UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO
Dipartimento di Chimica

POLITECNICO DI TORINO 1859 1906

HyMedPoly

CNRNANOTEC
Istituto di Nanotecnologia

PLASMA PROCESSED SURFACES FOR LIFE SCIENCES

polymer surface functionalisation

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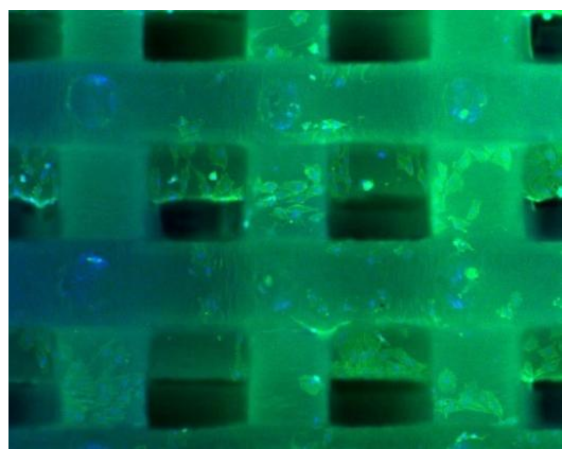

Biodegradable Polymers: Synthesis and Functionalisation
Workshop in the framework of the HyMedPoly ITN project *POLITO, Feb 10, 2016*

PLASMA PROCESSED SURFACES FOR LIFE SCIENCES

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OUTLINE

- Plasmas for Life Sciences
- Surface modification plasma processes for (bio)materials
- Selected examples
  - plasma processing of scaffolds for Tissue Engineering
  - nano/bio composite PE-CVD coatings
  - free standing PE-CVD coatings “NanoFilms”
- Conclusions
- Acknowledgements

1928
I. LANGMUIR INTRODUCES THE WORD "PLASMA"

I. Langmuir, *Oscillations in Ionized Gases*
Proc. Nat. Acad. Sci. 14, 627, Aug 1928

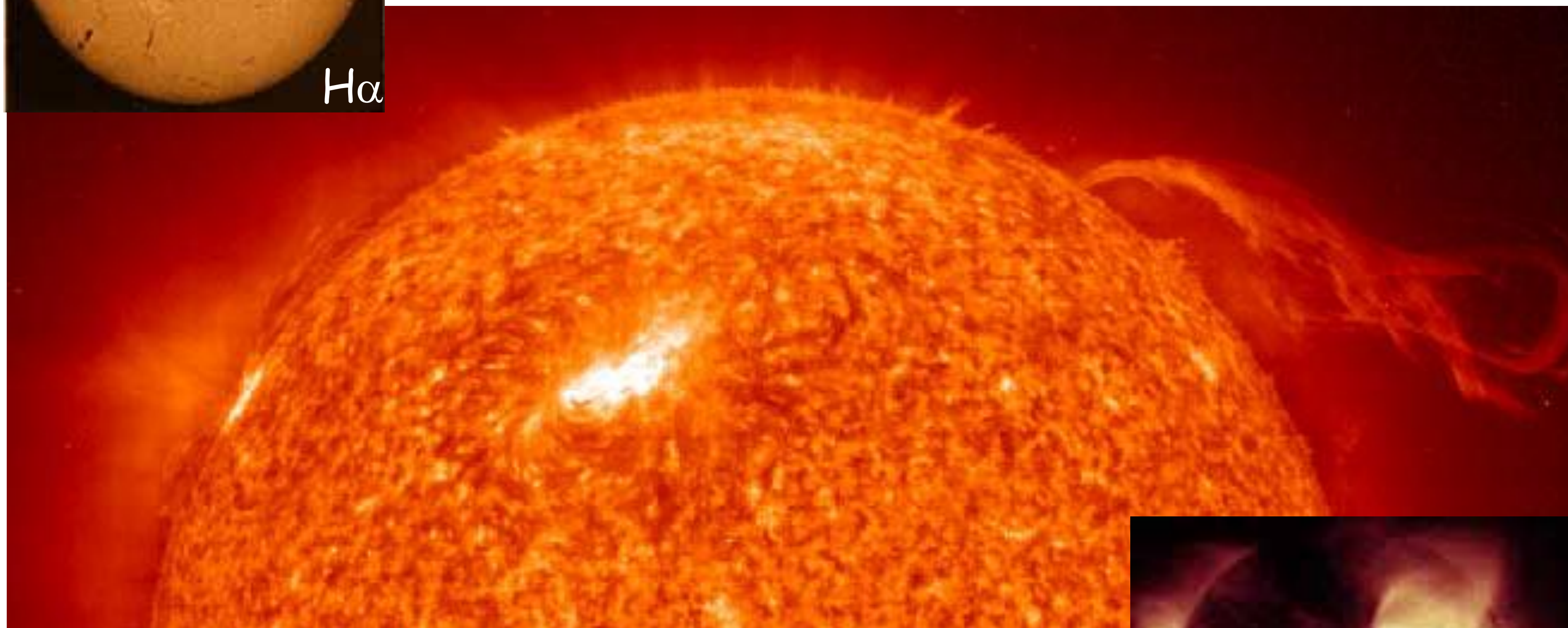
"Except near the electrodes, where there are sheaths containing very few electrons, the ionized gas contains ions and electrons in about equal numbers, so that the resultant space charge is very small. We shall use the name plasma to describe this region containing balanced charges of ions and electrons."

Irving Langmuir
(1881-1957)

Nobel Laureate in Chemistry 1932

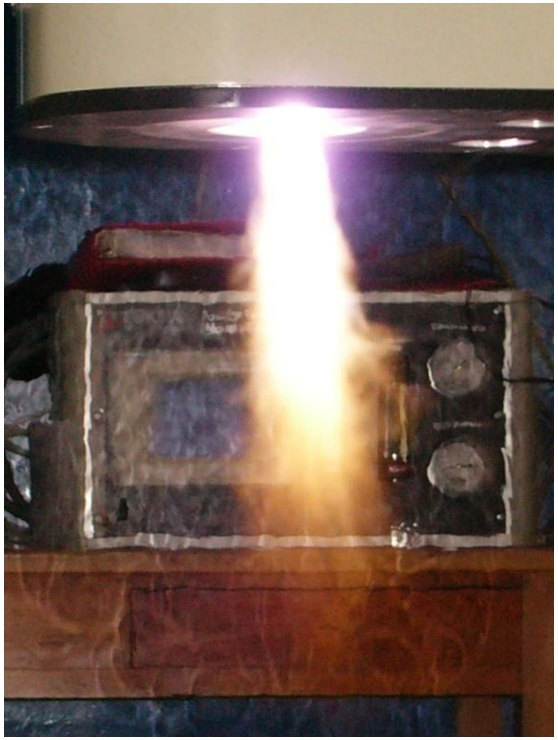
... for his discoveries and investigations in surface chemistry ...

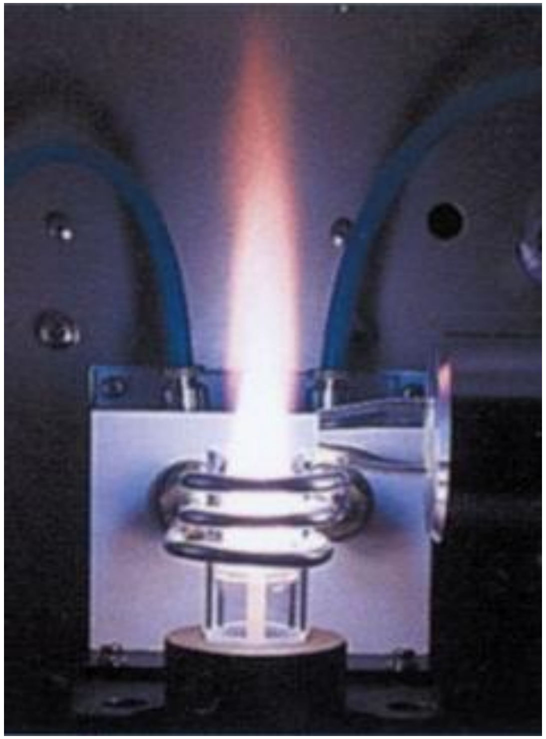
THERMONUCLEAR PLASMA

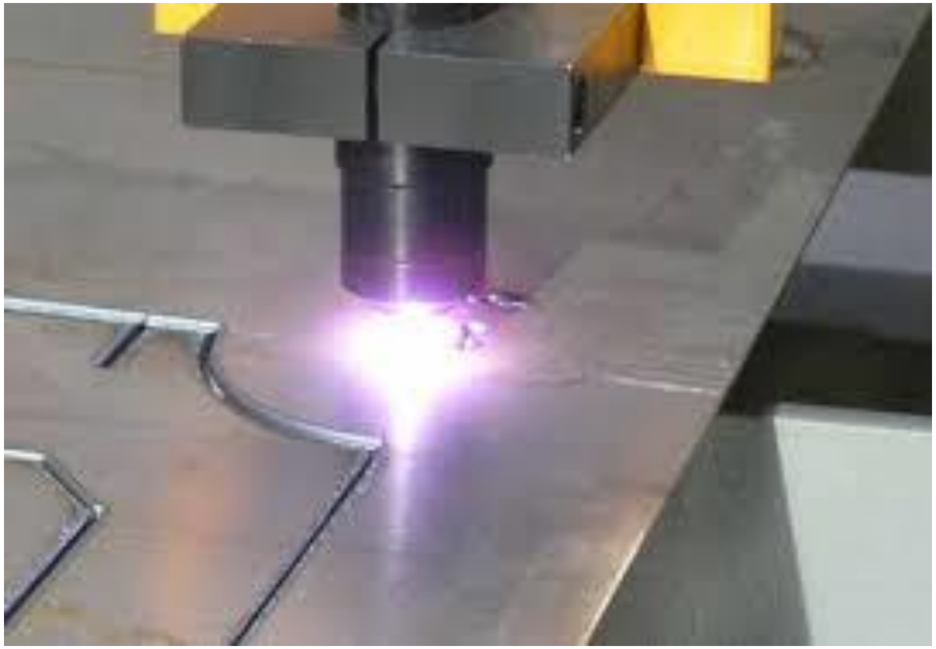


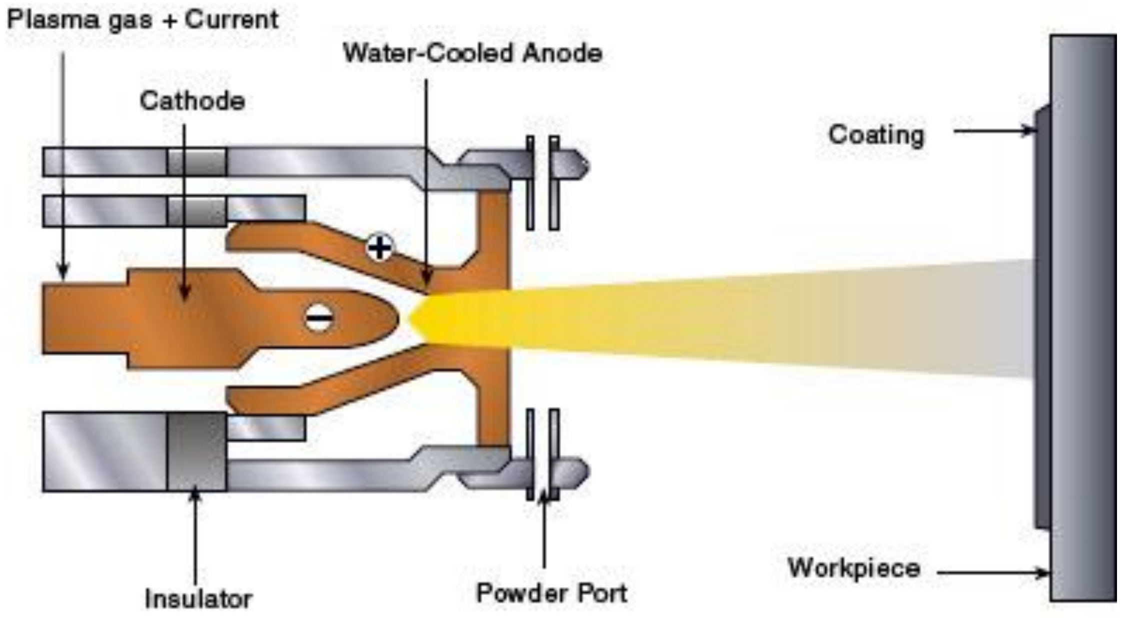
THERMAL PLASMAS

welding, cutting,
metallurgy,
plasma spray deposition,
ICP spectroscopy,
waste abatment



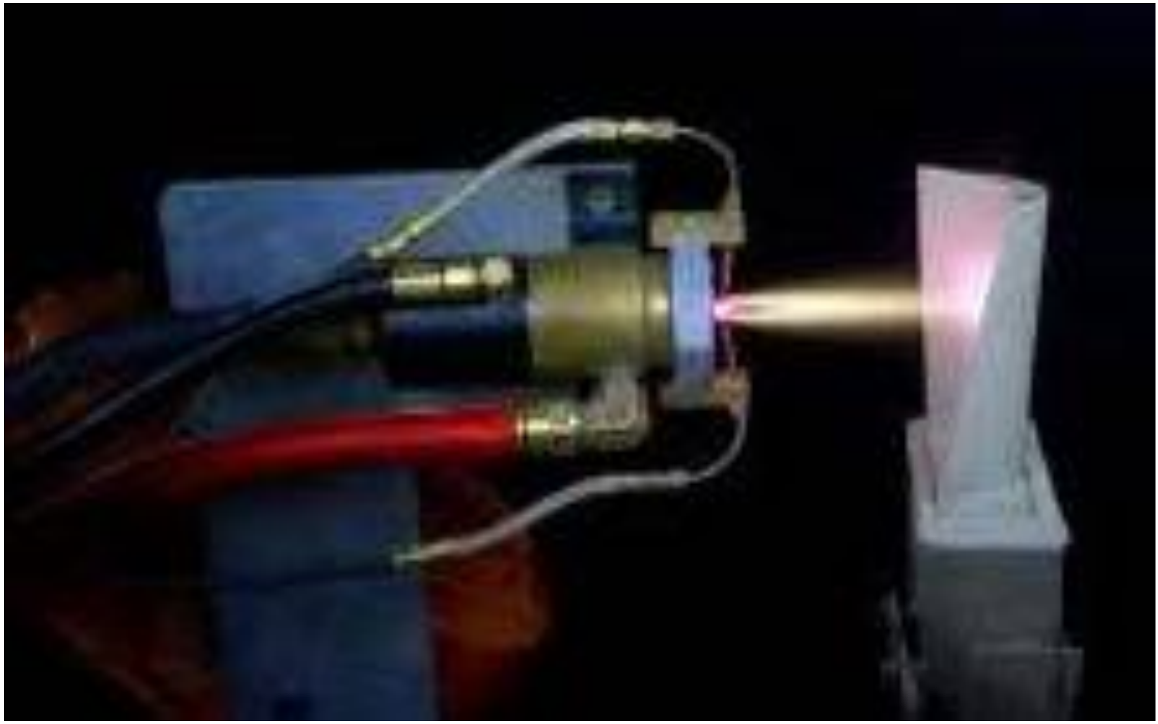




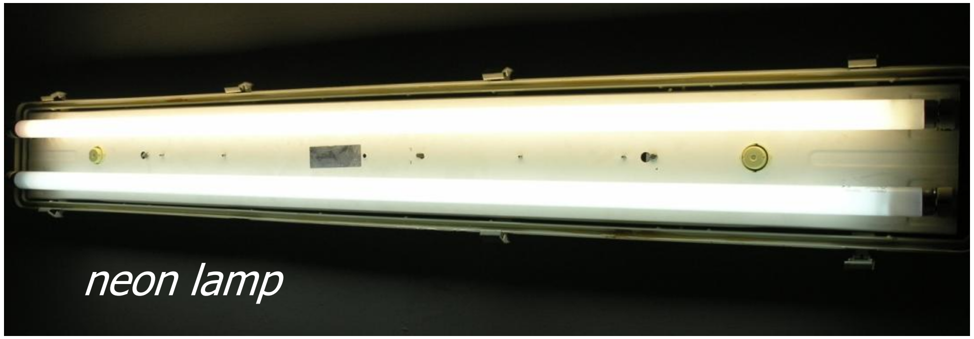


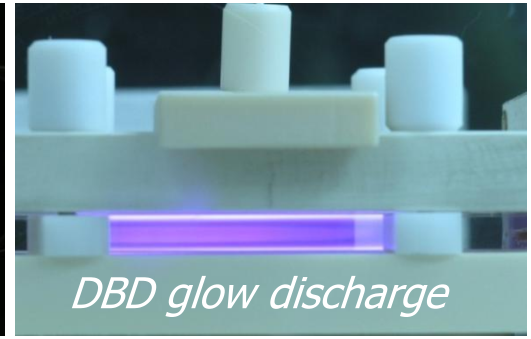
thermal plasmas for materials

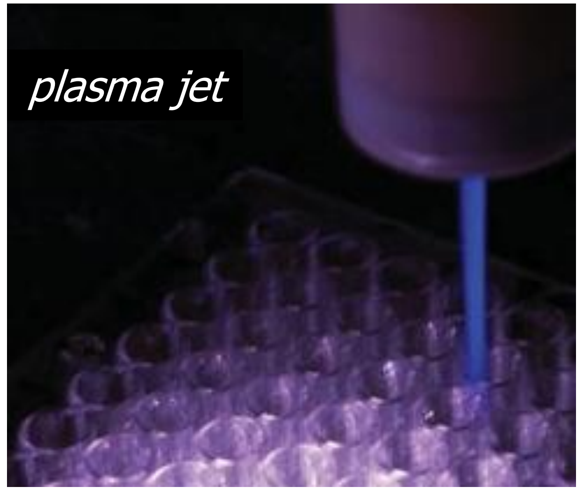
PLASMA SPRAY

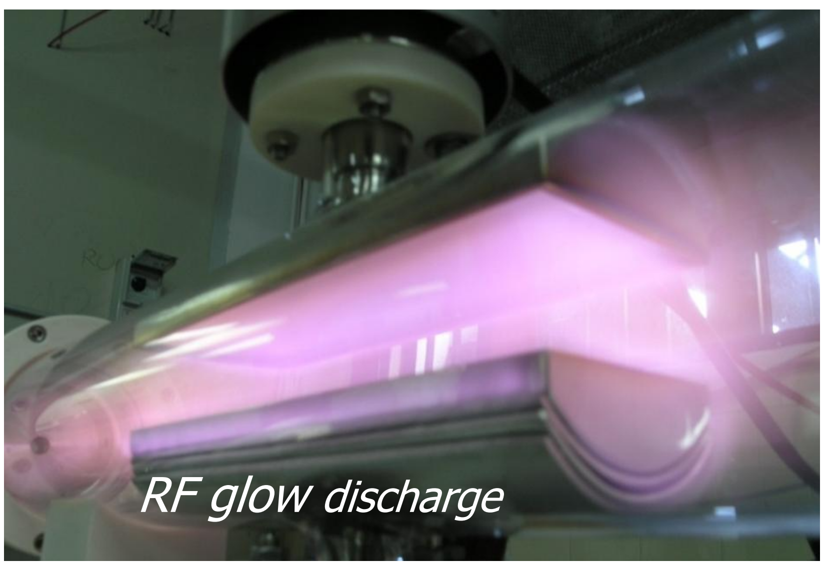


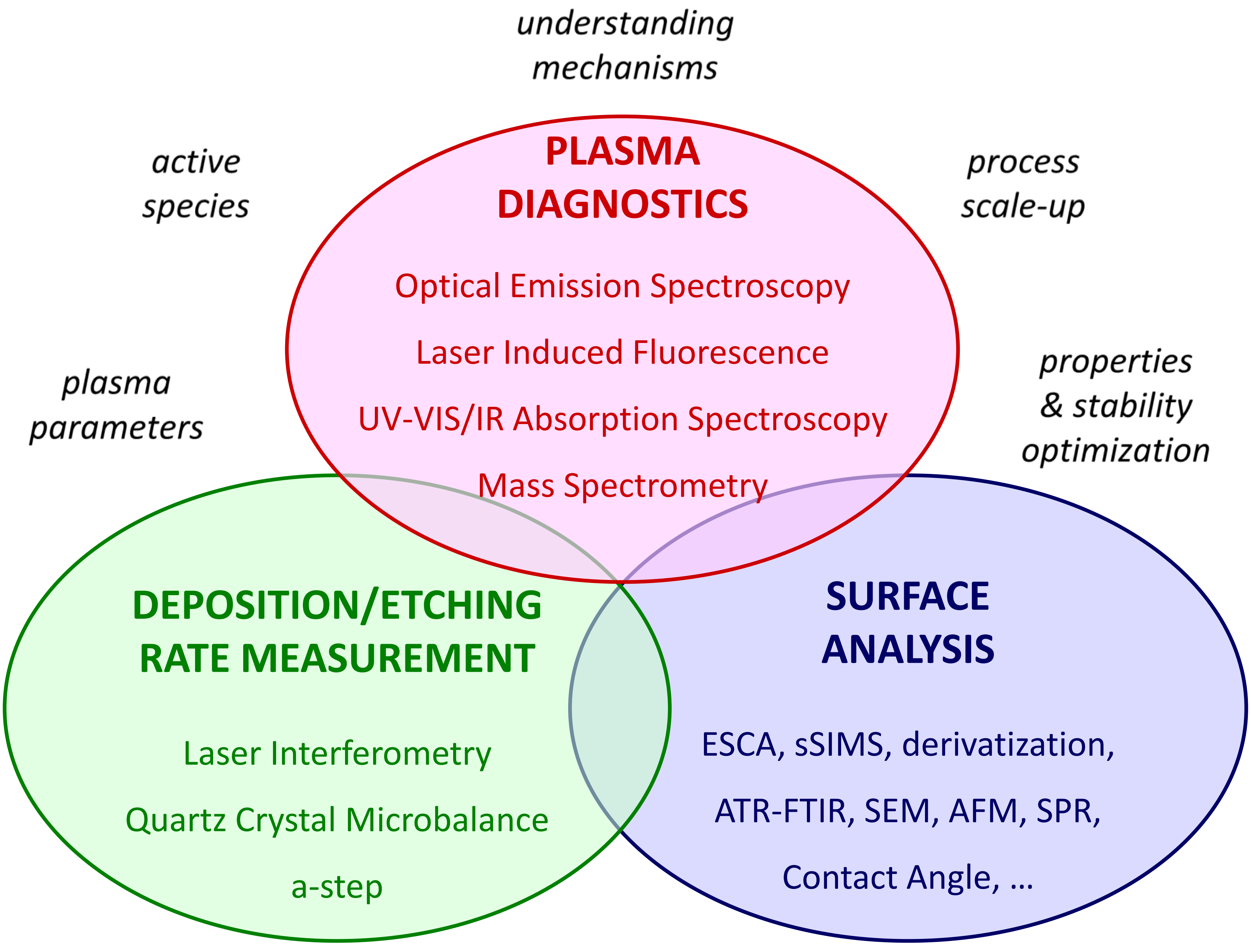
COLD PLASMAS









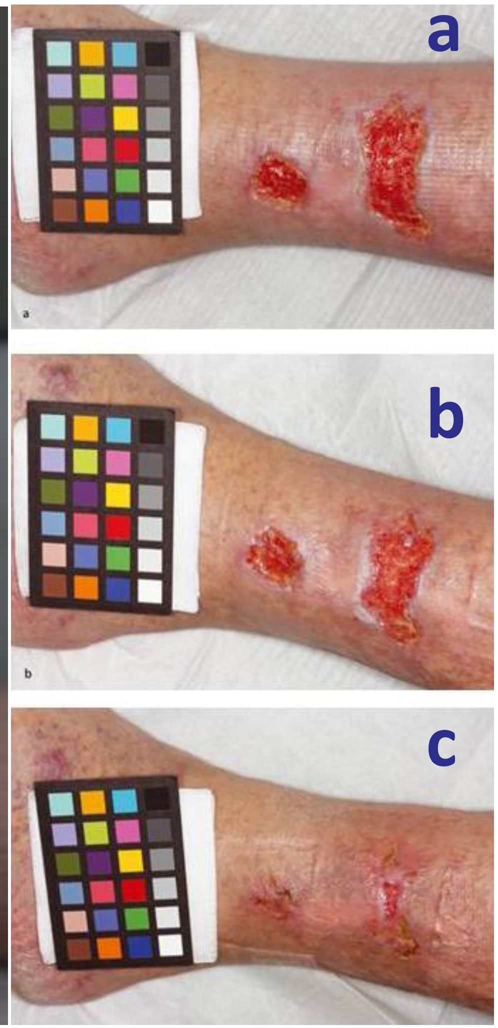

understanding mechanisms
active species
process scale-up
PLASMA DIAGNOSTICS
Optical Emission Spectroscopy
Laser Induced Fluorescence
UV-VIS/IR Absorption Spectroscopy
Mass Spectrometry
plasma parameters
properties & stability optimization
DEPOSITION/ETCHING RATE MEASUREMENT
Laser Interferometry
Quartz Crystal Microbalance
a-step
SURFACE ANALYSIS
ESCA, sSIMS, derivatization,
ATR-FTIR, SEM, AFM, SPR,
Contact Angle, …

WHAT PLASMAS CAN DO FOR LIFE SCIENCES

- **surface engineering of biomedical materials**
- **sterilization/decontamination of materials**
- **plasma medicine**
  and spin off applications (Food, Agriculture, …)

PLASMA MEDICINE

plasma is used directly on biological tissues in therapies for:
wound sterilization and healing, cancer treatments, dentistry, …



A 61-year-old patient with venous ulcers: wounds before plasma treatment (a), after 7 (b) and after 11 treatments (c). With a daily plasma therapy (MicroPlaster*) of 2 min. At the beginning of plasma treatment Klebsiella oxytoca and Enterobacter cloacae were detectable, after 11th treatment (23 days later) swabs were sterile*.

Shimizu et al, PPP 5, 577, 2008
Isbary et al, Brit. J. Derm. 163, 78,2010
Isbary et al, Clin. Plasma Med. 1, 19, 2013

PLASMA STERILIZATION

plasma is used on biomedical and other materials (solutions, food, vegetables, …) for sterilization and decontamination

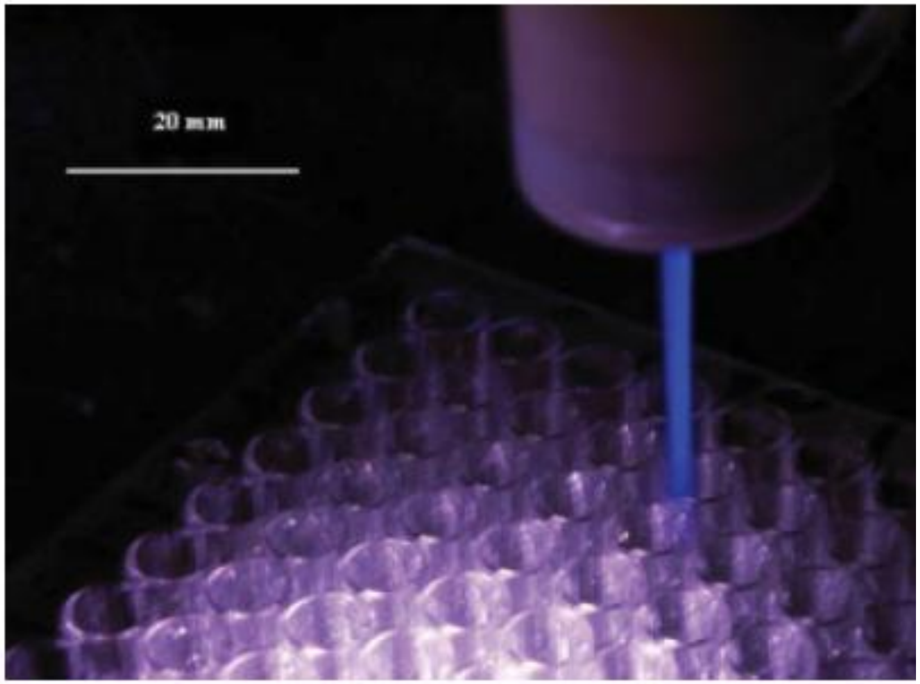


Figure 4. Photograph of the plasma pencil in operation

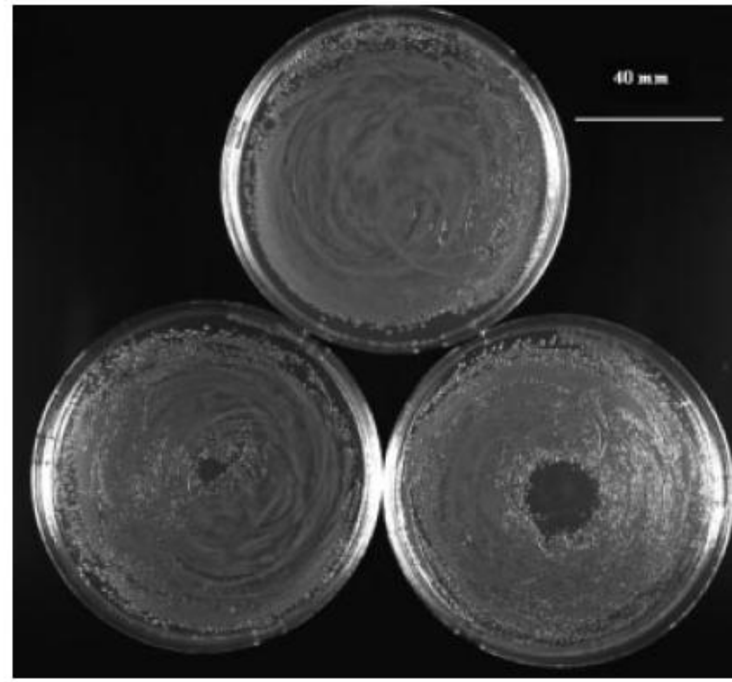


Figure 7. Localized inactivation of *E. coli* by the plasma pencil.[31] The top petri dish is the control, the left and right petri dishes represent 30 and 120 s plasma exposures, respectively. Helium is the operating gas.

Laroussi et al
PPP 3, 470, 2006
PPP 4, 777, 2007

SURFACE ENGINEERING PLASMA PROCESSES

plasma treatment, deposition and etching for tailoring surface composition, morphology and properties of (bio)materials, to the best interaction with cells, bacteria, tissues, blood, biological fluids

Time-lapse of Human Fibroblasts on PS plasma patterned with cell-adhesive (PEO15) tracks and non fouling (PEO5) domains. Cells grow confined within the 40 μm tracks and avoid the PEO-like domains.

Sardella et al PPP 3, 456, 2006

Plasma-treated PCL scaffold inplanted in an ovine condile (knee) for *in vivo* Regenerative Medicine experiments.

our lab, unpublished

plasma processes are investigated since the 60’s for adapting the surface of biomaterials

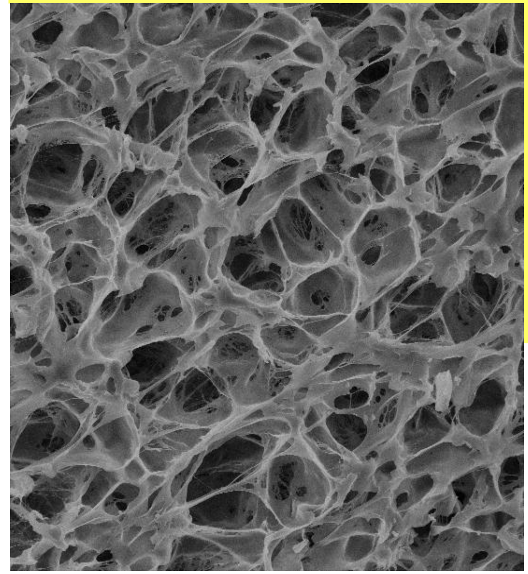
for

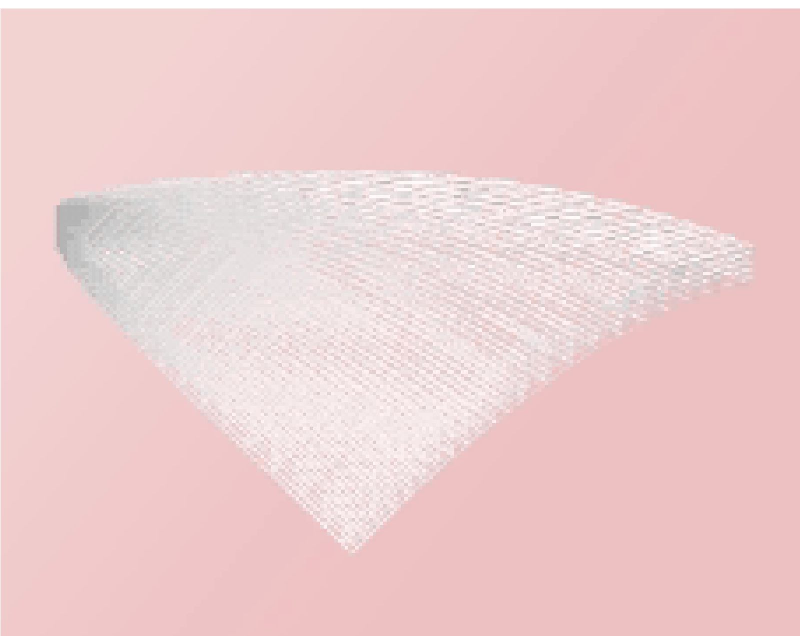
ex vivo in vitro in vivo

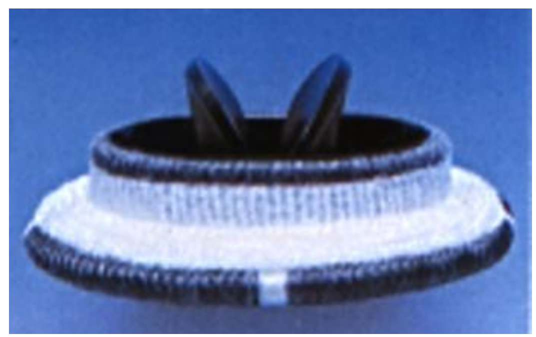
short medium long-term

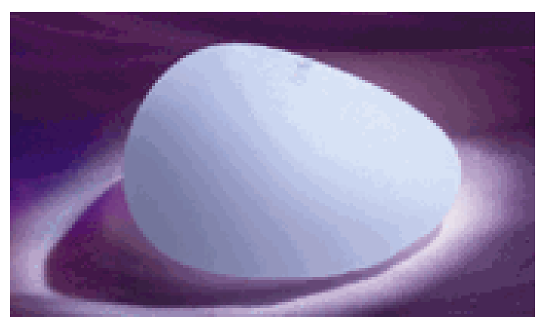
contact with

biological entities







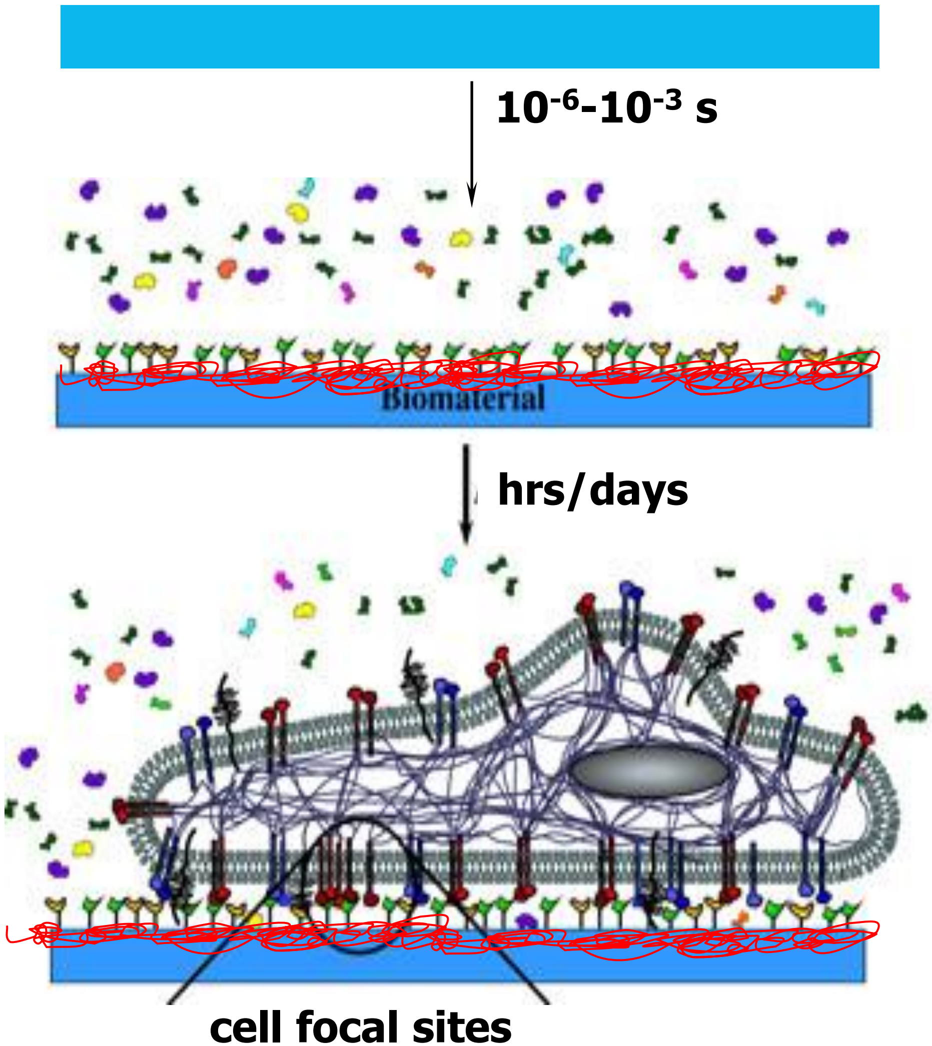


cold plasmas can tailor the CHEMICAL COMPOSITION of (bio)materials surfaces

cells and proteins in water medium

adsorbed protein layer

cell adhering to the protein layer



Proteins adhere to exposed surfaces IMMEDIATELY, in a DYNAMIC PROCESS.

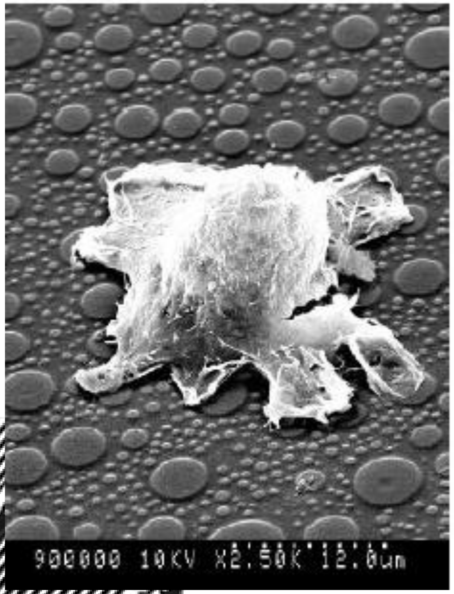
Density and conformation of proteins depend on the SURFACE CHEMISTRY of the substrate.

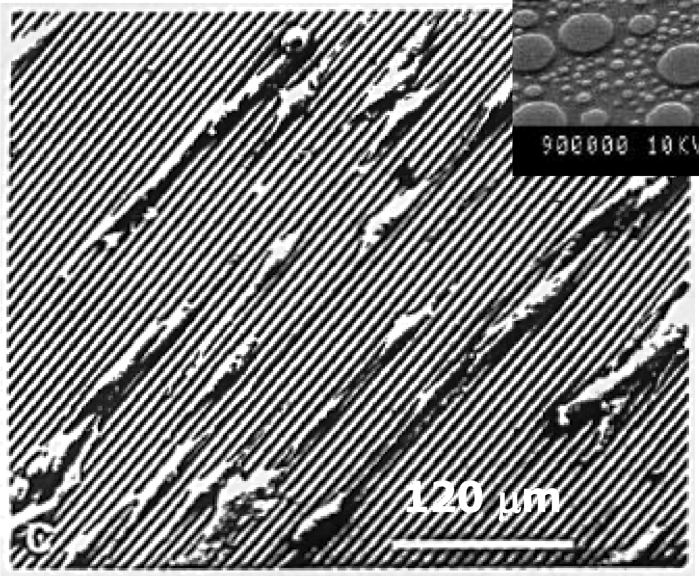
Within the FIRST DAY cells “interrogate” and “recognize” the protein layer through their FOCAL SITES (10-50 nm).

Cell attachment, adhesion, growth and behaviour is mediated by the surface protein layer.

cold plasmas can tailor also MORPHOLOGY and TEXTURE of (bio)materials surfaces

Dalby, Curtis *et al*
Univ. Glasgow, UK.





C Clark, A Curtis *et al*
Development, 108, 635, **1990**

CONTACT GUIDANCE

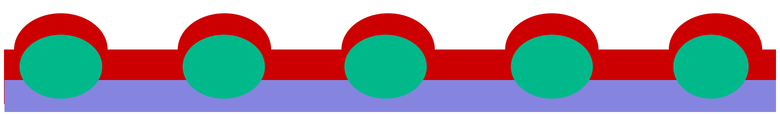
Cell adhesion, growth and behaviour is mediated also by constrains induced in the cytoskeleton by MORPHOLOGY, ROUGHNESS, TEXTURE and surface PATTERNS of the substrate material.

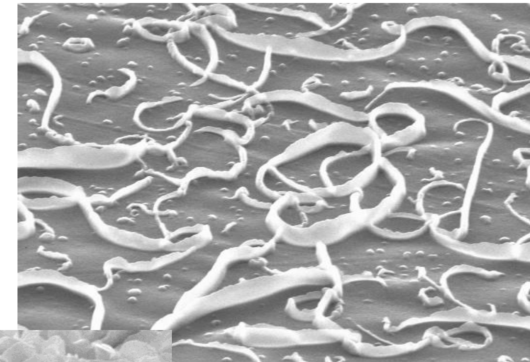
cold plasma can tailor INDEPENDENTLY surface composition and surface morphology of substrates

1- substrate

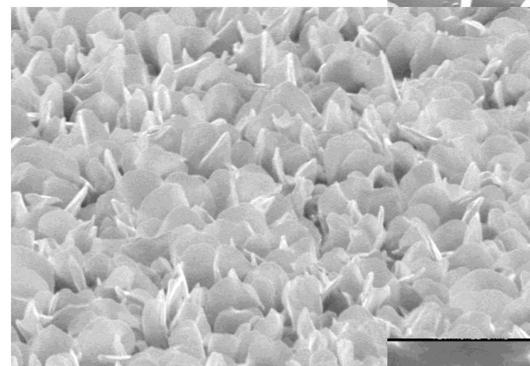
2- change surface morphology

3- change surface chemistry

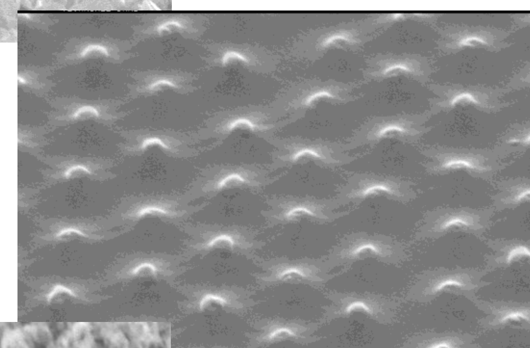




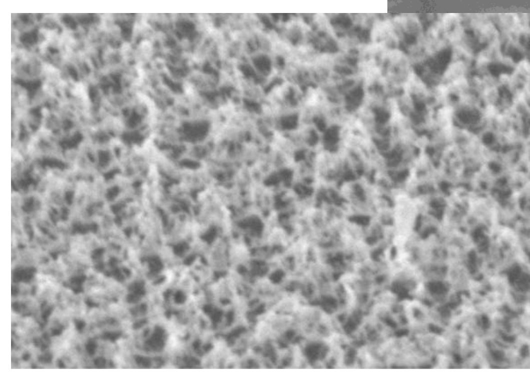
PTFE-like ribbon-like ctg



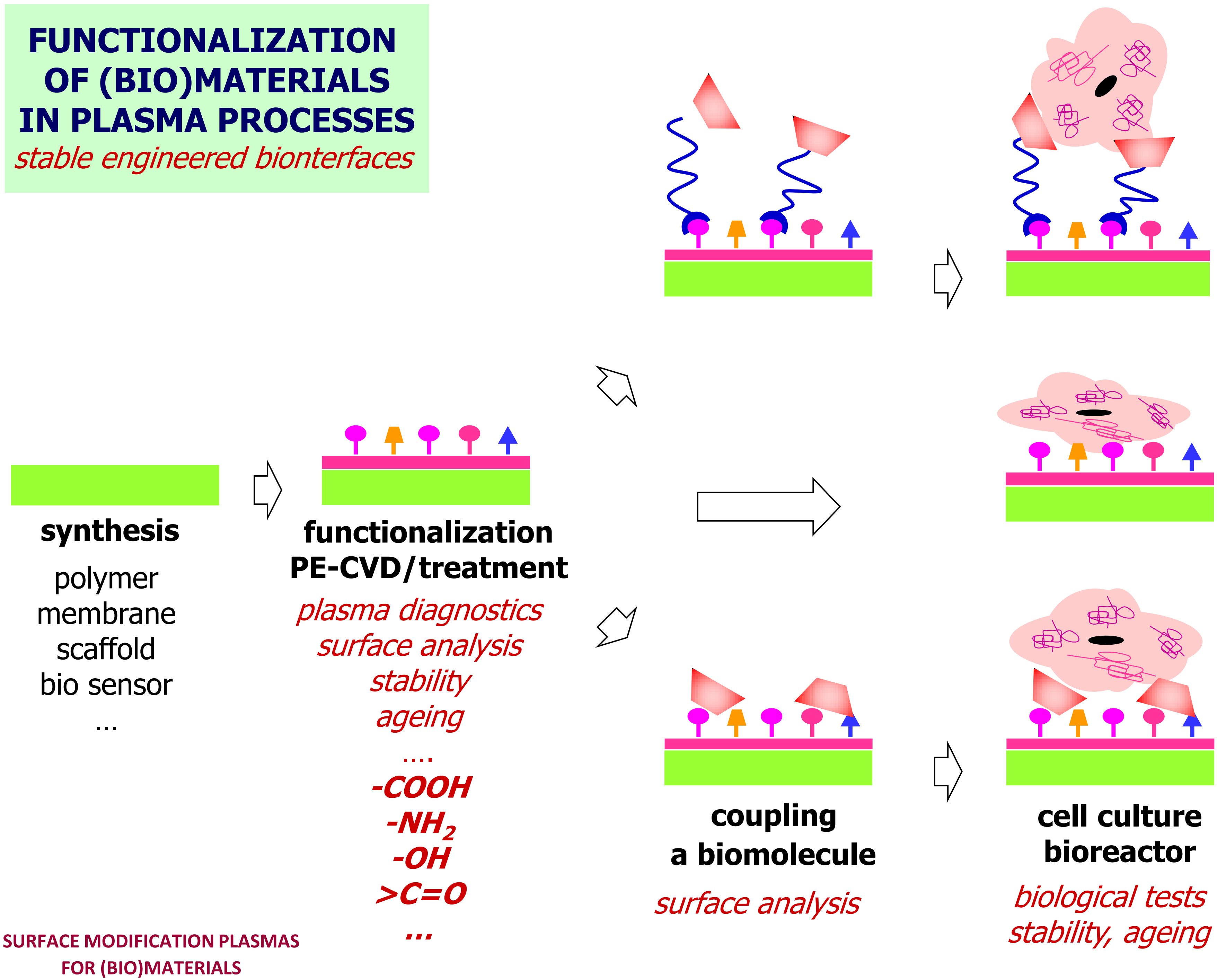
PTFE-like desert rose ctg



PET nanostructured by plasma-aided colloidal lithography



PS nanotextured CF_4/O_2 etch


FUNCTIONALIZATION OF (BIO)MATERIALS IN PLASMA PROCESSES
stable engineered biointerfaces
synthesis
polymer
membrane
scaffold
bio sensor
...
functionalization
PE-CVD/treatment
plasma diagnostics
surface analysis
stability
ageing
....
-COOH
-NH2
-OH
>C=O
...
coupling
a biomolecule
surface analysis
cell culture
bioreactor
biological tests
stability, ageing
SURFACE MODIFICATION PLASMAS
FOR (BIO)MATERIALS

SURFACE PROPERTIES OF BIOMEDICAL INTEREST THAT CAN BE TAILORED VIA PLASMA

- chemical composition
- roughness, morphology, texture, patterns
- hydrophobicity / hydrophilicity
- acid / basic character
- mechanical, elasticity,
- ….

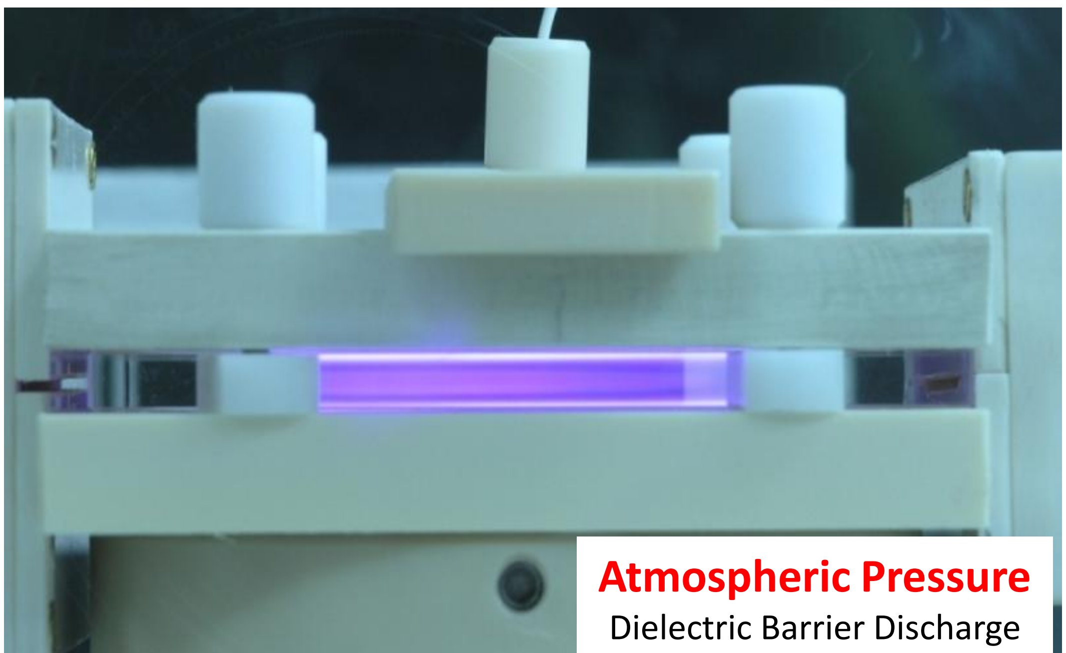
APPLICATIONS

- surfaces with improved (faster, selective, …) cell adhesion/growth
- surface immobilization of biomolecules (ECM, enzymes, peptides, …)
- protein/cell/bacteria repellent (unfouling) surfaces
- faster/better 3D colonized scaffolds for Regenerative Medicine
- improved membranes for dialysis and other purposes
- bactericidal surfaces
- drug release systems
- advances prostheses
- sensors
- ….

surface functionalization of materials in cold plasmas

PLASMA SOURCES

Low Pressure
parallel plate plasma reactor



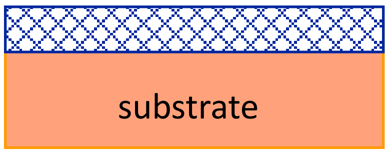
Atmospheric Pressure
Dielectric Barrier Discharge

Atmospheric Pressure
plasma jet

functionalization by PE-CVD

modified thickness 10 nm – 1 μm

dense, cross-linked

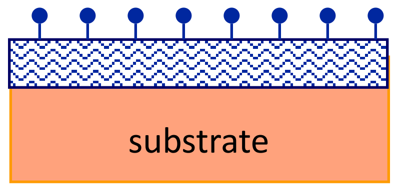


inorganic

DLC, SiOx, …

functionalized

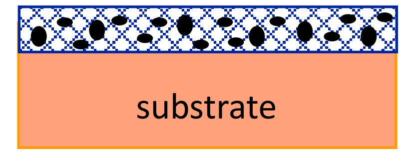
-COOH, $-NH_2$, -OH, >C=O …



organic

PEO-like, pdAA, teflon-like, silicone-like …

nano (bio) composite



(bio) organic/inorganic

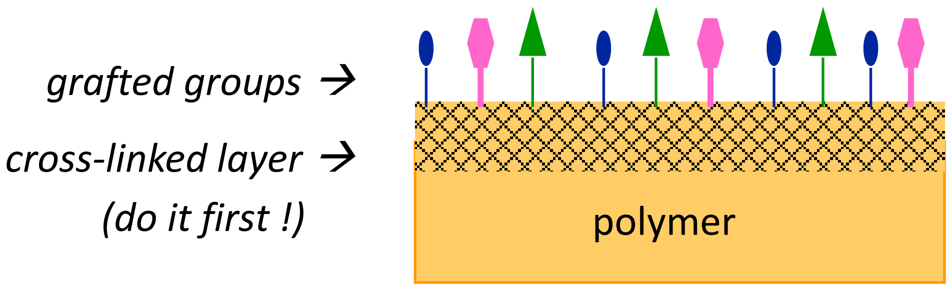
metal/ceramic clusters or biomolecules embedded in a matrix

- **careful optimization of plasma conditions**
- **Low vs Atm Pressure**
- **retention of the monomer structure**
- **ageing**
- **stability in water-based media**
- **adhesion to the substrate**
- **pre-treatments / graded coatings may be needed**

functionalization by Plasma Treatments

grafting of (polar) functional groups

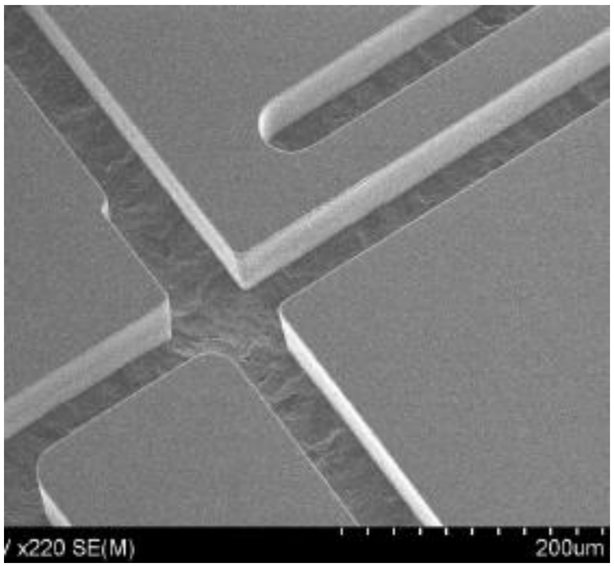
modified thickness 1 – 10 nm



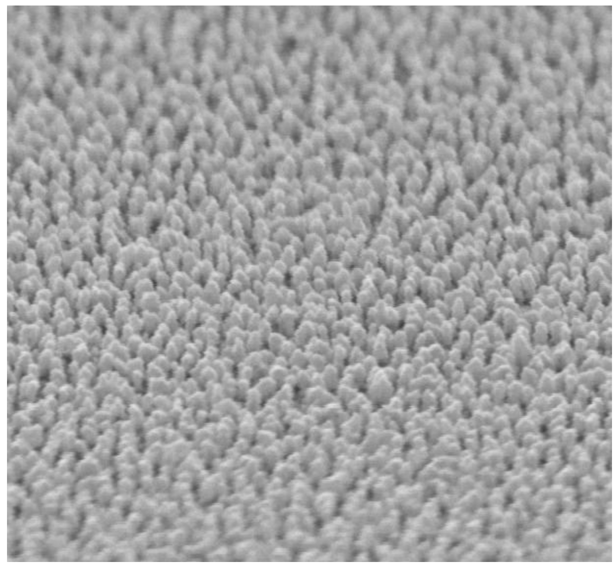
- **optimization of plasma conditions**
- **Low vs Atm Pressure**
- **ageing**
- **hydrophobic recovery**
- **stability in water-based media**
- **pre-treatments are generally needed**

Plasma Etching

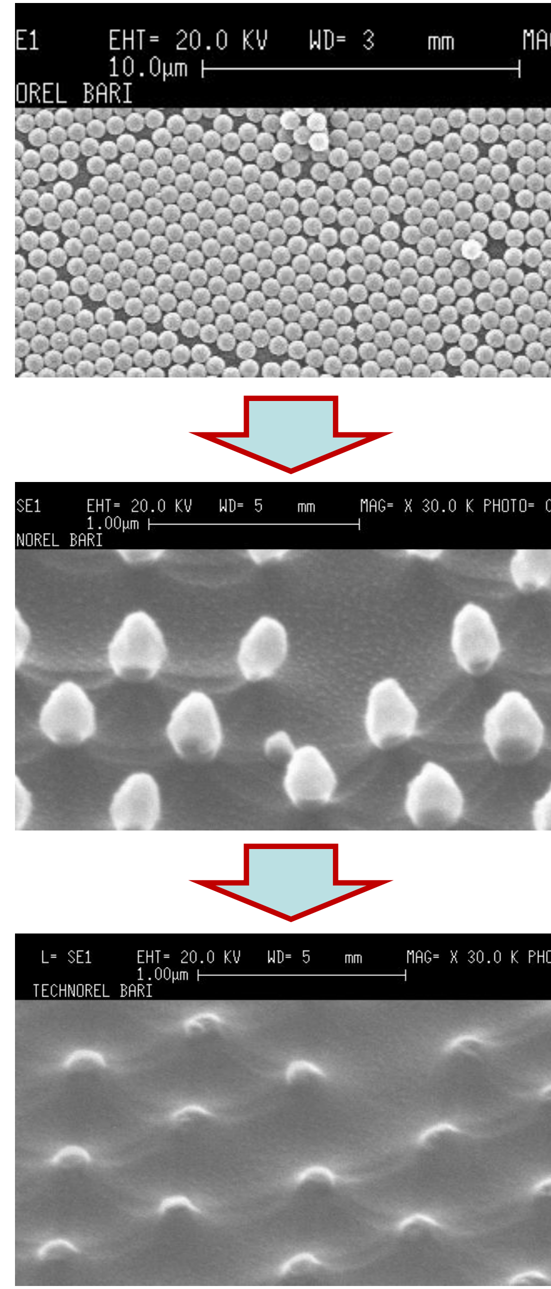
sculpting/patterning polymer "lab chips", μm
texturing of surfaces, μm – nm
plasma-aided coll. lithography & other methods, μm - nm



sculpted

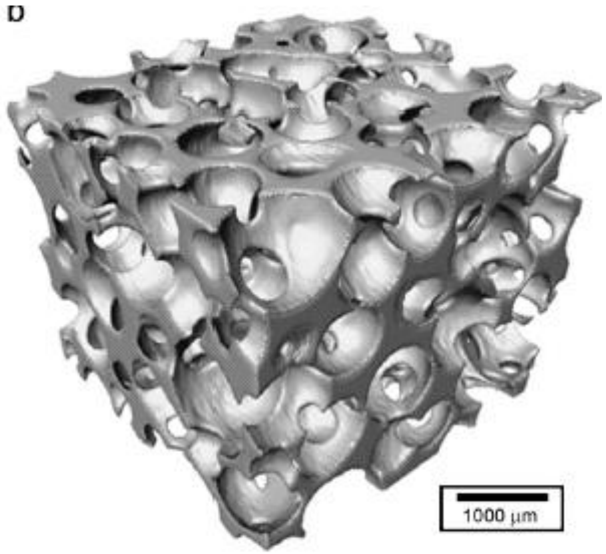


textured

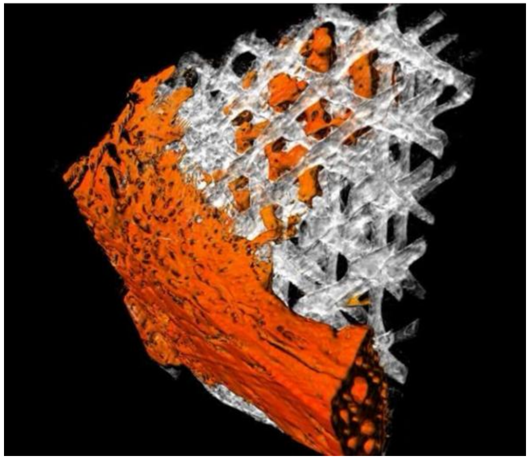


plasma-aided
colloidal lithography

scaffolds for
TISSUE ENGINEERING and REGENERATIVE MEDICINE



TE is an excellent alternative to artificial prosthesis and organ transplant to replace diseased or damaged organs. TE uses cells seeded in 3D scaffolds, that serve as temporary support for guiding tissue regeneration in vitro /in vivo.



REQUIREMENTS FOR SCAFFOLDS

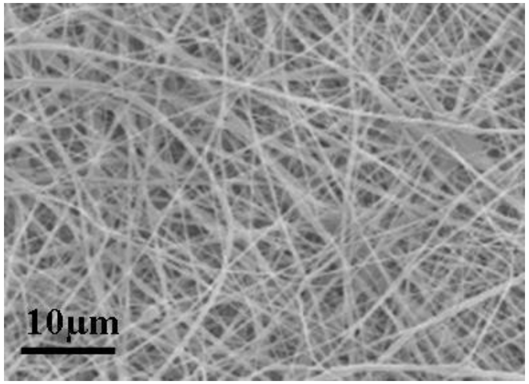
not toxic (biocompatible)

proper degradation rate (biodegradable)

high porosity, proper pore size, interconnected pores

proper mechanical properties

proper surface composition
(e.g. hydrobhopic --> hydrophilic)



Trizio, Intranuovo, Gristina, Dilecce, Favia
He/O_2 Atmospheric Pressure plasma jet treatments of PCL scaffolds
for Tissue Engineering and Regenerative Medicine; submitted 2015

ATMOSPHERIC PRESSURE

APPJ
He/O_2

Sardella, Fisher, Shearer, Garzia-Trulli, Gristina, Favia
N_2/H_2O plasma assisted functionalization of PCL porous scaffolds:
acidic/basic character vs cell behavior
Plasma Proc. Polym. accepted, 2015

Intranuovo, Gristina, Brun, Mohammadi, Ceccone, Sardella, Rossi, Tromba, Favia
Plasma modification of PCL porous scaffolds fabricated by
Solvent-Casting/Particulate-Leaching for Tissue Engineering
Plasma Proc. Polym. 11, 184, 2014

Brun, Intranuovo, Mohammadi, Domingos, Favia, Tromba
A comparison of 3D PCL Tissue Engineering scaffolds produced with conventional and
additive manufacturing techniques by means of quantitative analysis of SR μ-CT images
J Instr. 8, 1, art. n.C07001, 2013

Domingos, Intranuovo, Gloria, Gristina, Ambrosio, Favia, Bartolo
Improved osteoblast cell affinity on plasma-modified 3D extruded PCL scaffolds
Acta Biomaterialia 9, 5997, 2013

Intranuovo, Howard, White, Johal, Ghaemmaghami, Favia, Howdle, Shakesheff, Alexander
Uniform cell colonisation of porous 3D scaffolds achieved using radial control of surface chemistry
Acta Biomaterialia 7, 3336, 2011

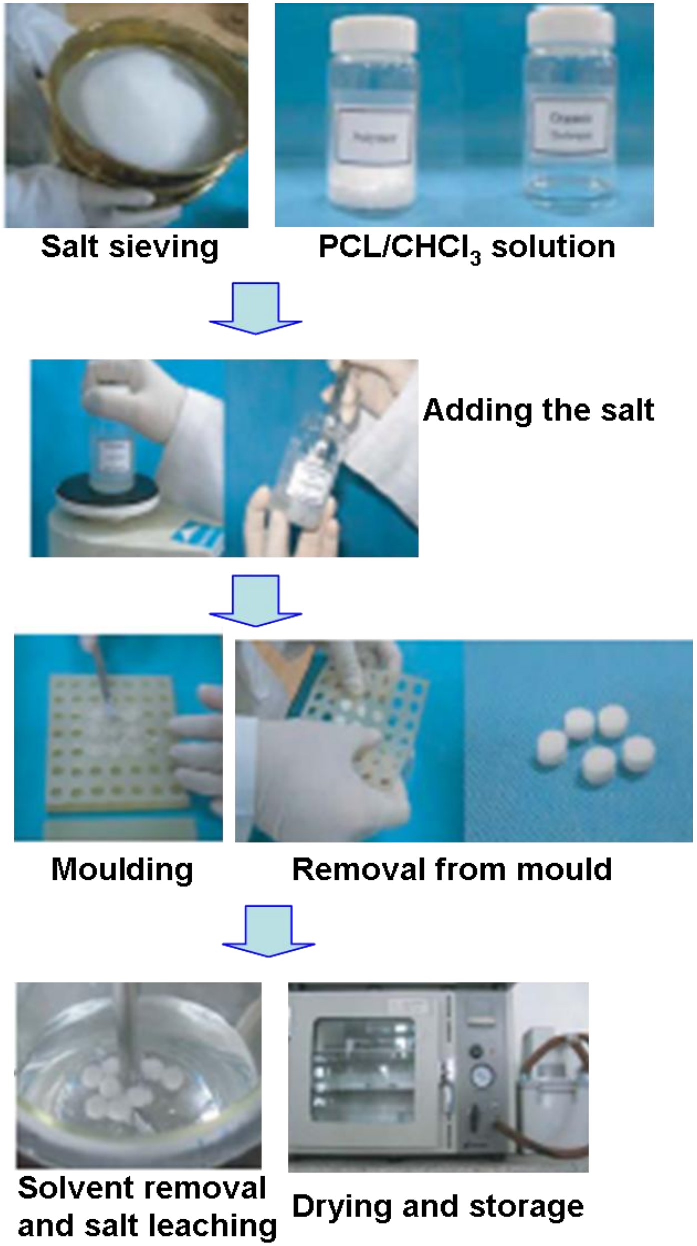
Intranuovo, Sardella, Gristina, Nardulli, White, Howard, Shakesheff, Alexander, Favia
PE-CVD processes improve cell affinity of polymer scaffolds for Tissue Engineering
Surf. Coat. Tech. 205, S548, 2011

LOW PRESSURE

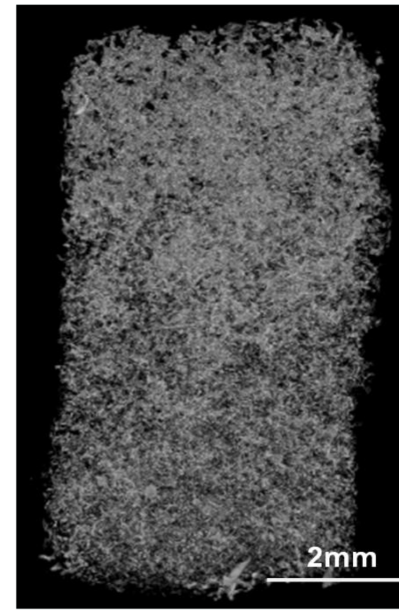
many configurations
13.56 MHz
N_2/H_2O
$C_2H_4/N_2 + H_2$
$C_2H_4/N_2 + C_2H_4$
C_2H_4/AA
O_2 + DEGDME

Solvent Casting/Particulate Leaching

poly-ε-caprolactone scaffolds



- Salt sieving, PCL/$CHCl_3$ solution
- Addition of NaCl
- Pouring into a PTFE mould
- Removal of scaffolds
- Solvent removal, salt leaching
- Drying, storage



Experimental parameters:

PCL/$CHCl_3$ <u>20/80</u> wt/wt

PCL/NaCl <u>5/95</u> - 8/92 - 10/90 wt/wt

NaCl crystal size: <u>300-500 µm</u>

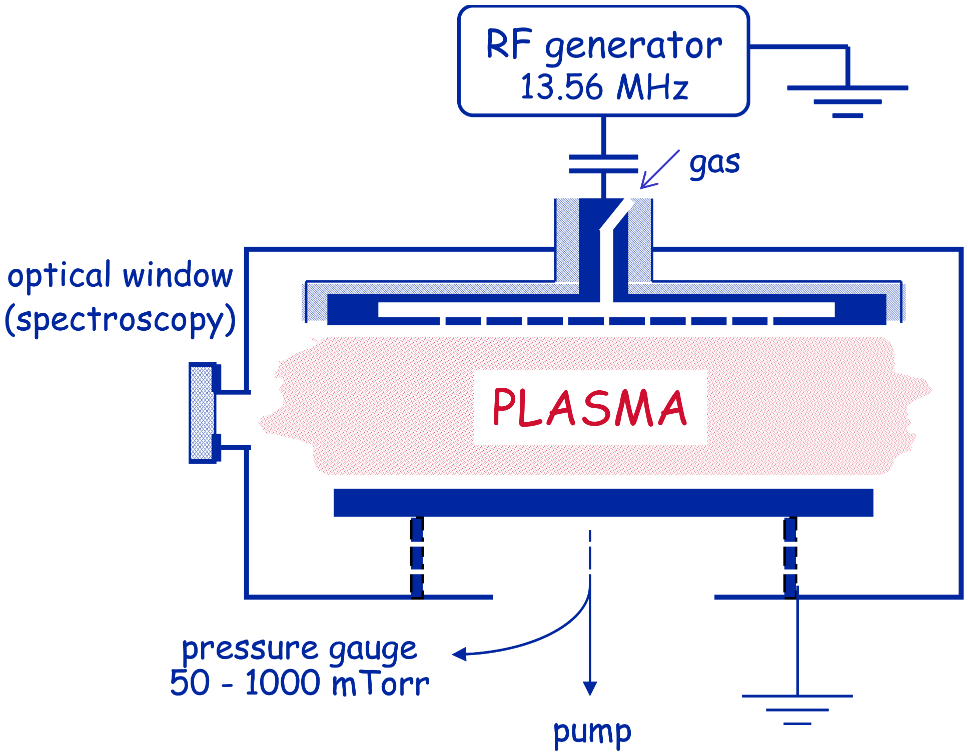
Scaffold size: 4 mm dia, 10 mm thick

Mean porosity 89 ± 3 %

Avg pore size 290 ± 90 µm

PE-CVD of functional coatings on/within PCL scaffolds

- flat PCL samples prepared with spin coating
- PCL scaffolds prepared with SCPL technique
- 2 PE-CVD processes investigated. WCA, water absorption and XPS to characterize the coatings
- 2 coatings tested for cyto-compatibility *in vitro*
- 1 coating selected for *in vivo* tests



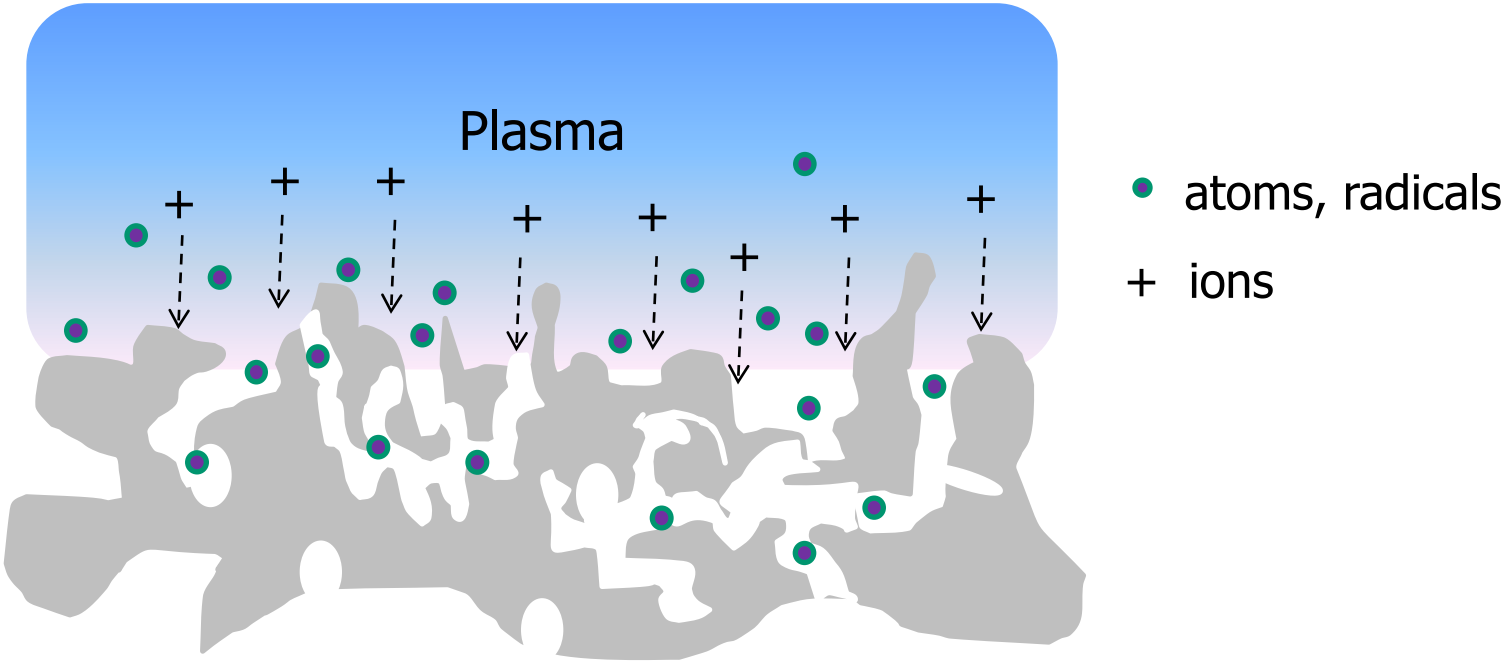
1) pdE:N/H_2 coating with nitrogen and oxygen containing functional groups

N_2/ethylene 5/1; 47 Pa; 50 W; 30 min; followed by H_2; 20 W; 3 min

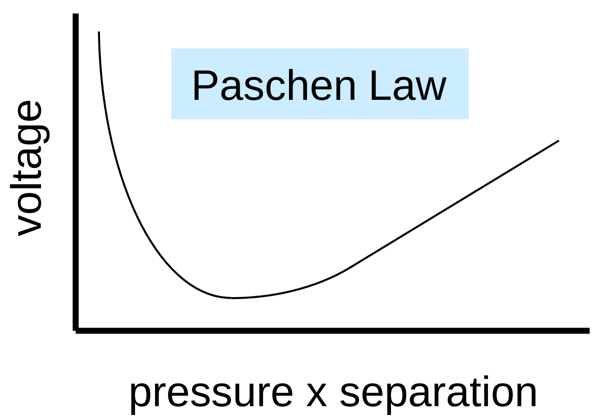
2) pdE:AA coating with oxygen containing functional groups

acrylic acid/ethylene/Ar 3/1/2; 33 Pa; 30 W; 20 min

plasma processing of porous substrates

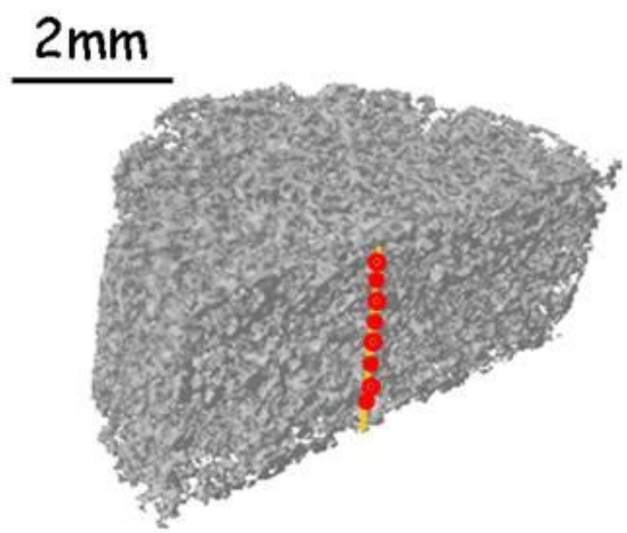


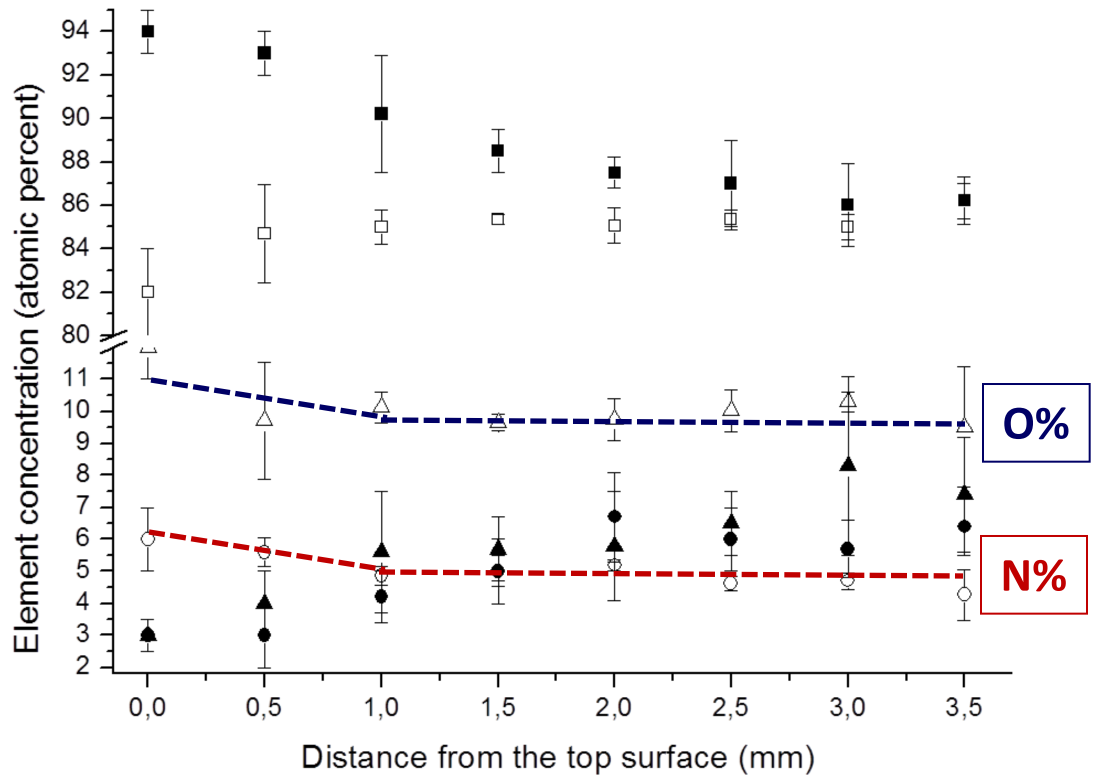
NO LOW P PLASMA
INSIDE PORES < 2 mm
(diffusion of active species)



chemical composition in depth

Micro-CT 3D rendering of the scaffold section



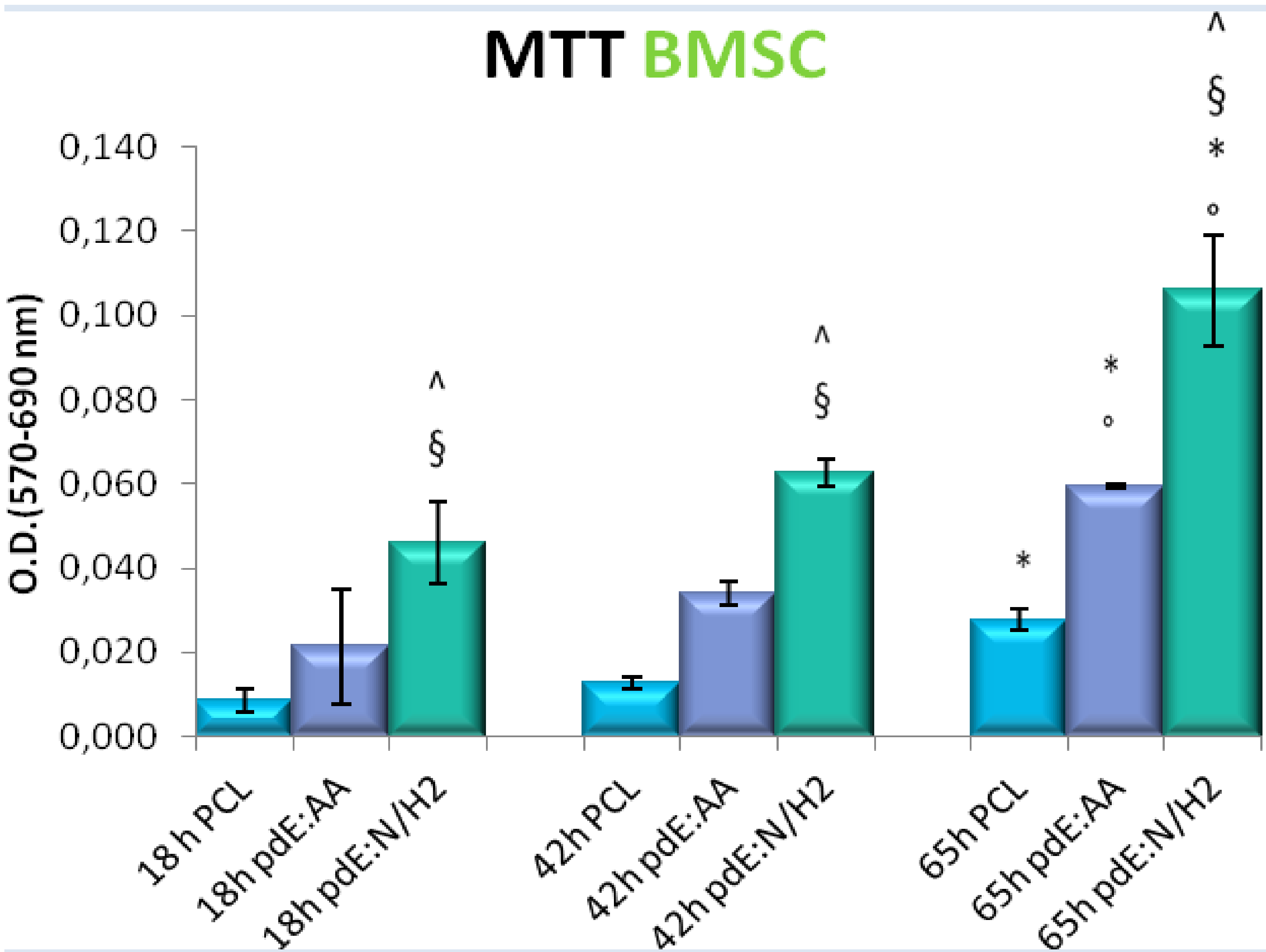


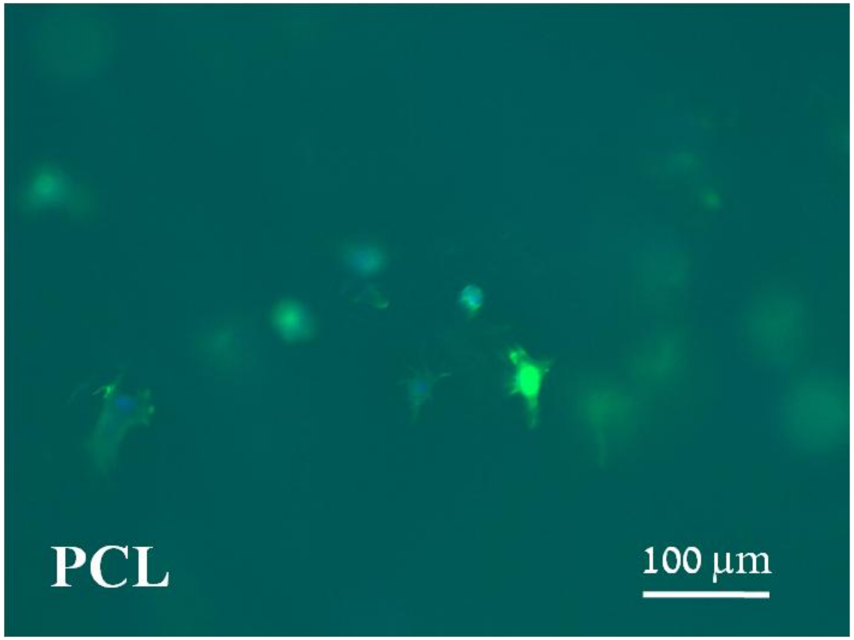
XPS chemical composition of scaffold sections at different depth of PdE:N/H_2(C%: □, O%: Δ, N%: ○) and ofPdE:N/C_2H_4 (C%: ■, O%: ▲, N%: ●) treated scaffolds.

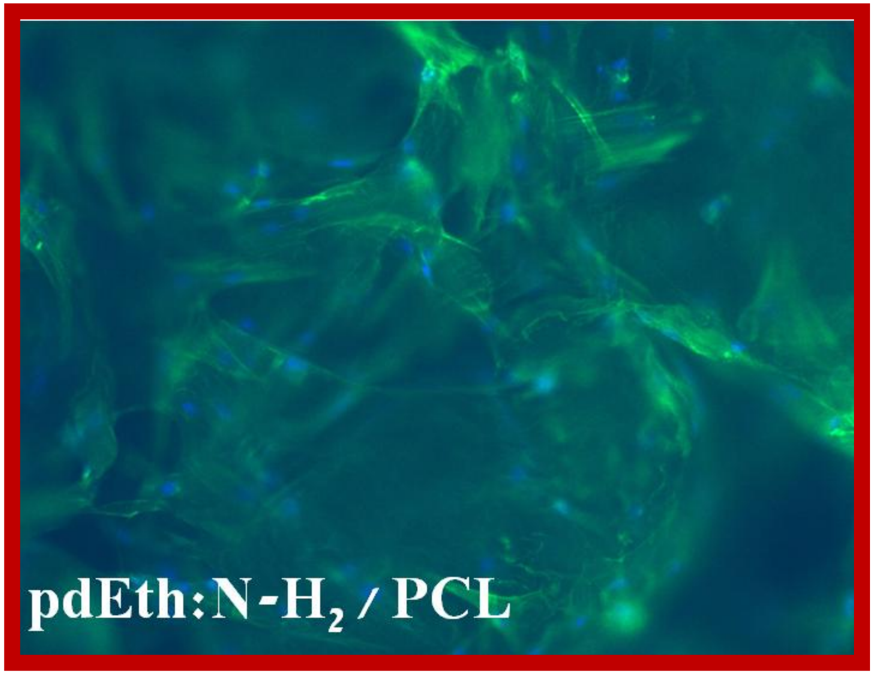
Intranuovo, et al, Plasma Proc. Polym. 11, 184, 2014

in vitro experiments

$1x10^4$ **BMSCs** were seeded on each 2D/3D PCL sample. Cell viability (**MTT**) and morphology (**actin cytoskeleton** fluorescence microscopy) were studied at 18, 42 and 65 h of culture.

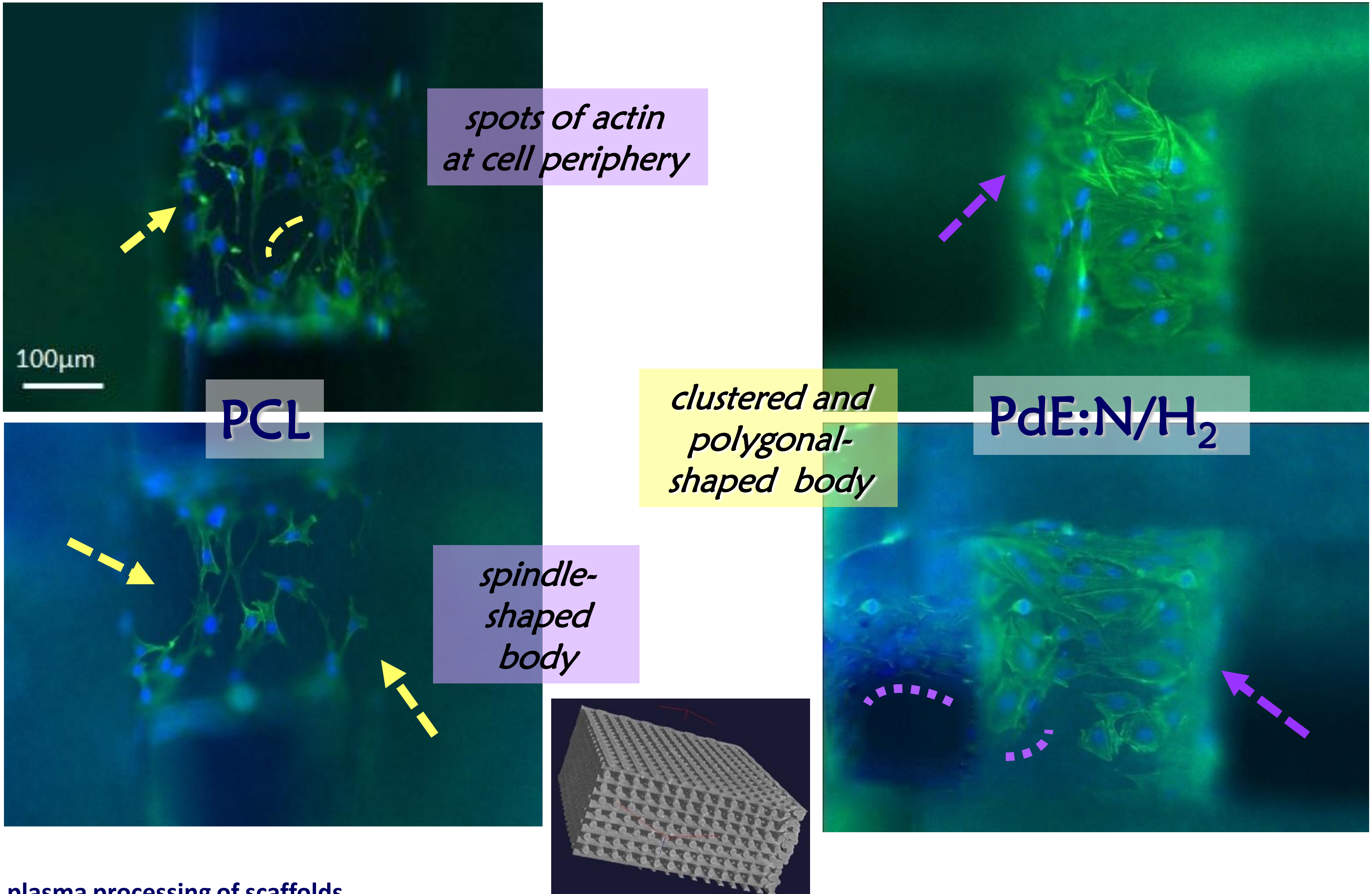






plasma processing of scaffolds

Saos2 Cells morphology on scaffolds



plasma processing of scaffolds

PdE:N/H_2 treated PCL scaffolds become:

- functionalized with polar -N and O containing groups outside and inside the 3D porous structure
  → BETTER CELL ADHESION & PROLIFERATION

- wettable and water absorbing
  → IMPROVED PENETRATION OF WATER & MEDIUM
  IMPROVED PENETRATION OF NUTRIENTS *in vivo*

TIMELINE

2 weeks
to get used to
the place

⇓

time 0
surgery

⇓

time 3 months
sacrifice GROUP 1

⇒

time 6 months
sacrifice GROUP 2

PCL 3 (2)
PCL + plasma 3 (4)
PCL + plasma + BMScells 3 (4)

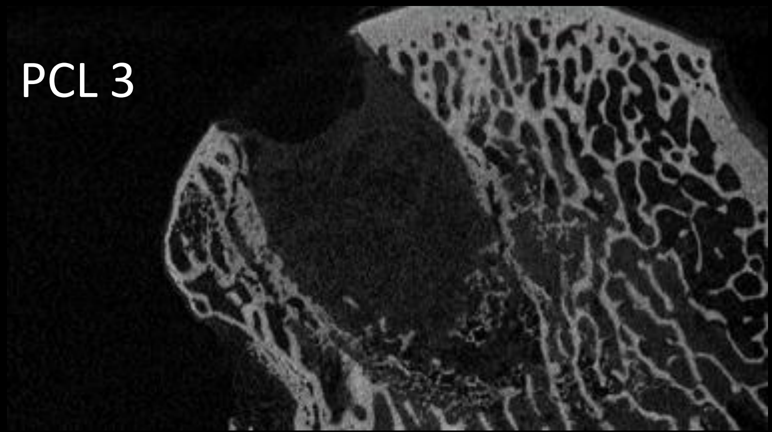
PCL 6 (2)
PCL + plasma 6 (4)
PCL + plasma + BMScells 6 (4)

in vivo experiments

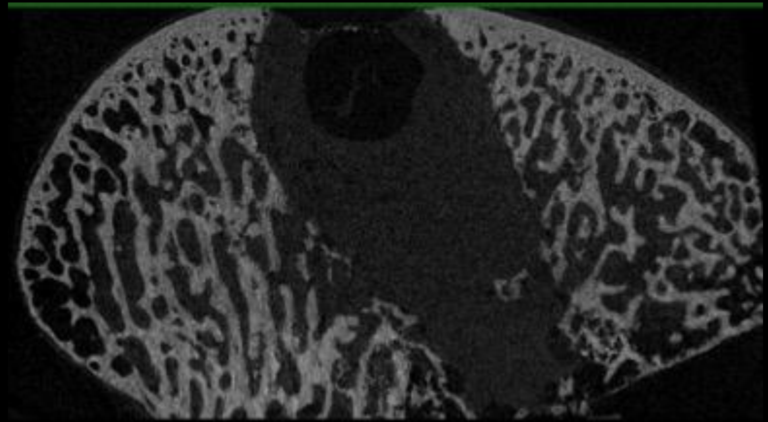
pdE:N/H_2 coated PCL scaffolds were implanted in ovine knees (sheep 9-10 yo, 40-50 Kg), in left lateral decubitus with the limb abducted. An osteochondral defect (4 mm dia) was sculpted in the medial condyle of the right femur and replaced with the scaffold.

Sez. Veterinaria, Dipartimento dell'Emergenza e Trapianto Organi, D.E.T.O.
Prof. Antonio Crovace, Università di Bari

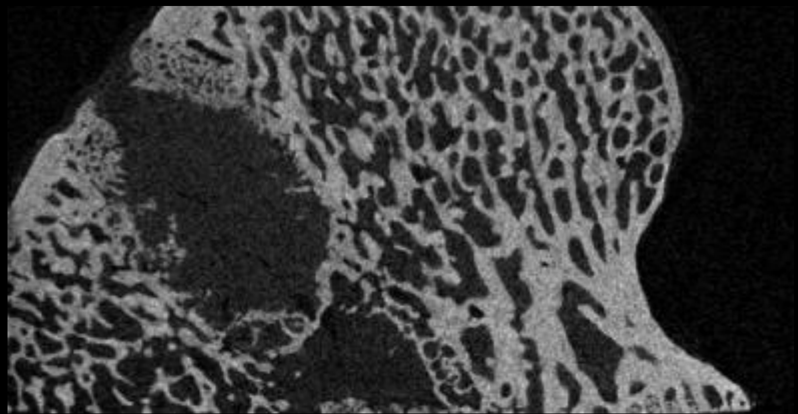
PCL 3



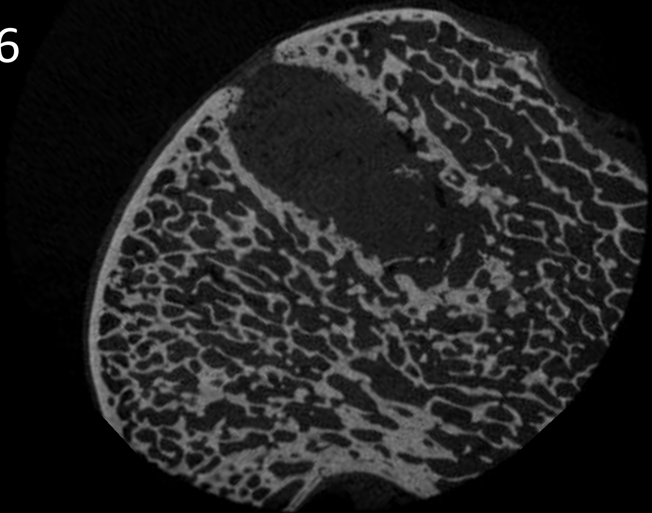
PCL 6



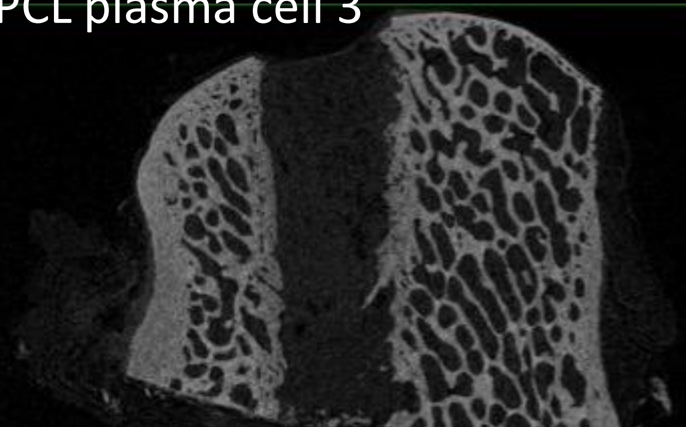
PCL plasma 3



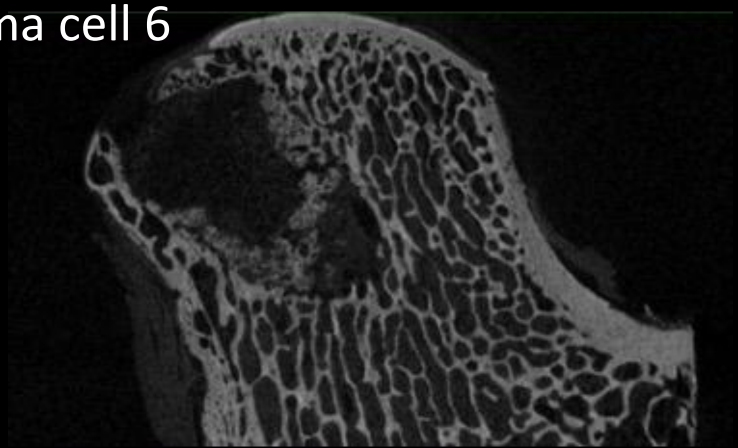
PCL plasma 6



PCL plasma cell 3



PCL plasma cell 6



low P *vs* atm P plasmas for materials

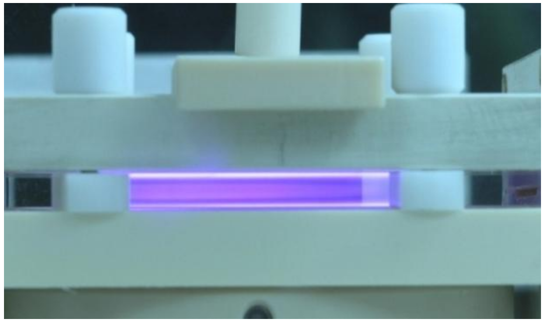
RF Glow Discharge system
Low Pressure

Surface modification LOW PRESSURE PLASMAS have about 45 years of tradition in biomaterials and biomedical devices (1st paper in 1969)

LOW P PLASMAS STILL OFFER MORE VERSATILE PROCESSES

high range of chemical compositions	anysotropic etching
space resolution	kind, size, shape of substrates
3D substrates	good coating/substrate adhesion

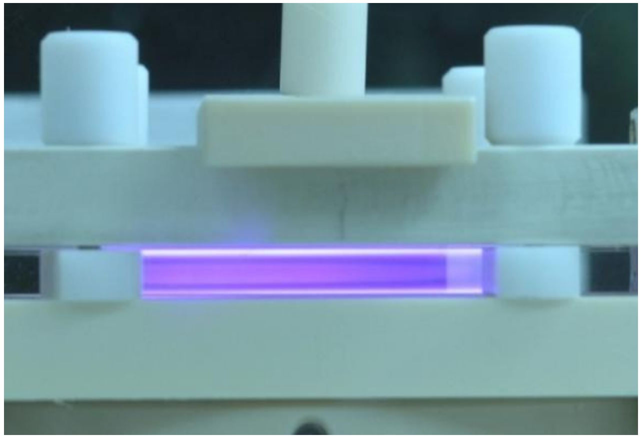
In recent years, however, ATMOSPHERIC PRESSURE PLASMAS started to produce surfaces formely synthesized only at low pressure



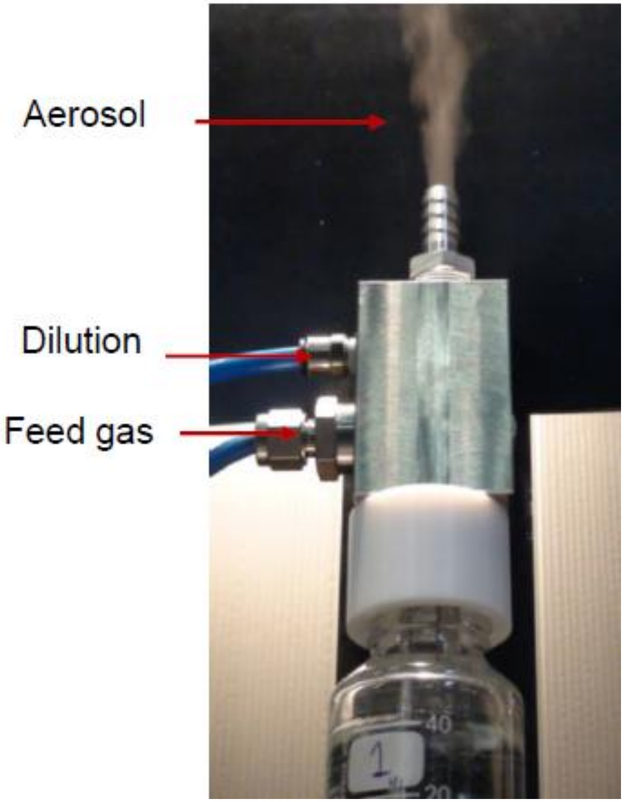
DBD system
Atmospheric Pressure

Favia et al, Eur. Phys. J. Appl. Phys. 56, 24023, 2011

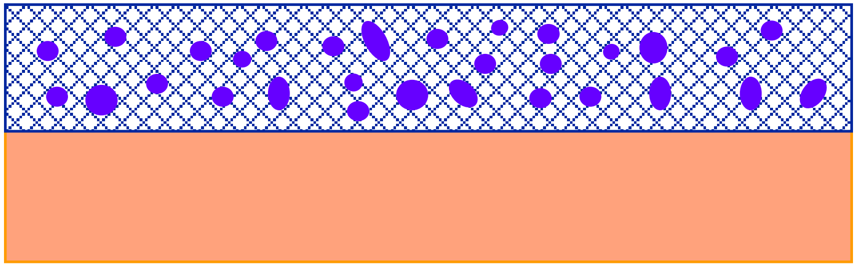
DBD system



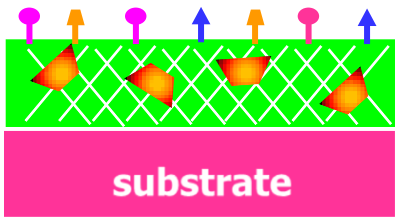
biomolecule loaded
drug release coatings
deposited by aerosol-assisted
atmospheric pressure plasma



nano/bio composite coating



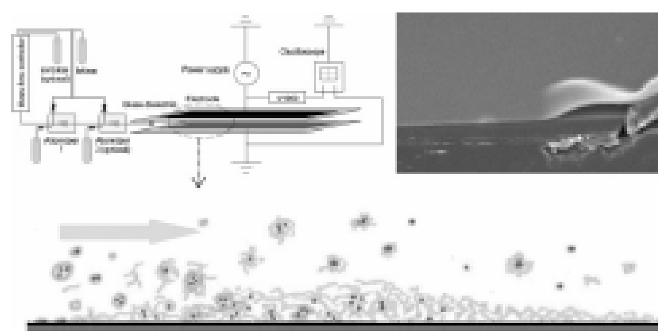
Plasma Process. Polym. **2011**, *8*, 965–974



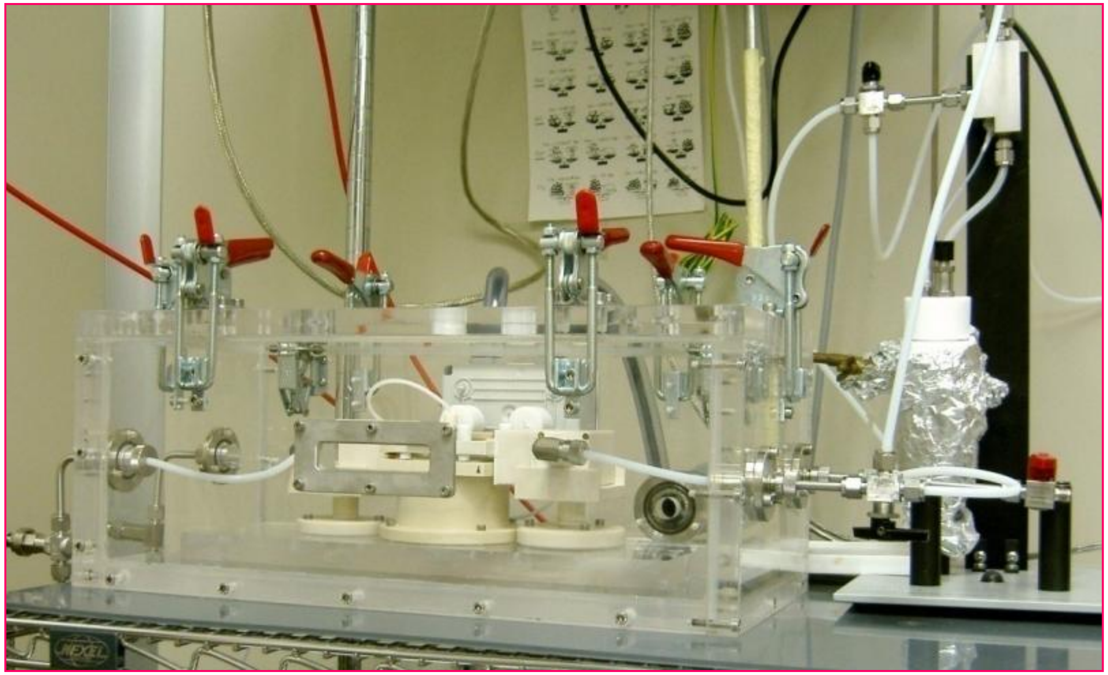
Exploration of Atmospheric Pressure Plasma Nanofilm Technology for Straightforward Bio-Active Coating Deposition: Enzymes, Plasmas and Polymers, an Elegant Synergy[a]

Pieter Heyse, Arne Van Hoeck, Maarten B. J. Roeffaers, Jean-Paul Raffin, Alexander Steinbüchel, Tim Stöveken, Jeroen Lammertyn, Pieter Verboven, Pierre A. Jacobs, Johan Hofkens, Sabine Paulussen,* Bert F. Sels

While protein or enzyme immobilization methodologies are readily applicable in a majority of industrial processes, some lacunas still remain. For example, the multi-step, wet-chemical nature of current immobilization reactions limits straightforward bio-film fabrication in continuous production units. As such, a fast and preferably single step immobilization technique, minimizing solvent use and decoupling deposition substrate from used method is awaited. In this research, an atmospheric pressure plasma reaction environment is chosen for its flexibility in terms of reactivity and the ease of coating depositions on a wide variety of substrates. Organic coating precursors such as acetylene or pyrrole are injected simultaneously with an atomized enzyme solution directly in the discharge. By atomizing the enzyme solution, the enzyme molecules are surrounded by a watery shell. It is envisioned that such droplet act as "shuttles", delivering the enzymes to the discharge while protecting them from the harsh plasma conditions. In the discharge, polymerization of the added organic coating precursor takes place and consequently, the enzyme molecules become trapped in the growing polymer network. In addition, atomization of the protein solution favors the spatial distribution of the proteins in the coating. Several enzymes are evaluated and enhanced temperature and solvent stability is observed. Moreover, single molecule fluorescence, enzyme activity and bio-recognition experiments demonstrate protein integrity after plasma assisted immobilization.



advantages of aerosol-assisted atmospheric pressure discharges



aerosol feed

thermally unstable precursors

high vapour tension precursors

high precursor concentration

no heating

use of solutions/suspensions of biomolecules, nanoparticles, …

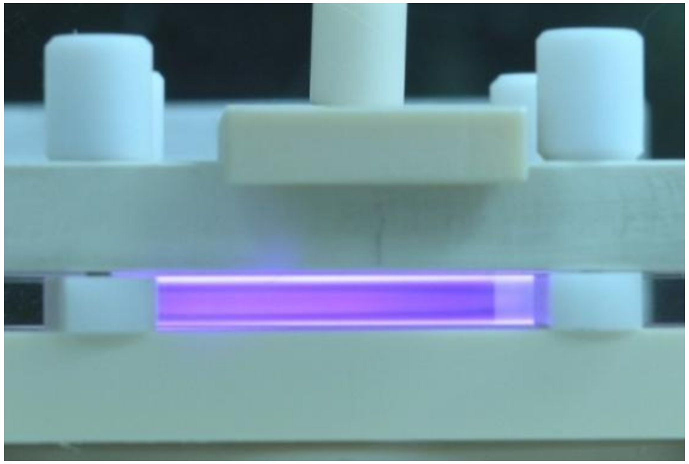
DBD (APP Jet)

no/reduced pumping system

easy processing of highly degassing substrates

easier integration in on-line systems

possible use of precursors in aerosol

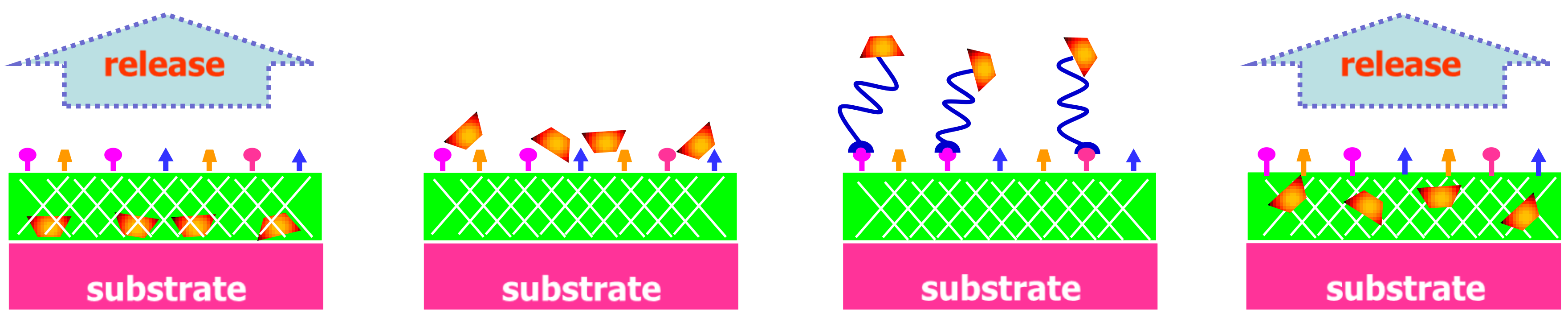


BIOMOLECULES
immobilized /embedded

enzymes
proteins, peptides
DNA
anti oxidant molecules
anti thrombotic molecules
growth factors
anti bacterial
drugs
……
nanoparticles

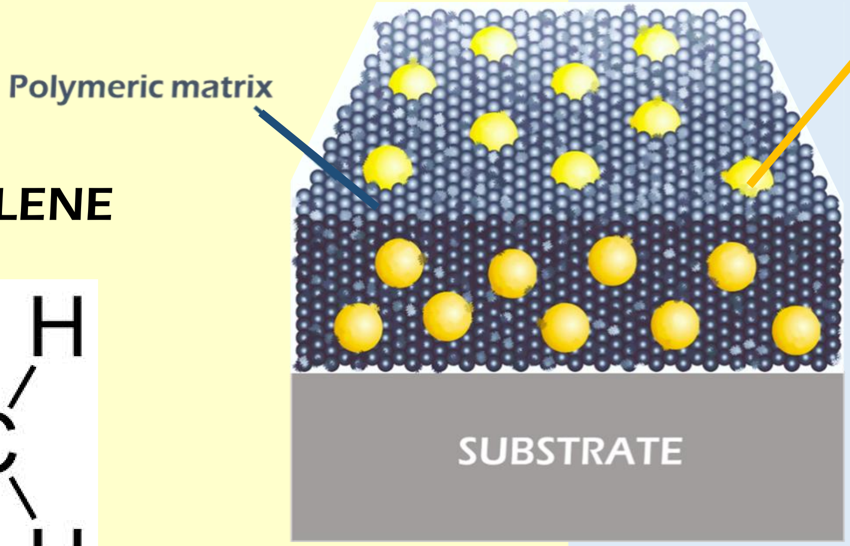
APPLICATIONS

biomaterials, prostheses
enhanced cell adhesion & growth
scaffolds for Regenerative Medicine
anti-bacterial surfaces
lab on chip
biosensors
drug release
drug testing
active packaging
food conservation

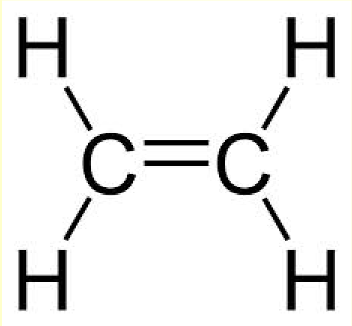


AIM OF THE WORK

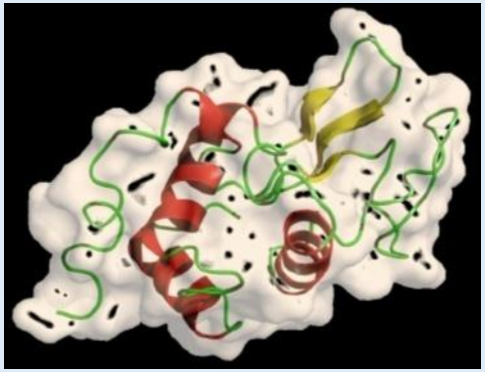
AP-PLASMA DEPOSITION



ETHYLENE



LYSOZYME



Reactive in plasma environment

Suitable source for CH_x matrix

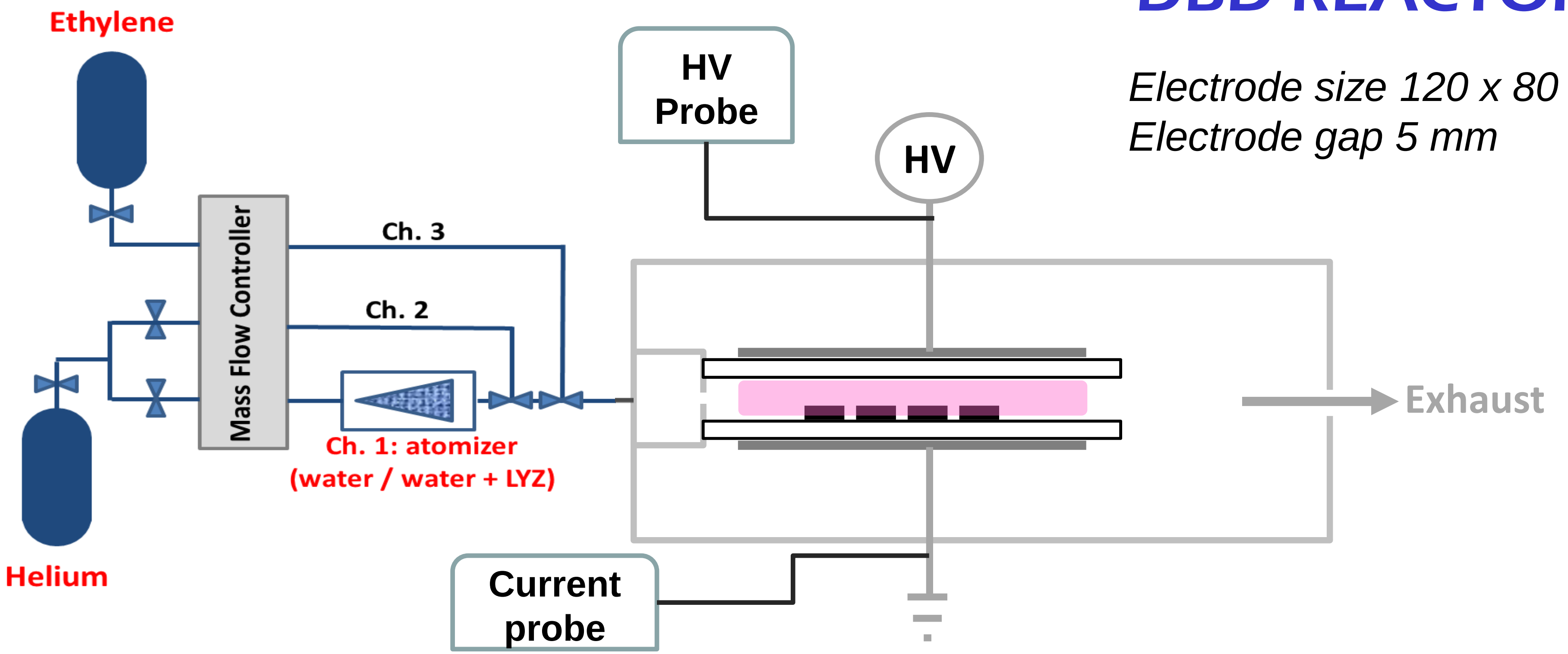
H_2O addition for tuning coating properties

natural antibacterial molecule

releasable

DBD REACTOR

Electrode size 120 x 80 mm
Electrode gap 5 mm

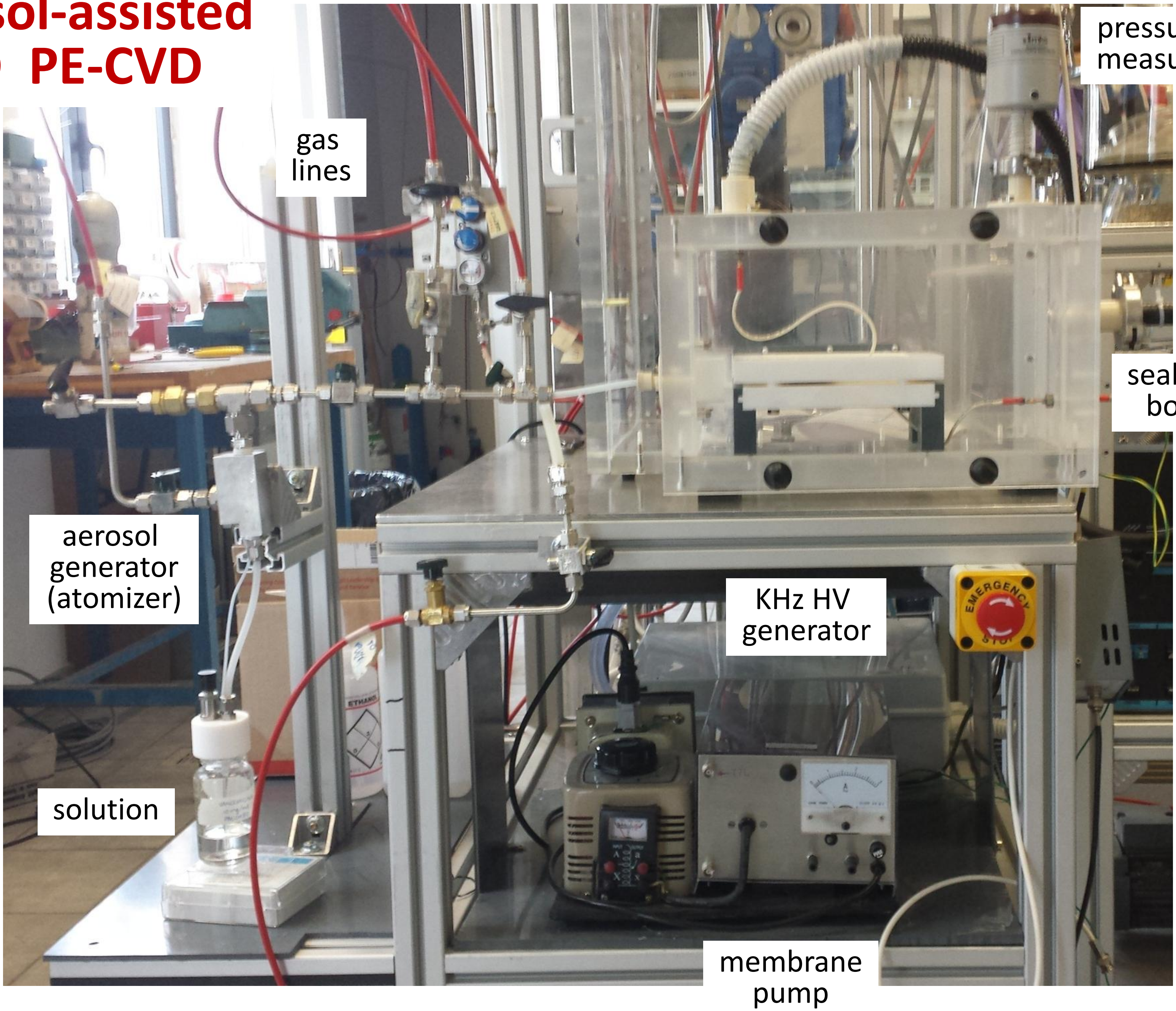


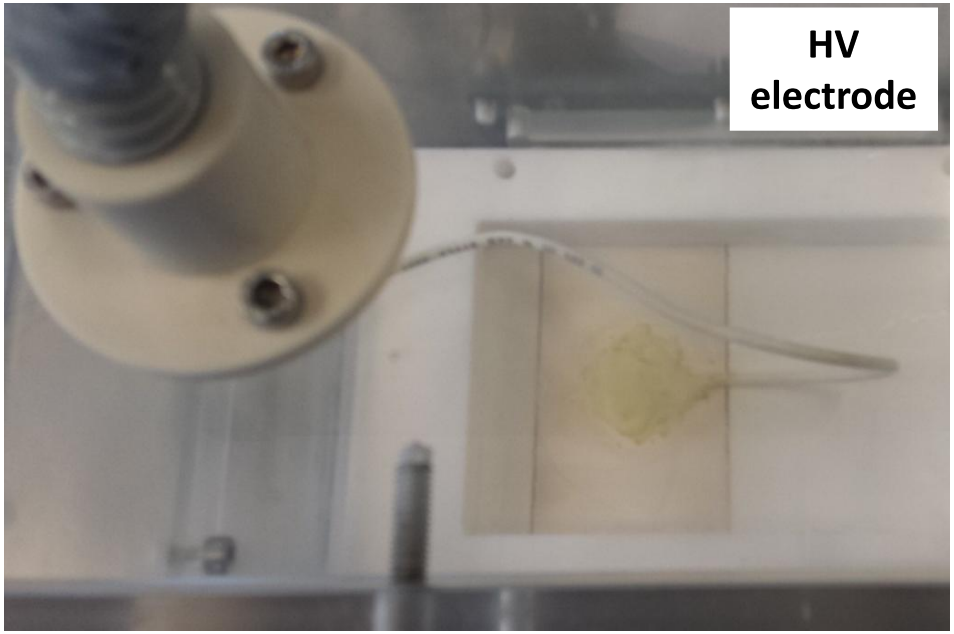
External parameters		
Frequency	4 kHz	11 kHz
Applied voltage	6 kV_{pp} (sinusoidal)	
Power	0.3 – 0.4 W cm^{-2}	0.9– 1.3 W cm^{-2}
C_2H_4 flow rate	10 sccm	
Total He flow rate	5 slm	
He flow rate through atomizer	0 – 5 slm (0 – 150 mg/min H_2O)	

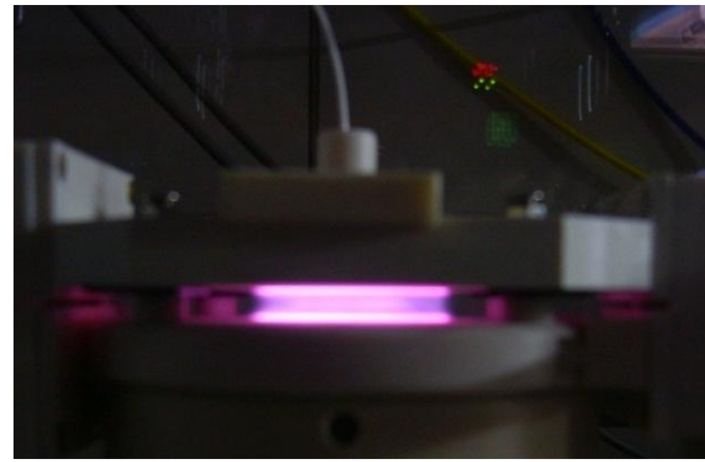
COATING ANALYSIS

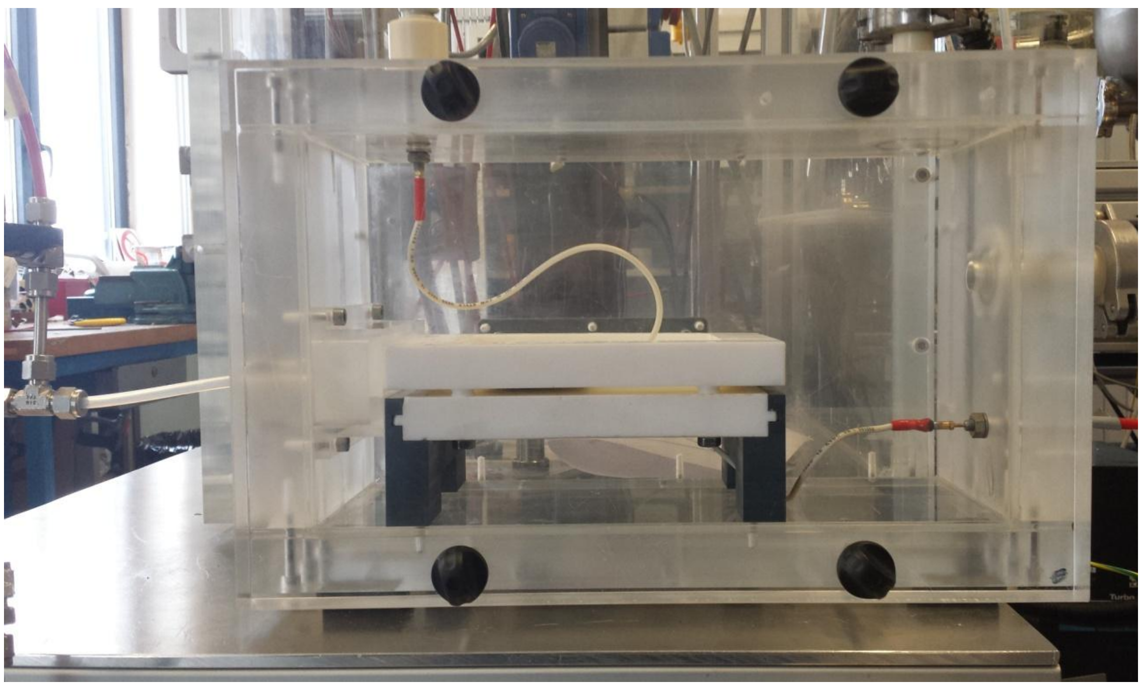
- FTIR
- XPS
- WCA
- Profilometry

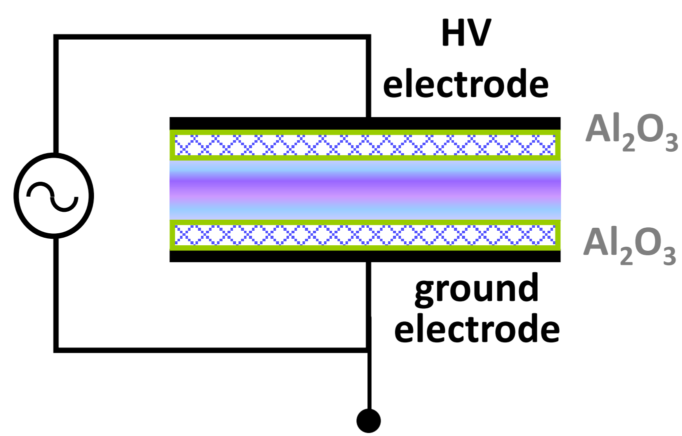
aereosol-assisted DBD PE-CVD




HV
electrode

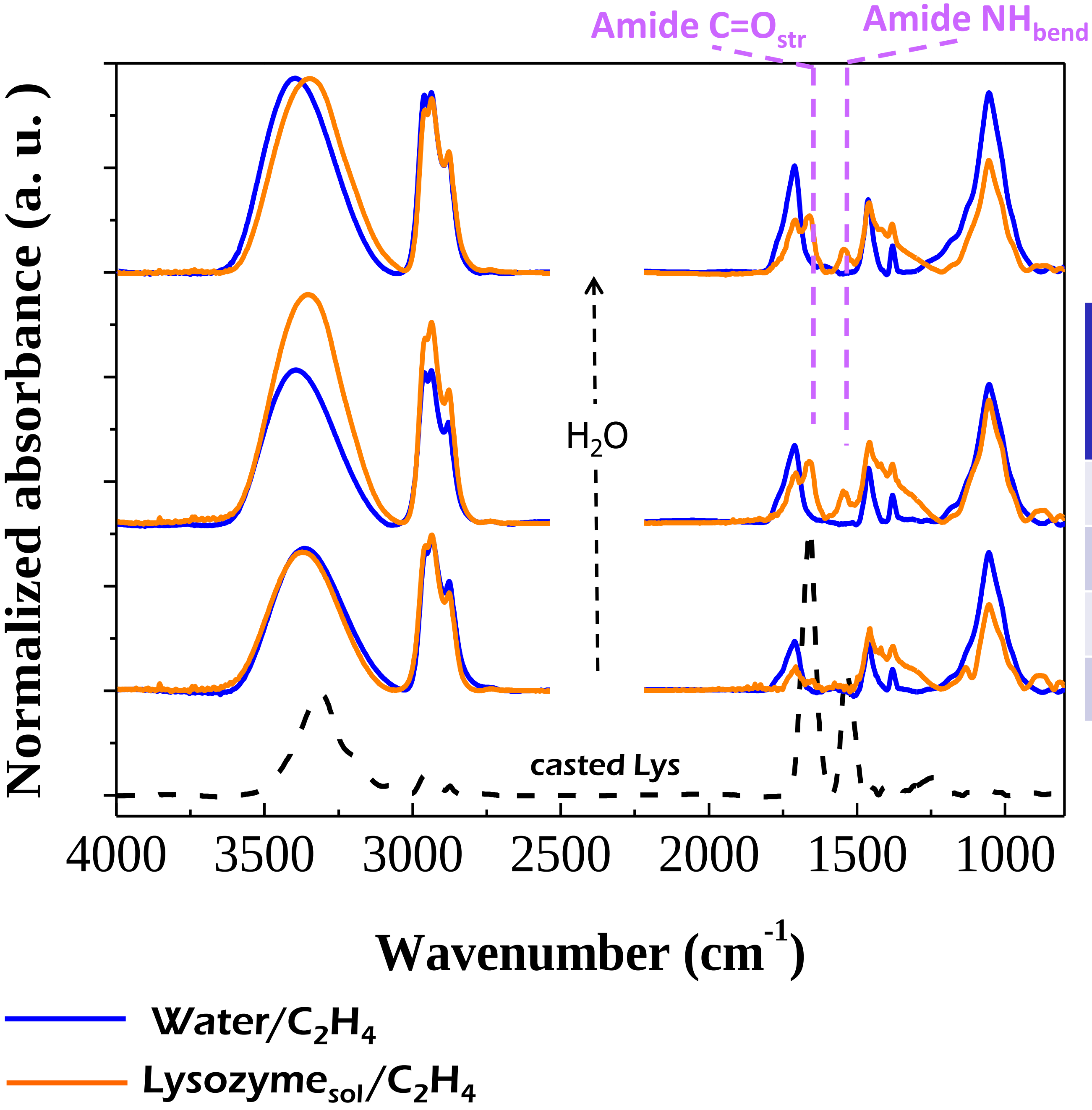





HV
electrode
Al_2O_3
Al_2O_3
ground
electrode

LYSOZYME$_{sol}$ / C_2H_4 - FTIR

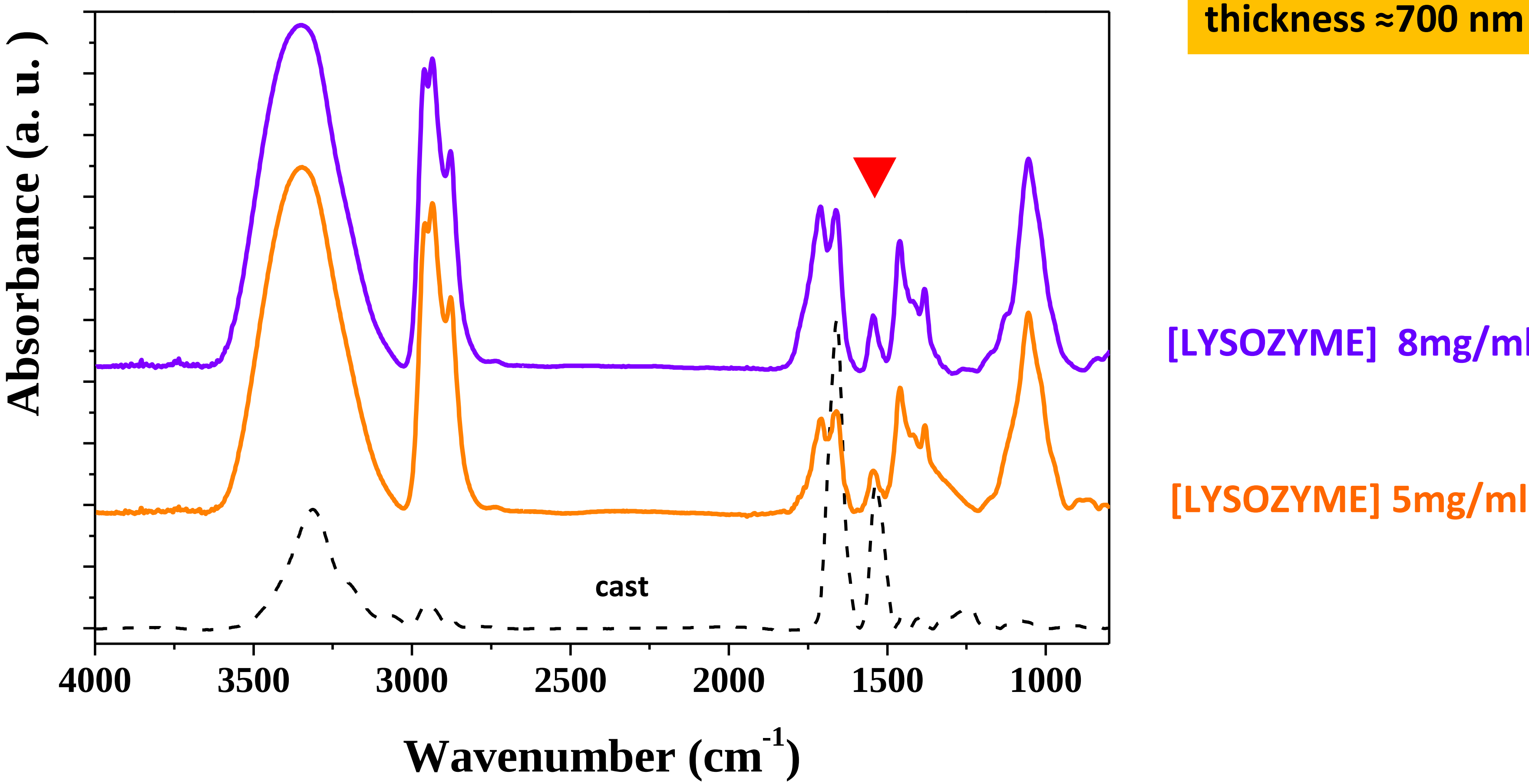
4 KHz / 6 KVpp
[Lys]$_{aerosol}$ 5 mg/ml
He: 5 slm
C_2H_4 10 sccm
thickness ≈700 nm



H_2O Flow Rate (ml/min)	Amide NH/CH
0	0.00
43	0.02
80	0.02
136	0.03

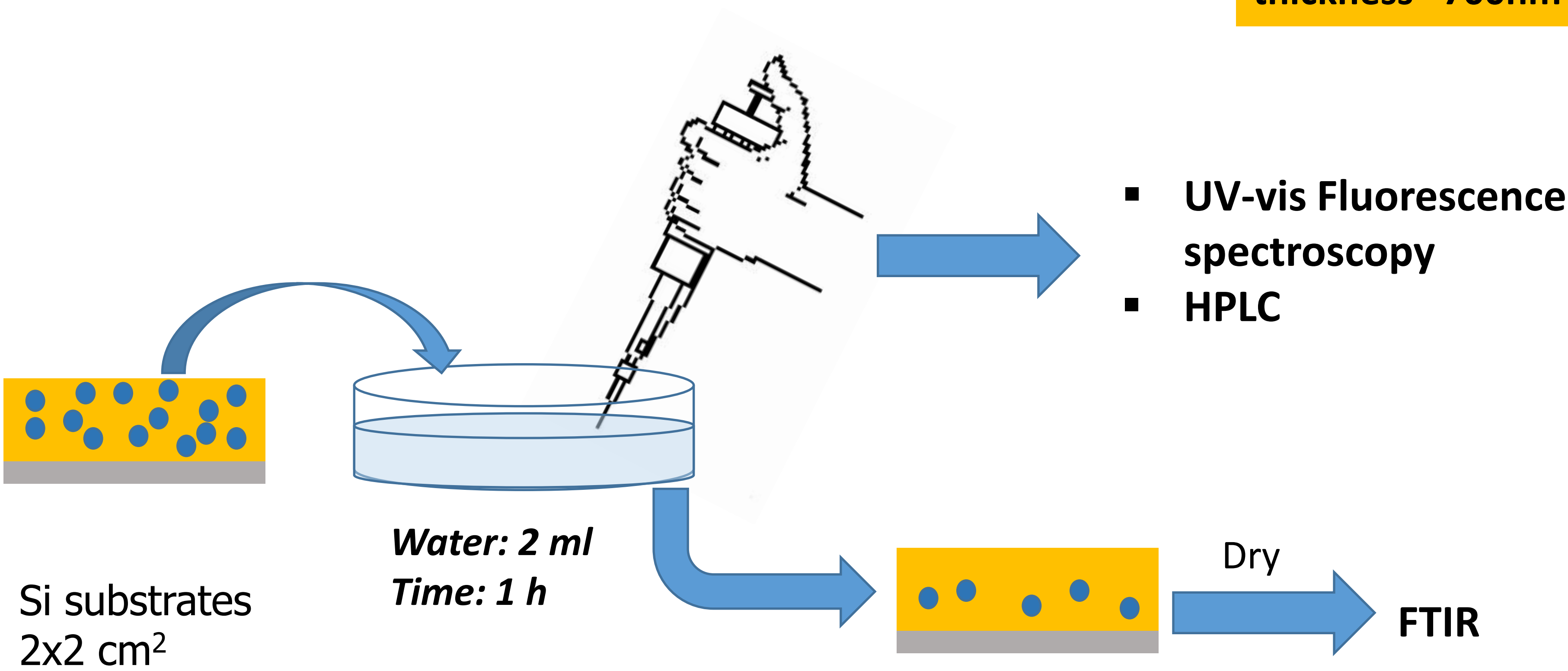
LYSOZYME$_{sol}$ / C_2H_4 - FTIR

4KHz / 6KVpp
[Lys]$_{aerosol}$ 5/8 mg/mL
He 5 slm
C_2H_4 10 sccm
H_2O 136 ml/min
thickness ≈700 nm



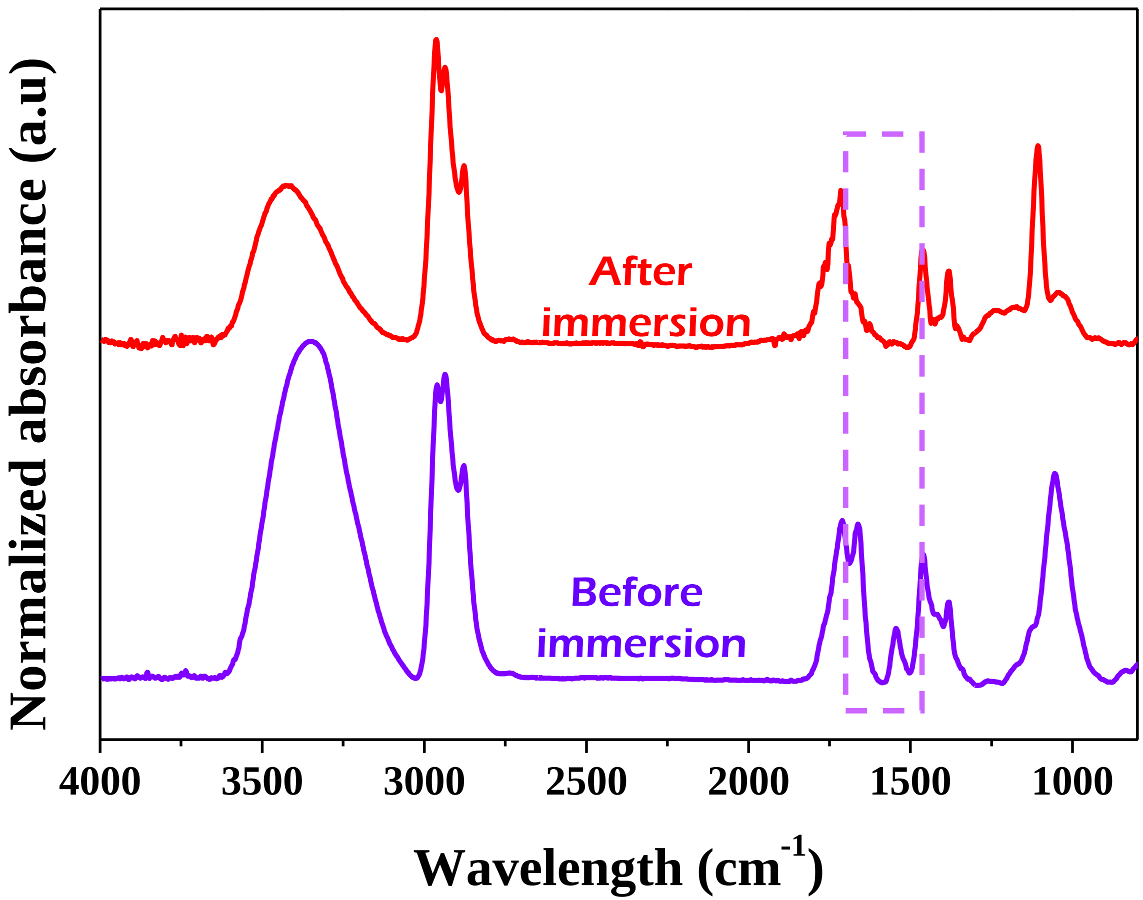
LYSOZYME$_{sol}$ / C_2H_4 – release test

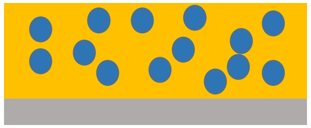
4KHz / 6KVpp
[Lys]$_{aerosol}$ 8 mg/ml
He 5 slm
C_2H_4 10 sccm
thickness ≈700nm

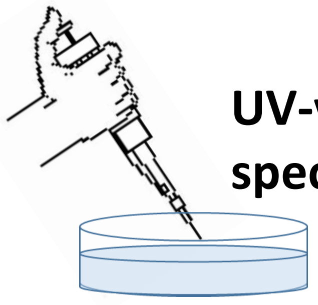


$LYSOZYME_{sol}$ / C_2H_4 – release test

4KHz / 6KVpp
$[Lys]_{aerosol}$ 8 mg/ml
He 5 slm
C_2H_4 10 sccm
thickness ≈700nm

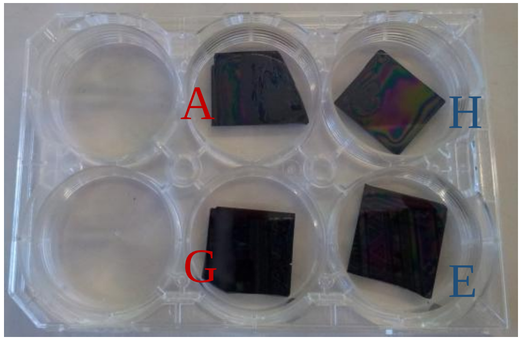






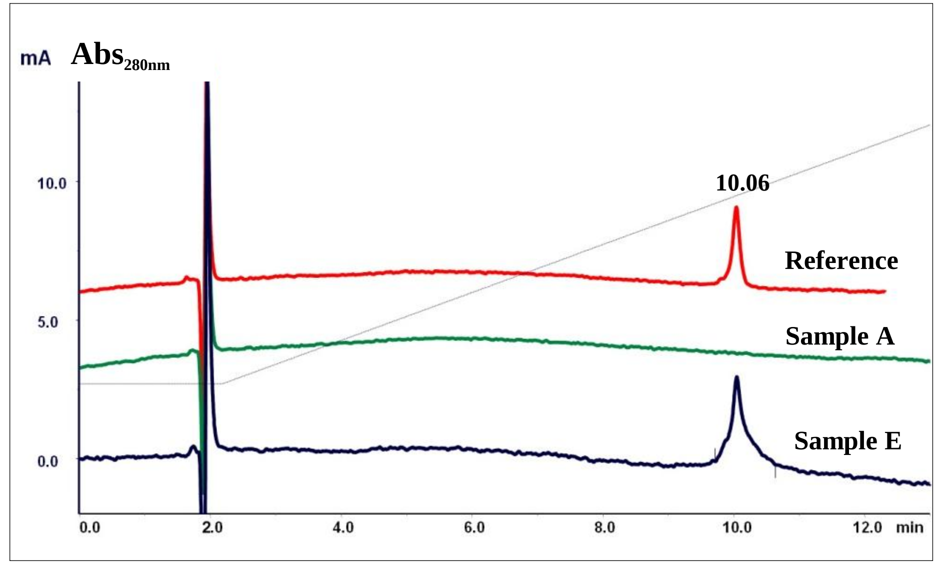
UV-vis Fluorescence spectroscopy → *About 30 µg/ml Lysozyme in the extraction liquid after 1 h immersion*

LYSOZYME$_{sol}$ / C_2H_4 – release test HPLC



RP-HPLC

Column: Zorbax SB300-C 18 (150 x 4.6 mm; i.d. 5 µm, 300 Å pore, Agilent). Linear gradient: 20 - 100% CH3CN with 0.1% TFA; flow rate: 1 mL/min for 20 min. Reference: Lysozyme 25 µg/ml



1. Addition of 2 ml of MilliQ water
2. Incubation at 25°C without shaking
3. Sampling at 15, 30, 45, 60 min.

Immersion time (min)	15	30	45	60
Lysozyme in the extraction liquid (µg/ml, cumulative)	1.8	23.1	25.0	27.5

HPLC confirms the presence of Lysozyme in the coating, not altered by the plasma

almost all Lysozyme embedded is released in 1h

is Lysozyme still «alive»?

Antimicrobial assay against *Micrococcus lysodeikticus*

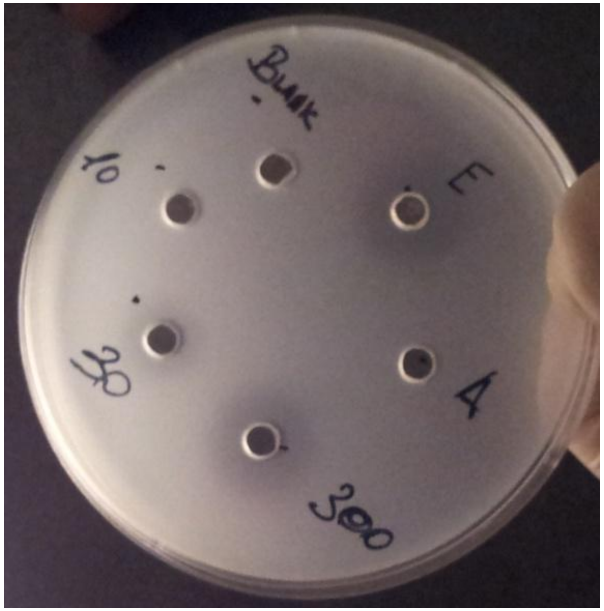
(Lie et al., Acta Veterinaria Scandinavica, 27(1): 23-32, 1986)

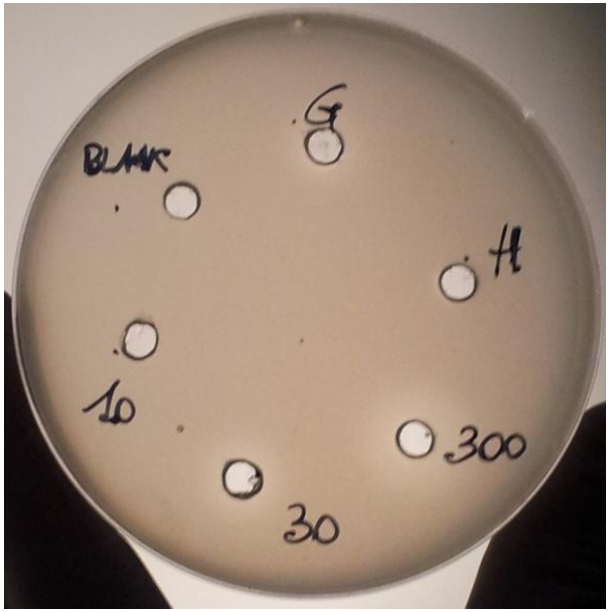
- Well plate diffusion 40 µl/well of enzyme solution (standard Lysozyme solution or extracts from Lysozyme biocomposite samples)
- buffered agar medium containing *M. lysodeikticus* incubated at 37 °C overnight

E, G spots from the estracts of the coatings

10, 30, 300 spots from standard Lys solutions

A, H, blank negative controls





Discoloration halo due to cell walls lysis for the lysozyme solution extracted from plasma deposited coatings

embedded Lysozyme is released in active antibacterial form

Table 5 Agar diffusion activity test results for the HiLyz coating.

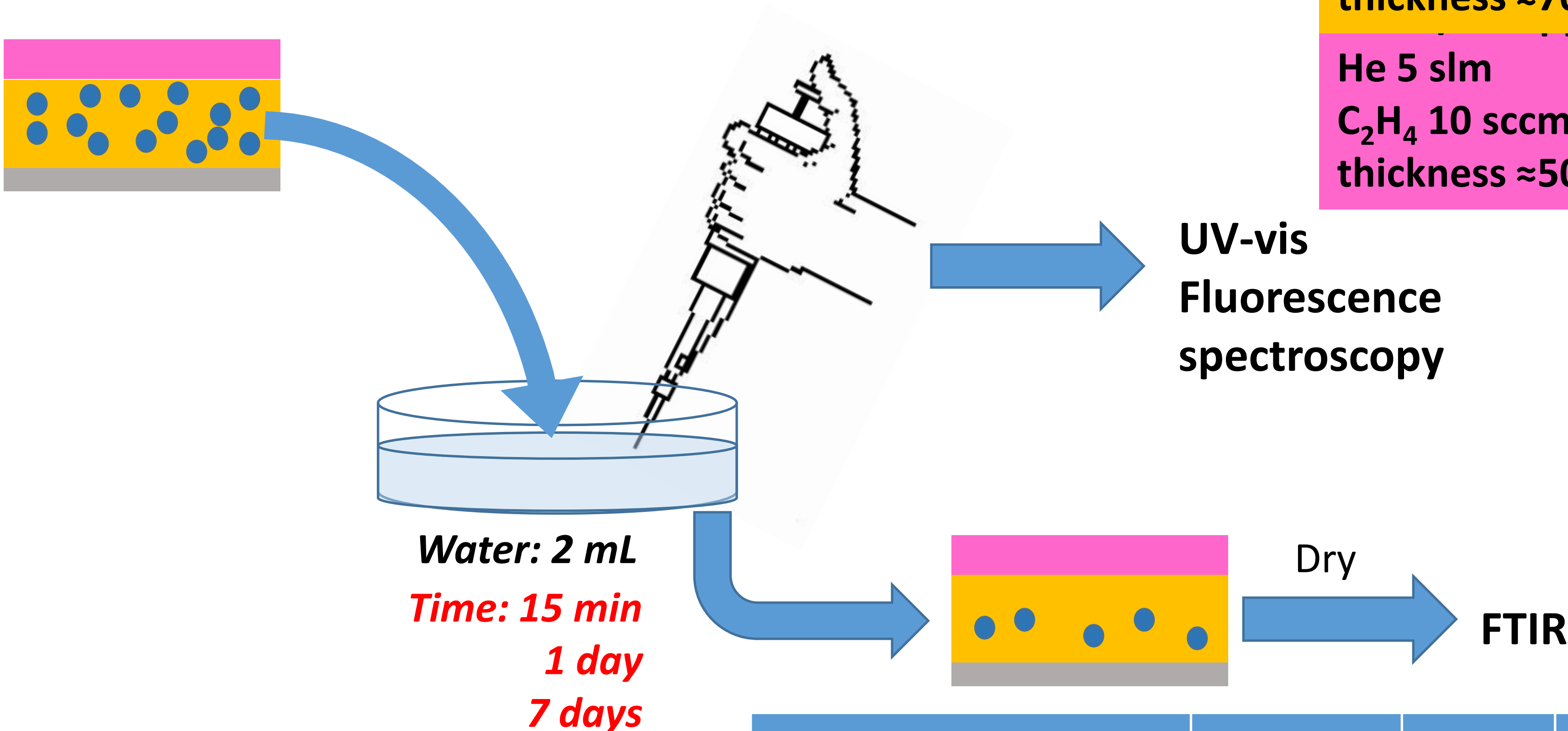
Well content	Inhibition halo diameter [mm]
C_2H_4/Lyz_{sol} HiLyz coating	8 ± 1
C_2H_4/H_2O plasma deposited coating (control)	0
Lyz standard solution *(10 μg/mL)*	0
Lyz standard solution *(30 μg/mL)*	6 ± 1
Lyz standard solution *(300 μg/mL)*	12 ± 1
Blank (negative control)	0

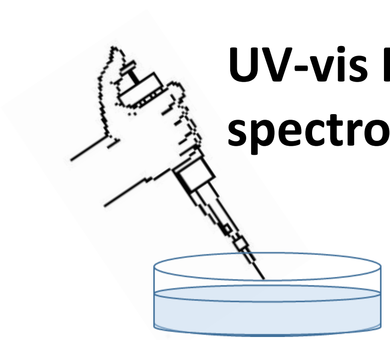
Palumbo et al, PPP 12, early view, 2015

LYSOZYME$_{sol}$ / C_2H_4 + diffusion barrier layer release test

4KHz / 6KVpp
[Lys]$_{aerosol}$ 8 mg/ml
He 5 slm
C_2H4 10 sccm
thickness ≈700 nm

He 5 slm
C_2H_4 10 sccm
thickness ≈50 nm





UV-vis Fluorescence spectroscopy

Immersion time	15 min	1 d	7 d
Lysozyme in the extraction liquid (µg/ml, cumulative)	1.5	15	18

IIT- Istituto Italiano di Tecnologia,
Center for Micro-BioRobotics @SSSA, Pontedera, Italy

Department of Chemistry,
University of Bari "Aldo Moro", Bari, Italy

free-standing bio-functional NanoFilms produced by plasma assisted technology

E Sardella, F Palumbo, R Gristina, P Favia
(CNR NANOTEC – UNIBA)

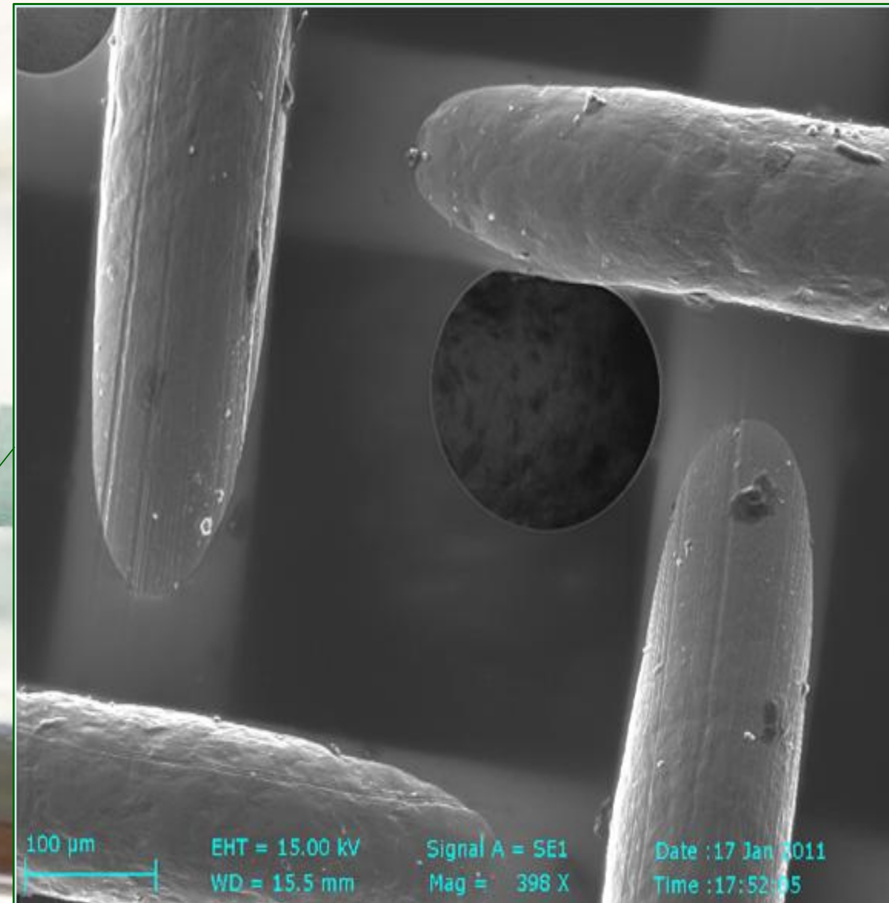
S Taccola, F Greco, V Mattoli
(IIT)

Daniela Pignatelli
grad st (IIT/SANT'ANNA – UNIBA)

polymeric free-standing NanoFilms

NanoFilms (polymeric ultrathin films)

polymer-based films
- with very large area (up to tens cm^2)
- few tens – hundreds nanometers thick
- with enormous (10^6) aspect ratio



MAIN FEATURES

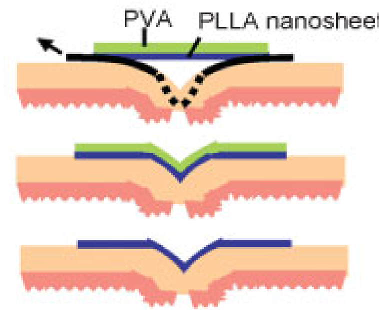
- ultra conformable
- high surface to volume ratio
- easy functionalization

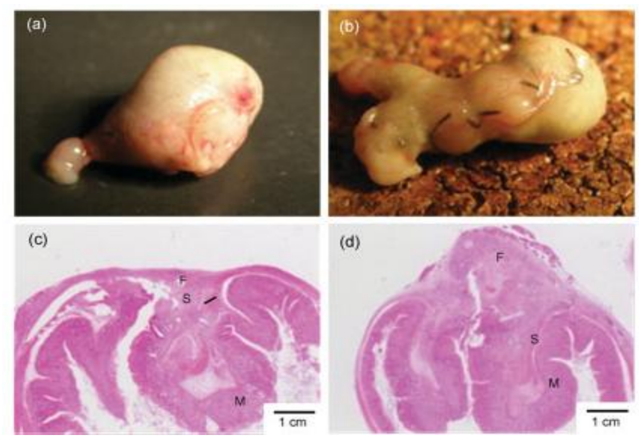
polymeric free-standing NanoFilms for biomedical applications

NanoFilms as patch/drug delivery systems

APPLICATIONS:
- ❑ drug delivery systems
- ❑ repair of tissue defects
- ❑ Internal surgical sutures

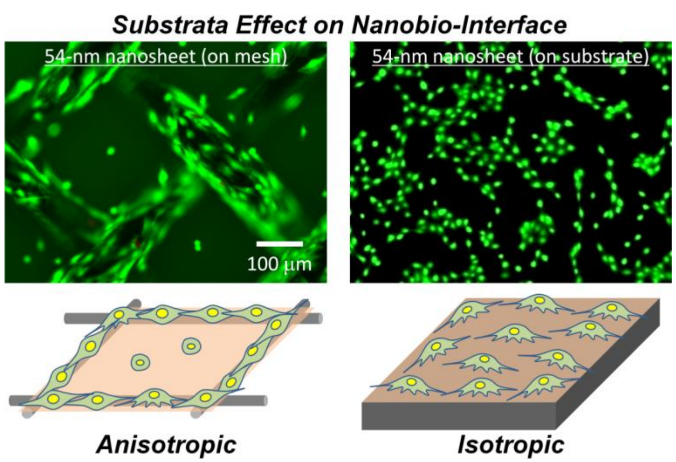
PLLA nanofilms for incision by sealing





Y. Okamura, K. Kabata, M. Kinoshita, D. Saito, S. Takeoka. Adv. Mater. 21, 4388–3492, 2007.

NanoFilms as cell culture substrates /scaffolds



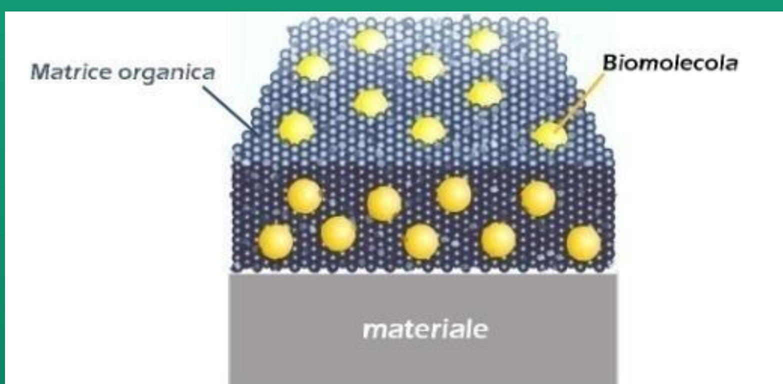
T. Fujie et al., “Evaluation of Substrata Effect on Cell Adhesion Properties Using Freestanding Polymeric Nanosheets”, Langmuir, vol. 27(21), pp. 13173-82, 2011

polymeric free-standing NanoFilms by Plasma Enhanced Chemical Vapor Deposition

NFs with non fouling properties, antibacterial features, drug-release capabilities, cell adhesion control

PE-CVD of C_2H_4/Vancomycin coatings

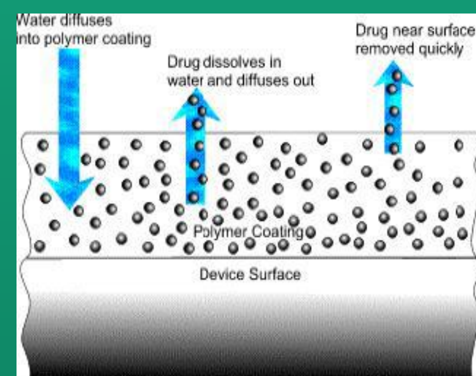
(aerosol assisted Atm P DBD)

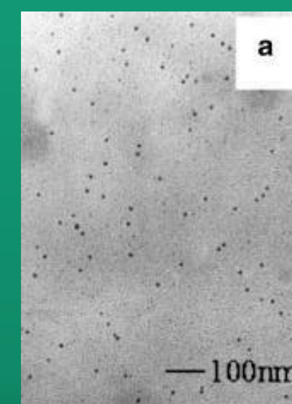


nano/biocomposite coatings

PE-CVD of $C_xH_yO_z$ (PEO-like) and of Ag:$C_xH_yO_z$ coatings

(sputter/deposition, Low P)



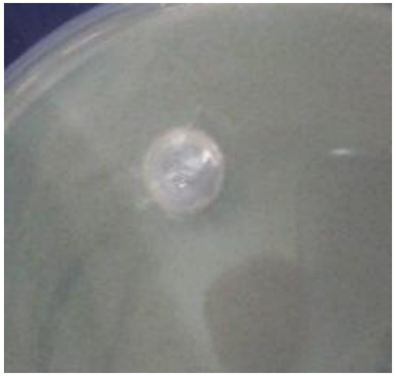


biomimetic, bioactive, antibacterial nanocomposite coatings

Antibacterial effect of C_2H_4/Vancomycin coatings

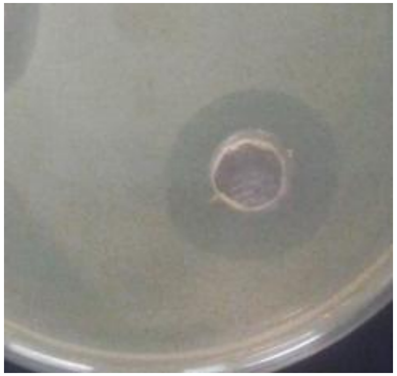
Agar diffusion test against Staphylococcus Aureus: Preliminary results

-Ti disc coated with Vancomycin containing films
-In contact with bacteria seeded agar for 6h



Uncoated Ti

Ti coated with C2H4/H2O

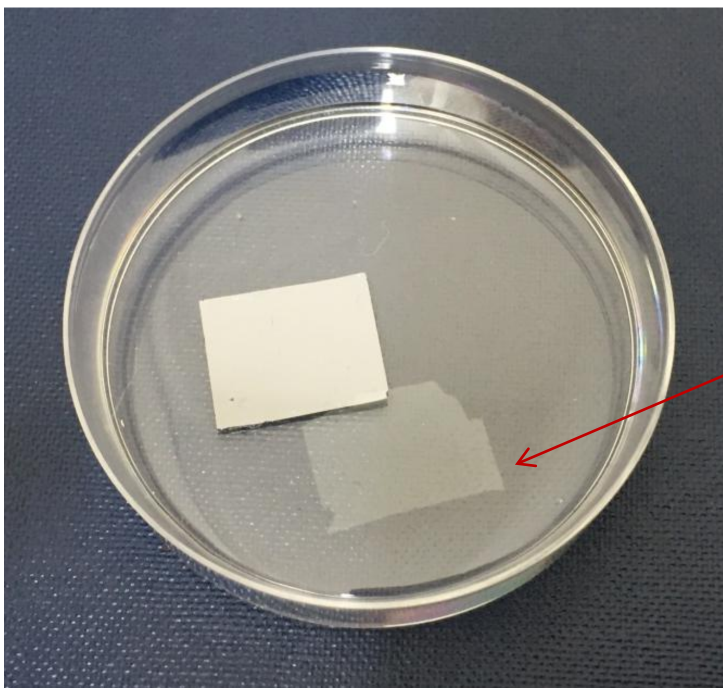


Ti coated with C2H4/Vancomycin(aerosol)

In collaboration with:
Biomaterials, Biomechanics and Tissue Engineering group
Dept. de Ciència dels Materials i Enginyeria Metallúrgica
Technical University of Catalonia (UPC)

ASPECT RATIO: 10^6

free standing PEO-like NanoFilm (53±7 nm thick) deposited directly on Si (no PVA)



delamination in water

Low P
parallel plate reactor, RF 13.56 MHz
5 sccm Ar, 0.4 sccm DEGME
5 W, 400 mTorr
90 min

PLASMA FUNCTIONALIZATION OF MATERIALS FOR BIO-ORIENTED APPLICATIONS *other hot topics*

- **membranes**
- **biosensors**
- **functionalization of NPs, CNTs, graphene**
- **μ-scale plasma printing/patterning**
- **probing nano-mechanical properties of thin coatings in liquids**
- **free standing plasma deposited NanoFilms**
- **PE-CVD/treatments of materials in liquids**
- **PECVD / treatments on biological tissues**
- **cell-containining coatings**

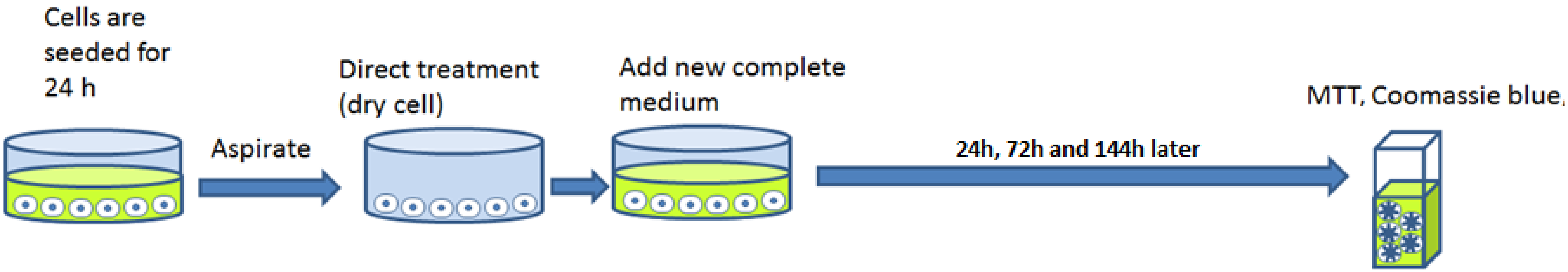
PLASMA-ACTIVATION OF CELLS … ON MATERIALS !?

CNR NANOTEC

INP Greifswald

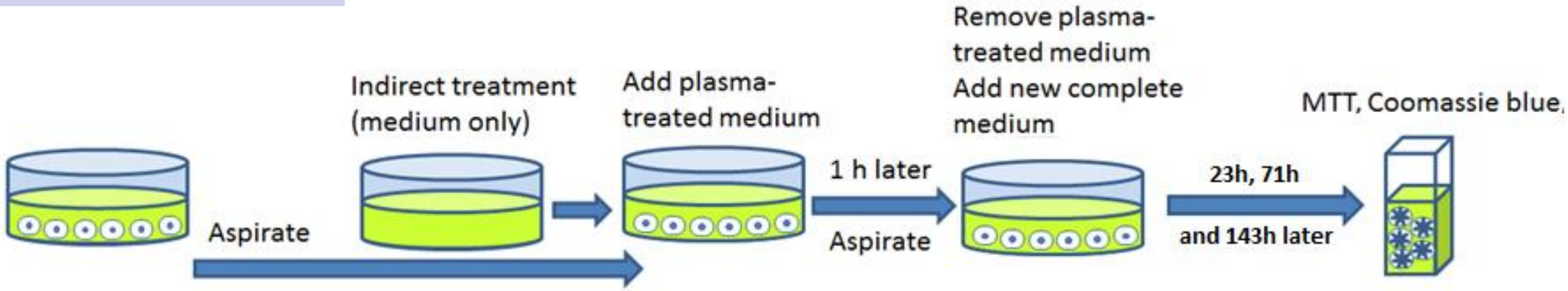
CPT

CELL PLASMA TREATMENT



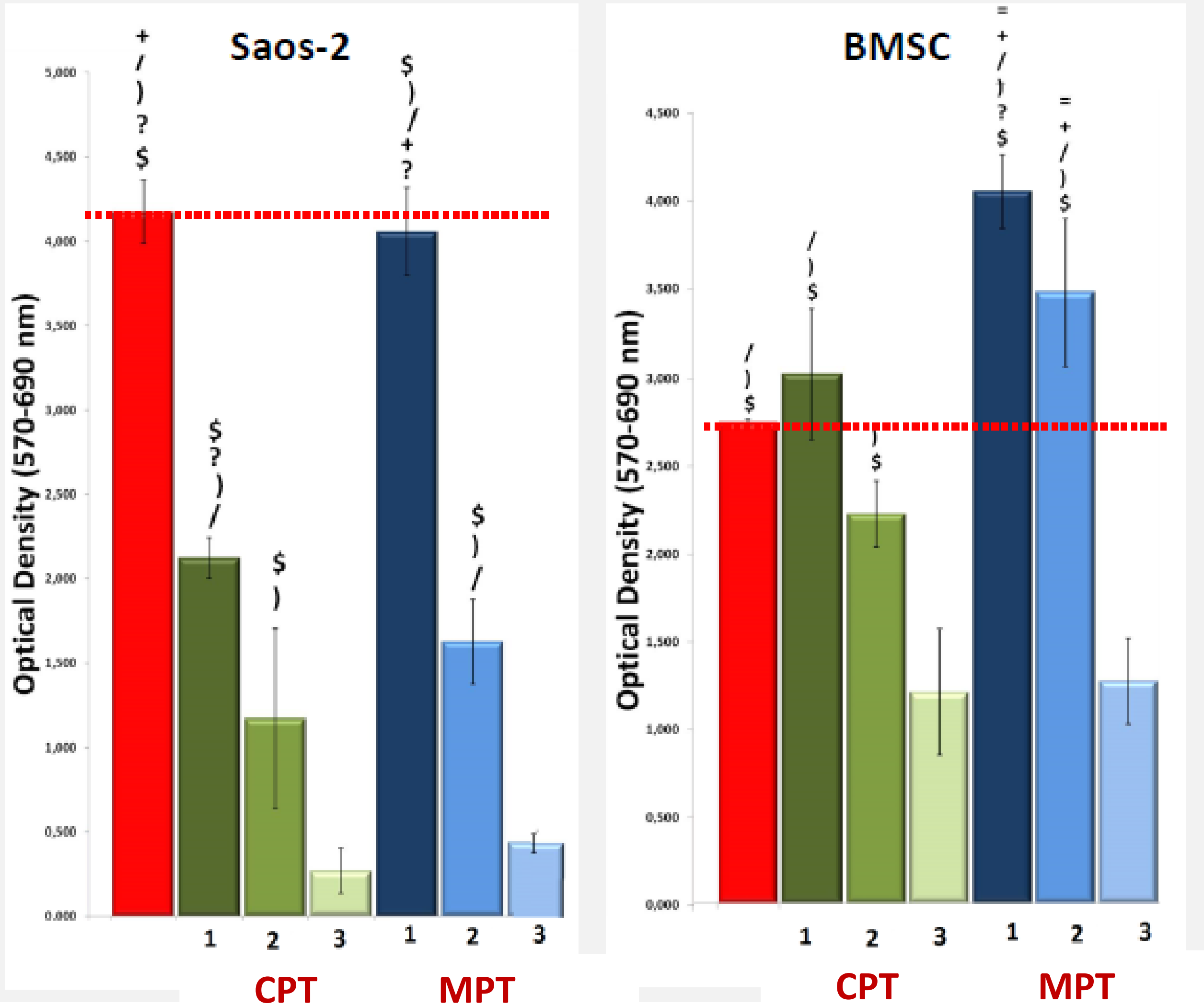
MPT

MEDIUM PLASMA TREATMENT



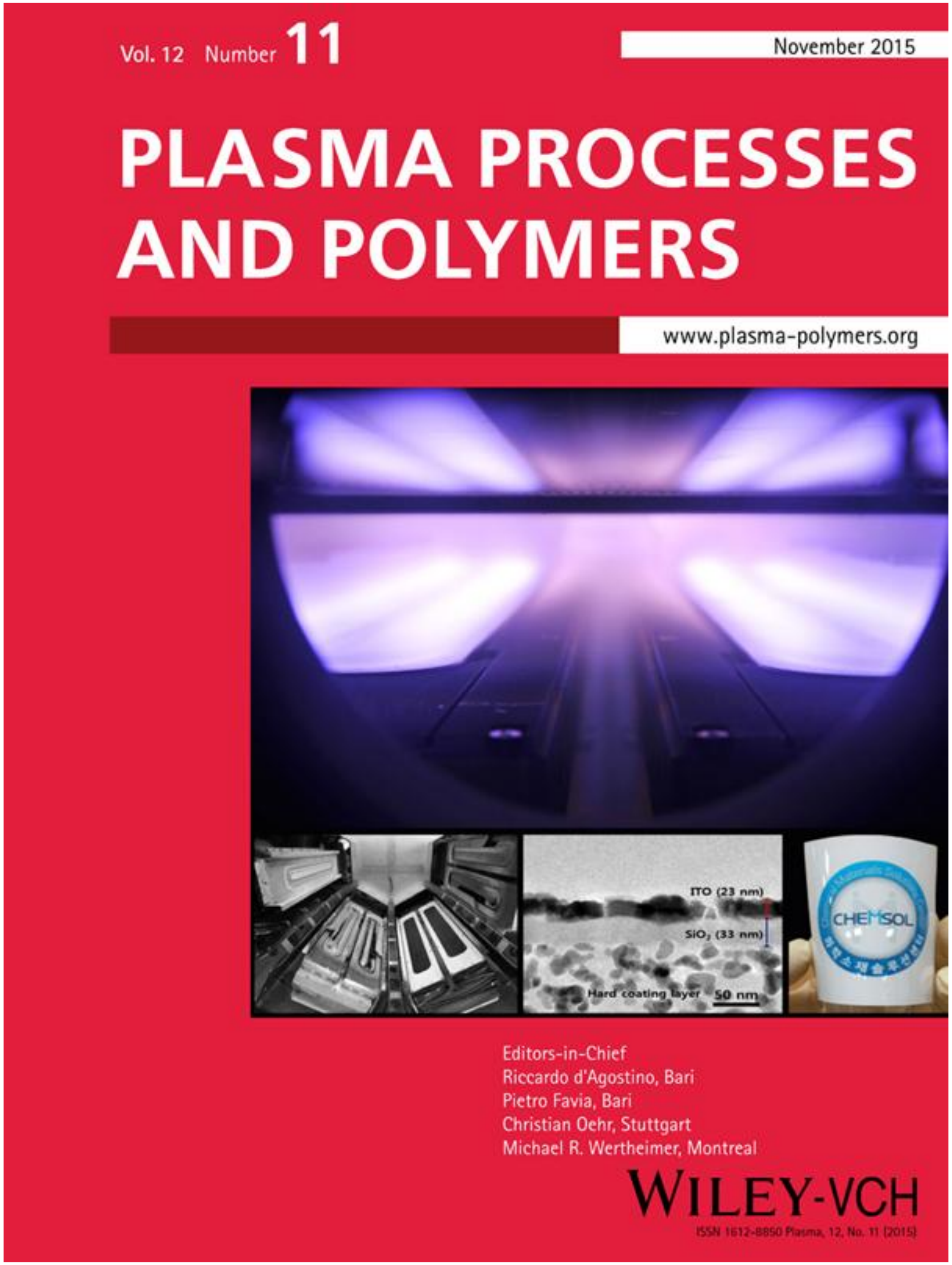
Trizio et al, ISPC-22, Best Poster Award

144h



TWO-WAY ANOVA
and Bonferroni's Post-Test:

=: p< 0.05 vs Control
+: p<0.05 vs Dir 1
/: p<0.05 vs Dir 2
): p<0.05 vs Dir 3
!: p<0.05 vs Ind 1
?: p<0.05 vs Ind 2
$: p<0.05 vs Ind 3



Dec 2015: Special Issue on Plasma vs Cancer

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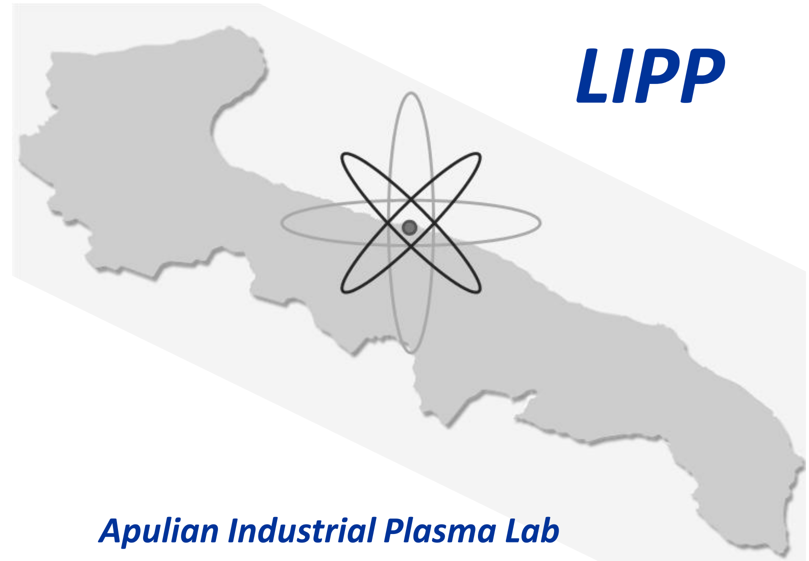
  (PON MIUR, Italy)
- **SISTEMA**
  (PON MIUR, Italy)
- **COST ACTION MP1101**
  biomedical applications of Atm P plasmas
- **MAIND**
  (PON MIUR, Italy)

LIPP



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PLASMA SOLUTION srl
spin off dell'Università di Bari

Schlumberger

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Action MP1101