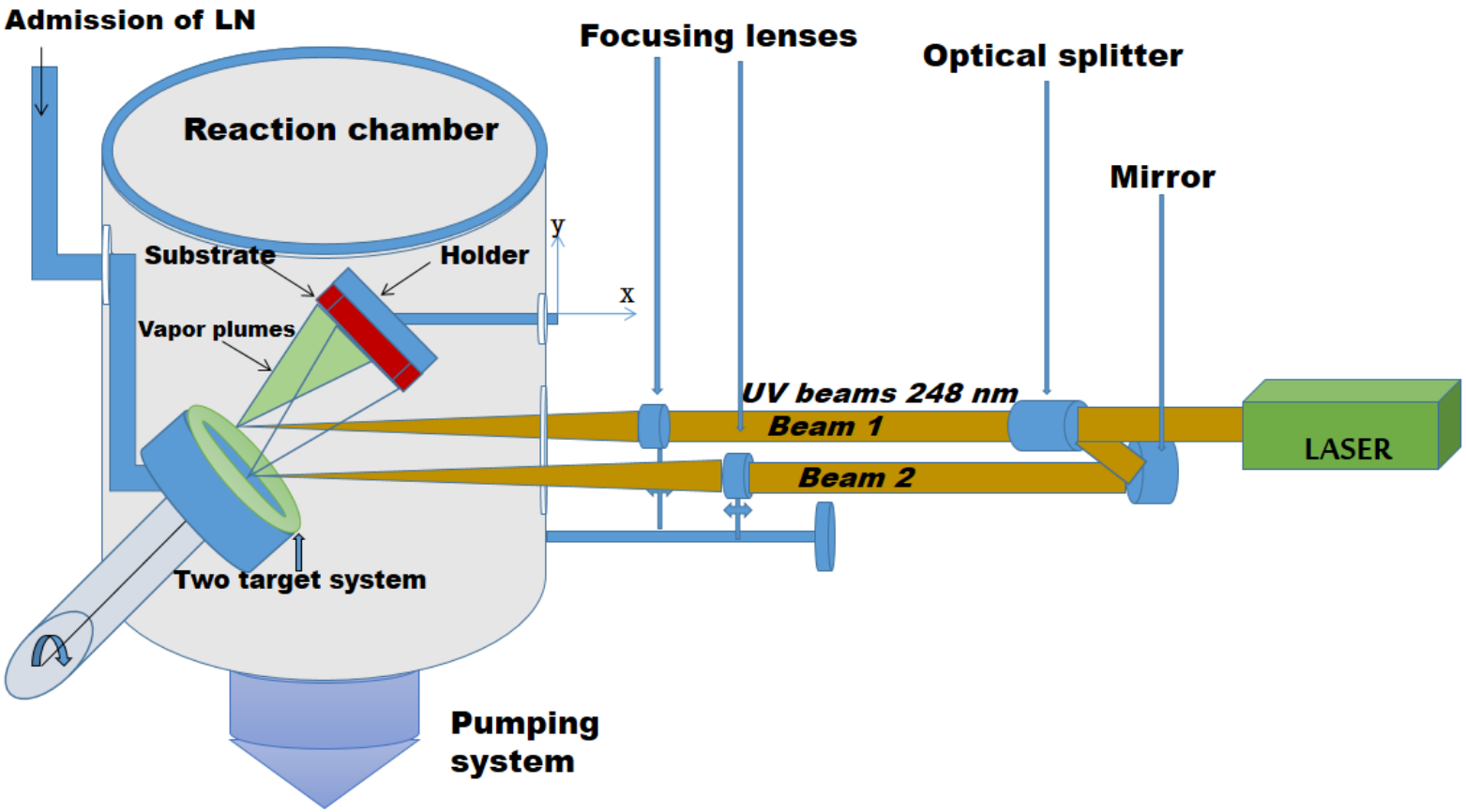
In-vitro tests demonstrate a much larger bioactivity of FN/HA/Ti and VN/HA/Ti as compared to HA/Ti or BSA/HA/Ti

Gradient Biopolymers by Combinatorial MAPLE

- hydrofile polymers with high biocompatibility



Scheme of C-MAPLE: reaction chamber and beam splitting



Schematic of the C-MAPLE set-up: two solutions are frozen and form the targets (reaction chamber, left side) in a concentrically two target system constantly fed by a LN flow. The laser beam is split in two beams which simultaneously vaporize the materials in frozen targets which are next deposited on a facing substrate.

Organic composite biomaterials assembled in specific configurations by C-MAPLE

Challenges:

1. **Find the optimum dosage from biological point of view between two materials with different structure and composition.**

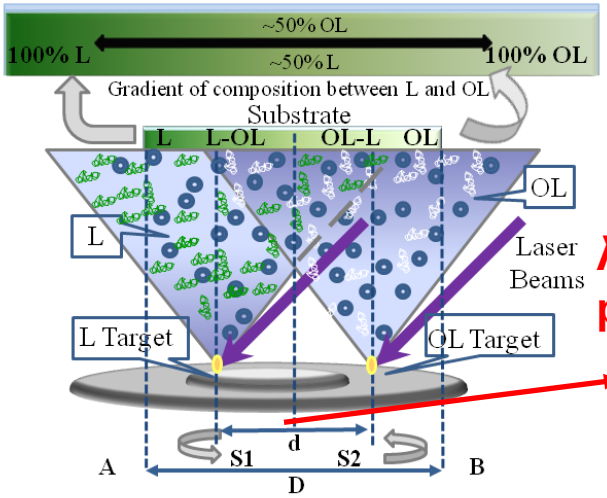
2. ***In situ*, single-step, doping of active substances (drugs, proteins or enzymes) in biodegradable polymeric matrices for local release.**

3. **Testing new compounds with the view to replace well known materials with innovative ones.**

1. **Find the best biological dosage between two biomaterials with different structure/composition**

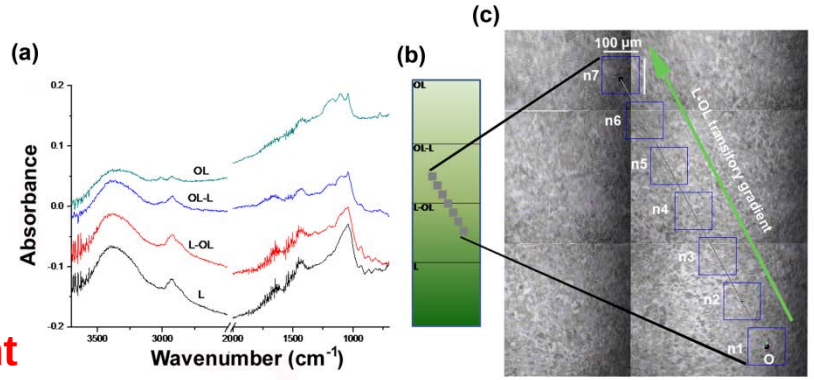
e.g. Levan and Oxidized Levan

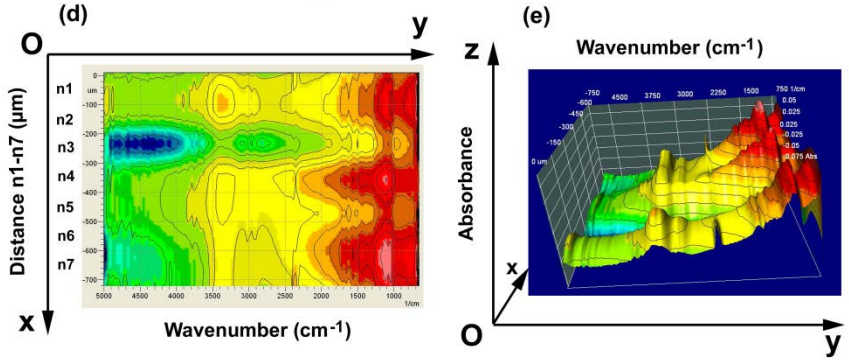
Compositional Gradient Biopolymers: μFTIR and fluorescence microscopy studies

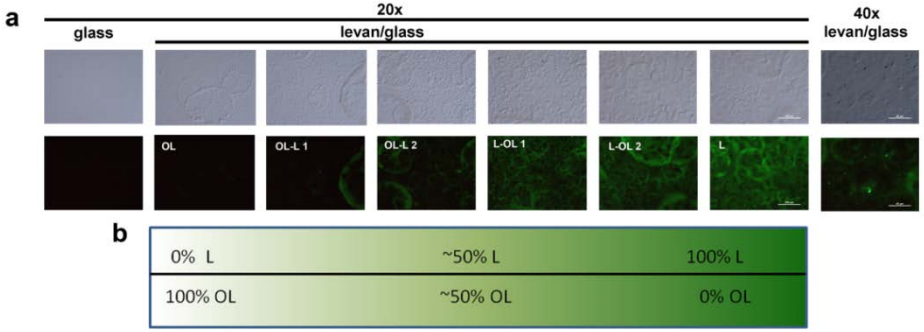


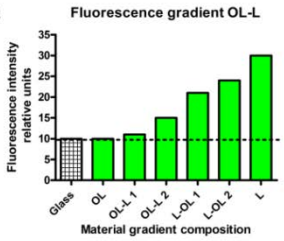
λ_{las}, polymer, solvent

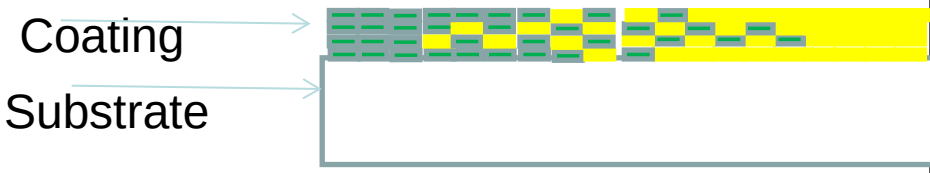
d_{s-s}





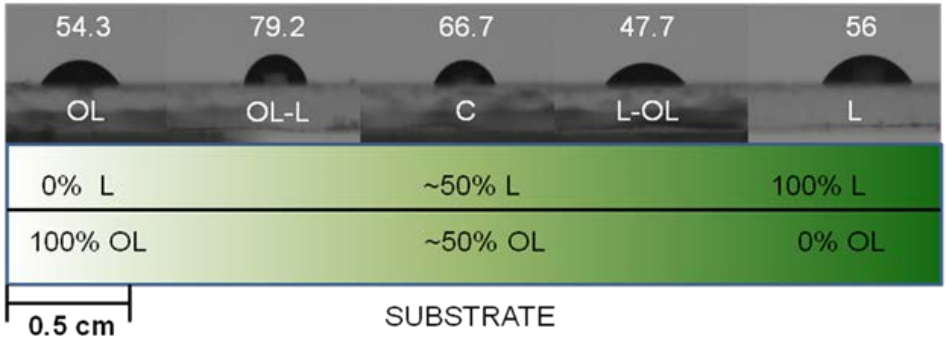


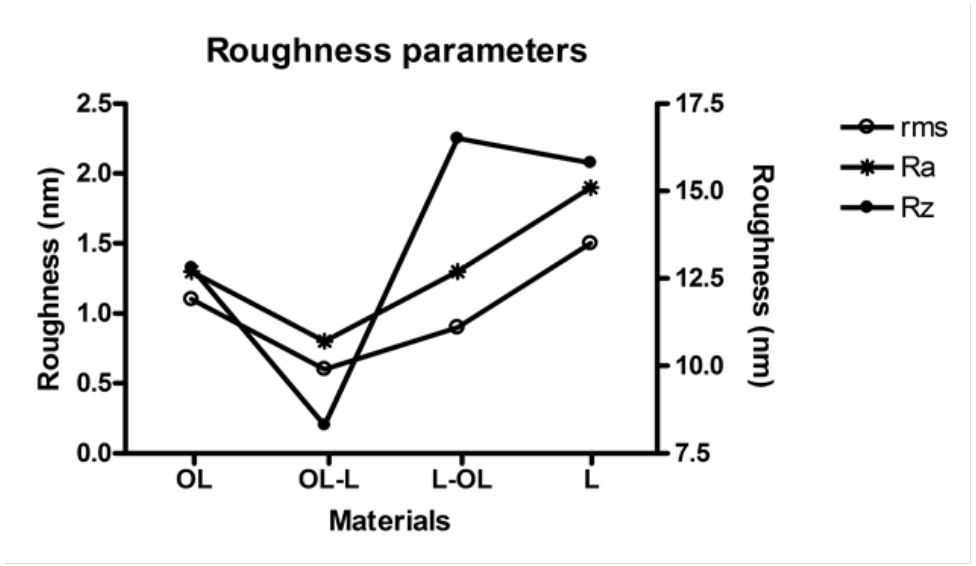




The gradient was demonstrated by μFTIR and fluorescence microscopy

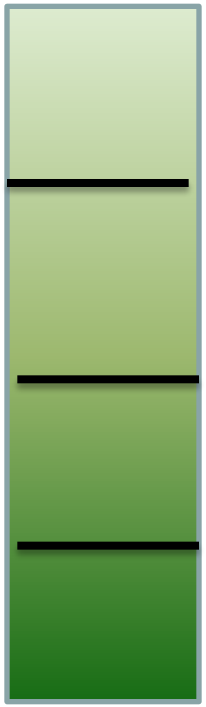
Wettability and roughness studies





Contact Angle (CA) reaches maximum (~80 degrees) in OL-L zone (top figure), where roughness is the smallest. The rougher the surface, the smaller is CA. Droplets either pursue the topography or penetrate inside texture.

In-vitro tests : 72 h



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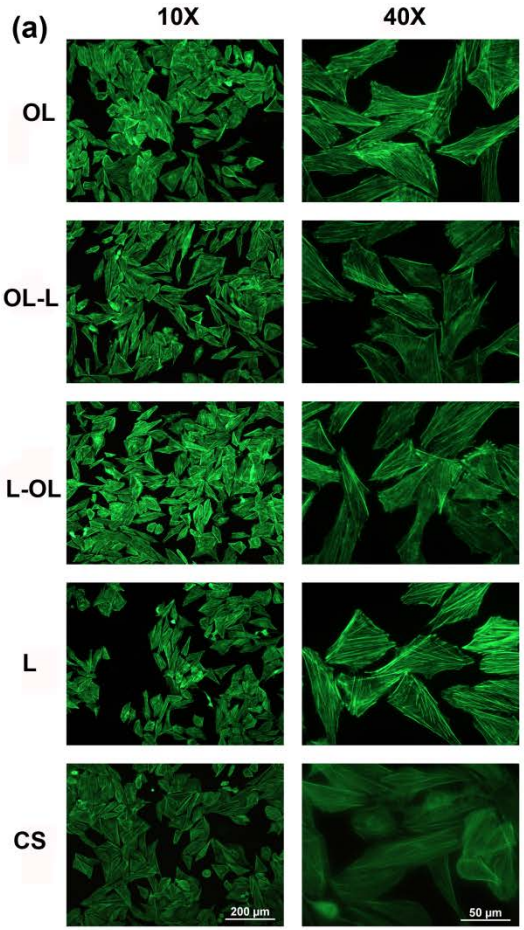
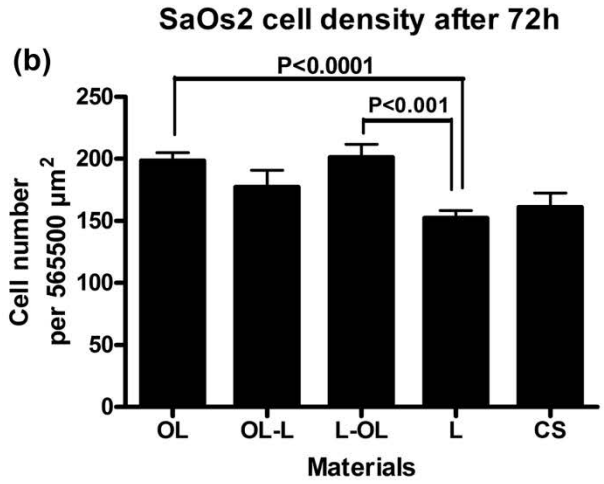
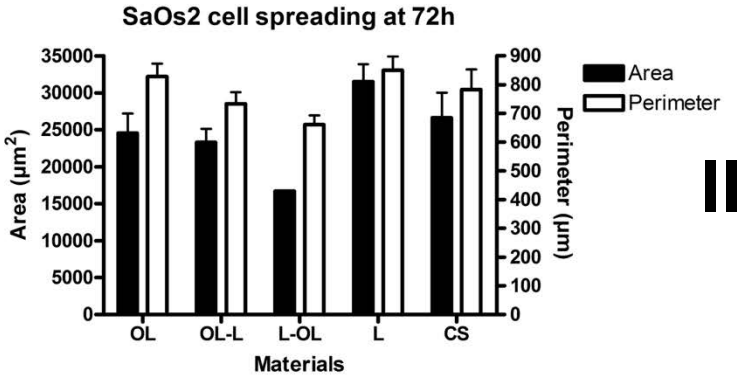




IIa



IIb

Cells preferred OL versus L regions. *Significant effect:* accumulation of cells on L-OL film region as compared to other areas (Fig. IIa).

Cell areas and perimeters correlate to spreading. A gradual decrease in the values associated to cell areas and perimeters was revealed (Fig. IIb) from OL to OL-L to L-OL regions.

2. *In-situ*, single-step, embedding of active substances (drugs, proteins or enzymes) in biodegradable polymeric matrices for local release;

e.g. Fibronectin in PDLLA

3. Comparison of two similar compounds with the view of replacing well known materials with new innovative ones

e.g. Heparin and Levan S (in progress)

Conclusions

1. We synthesized by MAPLE thin biomaterial films for applications

   (i) in drug delivery: triacetate pullulan, HA-alendronate, levan, oxidised levan;

   (ii) as biosensors: urease, IgG, TPP; or

   (iii) for biomimetic coatings of advanced implants: Lig(Ag):HA, PMMA-BG, pure and doped OCP, ECM proteins.

2. Multistructures can be deposited by subsequent application of different laser pulsed deposition techniques (PLD, MAPLE, LDW) for tissues and organs reconstruction.

3. We tested different solvents as deionized water, chloroform, 3-buthanol, DMSO, isopropanol, toluene, …. The proper choice of solvent and deposition conditions proved essential for getting the best possible compromise between films bioactivity and morphology.

4. The thickness of the buffer layer proved essential for obtaining the cold, clean and liquid-free transfer of organic compounds by BB-LDW.

Conclusions

5. Compositional and physical gradients with desired density of functional groups in nanometric scale were obtained by laser evaporation

6. Combinatorial MAPLE applied to tailor biodegradable properties of polymers with embedded FN

7. All deposited films resemble in composition, structure, morphology, and most likely functionality the base material and replacing tissues, as proved by physical-chemical characterization and *in-vitro* testing.

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Thank you for your attention!