PROCESSI VIA PLASMA
per applicazioni biomediche

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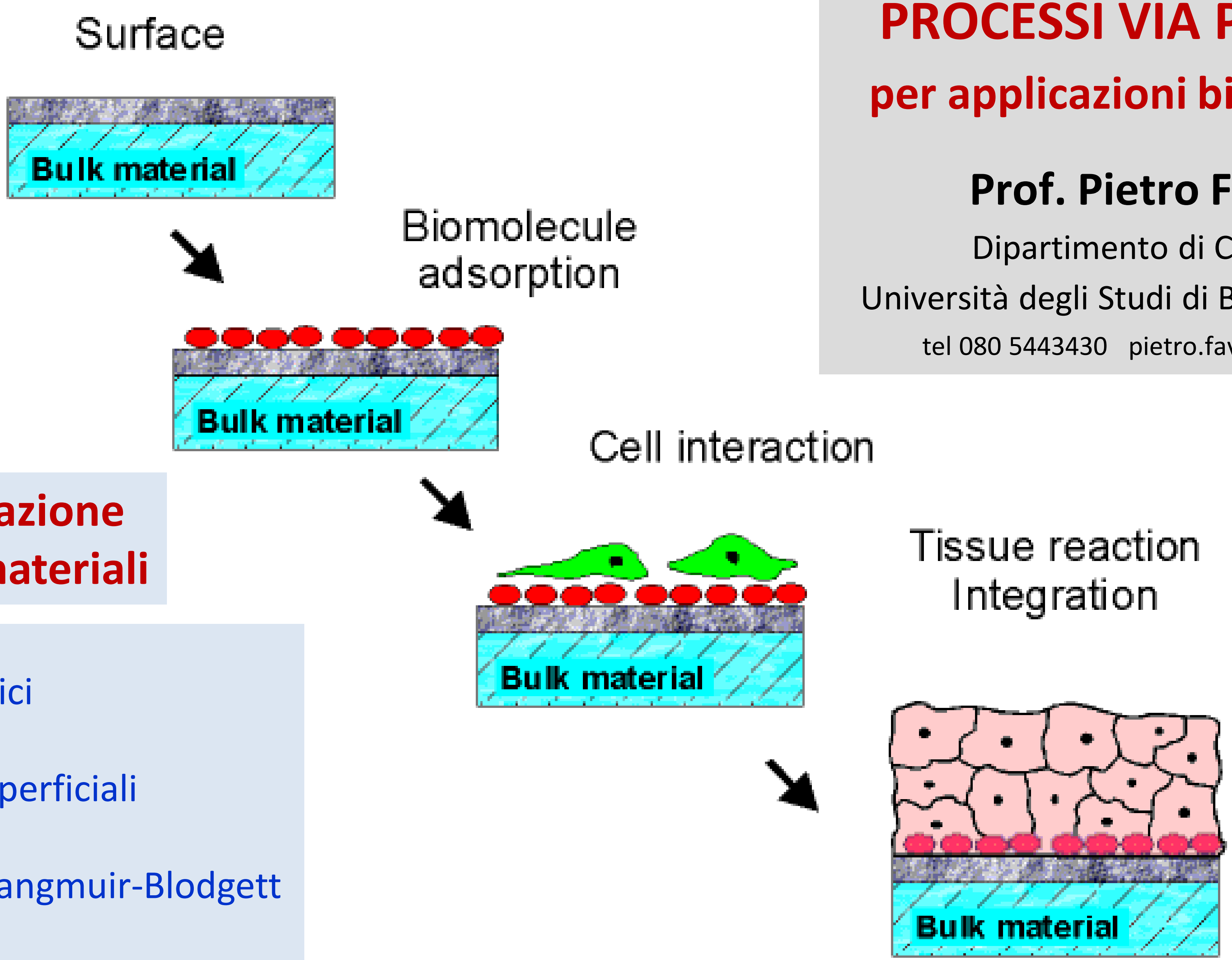


metodi di modificazione superficiale di biomateriali

- Processi plasmochimici
- Reazioni chimiche superficiali
- Deposizione di film Langmuir-Blodgett
- Self-assembled monolayers (SAMs)

Modificazioni superficiali di biomateriali: obiettivi specifici

1. PULIRE UNA SUPERFICIE

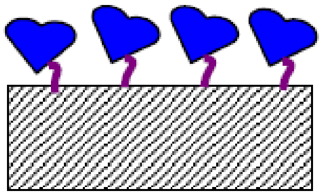
2. RIDURRE/ELIMINARE L'ADSORBIMENTO DI PROTEINE/CELLULE

- Es.: ridurre risposte indesiderate/incontrollate agli impianti e dispositivi extracorporei
- Es.: ridurre adsorbimenti aspecifici su biosensori (rumore e contaminazioni)
- **Superfici idrofile** (film di PEO ''gold standard''), **$-(CH_2CH_2O)n$**
- Superfici **NON FOULING**

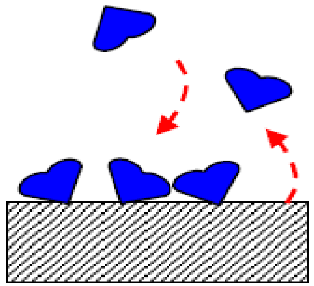
3. RIDURRE LA TROMBOGENICITA'

- **Superfici idrofile:** impediscono l'adsorbimento di proteine
- **Superfici idrofobe**: interazione superficie/cellula intrinsecamente debole
- **Superfici funzionalizzate con eparina:** si lega naturalmente all'antitrombina, inattivando il fattore X, la trombina e altre proteasi coinvolte nella coagulazione del sangue

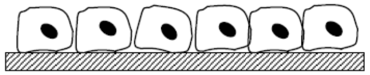
3. RIDURRE LA TROMBOGENICITA’



• **Superfici funzionalizzate con albumina:** nessun legante per le piastrine

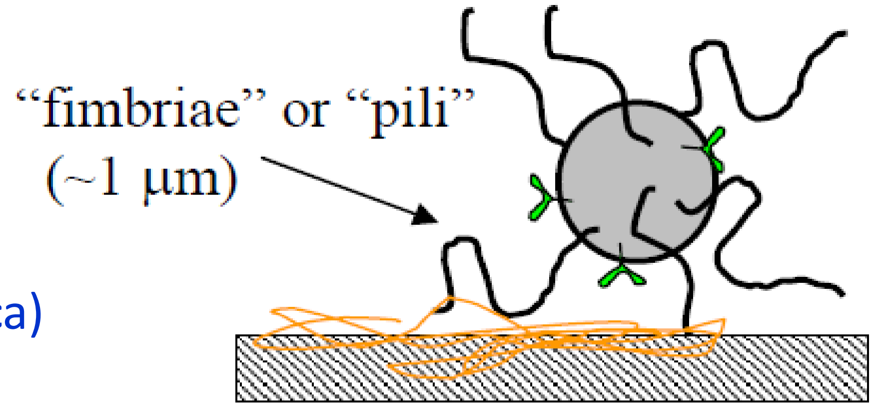


• **Rivestimenti affini all’albumina:** superfici che, promuovendo forte adsorbimento di albumina dal sangue, formano un rivestimento ‘’passivante’’ (es. bilirubina)



• **Adesione di cellule endoteliali:** rivestimento naturale dei vasi sanguigni in grado di idrolizzare la fibrina

4. RIDURRE L'ADESIONE BATTERICA



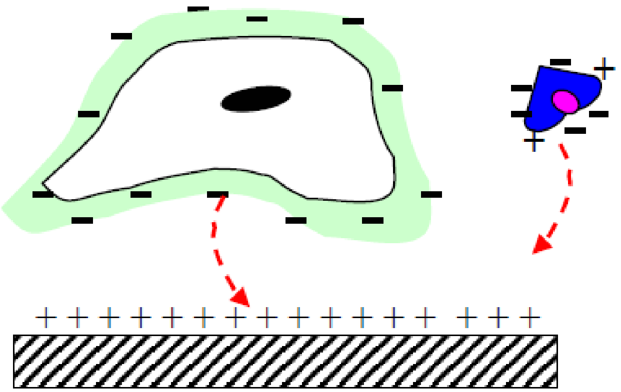
Adesione batterica

- Via proteine e polisaccaridi della membrana cellulare (aspecifica)
- Via specifici recettori per proteine plasmatiche
- I pili facilitano l'iniziale adesione alla superficie

- **Rivestimenti passivanti:** polimeri idrofili
- **Agenti battericidi:**
  (i) argento;
  (ii) antibiotici (es. film a rilascio di gentamicina);
  (iii) agenti che alterano la membrana cellulare (es. peptidi antimicrobici e polimeri cationici, carichi positivamente, che interagiscono con le strutture di membrana a carica netta negativa)

5. PROMUOVERE L'ADESIONE CELLULARE

- **Modificare la chimica superficiale:** promuovere l'adesione proteica
- **Creare cariche superficiali positive:**

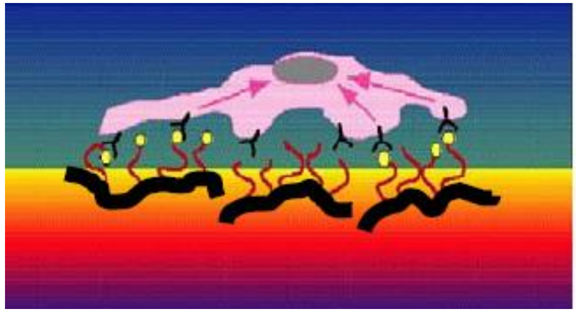


- Molte proteine hanno carica superficiale netta negativa (aumenta l'adsorbimento proteico);
- Il glicocalice è carico negativamente (attrazione aspecifica)
- Una superficie molto carica positivamente, però, inibisce l'adesione cellulare

- **Aumentare la rugosità/porosità superficiale:**
  - promuove l'attacco cellulare (maggiore area di contatto)
  - può inibire la crescita cellulare

5. PROMUOVERE L'ADESIONE CELLULARE

- **Funzionalizzare la superficie con ligandi che promuovano l'adesione cellulare:**

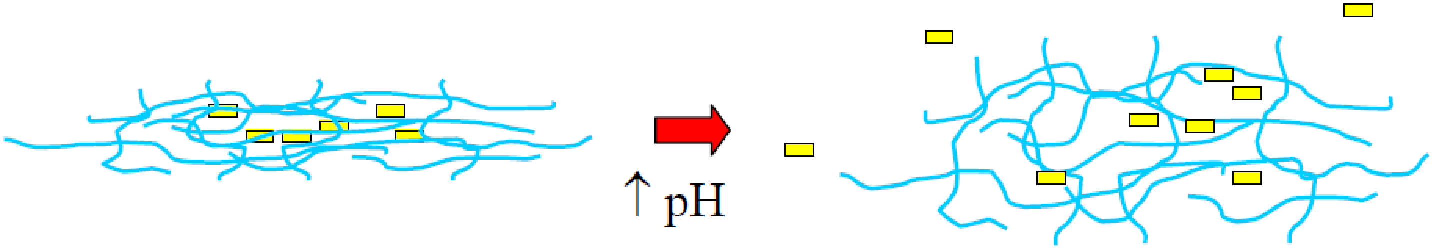


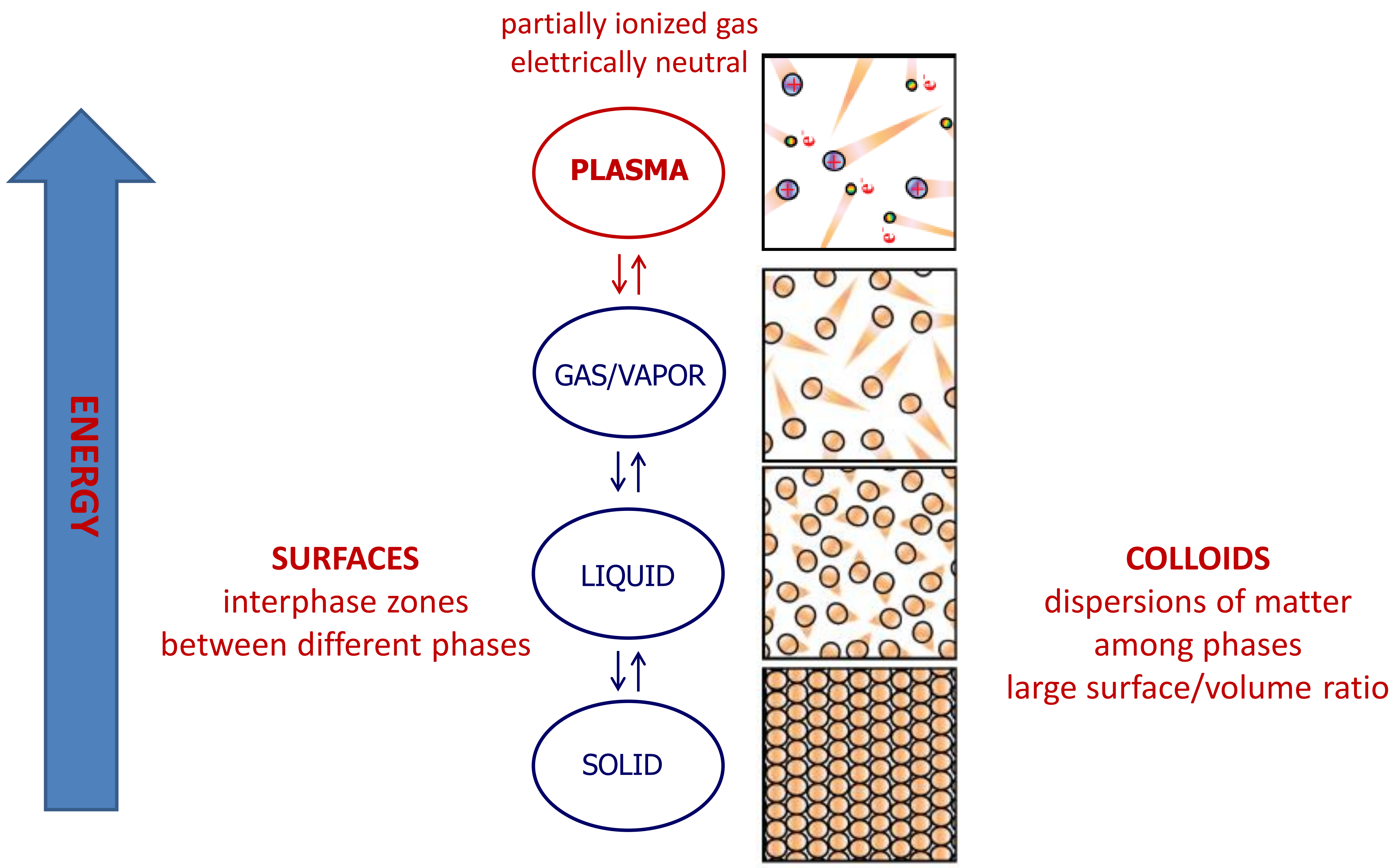
- proteine di adesione (fibronectina)
- sequenze amminoacidiche: es. RGD (Arginina – Glicina - Acido aspartico), ligando di proteine di membrana coinvolte nel processo di adesione cellulare

6. MODIFICARE PROPRIETA' DI TRASPORTO

Regolare il passaggio di acqua, agenti terapeutici, etc.

- **Crosslinkg superficiale (passivo) o film che rispondono a stimoli esterni (attivi):**




partially ionized gas
elettrically neutral
PLASMA
GAS/VAPOR
LIQUID
SOLID
ENERGY
SURFACES
interphase zones
between different phases
COLLOIDS
dispersions of matter
among phases
large surface/volume ratio

1928
I. LANGMUIR INTRODUCES THE WORD “PLASMA”

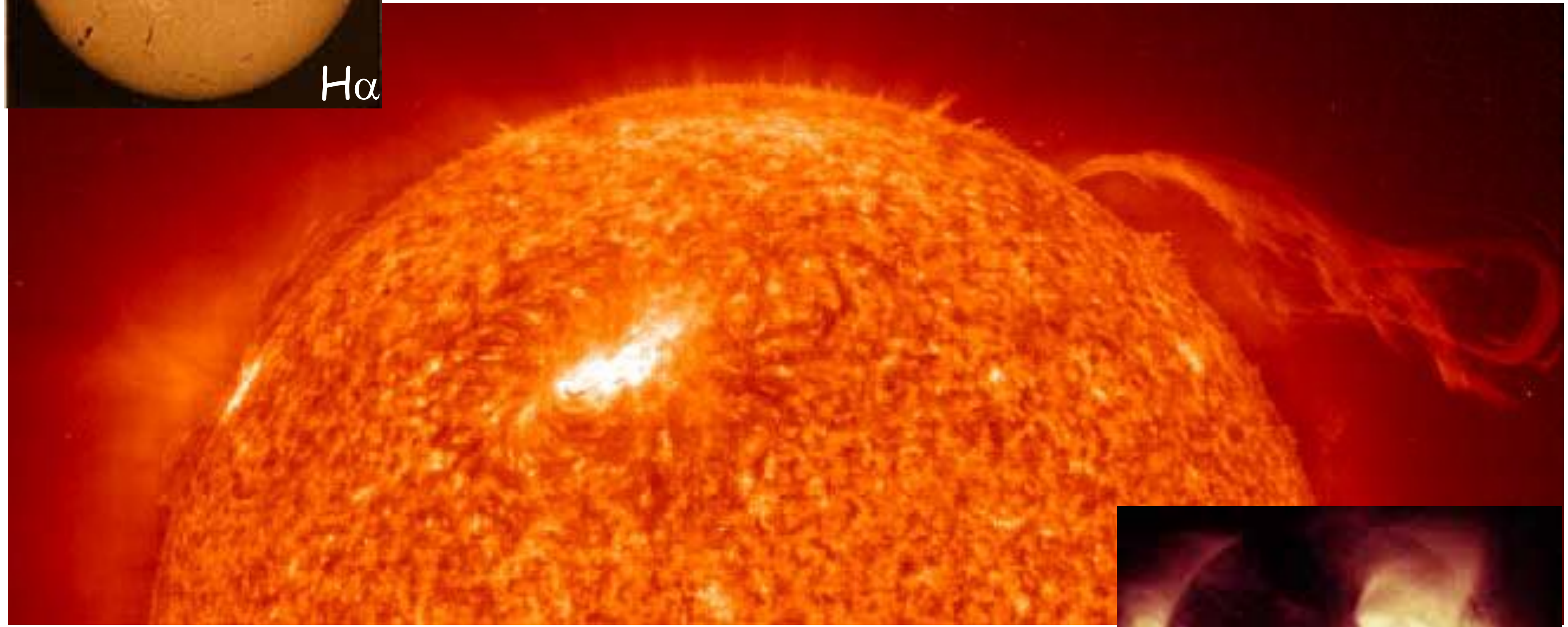
I. Langmuir, *Oscillations in Ionized Gases*
Proc. Nat. Acad. Sci. 14, 627, Aug 1928

“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

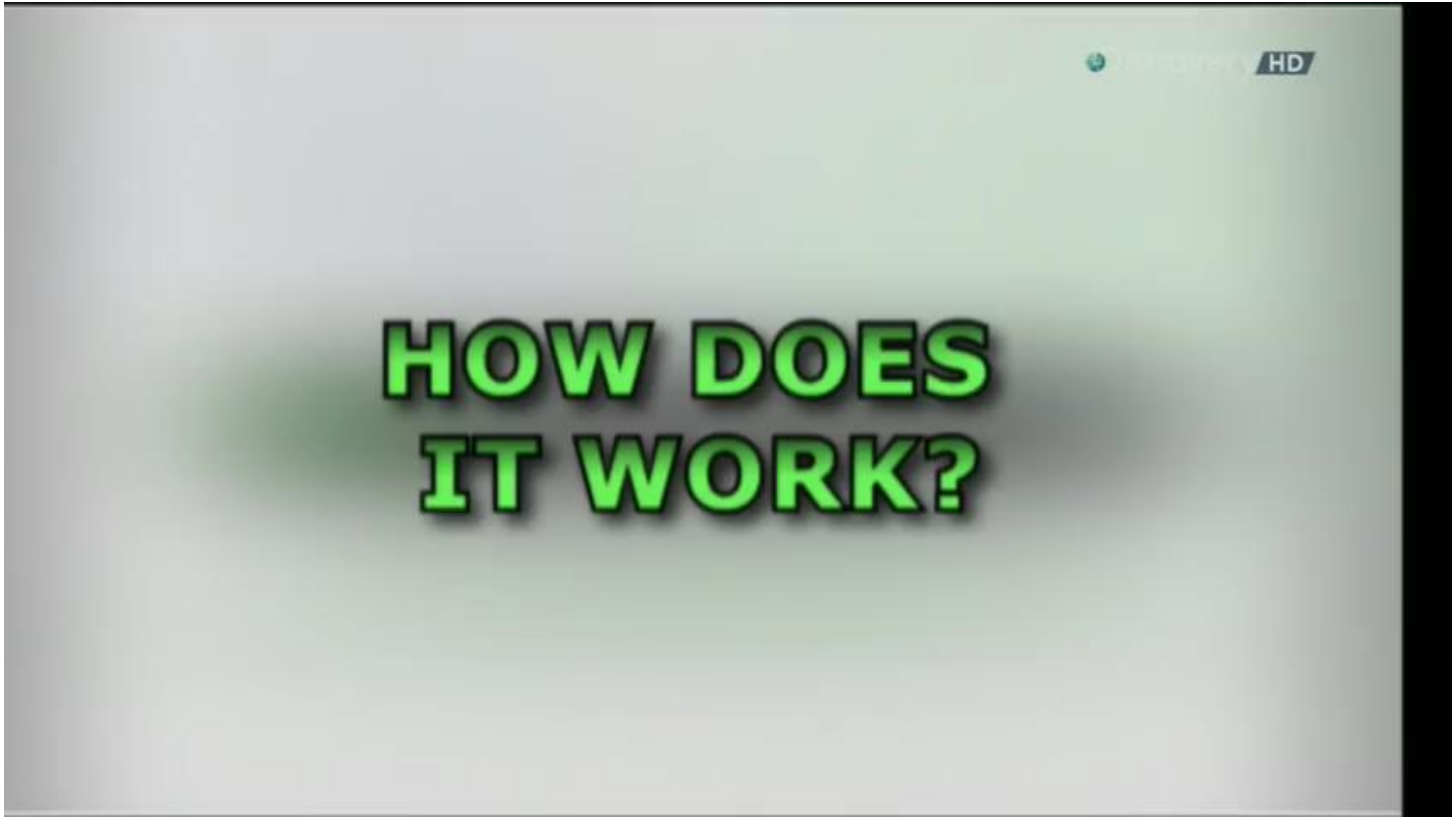


PLASMA CALDO

PLASMA FREDDO

PLASMA CALDO

taglio al plasma
video



PLASMA FREDDO

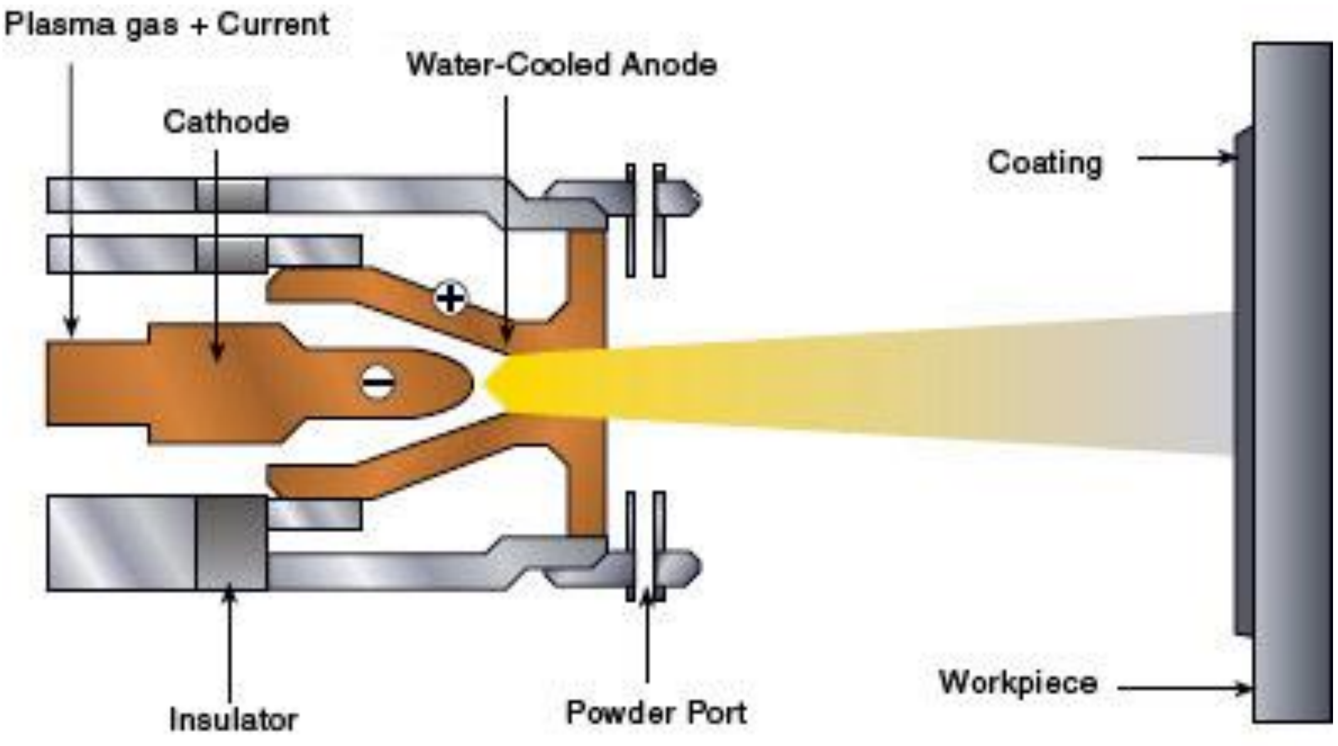
PLASMA CALDO

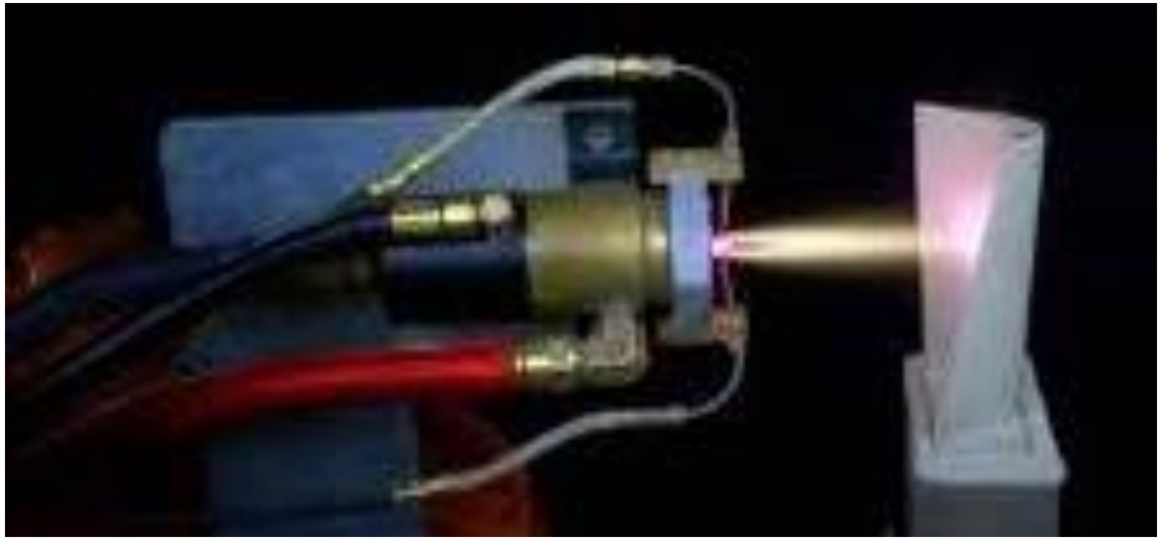
PLASMA FREDDO

PLASMA SPRAY

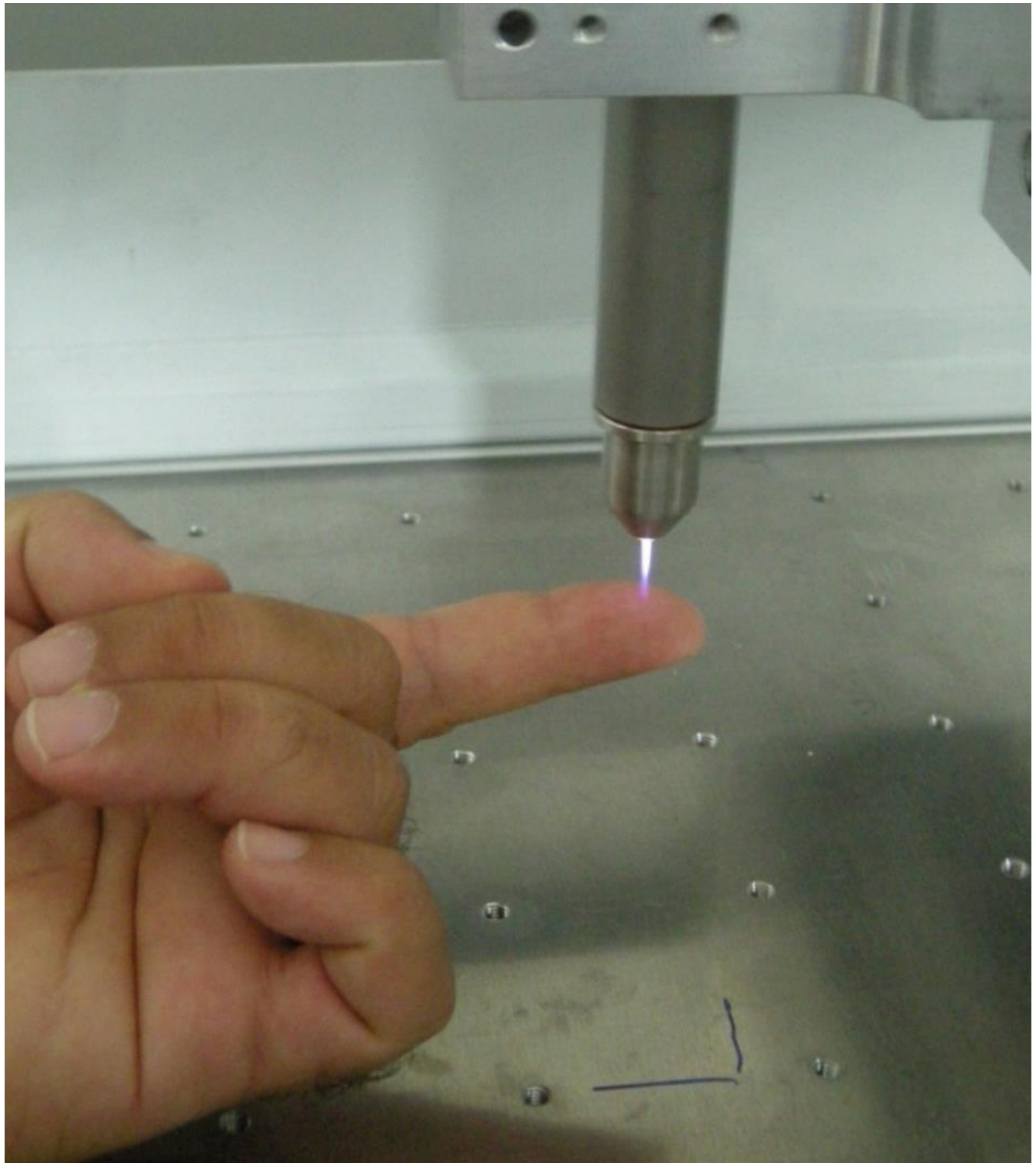
thermal plasmas
for materials

hydroxyapatite coatings for orthopedic and dental implants



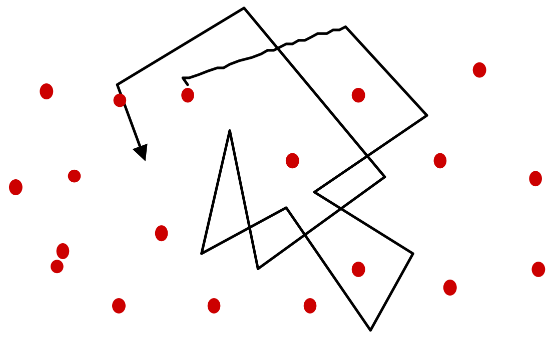


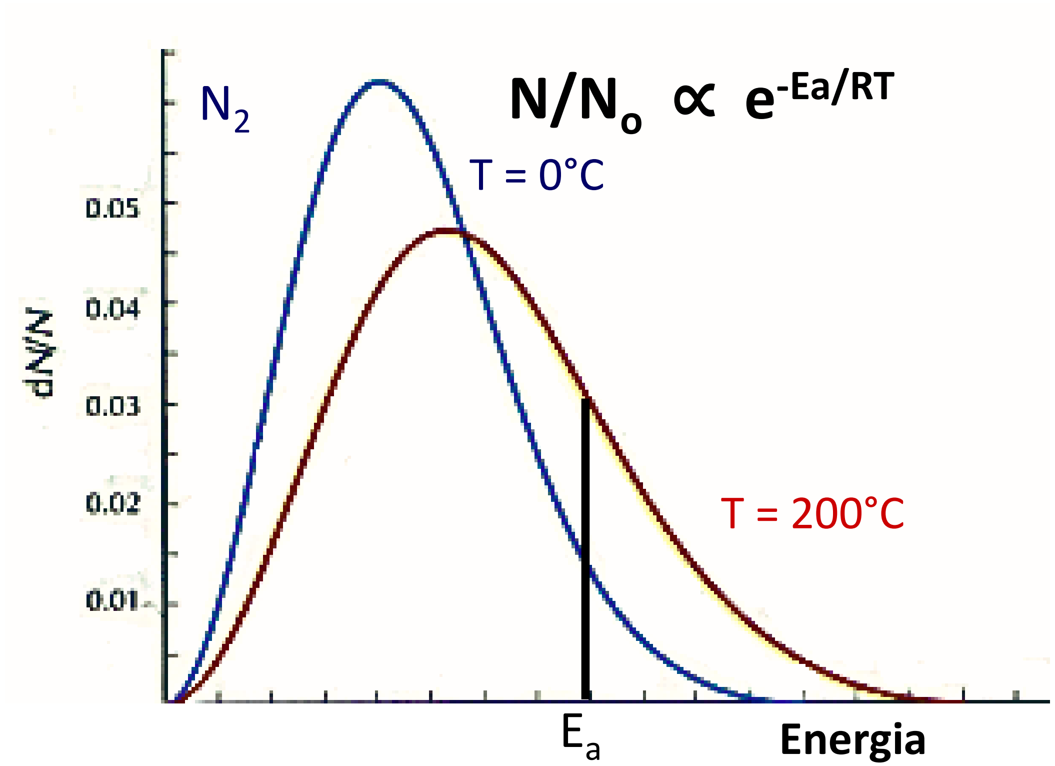
PLASMA FREDDO



stato gassoso

Distribuzione delle velocità di Maxwell-Boltzman





Equazione di stato dei gas ideali

$$PV = nRT$$

R = 0.082 l atm / mole K

$N_A = 6.023 \times 10^{23}\ mol^{-1}$

La temperatura del gas misura la sua energia cinetica

$E_k = ½\, mv^2 = 3/2\ k_B T$

(x molecola)

$k_B = R/N_A \rightarrow E_k = 3/2\ RT$ (x mole)

1 eV ≈ 11,600 K ≈ 23 Kcal /mole

$k_B = 1.38 \times 10^{16}$ J/K

Cammino libero medio (λ)

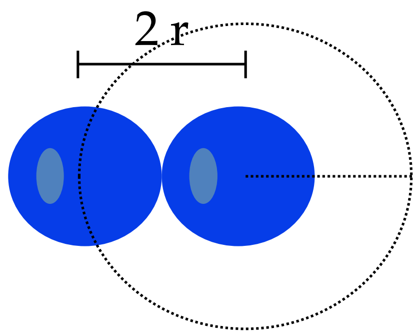
Distanza media percorsa da una particella tra due collisioni

$$\lambda = \frac{k_B T}{1.41\ \sigma^2\ P}$$

Sezione d'urto

probabilità che l'urto avvenga

$$\sigma^2 = \pi\,(R^2 - r^2)$$

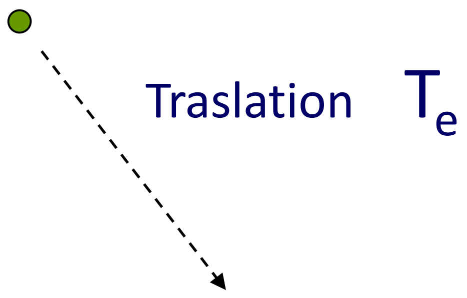


Vacuum range	Pressure (mbar)	Mean free path
Ambient pressure	1013	68 nm
Low vacuum	300 - 1	0.1 - 100 µm
Medium vacuum	1 - 10^{-3}	0.1 - 100 mm
High vacuum	10^{-3} - 10^{-7}	10 cm - 1 km
Ultra high vacuum	10^{-7} - 10^{-12}	1 km - 10^5 km
Extremely high vacuum	<10^{-12}	>10^5 km

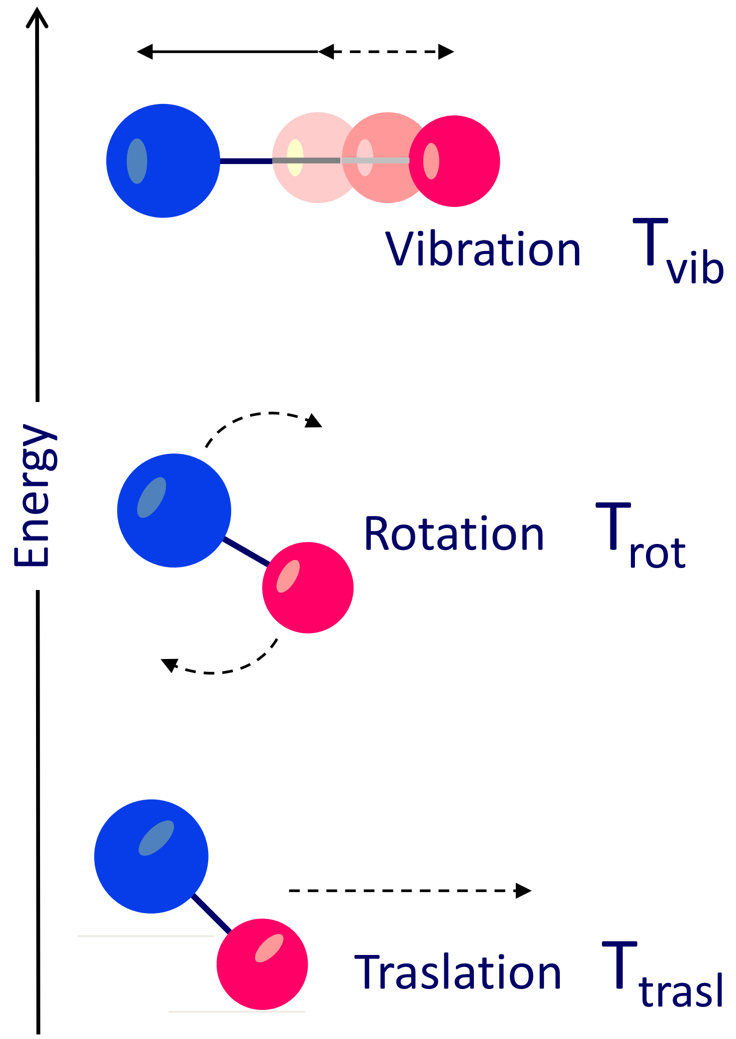
Il cammino libero medio diminuisce all'aumentare della pressione (più collisioni)

Il cammino libero aumenta con T perché il gas diventa più rarefatto (il volume occupato aumenta → meno collisioni)

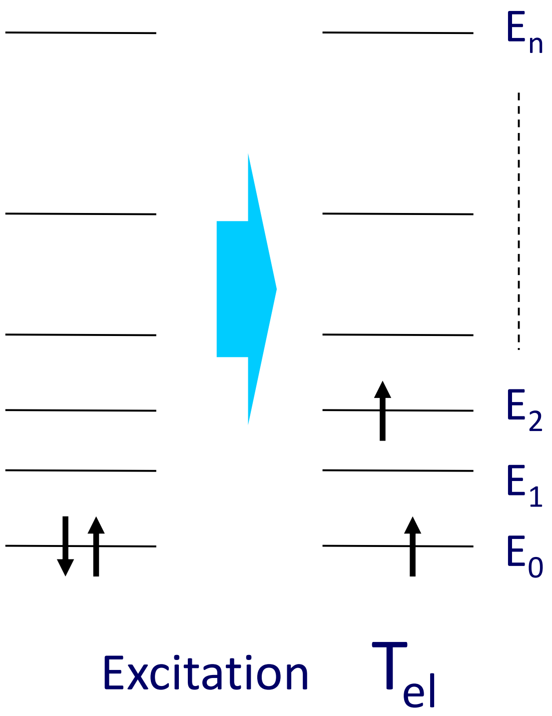
free electron motions



molecular motions



electron levels in atoms/molecules



GAS PHASE: collisions

Elastic collisions

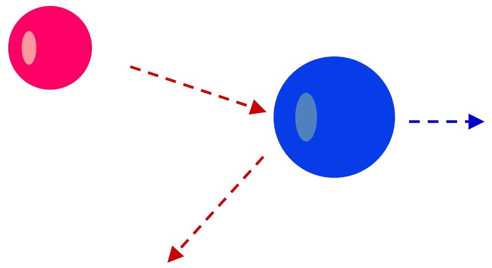
transfer of kinetic energy

$$F \propto \frac{m_1 \; m_2}{(m_1 + m_2)^2}$$

Efficient

high F value

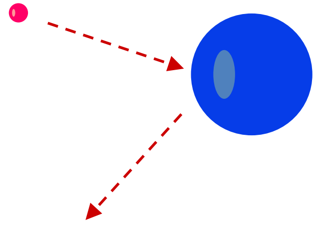
$m_1 \approx m_2$



Non efficient

low F value

$m_1 << m_2$

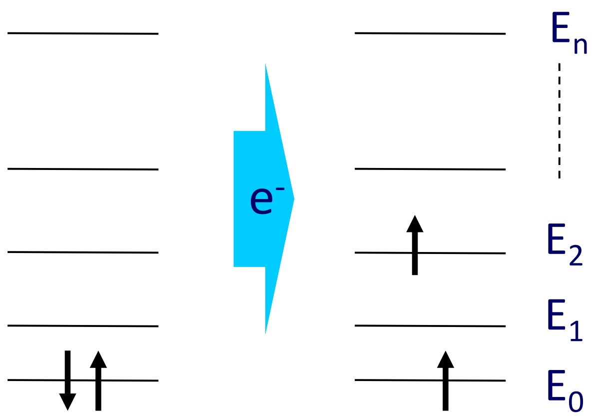


Anelastic collisions

kinetic energy not conserved

transfer of internal energy

(excitations, ionizations, bond cleavage)



generazione di un plasma

SI APPLICA UN CAMPO ELETTRICO AD UN GAS

⬇

ELETTRONI E PARTICELLE CARICHE* VENGONO ACCELERATE DAL CAMPO ELETTRICO

⬇

COLLISIONI ELASTICHE E ANELASTICHE,

SI FORMANO PIU' ELETTRONI E IONI,

IL GRADO DI IONIZZAZIONE AUMENTA,

LE MOLECOLE VENGONO FRAMMENTATE,

AVVENGONO REAZIONI OMOGENEE ED ETEROGENEE

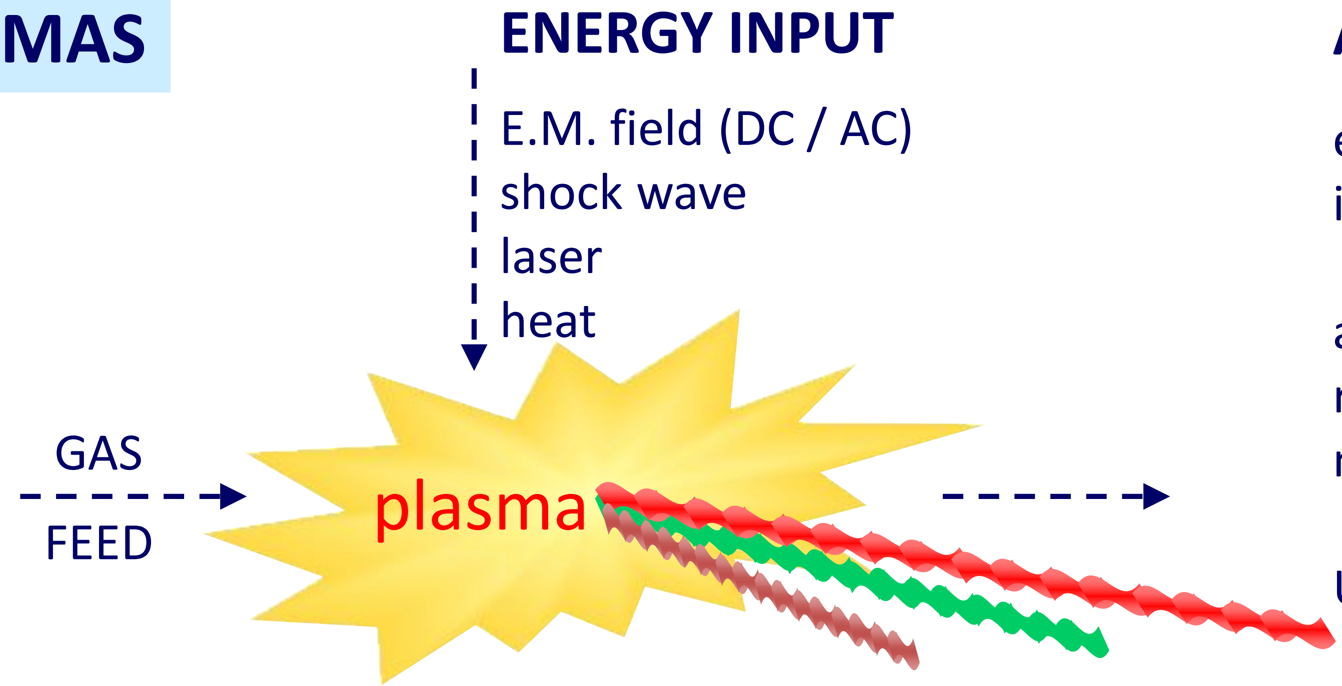
AVVENGONO ANCHE REAZIONI DI RICOMBINAZIONE

⬇

ALLO STATO STAZIONARIO IL PLASMA E' SOSTENUTO DAL BILANCIO TRA PRODUZIONE E PERDITA DI SPECIE CARICHE

In condizioni normali solo una minima frazione di molecule/atomi del gas è ionizzata (UV, radiazioni, vento solare, sorgenti γ), per un grado di ionizzazione α $10^{-11} – 10^{-12}$. Il campo elettrico agisce inizialmente su questi pochi elettroni, e aumenta in breve il grado di ionizzazione fino a $10^{-3} – 10^{-7}$

COLD PLASMAS



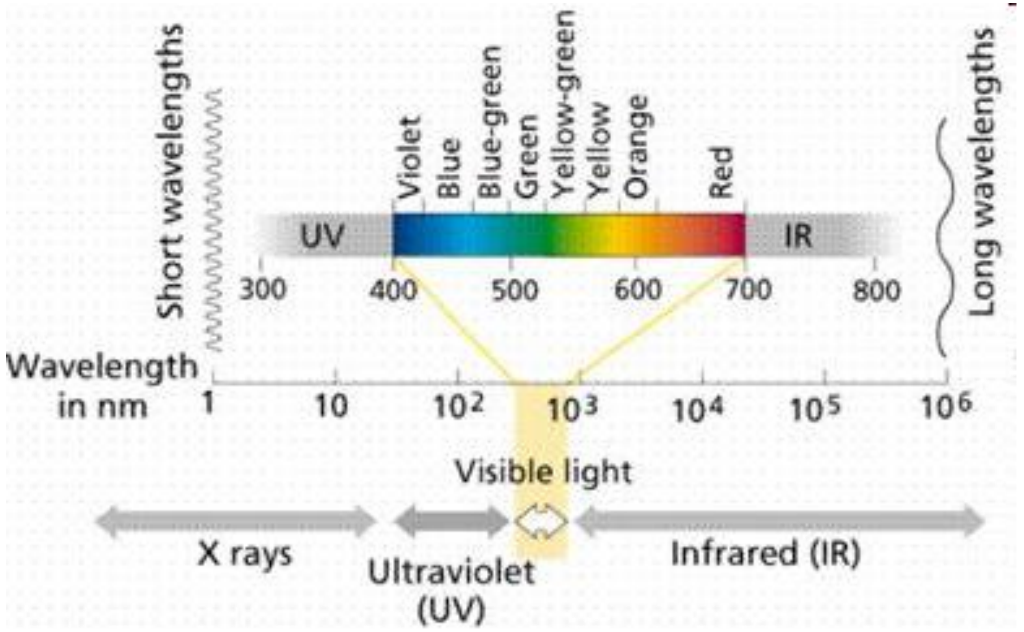
ACTIVE SPECIES

electrons (0 - 10 eV)
ions

atoms
radicals
molecules
in different states

UV – VIS radiation

Bond	Energy (eV)	gas	Ionization Energy (eV)
C-H	4.3	O_2	12.5
C-N	3.2	O	13.6
C-Cl	3.5	N_2	15.6
C-F	5.5	N	14.5
C-C	3.6	H_2	15.4
C=C	6.3	H	13.5
C=O	7.0	H_2O	12.6

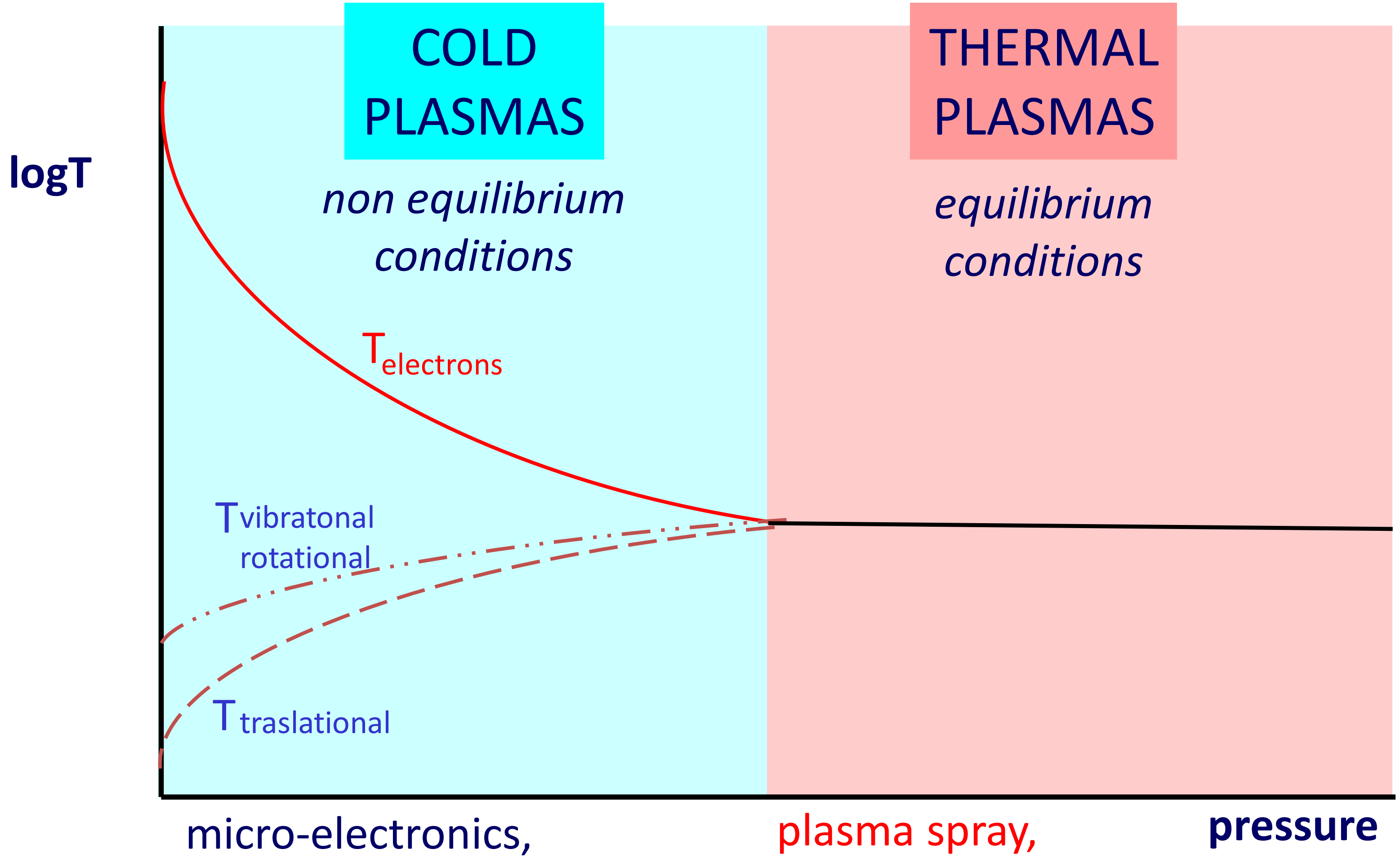


LOW PRESSURE

ATMOSPHERIC PRESSURE

COLD PLASMAS

THERMAL PLASMAS



micro-electronics, solar cells, packaging, polymers, biomaterials, textiles, paper …

plasma spray, plasma torches, waste distruction, ICP, welding …

non eq. conditions can exist also at atmospheric pressure*e.g.*, APGD, DBD plasmas

EQUILIBRIUM

HIGH PRESSURE (> 10 T
HOT, THERMAL PLA

flames, torches, a
sparks, stars, …

A sufficient number of e – neutral inelastic collisions occurs. In spite of their low efficacy, they can distribute the energy of the electrons among all species.

Electron energy lowers,
gas temperature increases.

$T_{trasl} \approx 5\text{-}10 \times 10^3$ K

$T_e \approx T_{el} \approx T_{vib} \approx T_{rot} \approx T_{trasl}$

Dielectric Barrier Discharges

Atmospheric Pressure
Glow Discharges

NON EQUILIBRIUM

V PRESSURE (< 10 Torr),
COLD PLASMAS

scharges, neon lamps,
iosphere, aurora, …

he number of e – neutral inelastic collision is too low to efficiently distribute the energy of the electrons among all species.

Electron energy remains high,
the gas remains at room T.

$T_e \approx 10^5$ K $T_{trasl} \approx$ room T

$T_e >> T_{el} > T_{vib} \approx T_{rot} > T_{trasl}$

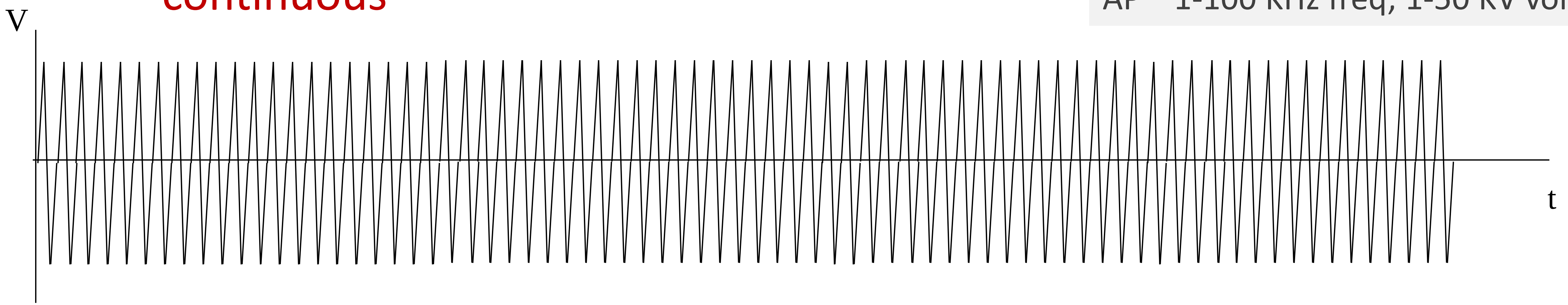
Most applications of non equilibrium plasmas requires that the gas remains at room T. Since the low efficiency and number of elastic collisions at low P limit the energy transfer from free electrons to heavier species, it is quite easy to produce cold Low P gas discharges. With increasing pressure, however, the electron-species collision frequency increases, the energy transfer becomes more efficient, resulting in gas heating and plasma instabilities (e.g., sparks and arcs).

Many approaches are used to keep the gas cold in Atmospheric P discharges, namely:

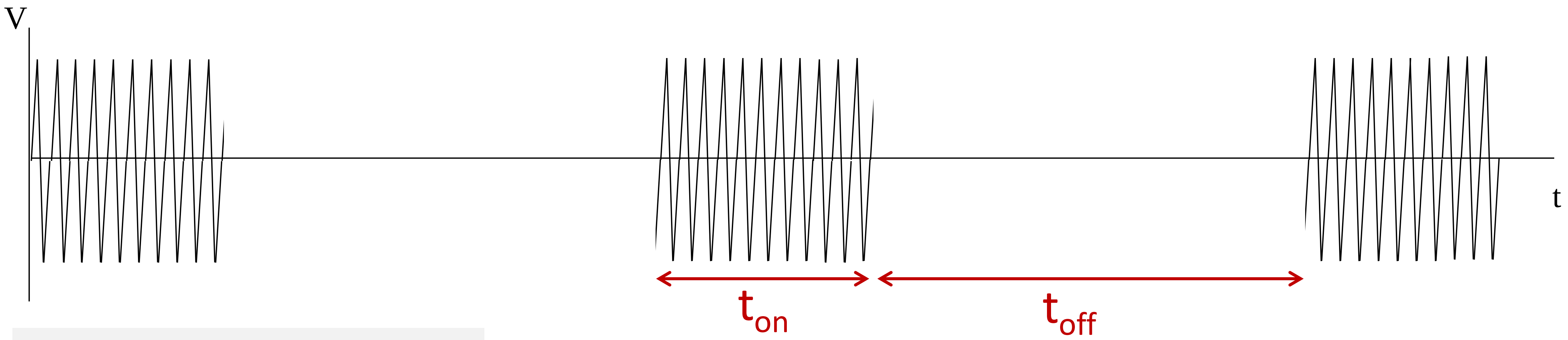
- **sharp electrodes, as in corona discharges;**
- **pulsing the plasma; μs-ns wide plasma pulses**
- **improved heat transfer;**
- **using gases (e.g., He) with high thermal conductivity;**
- **reduce the size of plasmas (e.g., micro-discharges);**
- **reduce the current with dielectric layers on the electrodes, as in Dielectric Barrier Discharges (DBD)**

AP 1-100 KHz freq; 1-50 KV volt.

continuous



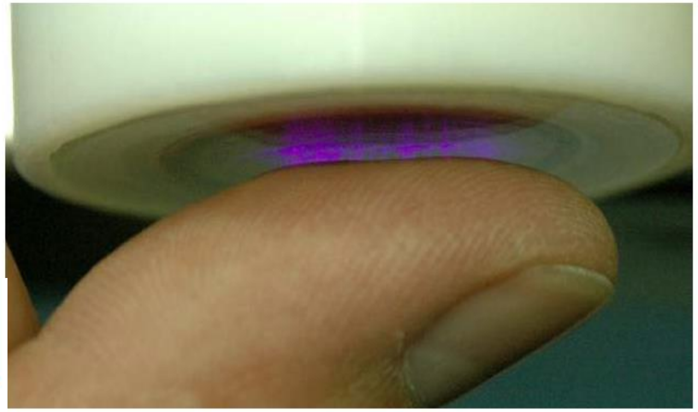
pulsed

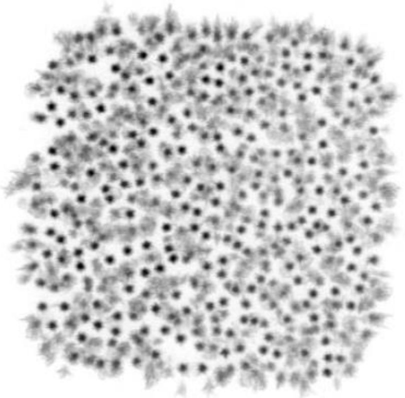


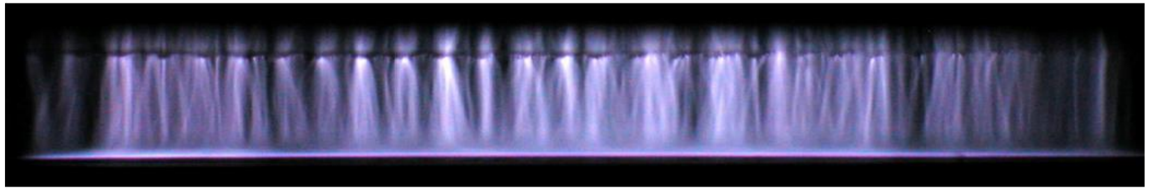
lower pulse width (μs, ns)
and higher V-rise rate
are desired to keep the gas cold

milli
micro
nanoseconds

Microdischarge Interaction and Structuring in Dielectric Barrier Discharges

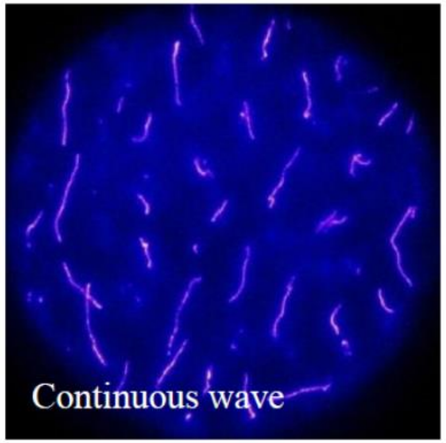


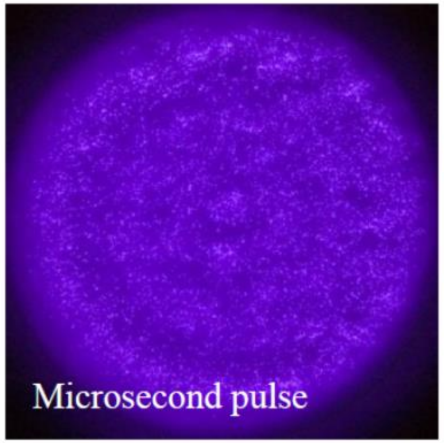


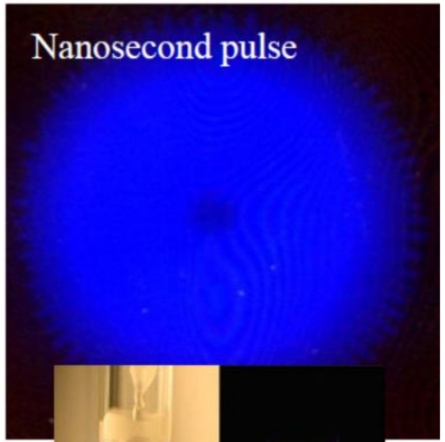


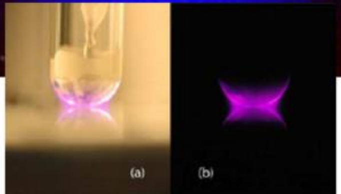
FE-DBD: Sinusoidal, Micro-pulsed, Nano-pulsed

Continuous (sinusoidal)	Microsecond-pulsed	Nanosecond-pulsed
Rise time: ~1 V / nsec	Rise time: ~5 V / nsec	Rise time: ~3,000 V / nsec
Sinusoidal wave	Pulse duration: ~2 µsec	Pulse duration: ~40 nsec
Filament temperature: 350-450K	Filament temperature: 320-420K	Rotational temperature: ~300K







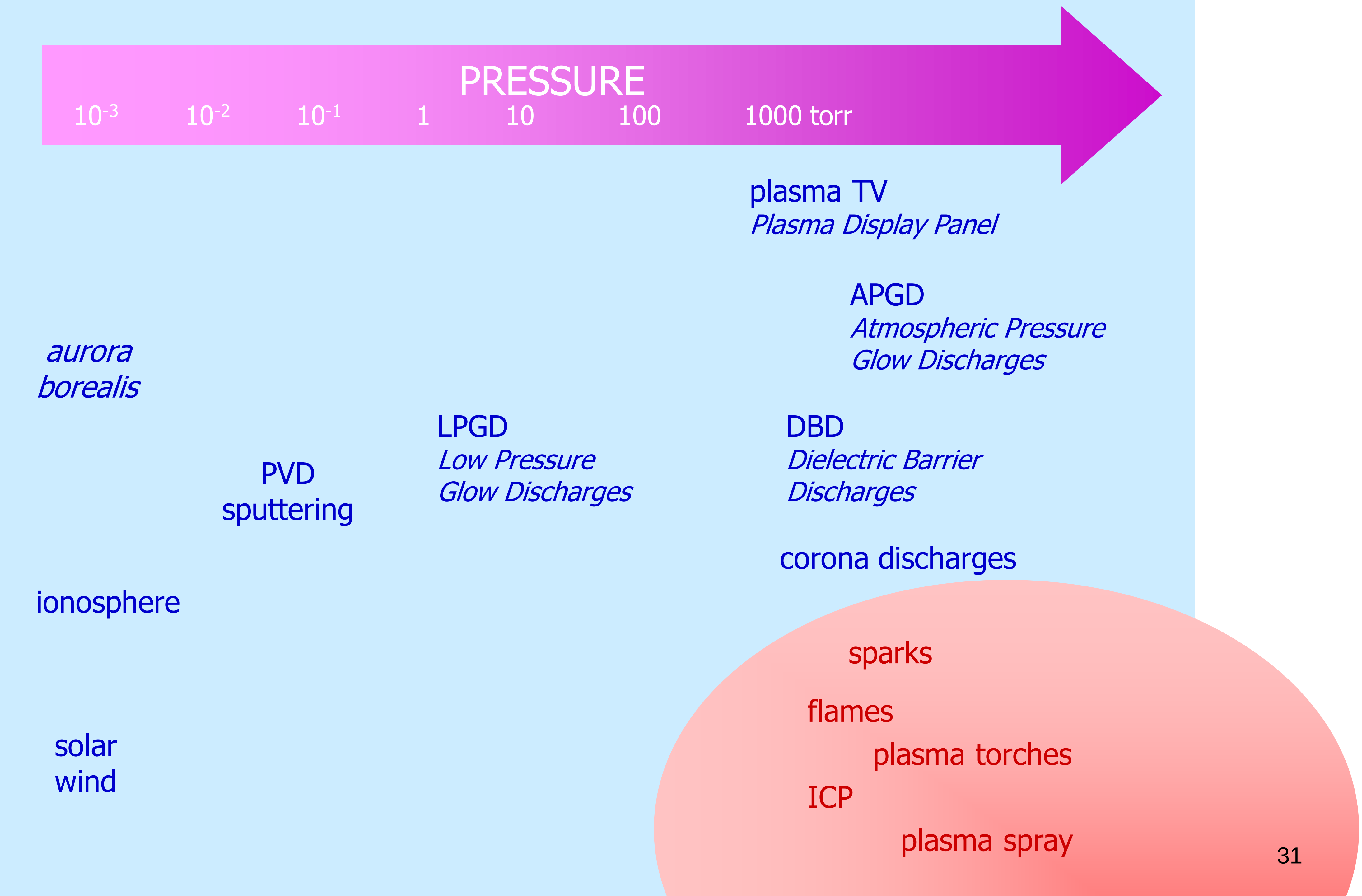


parametri plasma freddo

pressure range	P	0.01 – 10 Torr
Debye lenght	λ_D	10^{-2} – 1 mm
plasma density	n_e	$10^8 – 10^{12}$ cm^{-3}
density of neutrals	N_X	$10^{13} – 10^{17}$ cm^{-3}
ionization degree	n_e/N_X	$10^{-5} – 10^{-7}$
electron temperature	T_e	1 – 10 eV
ion temperature *(plasma bulk)*	T_I	10^{-2} – 2 eV
ion temperature *(plasma edge)*	T_I	1 – 10^3 eV
rot/vib temperature	$T_{rot/vib}$	10^{-2} – 1 eV
gas temperature	T_{trasl}	room T

1 eV ≈ 11,600 K

1 mole ≈ 6 x 10^{23} particles


PRESSURE
10^{-3}
10^{-2}
10^{-1}
1
10
100
1000 torr
plasma TV
Plasma Display Panel
APGD
Atmospheric Pressure Glow Discharges
aurora borealis
LPGD
Low Pressure Glow Discharges
DBD
Dielectric Barrier Discharges
PVD sputtering
corona discharges
ionosphere
sparks
flames
plasma torches
ICP
plasma spray
solar wind

THE 4th STATE OF THE MATTER:
birth of a concept

V sec B.C. Empedocles defines EARTH, AIR, WATER, FIRE as the 4 elements

XVII century First observations of lightnings

XIX century German scientists find that electric discharges in hydrocarbon gases originate oily droplets

1857 Siemens develops the first Ozone generator, mainly used for water purification

1879 W. Crookes defines the state of a ionized gas as "… a world where matter may exist in a 4th state …".

1910 G. Claude exhibits neon lights in public at the Paris Motor Show

1928 I. Langmuir uses the word plasma to define a neutral ionized gas made of electrons, ions atoms and molecules as the "4th state of the matter".

late XIX, first half XX sec DC/AC low pressure gas discharges and flames are used to investigate the structure of atoms and molecules by means of Emission Spectroscopy

50’-60’s

- Plasma chemistry for producing chemicals
- First depositions of thin films
- Miller experiment

70’s

- First plasma etching processes
- Equilibrium/non equilibrium debate
- Deposition of α-Si:H

80’s

- Solar cells (α-Si:H) produced
- Microelectronics at large
- Other applications start

90’s

- Extreme miniaturization in microelectronics
- Polymers, textiles, packaging, biomaterials, paper, composites, MEMS,… sterilization …

2000

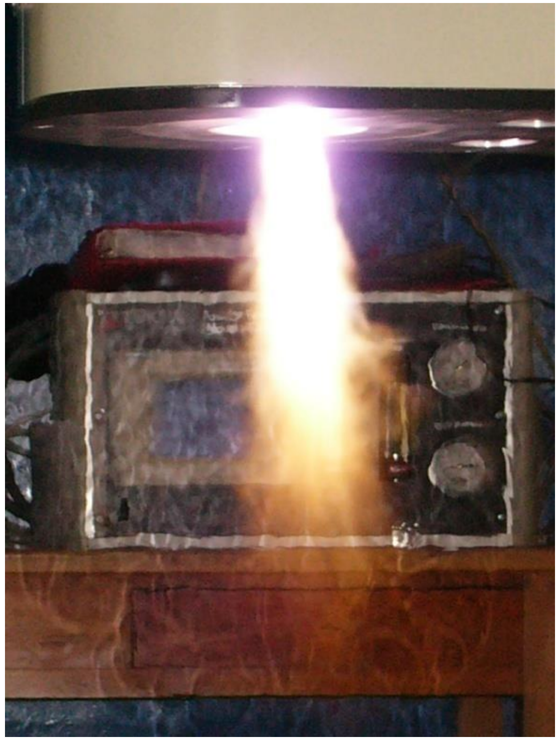
- Micro- , nano- surface plasma-engineering in different fields
- Large area easy processing
- Plasmas very common also in low-tech fields

THE 4th STATE
OF THE MATTER:
developments and maturity

Il quarto stato *Giuseppe Pellizza da Volpedo*

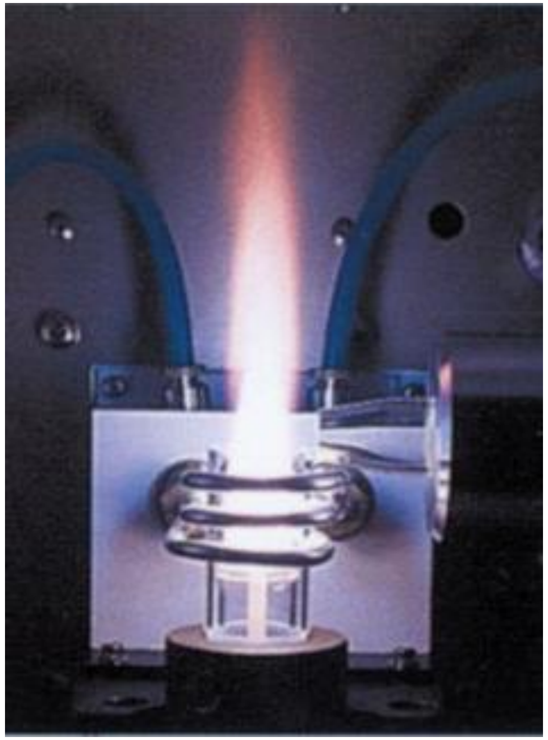
2010 →

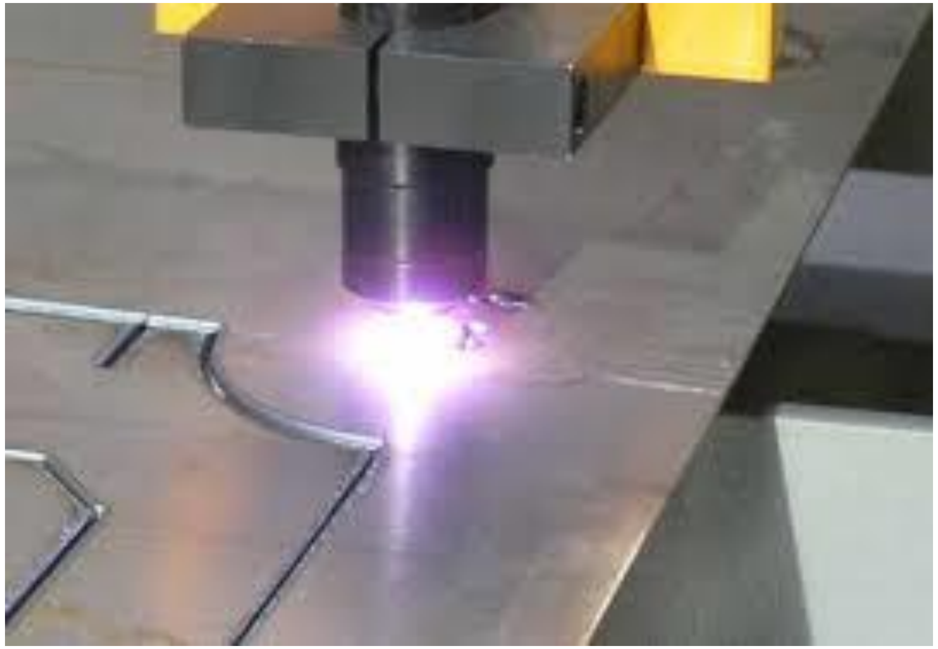
- Plasma Medicine (Agriculture, Food, …)



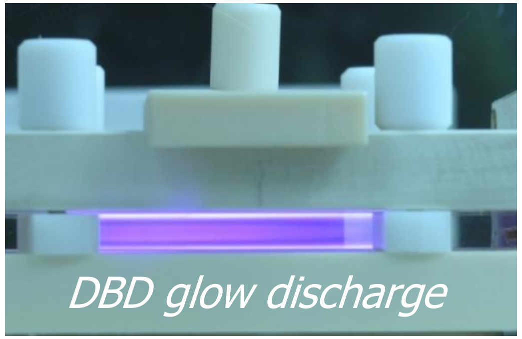
THERMAL PLASMAS

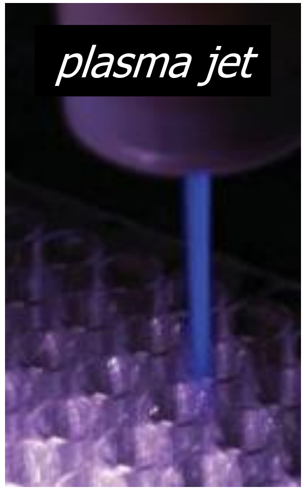
welding, cutting,
metallurgy,
plasma spray deposition,
ICP spectroscopy,
waste abatment



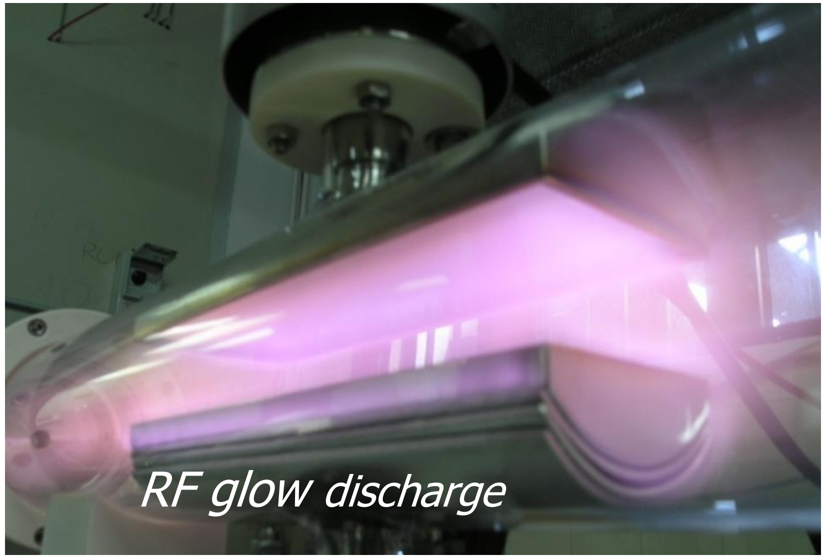


COLD PLASMAS


DBD glow discharge


plasma jet

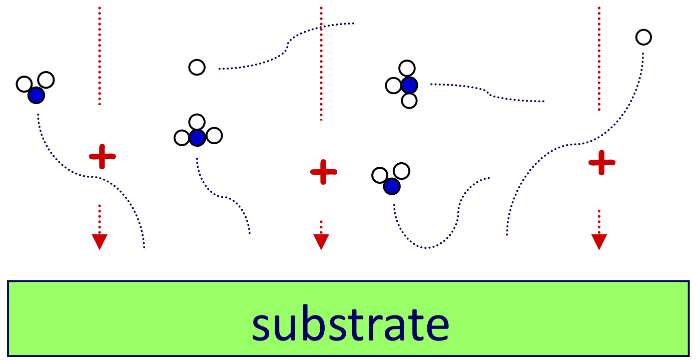

aurora borealis


RF glow discharge

plasma TV

PLASMA and MATERIALS

plasma – surface interactions

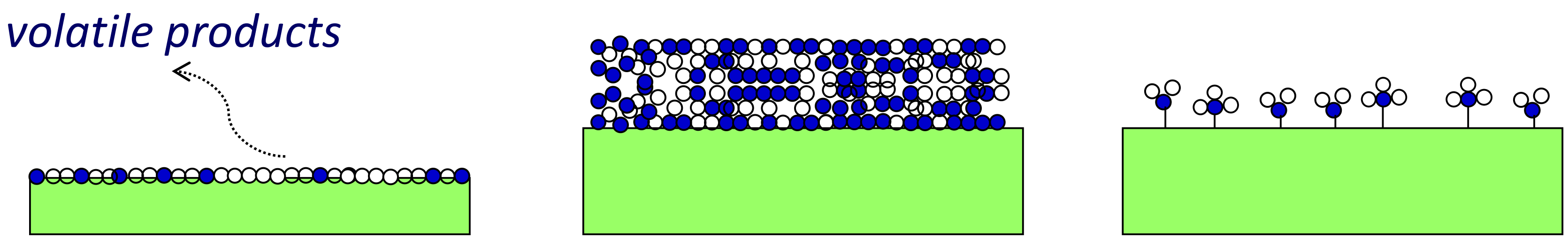


*synergistic action of active species and ion bombardment at **low P***

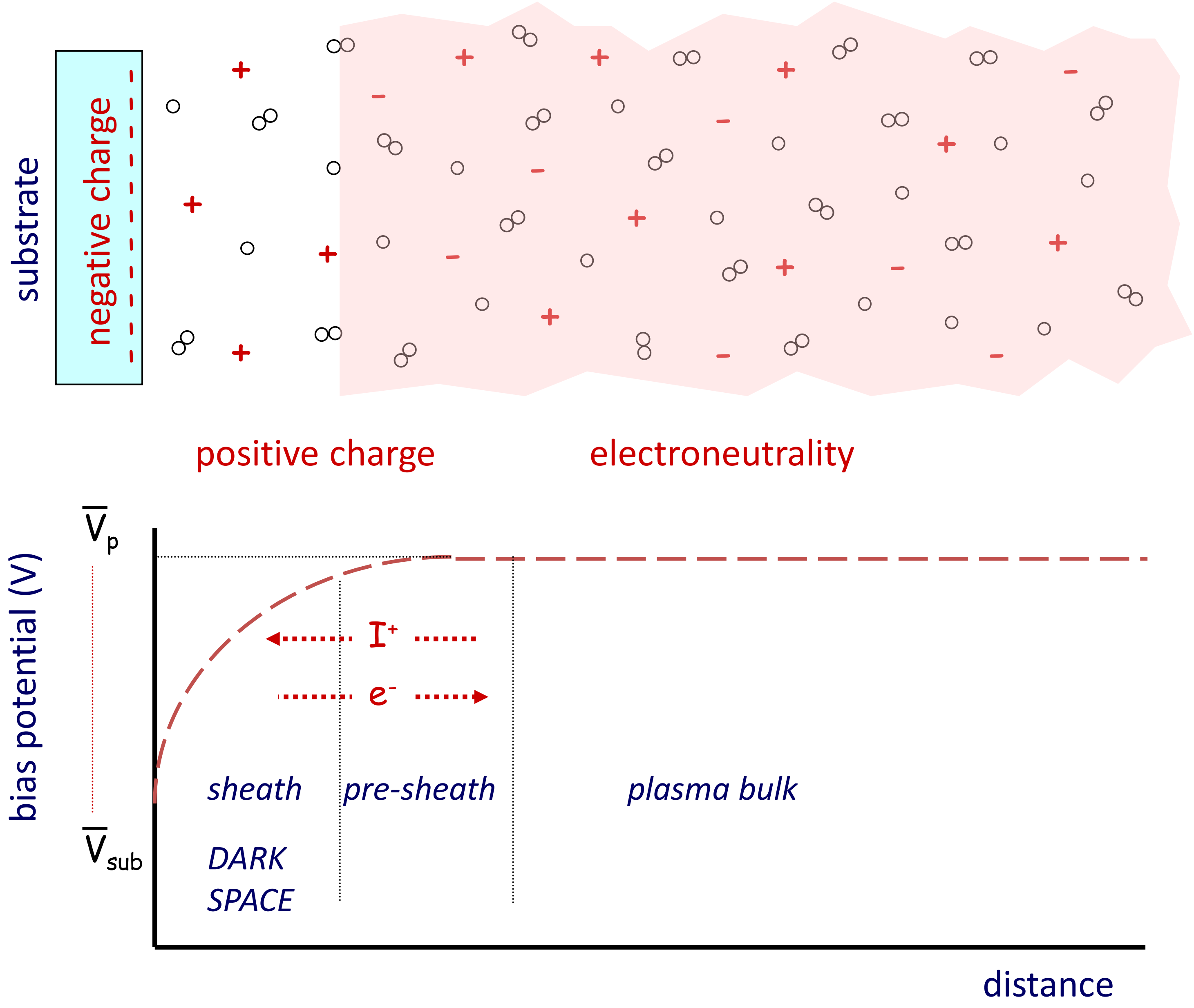
high density | low ionization degree

active species **+** ion bombardment

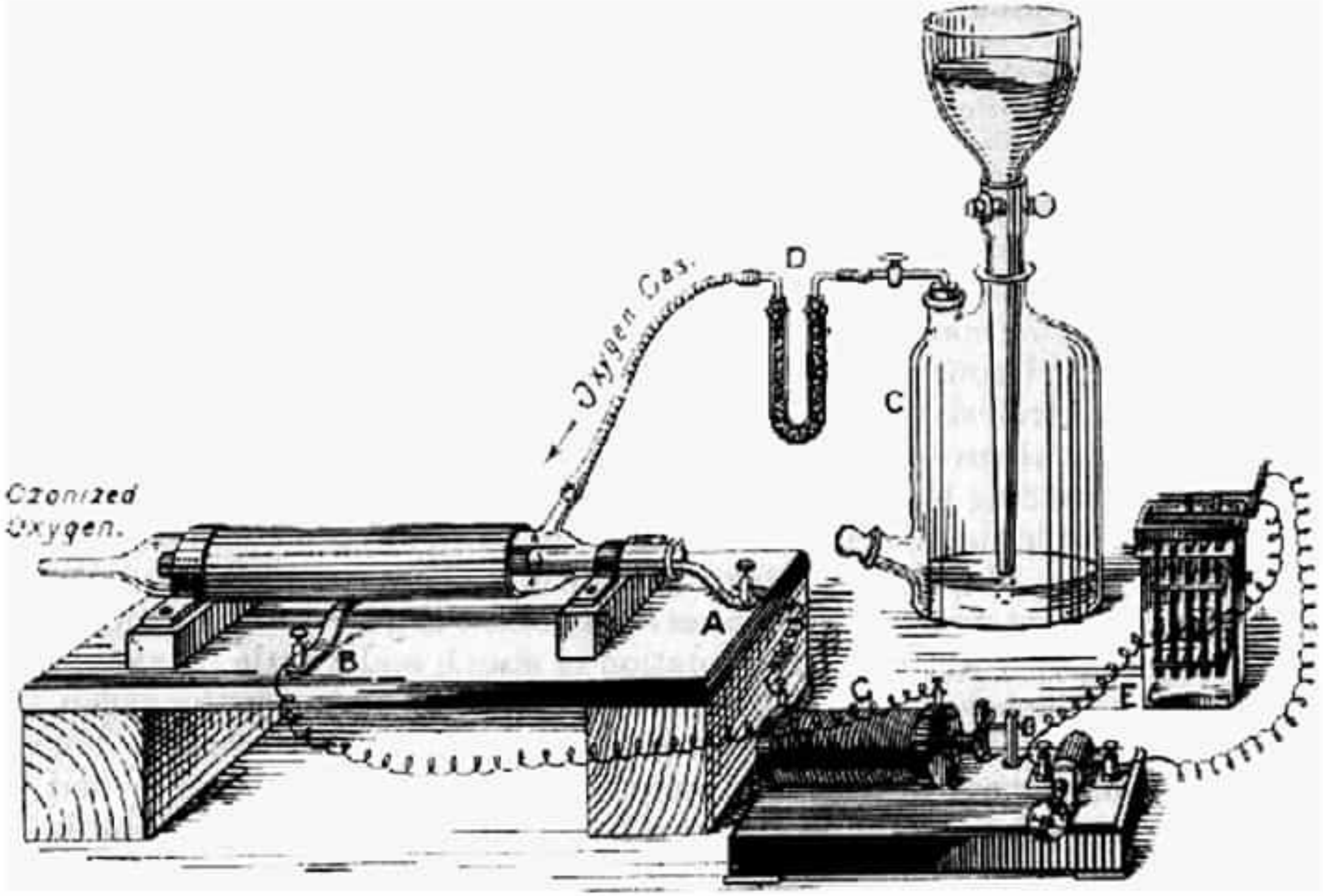
etching | *deposition* | *treatment*

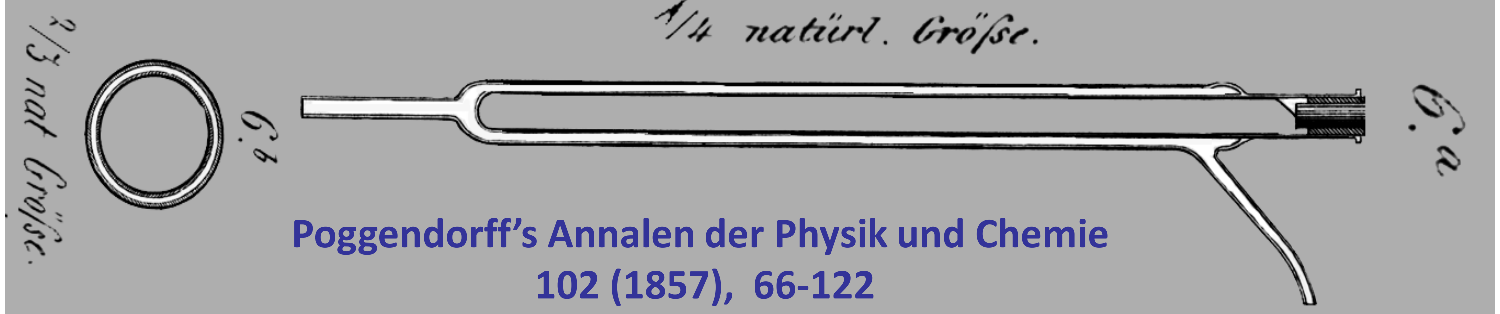


bias potential at Low P



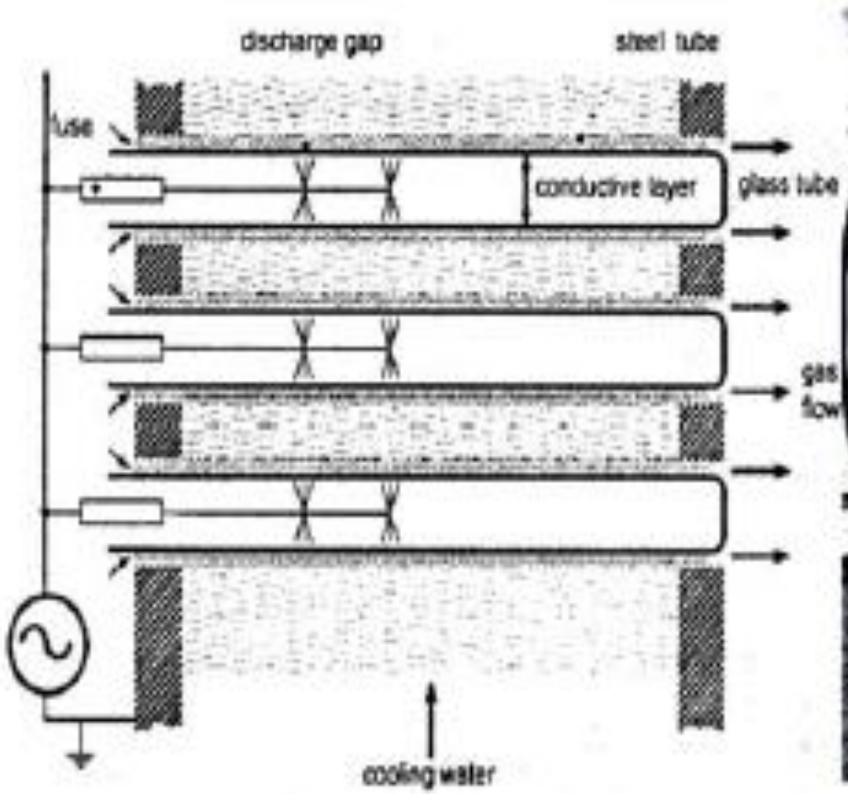
Historical Ozone Tube
W. Siemens 1857

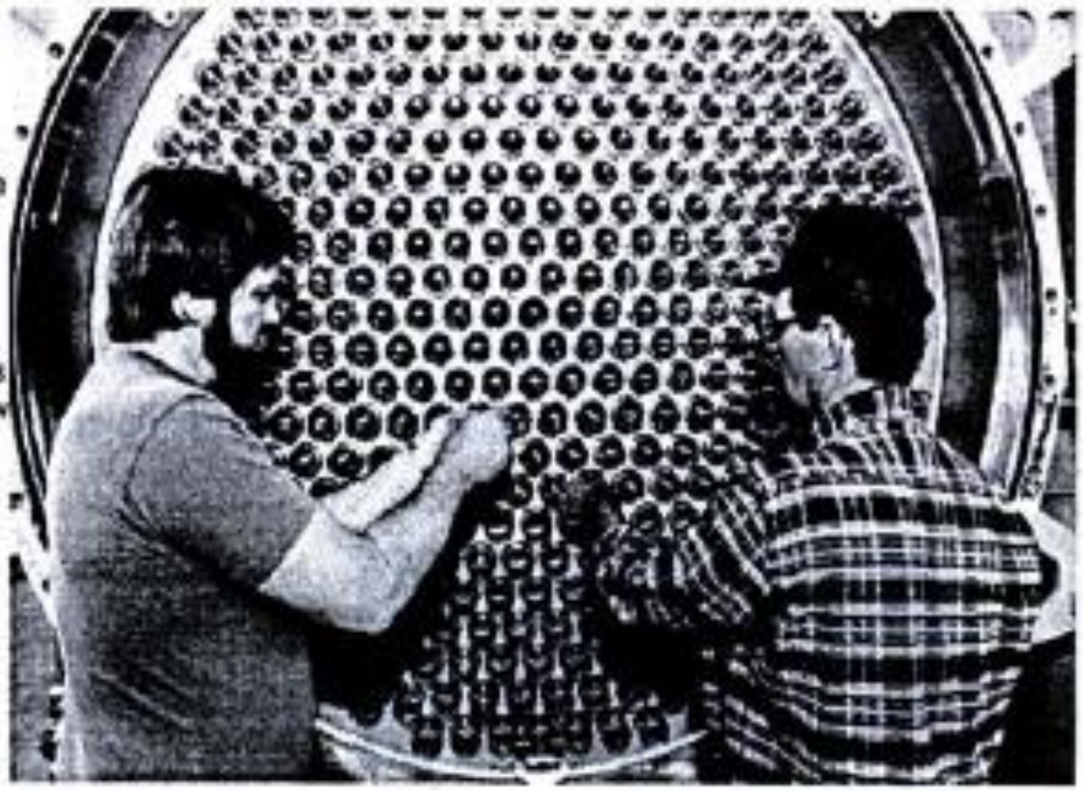




Poggendorff's Annalen der Physik und Chemie
102 (1857), 66-122

applicazioni delle Dielectric Barrier Discharge: O_3





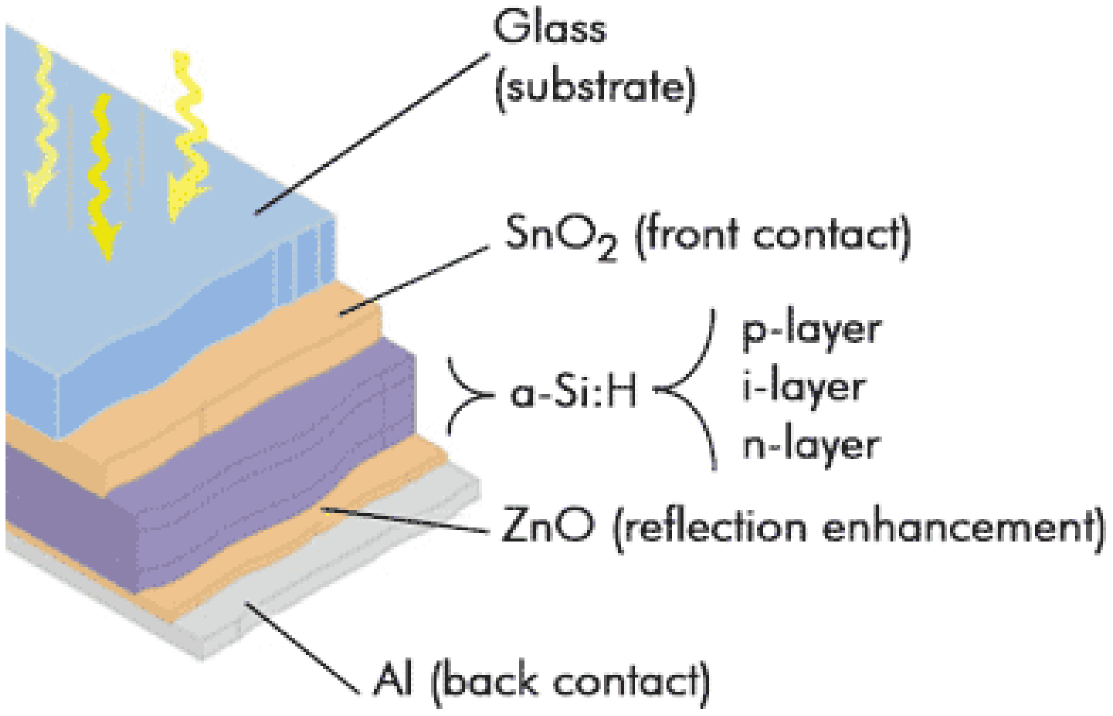
generatore di O_3
Los Angeles
Aqueduct Filtration Plant

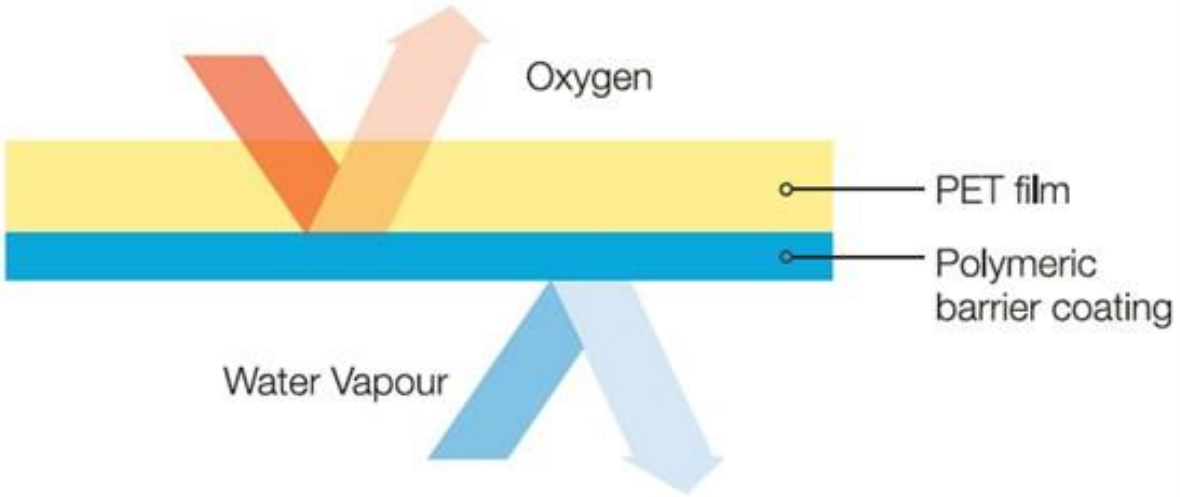
$$O_2 + O + M \rightarrow O_3^* + M \rightarrow O_3 + M$$

i generatori sono in grado di produrre centinaia di Kg/h di ozono

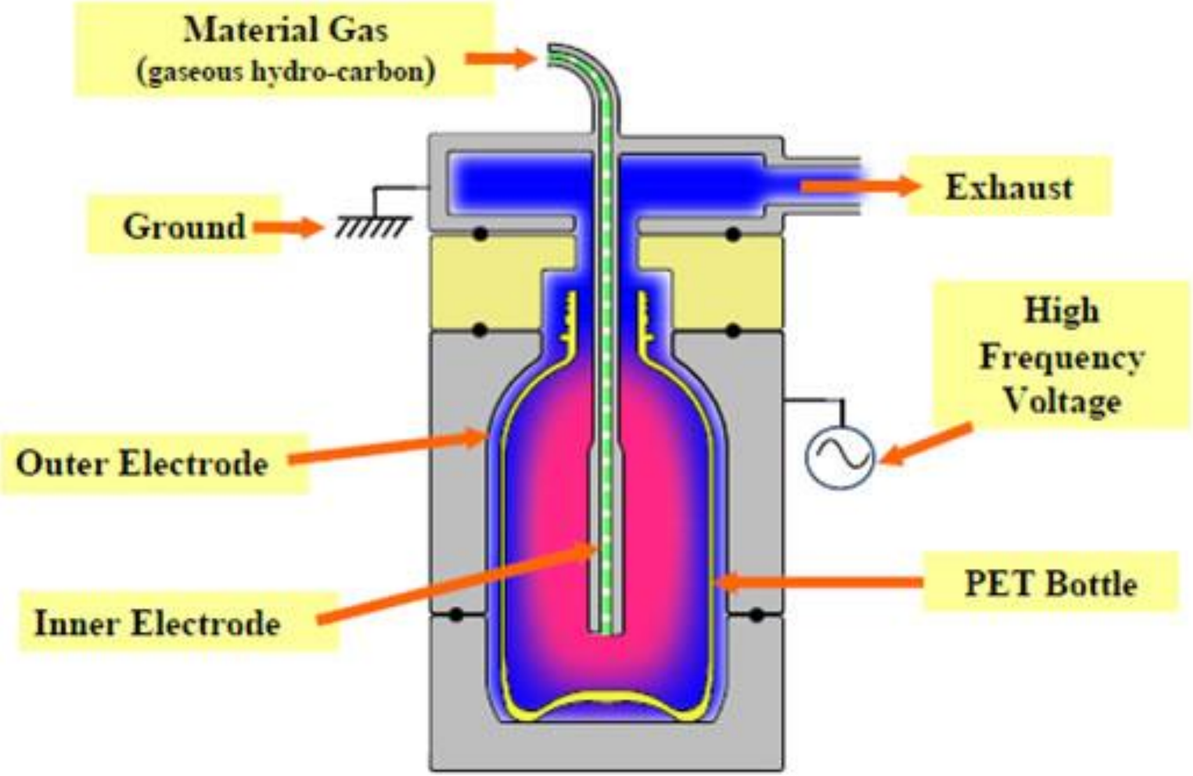
per trattamento delle acque e sbiancamento della polpa di legno

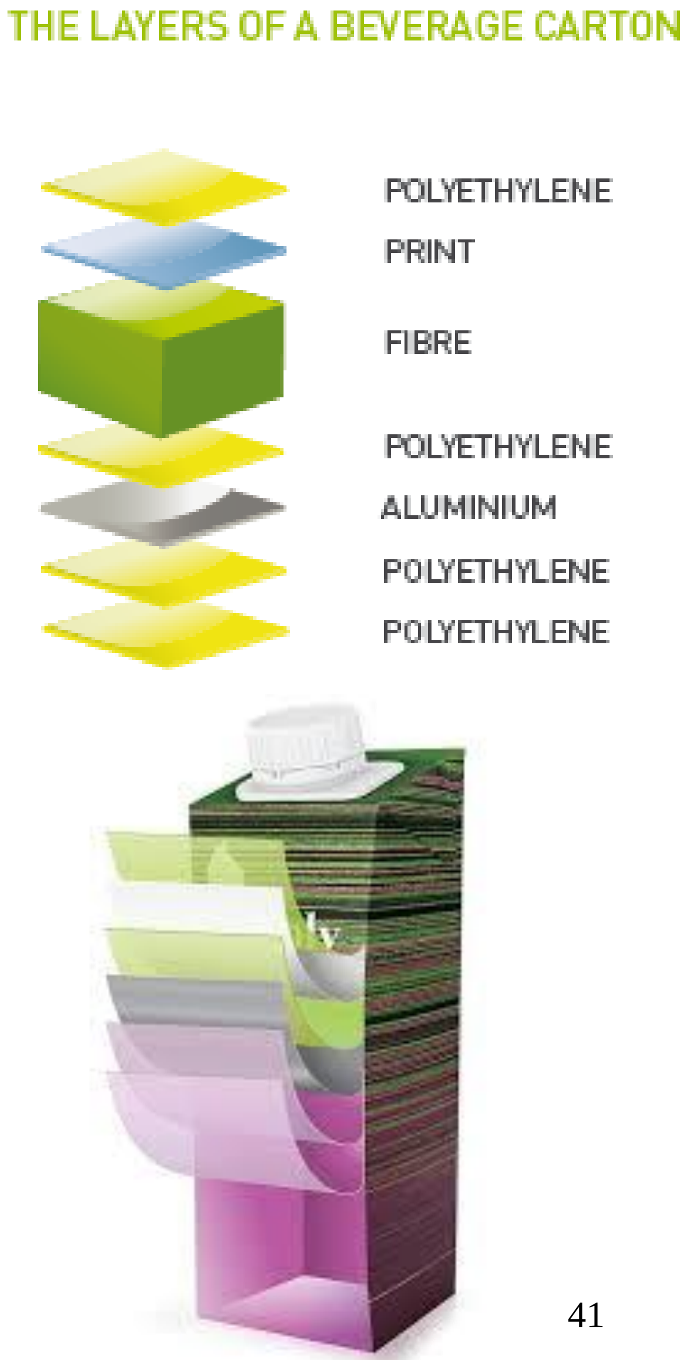
PLASMA DEPOSITED ACTIVE LAYERS IN SOLAR CELLS

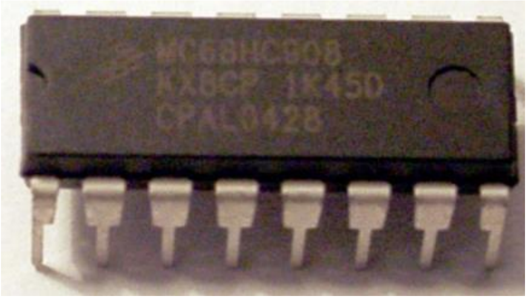


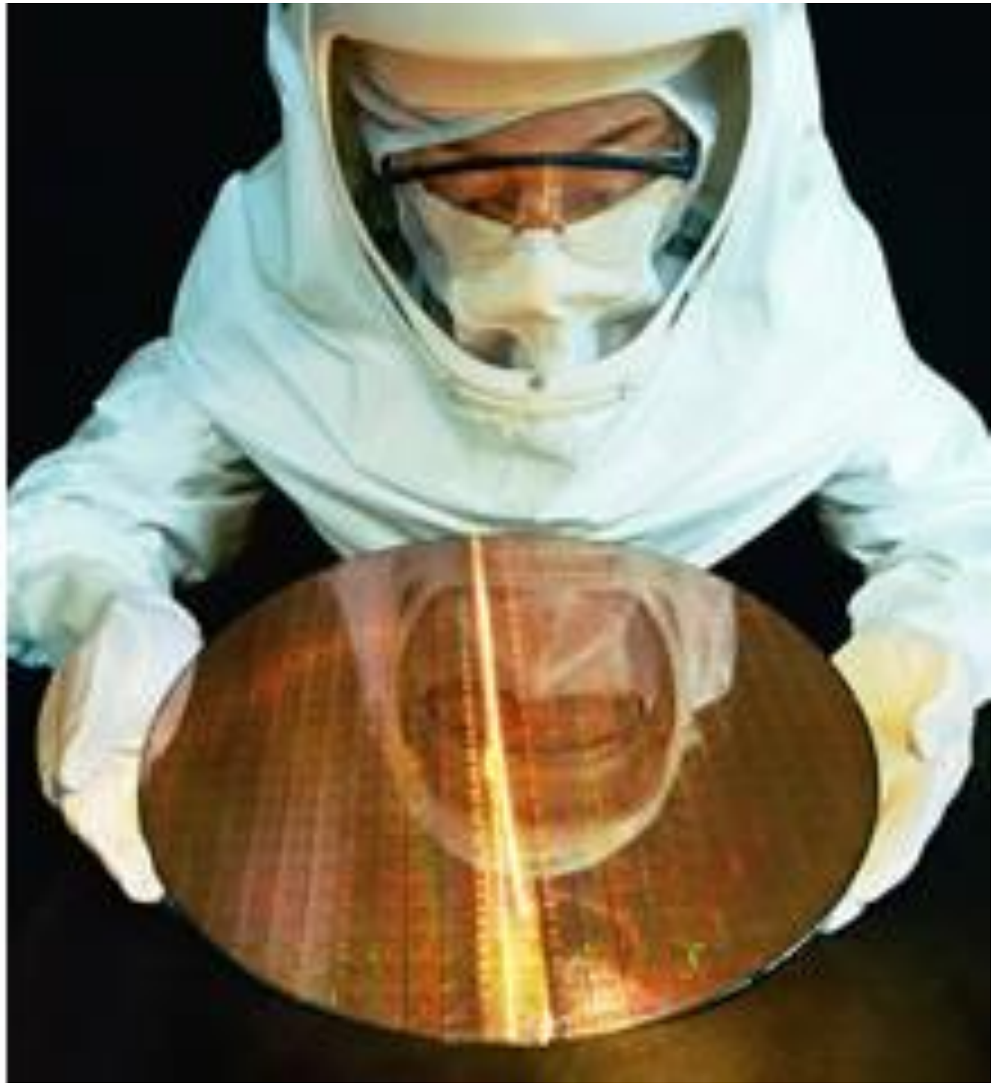


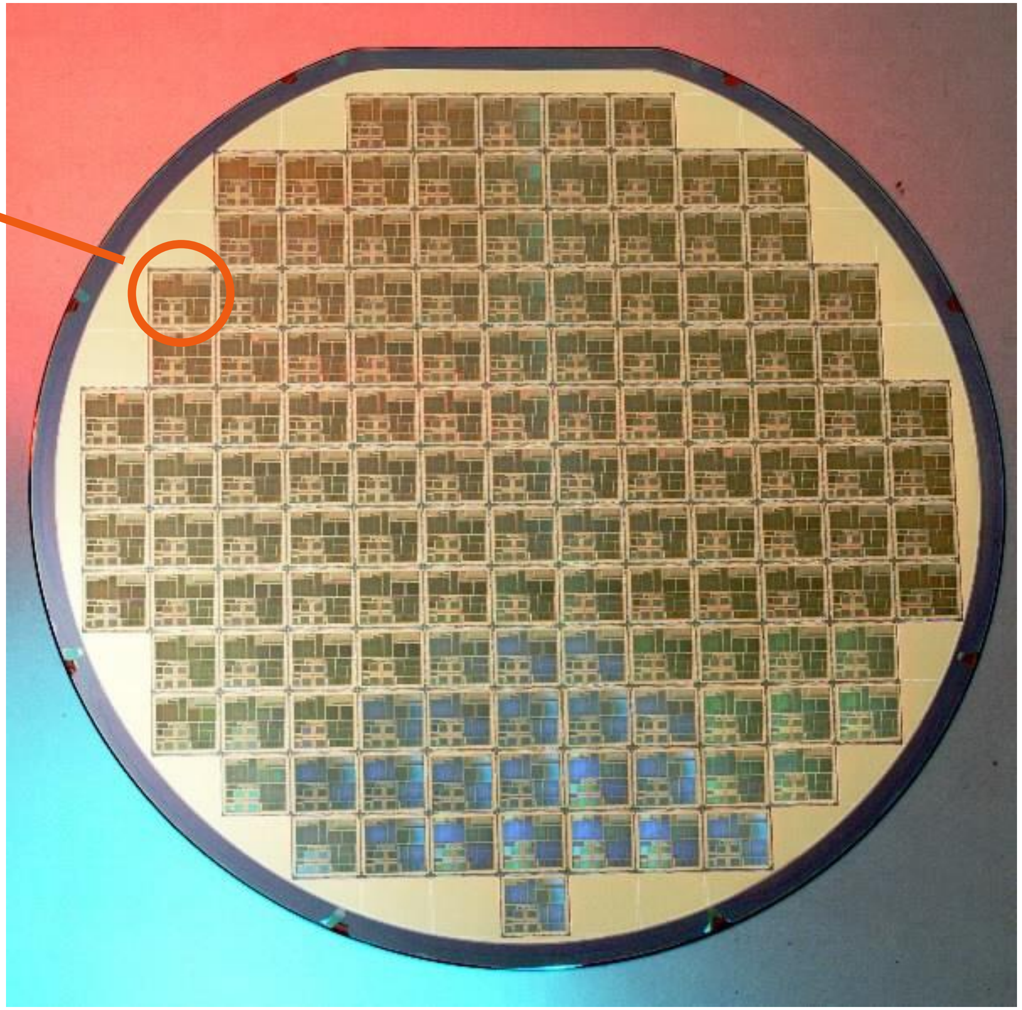
gas barrier coatings





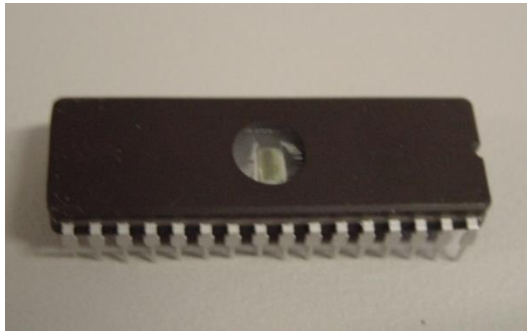


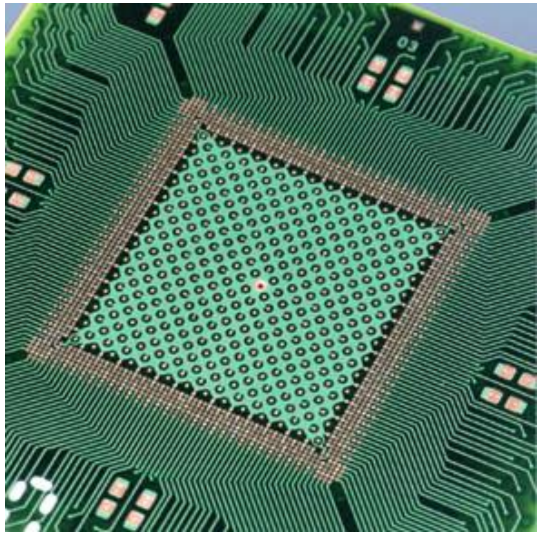


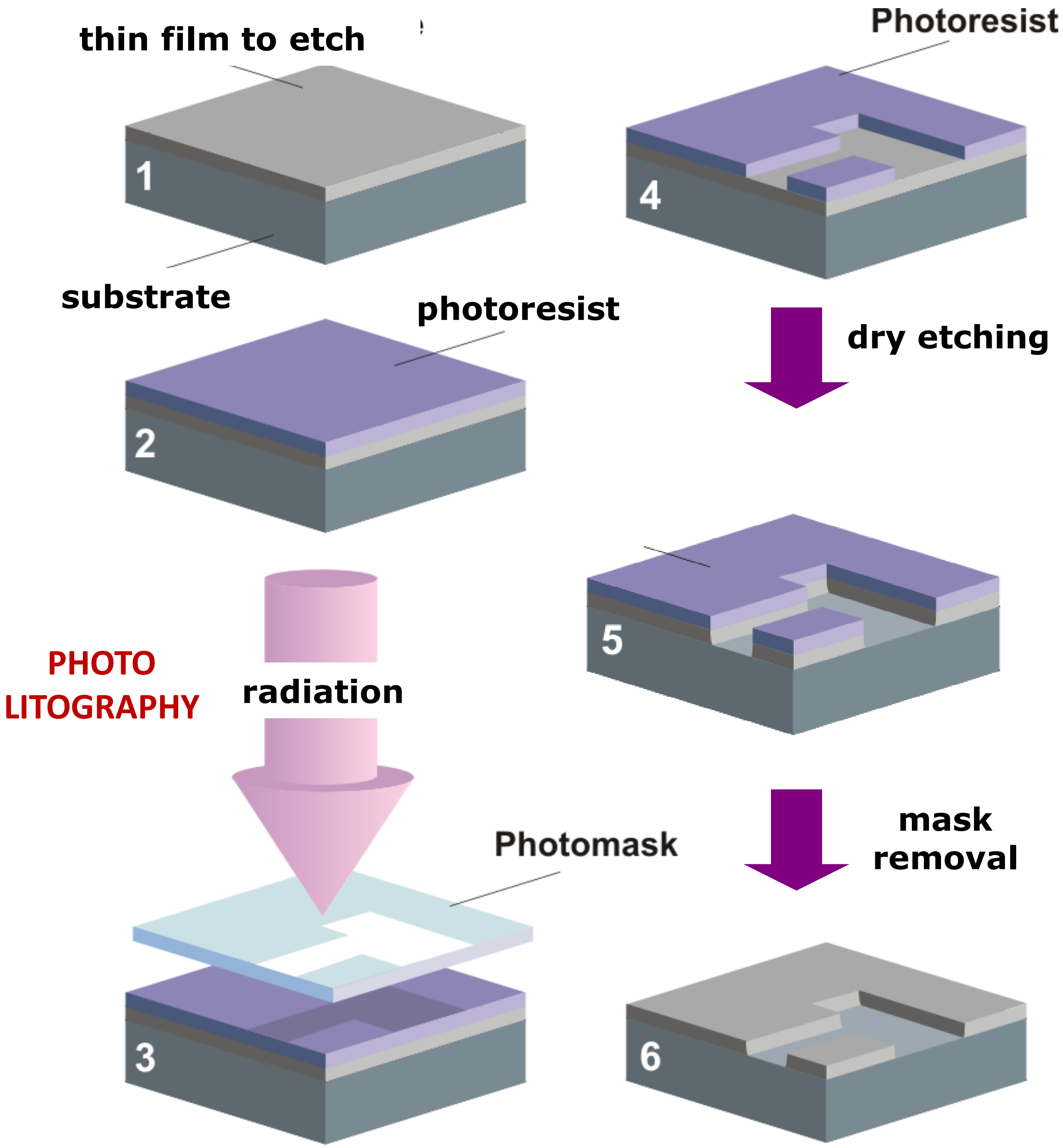


CIRCUITI INTEGRATI
IN MICRO ELETTRONICA

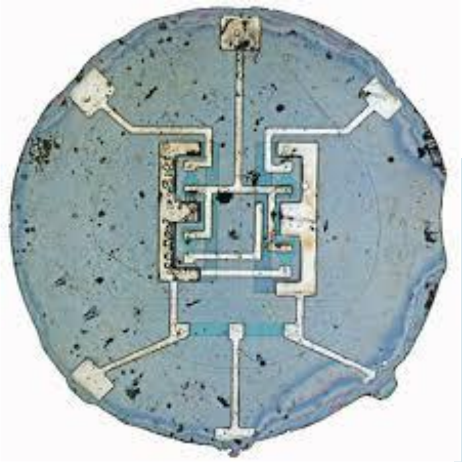
PLASMA (DRY) ETCHING

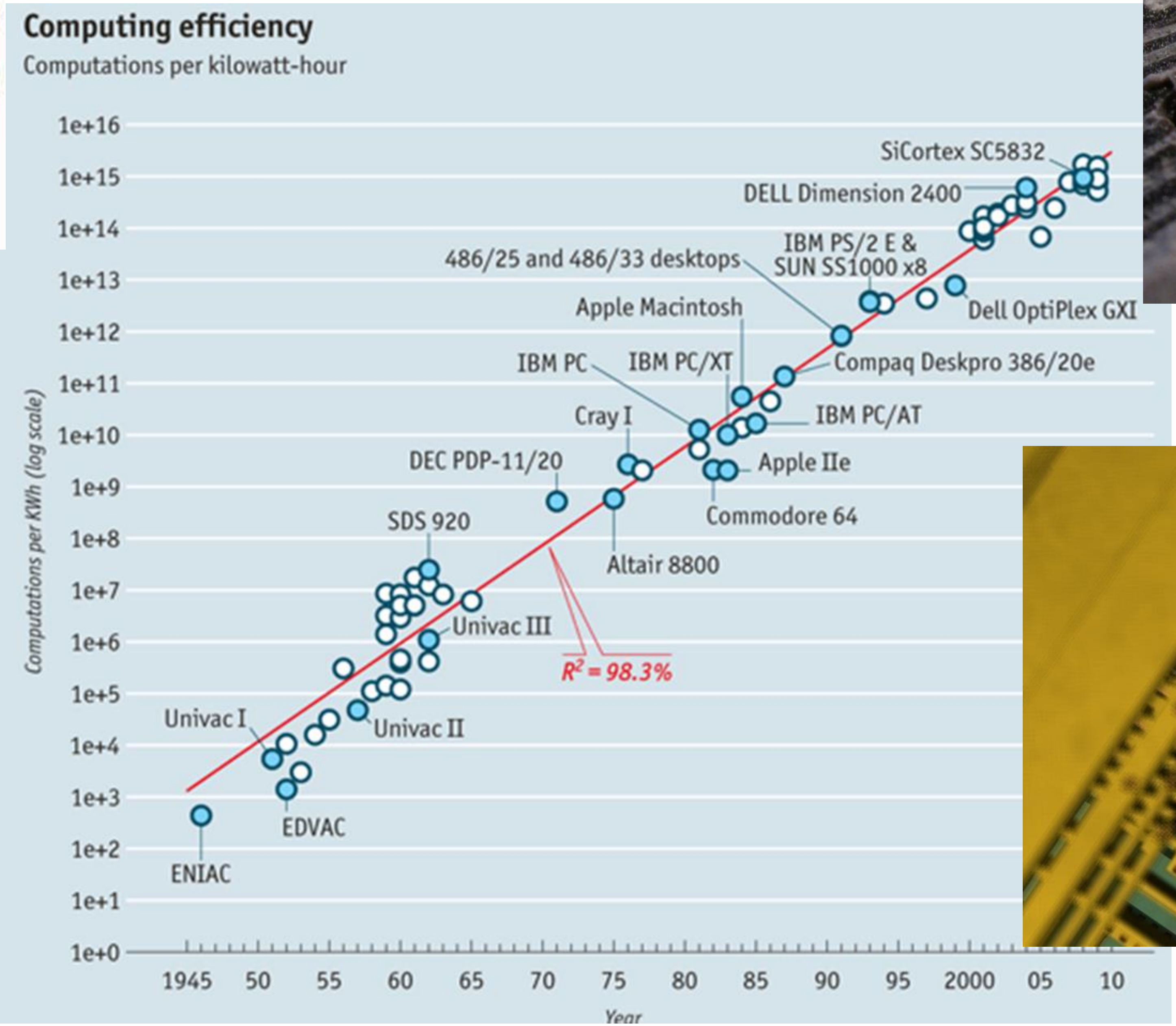


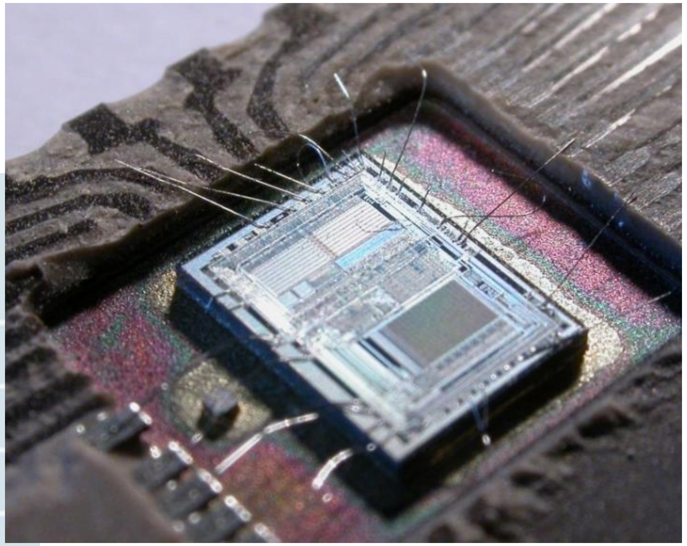


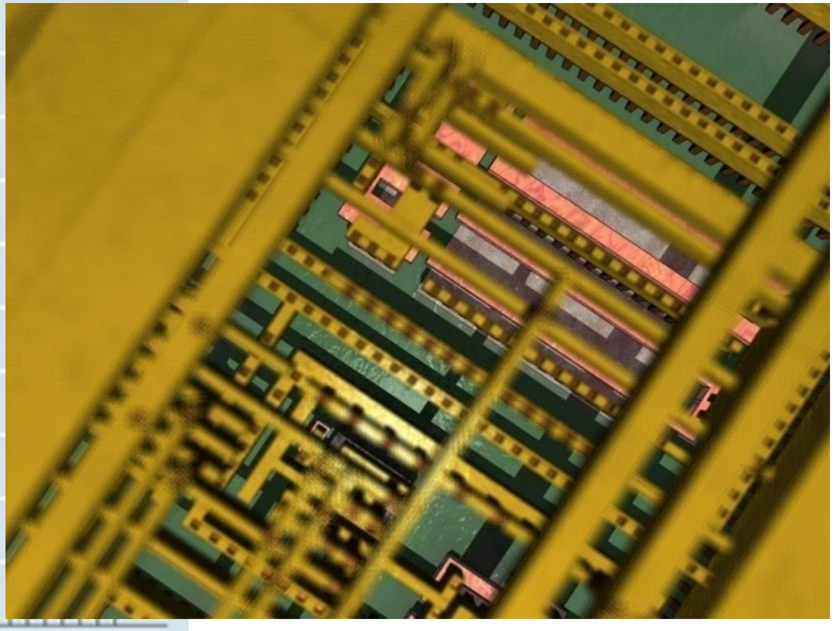


Integrated Circuits

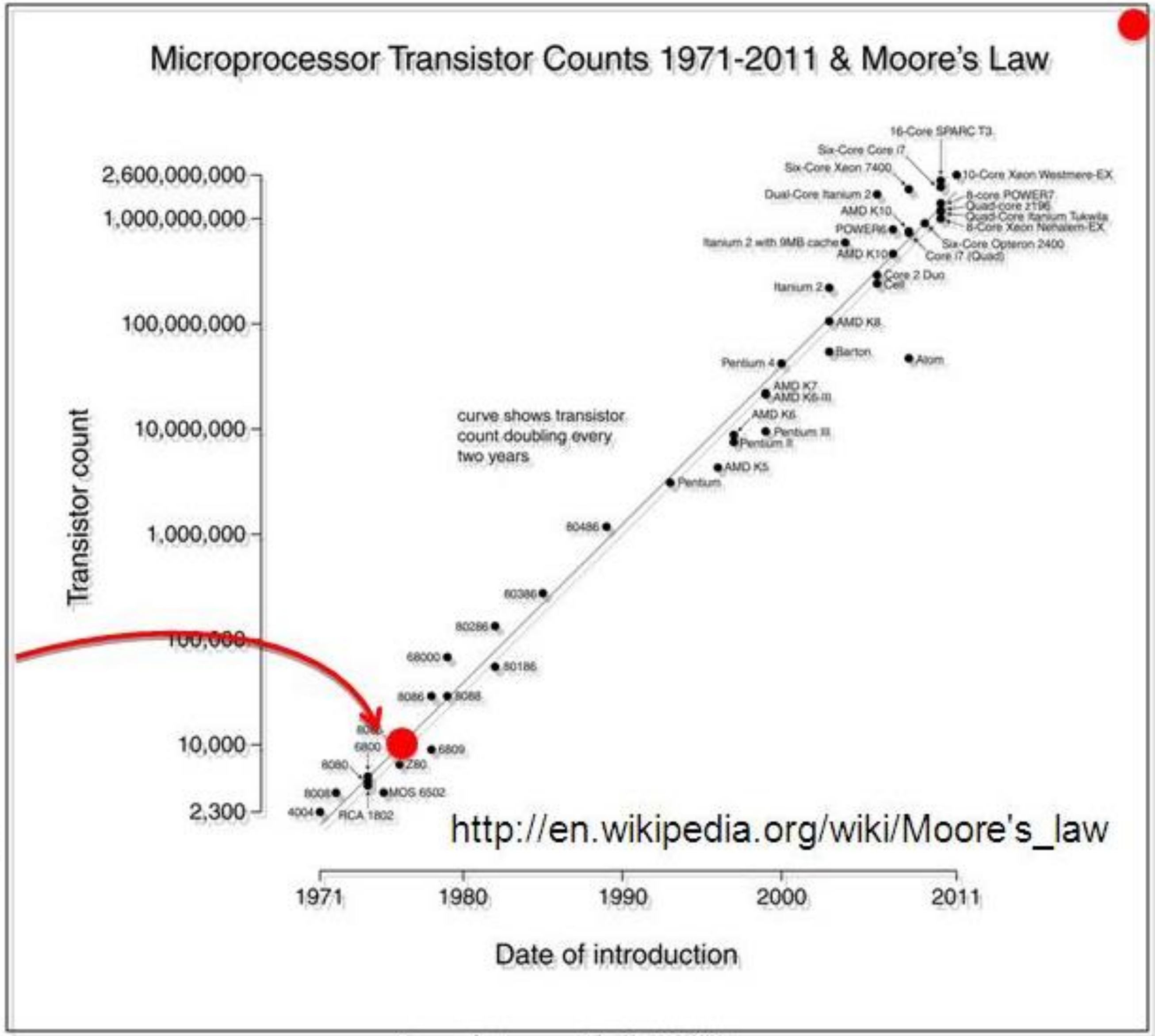








Moore's Law



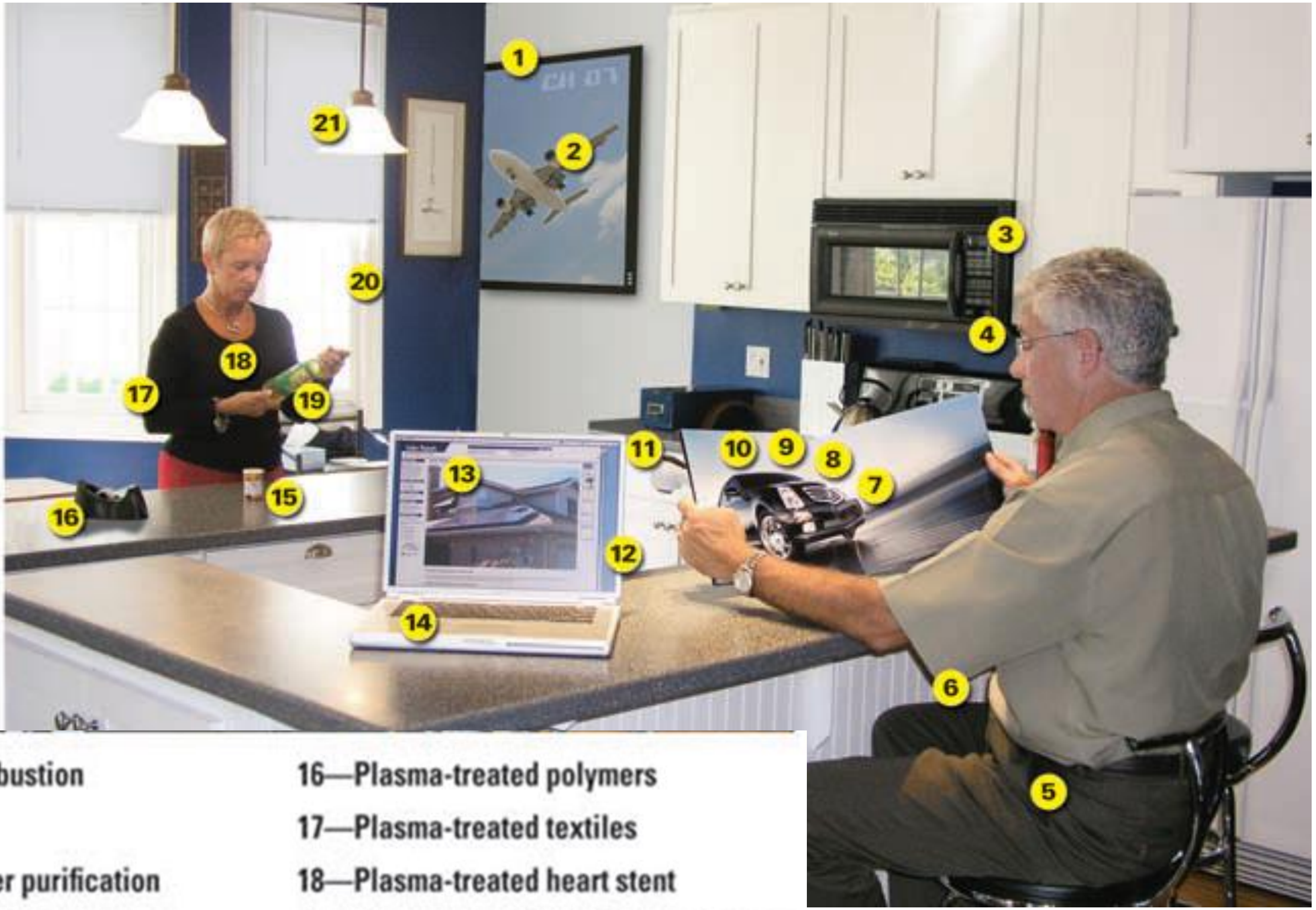
$L = 5000$ nm

Micro-electronics

$L = 5$ nm

Nano-electronics

1993 vs 2013



01—Plasma TV
02—Plasma-coated jet turbine blades
03—Plasma-manufactured LEDs in panel
04—Diamondlike plasma CVD eyeglass coating
05—Plasma ion-implanted artificial hip
06—Plasma laser-cut cloth
07—Plasma HID headlamps
08—Plasma-produced H_2 in fuel cell
09—Plasma-aided combustion
10—Plasma muffler
11—Plasma ozone water purification
12—Plasma-deposited LCD screen
13—Plasma-deposited silicon for solar cells
14—Plasma-processed microelectronics
15—Plasma-sterilization in pharmaceutical production
16—Plasma-treated polymers
17—Plasma-treated textiles
18—Plasma-treated heart stent
19—Plasma-deposited diffusion barriers for containers
20—Plasma-sputtered window glazing
21—Compact fluorescent plasma lamp

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https://doi.org/10.1088/1361-6463/aa76f5

Topical Review

The 2017 Plasma Roadmap: Low temperature plasma science and technology

I Adamovich[1], S D Baalrud[2], A Bogaerts[3], P J Bruggeman[4], M Cappelli[5], V Colombo[6], U Czarnetzki[7], U Ebert[8,9], J G Eden[10], P Favia[11], D B Graves[12], S Hamaguchi[13], G Hieftje[14], M Hori[15], I D Kaganovich[16], U Kortshagen[4], M J Kushner[17], N J Mason[18], S Mazouffre[19], S Mededovic Thagard[20], H-R Metelmann[21], A Mizuno[22], E Moreau[23], A B Murphy[24], B A Niemira[25], G S Oehrlein[26], Z Lj Petrovic[27], L C Pitchford[28], Y-K Pu[29], S Rauf[30], O Sakai[31], S Samukawa[32], S Starikovskaia[33], J Tennyson[34], K Terashima[35], M M Turner[36], M C M van de Sanden[9,37] and A Vardelle[38]

WHY NON EQUILIBRIUM PLASMAS ?

LOW TEMPERATURE PROCESSES FOR THERMOLABILE MATERIALS

SURFACE MODIFICATIONS, NO BULK ALTERATIONS

polymers, paper, textiles, ...

ADAPTABLE TO ANY SHAPE AND MATERIAL SUBSTRATE

webs, inside of small tubes, powders, granules, fibers, ...

HIGH DENSITY OF ACTIVE SPECIES

comparable with high T gases and flames

TUNEABLE ION BOMBARDMENT

DRY TECHNOLOGY, NEGLIGIBLE IMPACT TO THE ENVIRONMENT

ATMOSPHERIC PRESSURE PROCESSES

SYNTHESIS OF AN ENTIRELY NEW CLASS OF SURFACES

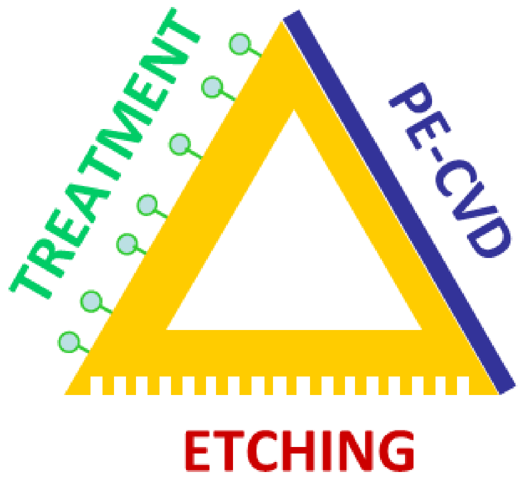
TRANSFER TO INDUSTRIAL SCALE

PROCESS CONTROL POSSIBLE

PLASMA SCIENCE AND TECHNOLOGY

first applications

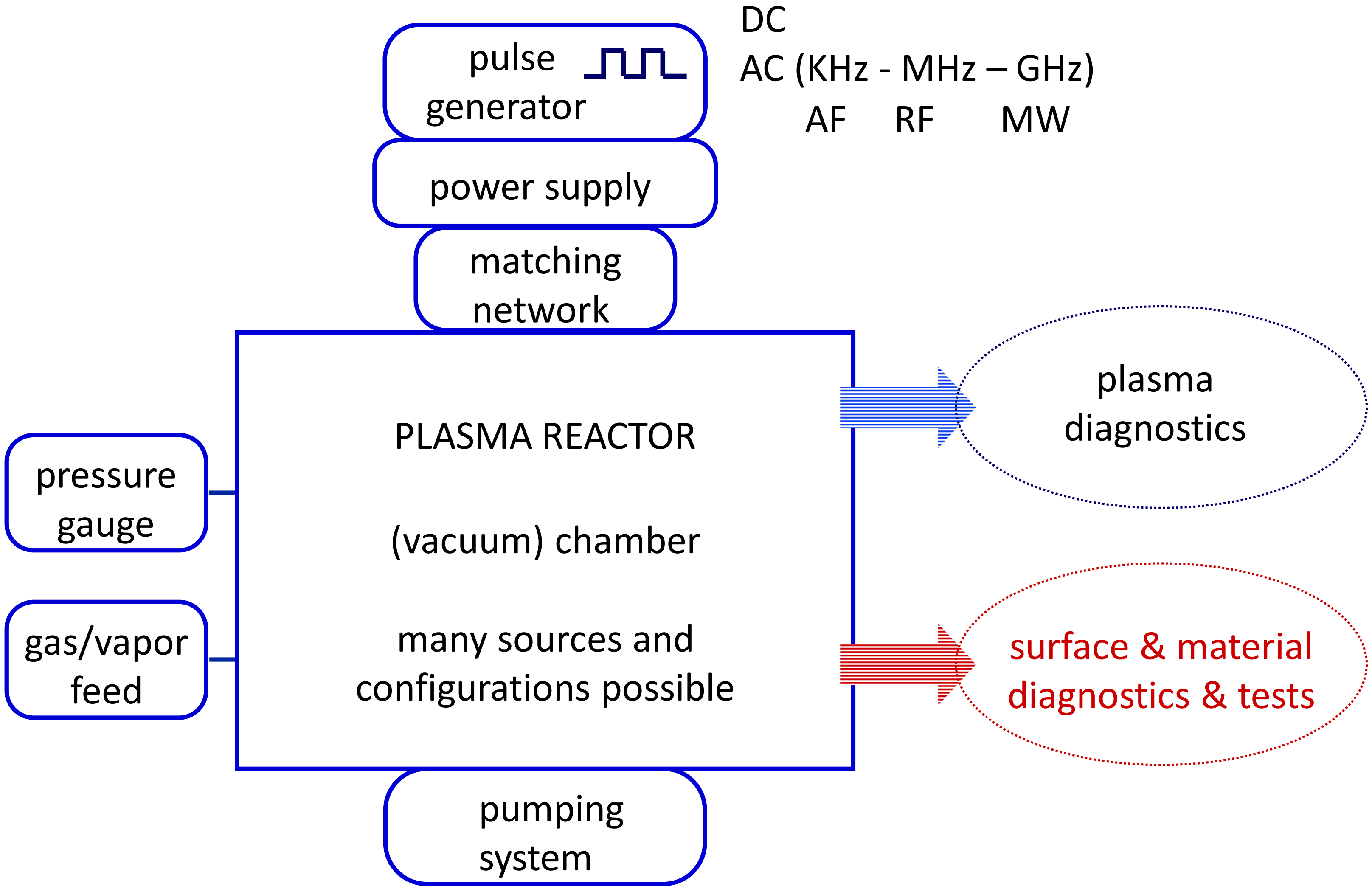
LIGHT SOURCES	CATALYSIS
OZONE PRODUCTION	MEDICINE
MICROELECTRONICS	POLYMERS
SEMICONDUCTORS	PAPER
SOLAR CELLS	WETTABILITY
AUTOMOBILE	ADHESION
FOOD PACKAGING	METALLIZATION
TEXTILE	PRINTING, DYEING
BIOMATERIALS	CORROSION PROTECTION
MICROFLUIDICS	CULTURAL HERITAGE
MEMS	COMPOSITES
CLEANING	SENSORS
STERILIZATION	OPTICS
BIOLOGY	BUILDINGS
ENVIRONMENT	AGRICULTURE



IN MATERIAL SCIENCE TECHNOLOGY NON EQUILIBRIUM «COLD» PLASMAS ALLOW

- **SURFACE ALTERATIONS OF PROPERTIES**
- **AT ROOM TEMPERATURE**
- **WITH NO BULK MODIFICATION**

PLASMA REACTORS LP/AP



Plasma Processes for Life Sciences

Ilaria Trizio, Marta Garzia Trulli, and Chiara Lo Porto, Department of Chemistry, University of Bari, Bari, Italy
Daniela Pignatelli, Italian Institute of Technology, Pontedera, Italy
Giuseppe Camporeale, Department of Chemistry, University of Bari, Bari, Italy
Fabio Palumbo, Eloisa Sardella, and Roberto Gristina, CNR Institute of Nanotechnology, Bari, Italy
Pietro Favia, CNR Institute of Nanotechnology, Bari, Italy; Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Bari, Italy

Elsevier Reference Module in Chemistry, Molecular Sciences and Chemical Engineering, (2018)

parallel plate reactor (Low P)

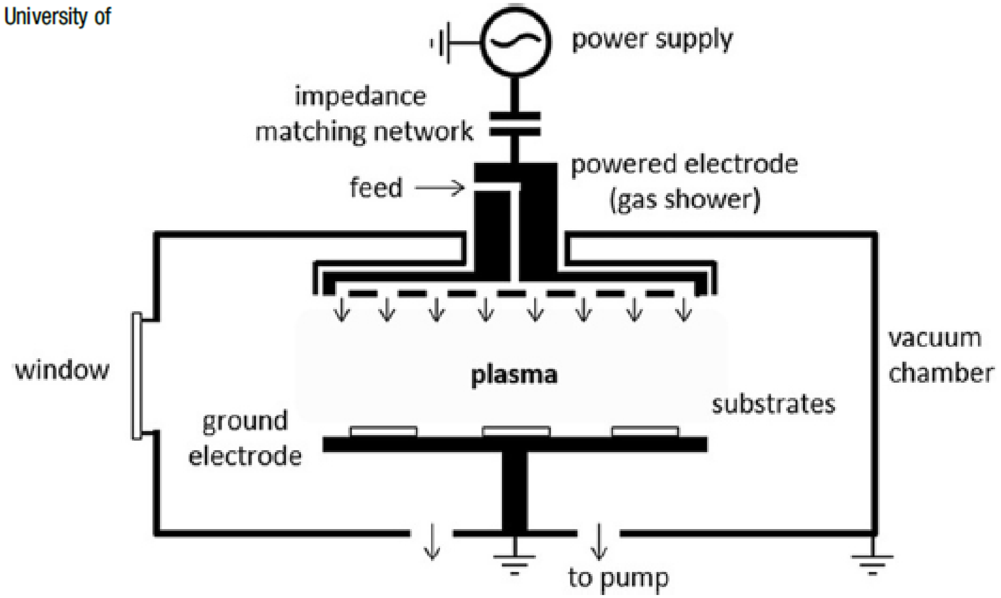


Fig. 1 General sketch of a LP parallel plate plasma reactor.

Dielectric Barrier Discharge (Atm P)

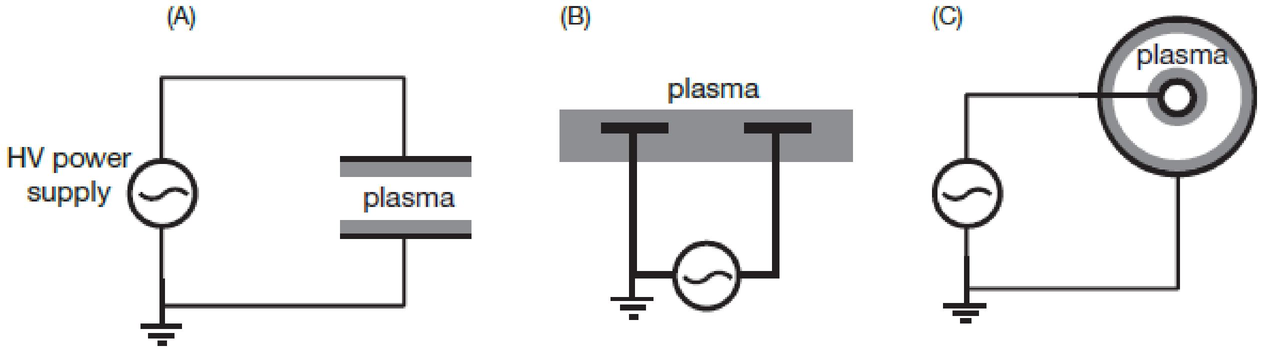


Fig. 2 Design of the three possible configurations of AP-DBD: (A) parallel-plate DBD; (B) coplanar surface DBD; (C) coaxial DBD. The dielectric layer that covers the electrodes is represented in *gray*.

Atmospheric Pressure Plasma Jet (Atm P)

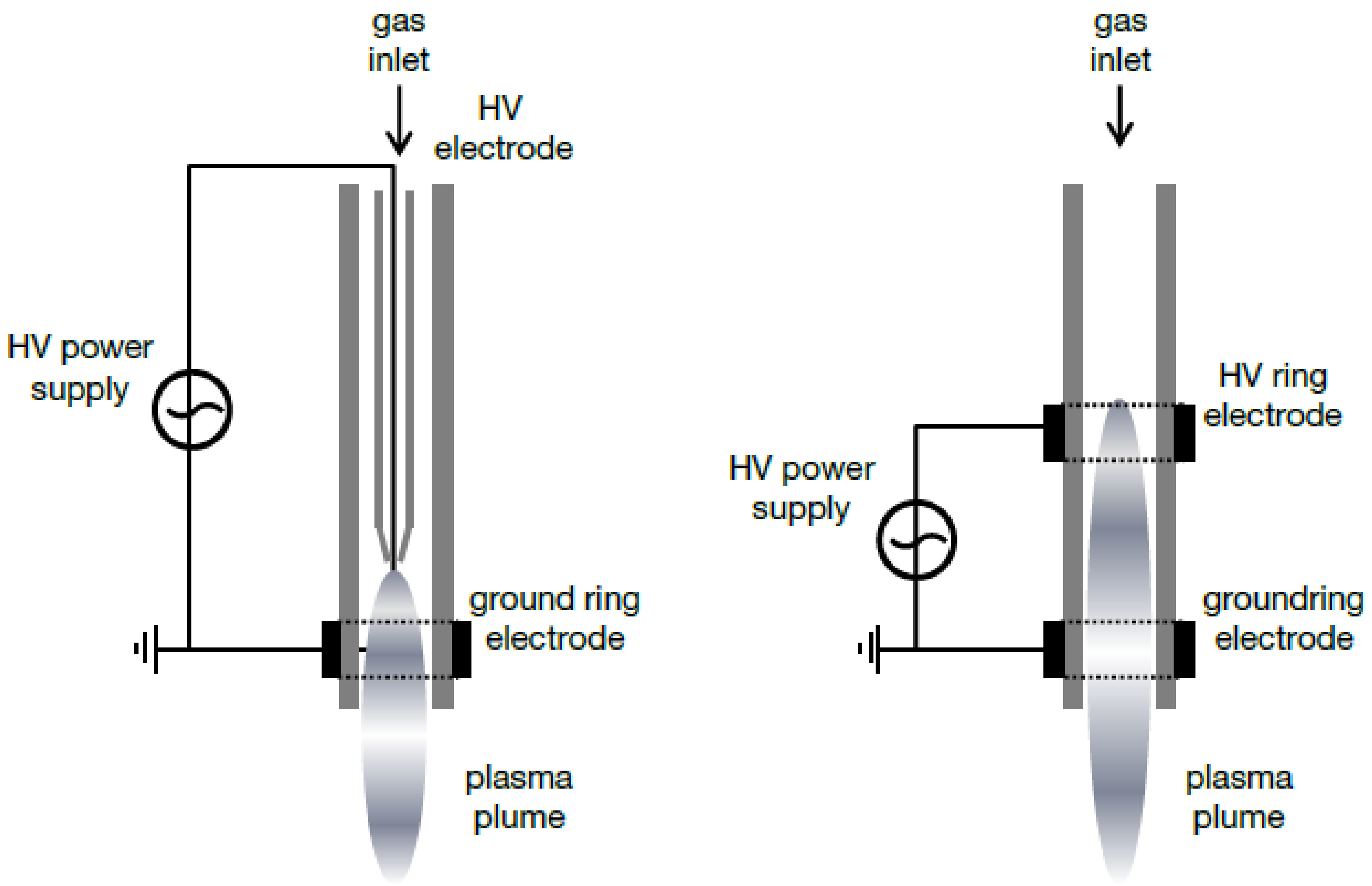


Fig. 3 Design of two APPJ plasma sources. Glass (dielectric) tubes are in *gray*.

PLASMA PARAMETERS: external and internal

"external" PARAMETERS

imposed from the operator

Pressure

Feed composition, flow rate, leaks

Field frequency, power density

Reactor configuration, materials, electrode geometry

Substrate position (*e.g.* glow *vs*. afterglow)

Duty cycle %, *time on*, *time off* in pulsed plasmas

Substrate temperature

Substrate *bias* potential

"internal" PARAMETERS

output from diagnostics

Fragmentation degree of the feed

Density and distribution of neutrals

Distribution energy (EEDF) and density (n_e) of electrons

Ionization degree

Residence time of the species

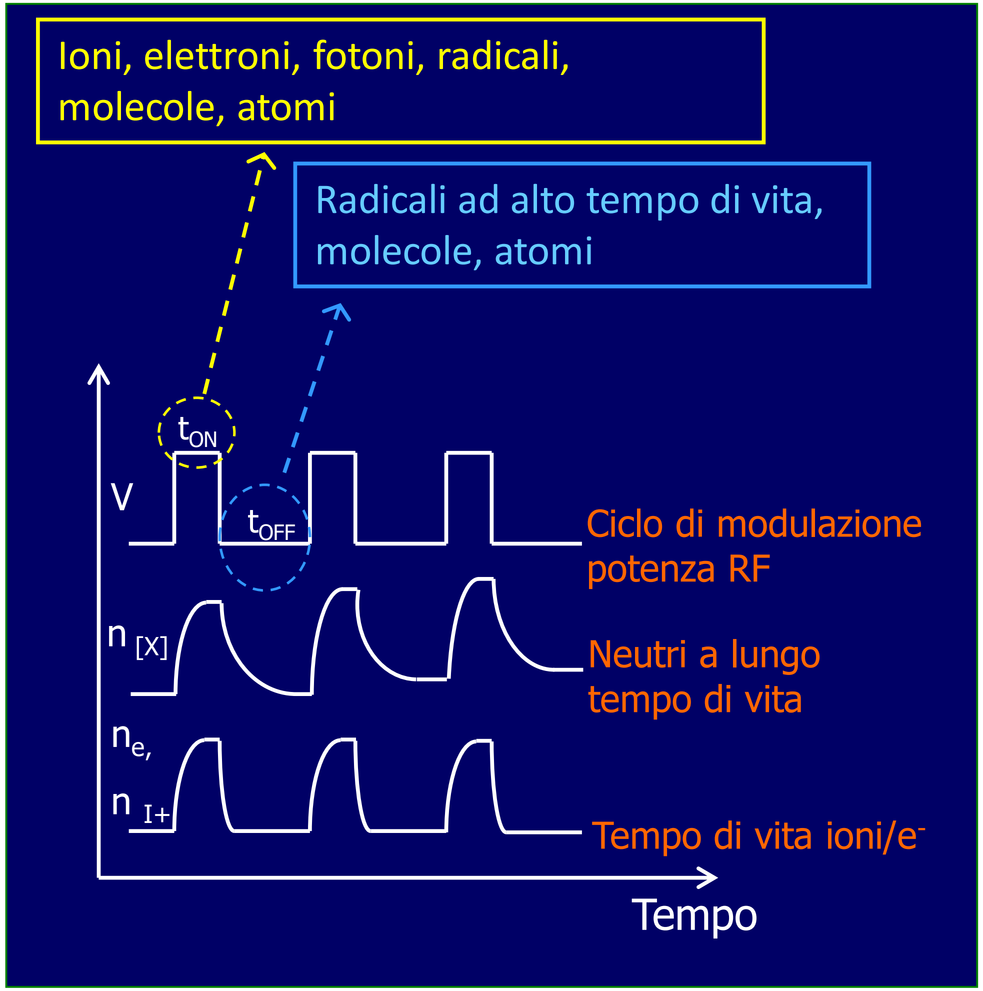
Process homogeneity

Positive-ion bombardment, sputtering

Deposition, etching, treatment rate

Contaminations

plasmi continui vs plasmi pulsati



Parametri di modulazione

Periodo = $t_{ON} + t_{OFF}$

Duty Cycle (DC) = $(t_{ON}/\text{periodo}) * 100$

$W_{pulsato} = DC * W_{continuo}/100$

- Elevata ritenzione di struttura del monomero
- Durante il tempo off → polimerizzazione radicalica convenzionale

processi plasmochimici di modificazione superficiale

PLASMA (Dry) ETCHING

Ablazione di materiali (Si, SiO_2, resist, polimeri, metalli, ecc.) attraverso reazioni con specie attive del plasma a dare prodotti volatili

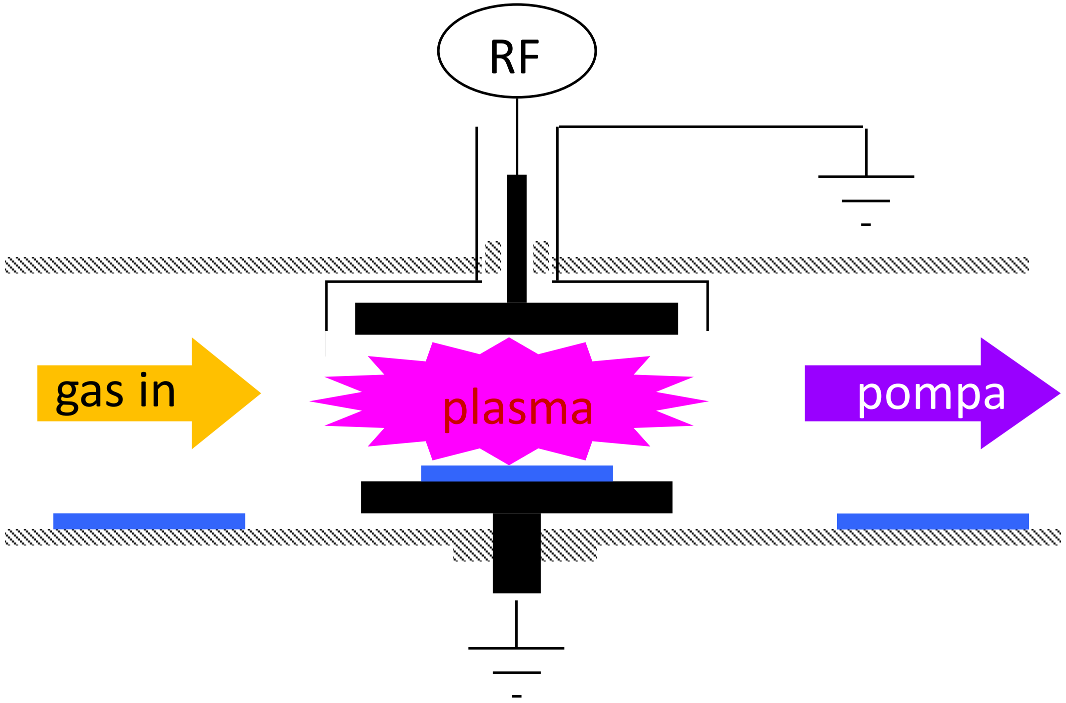
PLASMA ENHANCED - CHEMICAL VAPOR DEPOSITION (PE-CVD)

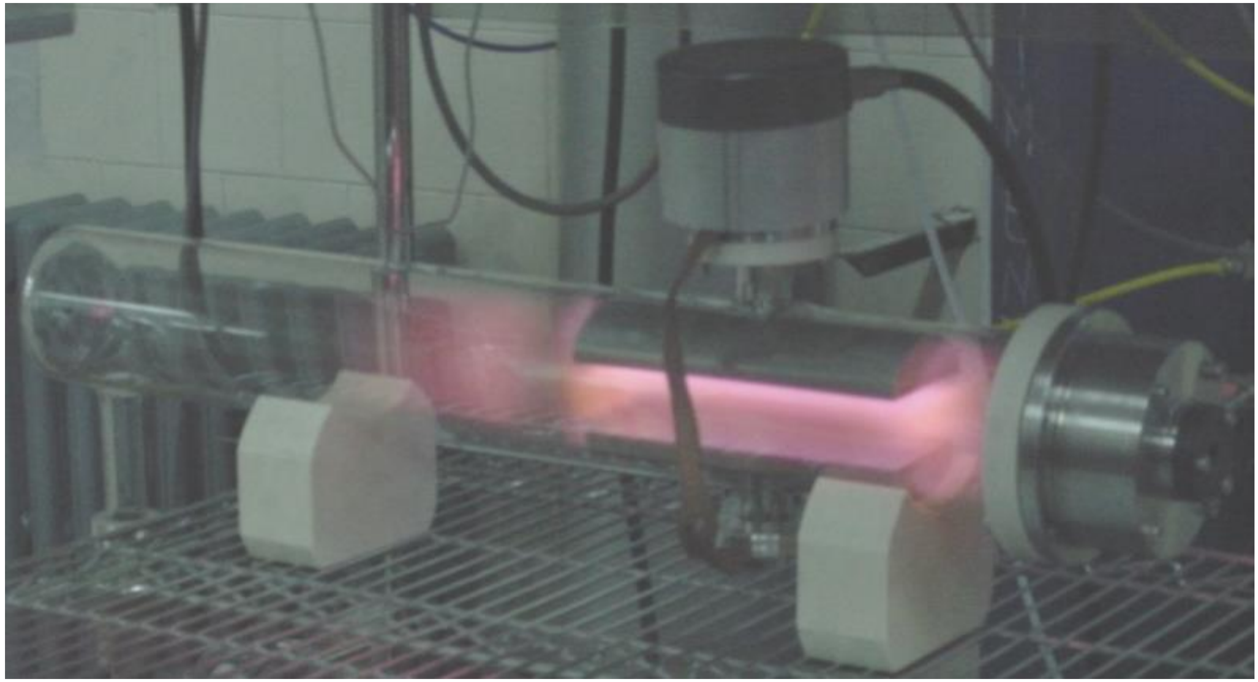
Permette di depositare film inorganici (SiO_2, film simili al diamante, a-Si:H, ecc.), organici (silicone-, PEO-like, PTFE-like, etc.)
PLASMA POLYMERIZATION è il nome comune per PE-CVD di film organici in cui si usa un monomero per alimentare un plasma

TRATTAMENTI VIA PLASMA

Modificazione degli strati più esterni dei materiali attraverso l'innesto di gruppi funzionali ($-NH_2$, -COOH, -F, -OH …) e/o reticolazione della superficie con gas reattivi (NH_3, CF_4, O_2, …) o inerti (Ar, He,..)

posizione substrato





Preglow
No trattamento

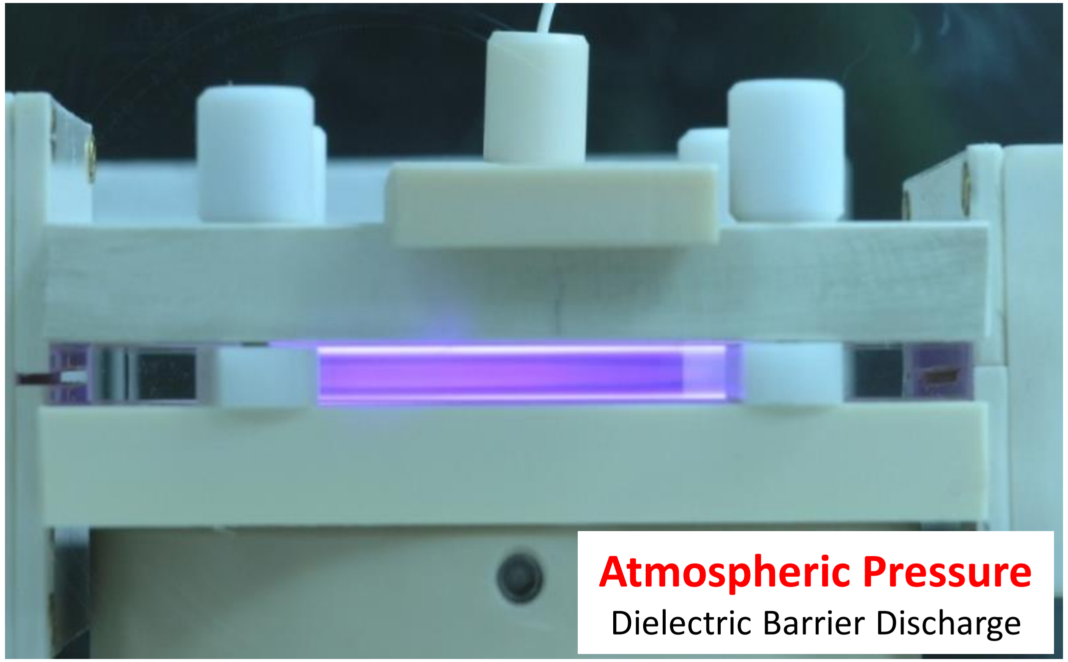
Glow
Specie attive
Bomb. ionico
Crosslinking
Frammentazione
monomero
alte velocità etc. dep.

Afterglow
No elettroni, ioni, bias
Specie ad elevato tempo di vita
Trattamenti poco energetici
Selettività
Ritenzione di struttura
basse velocità etc. dep

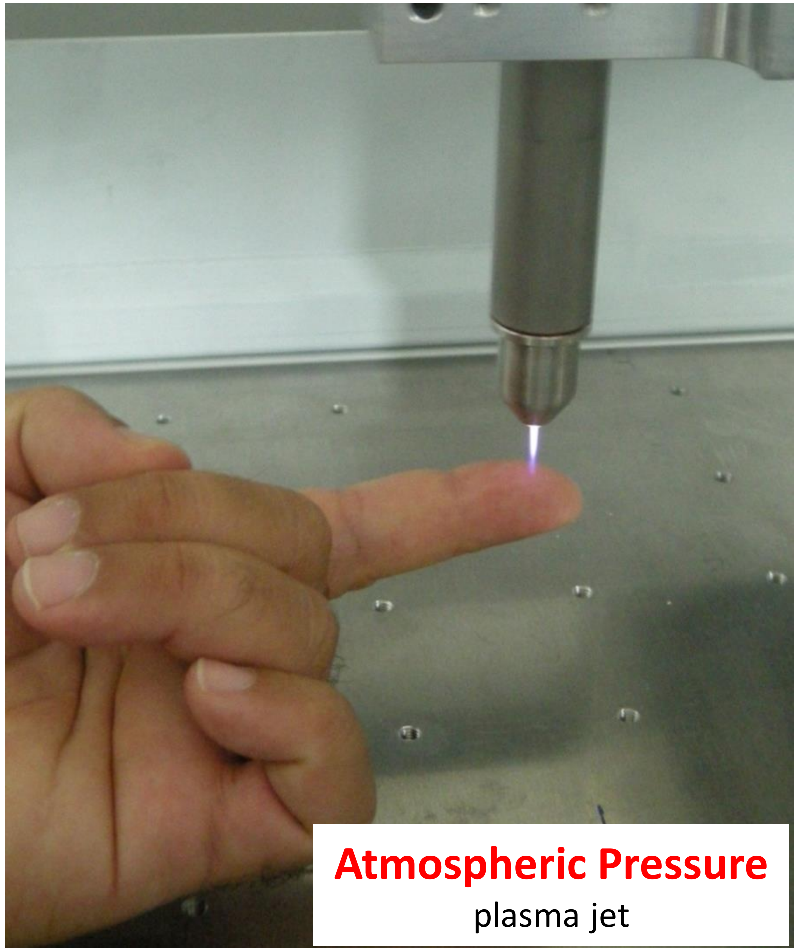
surface functionalization of materials in cold plasmas

PLASMA SOURCES

Low Pressure
parallel plate plasma reactor



Atmospheric Pressure
Dielectric Barrier Discharge



Atmospheric Pressure
plasma jet

CVD, Chemical Vapor Deposition

The precursor of the coating is in the gas phase.
The deposition/polymerization process can be initiated by an initiator molecule and/or by a hot filament, or by heating the substrate.

PE-CVD, Plasma-Enhanced CVD

The precursor of the coating is in the gas phase.
The deposition process is initiated by fragmenting the “monomer” with an electric field (glow discharge).

PVD, Physical Vapor Deposition

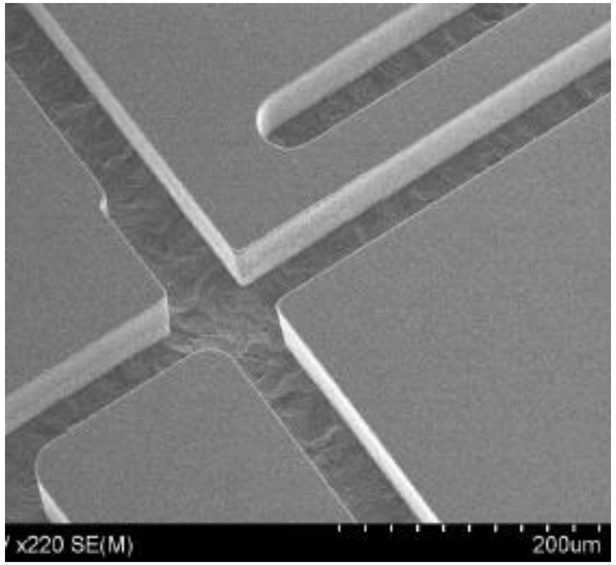
The precursor of the coating is in the solid phase (filament, electrode).
The deposition process is initiated by heating a filament (evaporation) or by sputtering from an electrode bombarded by positive ions (glow discharge, ion gun, etc).

Plasma Etching

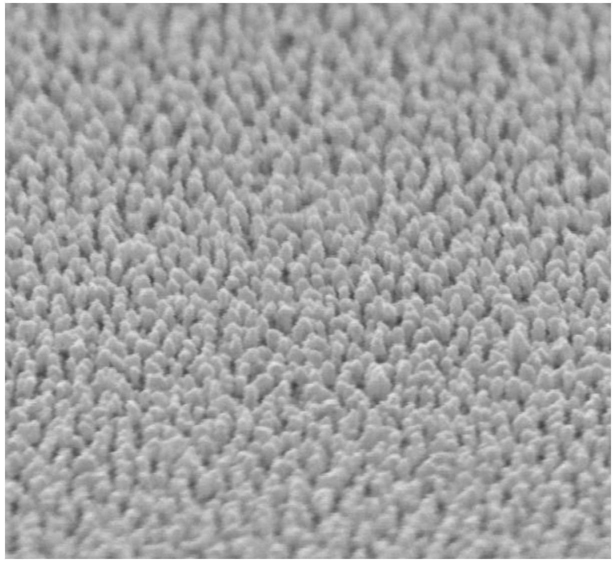
sculpting/patterning polymer "lab chips", µm

texturing of surfaces, µm – nm

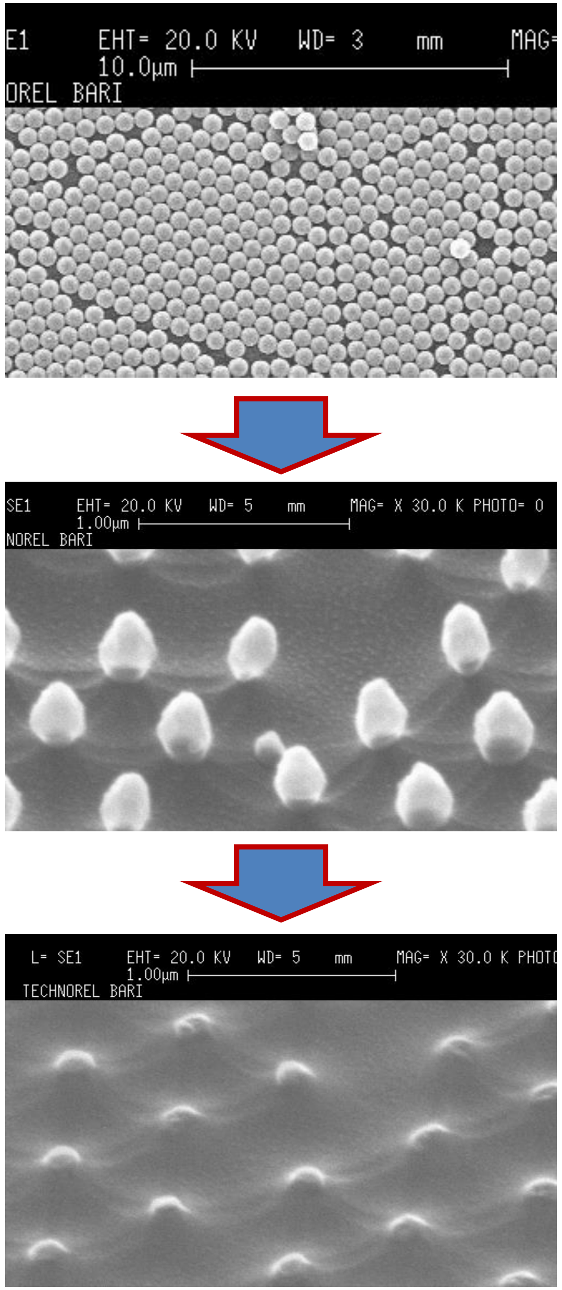
plasma-aided coll. lithography & other methods, µm - nm



sculpted



textured

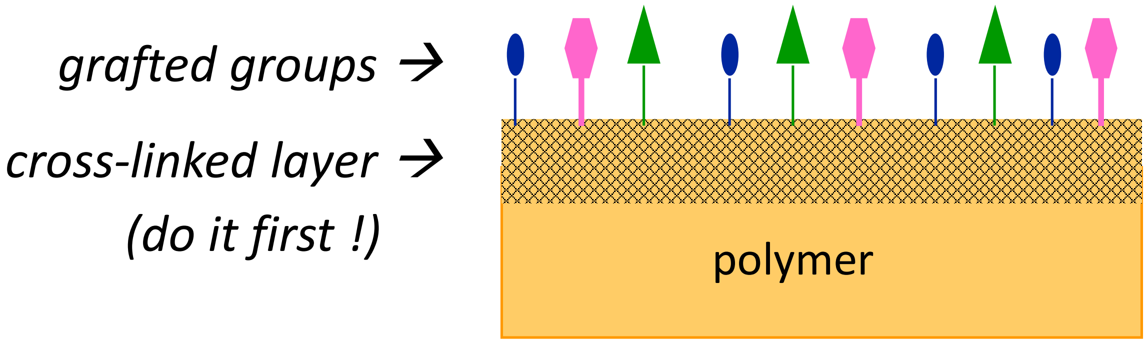


plasma-aided
colloidal lithography

functionalization by Plasma Treatments

grafting of (polar) functional groups

modified thickness 1 – 10 nm

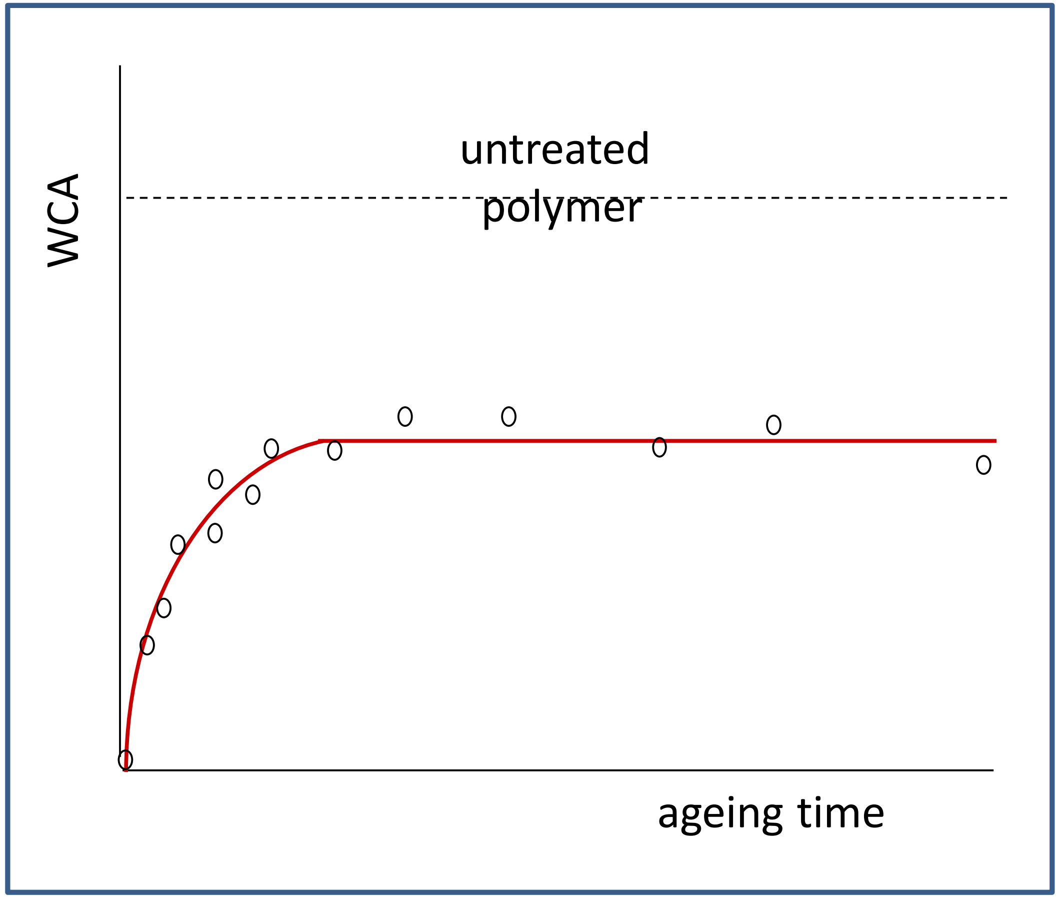


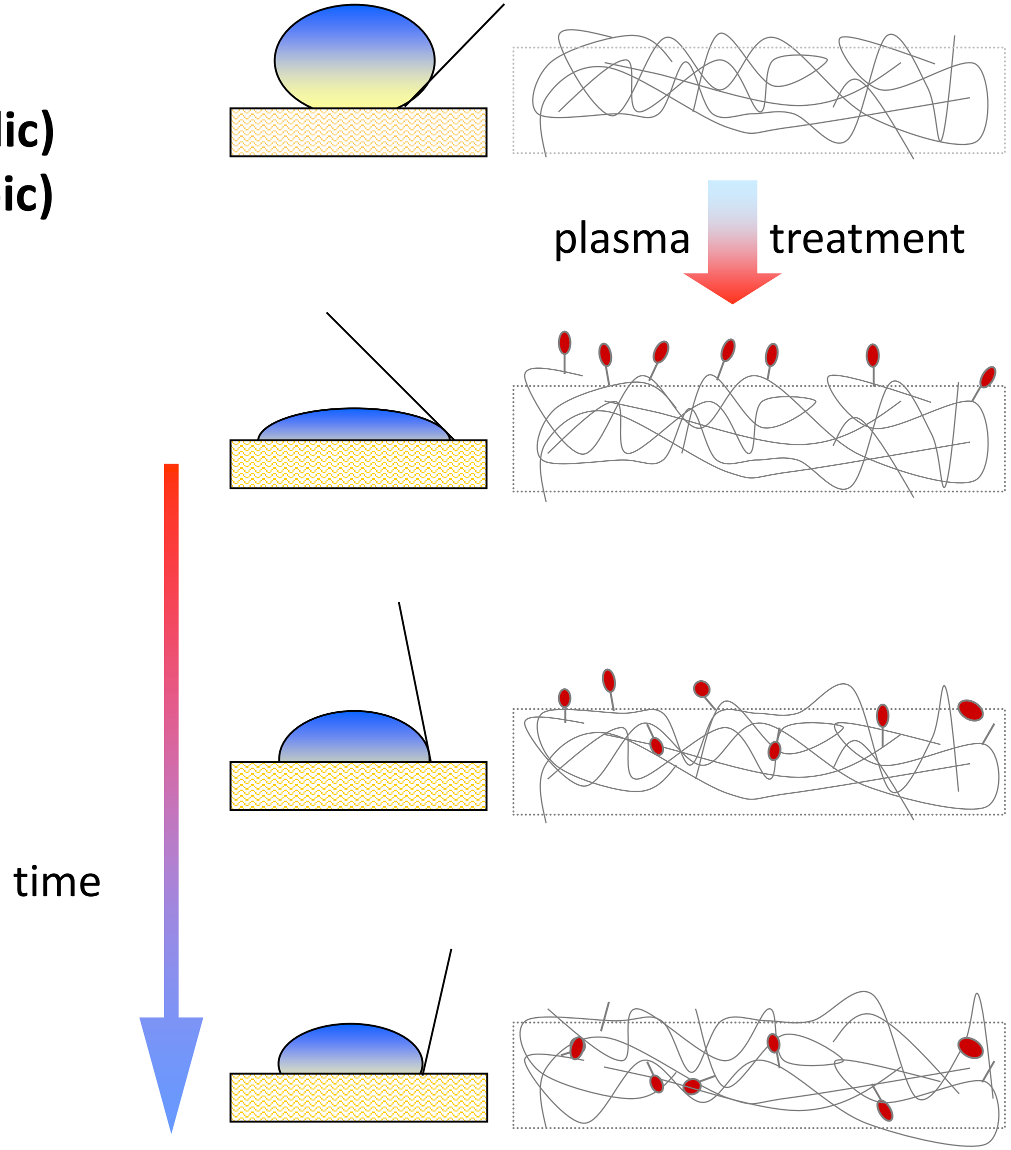
- **optimization of plasma conditions**
- **Low vs Atm Pressure**
- **ageing**
- **hydrophobic recovery**
- **stability in water-based media**
- **pre-treatments are generally needed**

surface modification (deposition, etching, grafting) plasma processes can be considered nanotechnologies for the z axis

HYDROPHOBIC RECOVERY

hydrophobic polymers, grafted with polar (hydrophilic) groups, loose properties with time in air (hydrophobic)





Chatelier et al
Langmuir 11, 2576, 1995
Langmuir 11, 2585, 1995

Favia et al
PPP (the book, Wiley-WCH) 271, 2005

Trattamenti via plasma

- Differentemente dalla deposizione (PE-CVD) in cui aggiungiamo materiale sul substrato in modo significativo, i trattamenti via plasma permettono di modificare la struttura e la composizione chimica dei primi strati superficiali (1-10 nm)

- I plasmi utilizzati per questo tipo di processi sono quelli di gas inerti (es. He o Ar) o gas non polimerizzabili (es. N_2, NH_3, O_2, H_2O).

- Durante i trattamenti sulla superficie si verificano quattro tipi di effetti. Ciascuno è sempre presente, ma uno può essere favorito rispetto agli altri a seconda del tipo di substrato, della chimica del plasma, del tipo di reattore e dei parametri di processo:

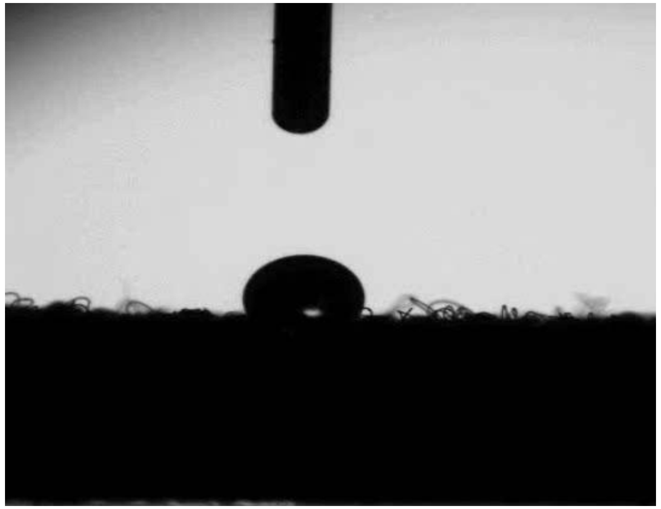
1. Pulizia superficiale (rimozione di contaminanti organici dalla superficie)
2. Etching (maggiore quantità di materiale rimossa)
3. Cross-linking (scissione, riarrangiamento e reticolazione delle catene polimeriche)
4. Funzionalizzazione (innesto di gruppi funzionali)

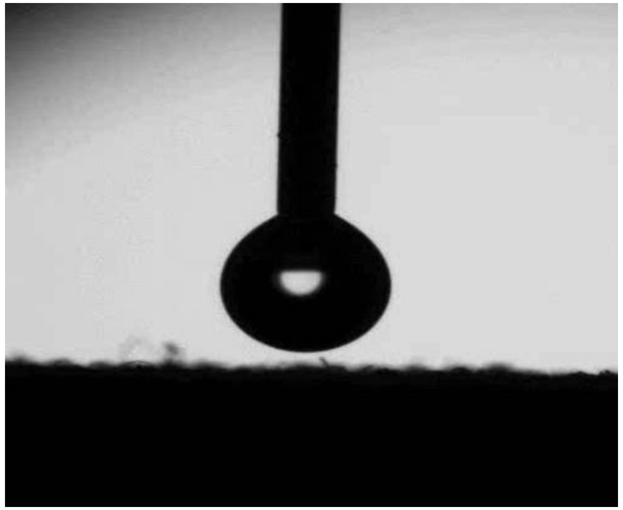
HYDROPHILIC TEXTILE

untreated

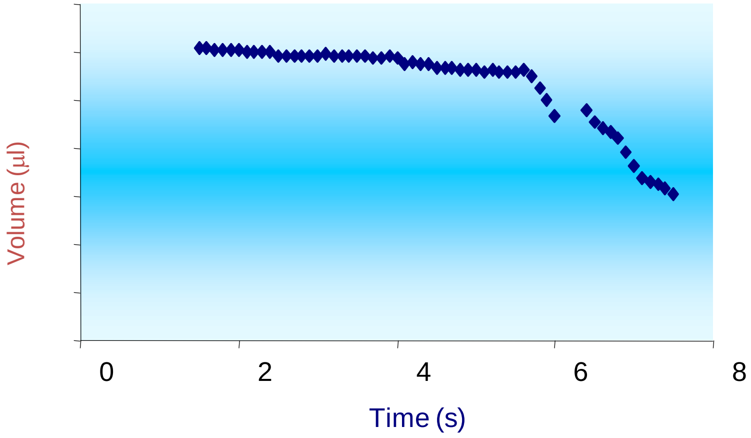
CF_4 plasma treated

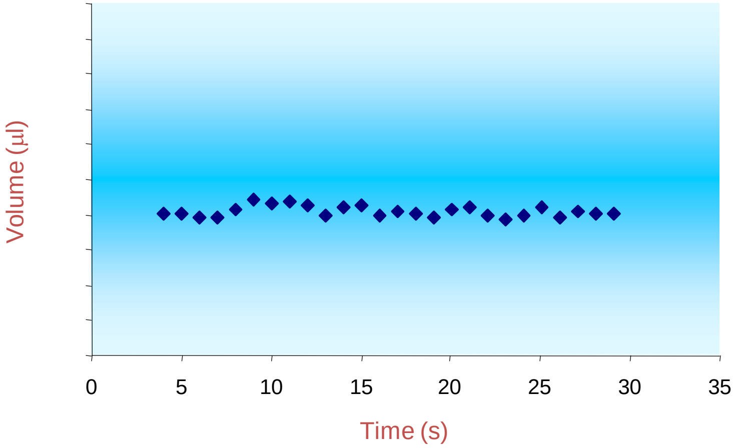
WCA
122±3°





water adsorbtion kinetics





Trattamenti via plasma

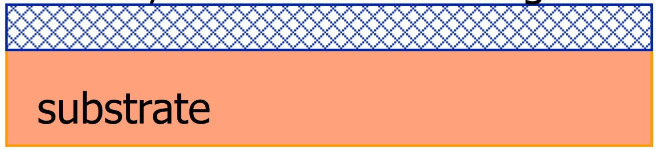
Attivazione polimeri

Polystyrene cell-culture plates plasma-hydrophilized with an Atmospheric Pressure corona discharge in air.

M. J. Lerman et al, Tissue. Eng. Part. B. Rev. 2018, 24, 359.

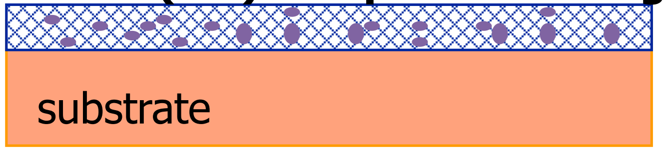
PE-CVD

dense, cross-linked coating



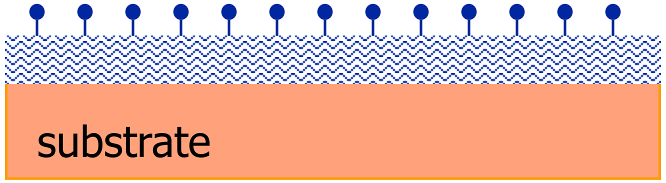
inorganic
DLC, SiOx, …

nano (bio) composite coating



organic/inorganic
metal/ceramic cluster
or biomolecules
embedded in a matrix

functional coating **(-COOH, -NH2, -OH, >C=O, …)**

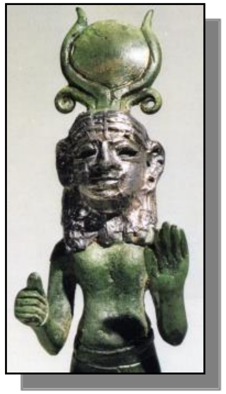


organic
PEO-like, pdAA, teflon-like,
silicone-like …

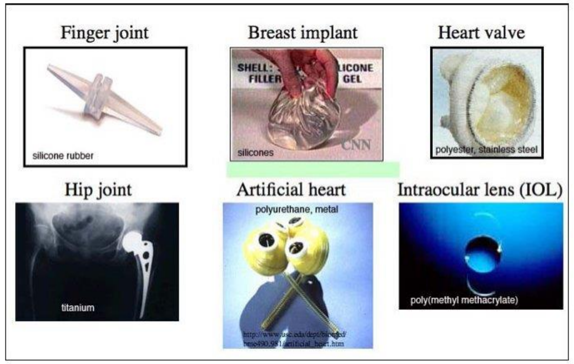
modified thickness
10 – 1000 nm

PLASMI

beni culturali



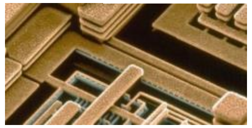
biomateriali



energia

elettronica

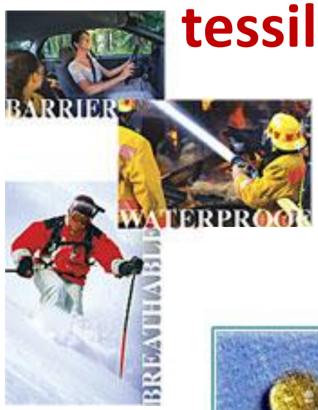
microelettronica

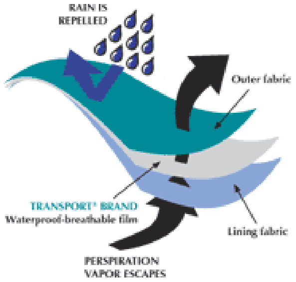


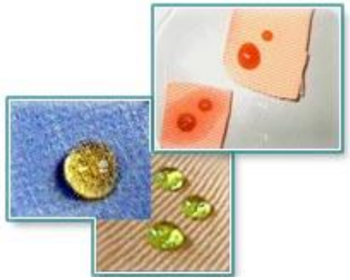
imballaggio alimentare e farmaceutico

corrosione

tessile







Plasma Processes for Life Sciences

Ilaria Trizio, Marta Garzia Trulli, and Chiara Lo Porto, Department of Chemistry, University of Bari, Bari, Italy
Daniela Pignatelli, Italian Institute of Technology, Pontedera, Italy
Giuseppe Camporeale, Department of Chemistry, University of Bari, Bari, Italy
Fabio Palumbo, Eloisa Sardella, and Roberto Gristina, CNR Institute of Nanotechnology, Bari, Italy
Pietro Favia, CNR Institute of Nanotechnology, Bari, Italy; Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Bari, Italy

Chemistry Molecular Sciences and Chemical Engineering https://doi.org/10.1016/B978-0-12-409547-2.12271-1
Elsevier Reference Module in Chemistry, Molecular Sciences and Chemical Engineering, (2018)

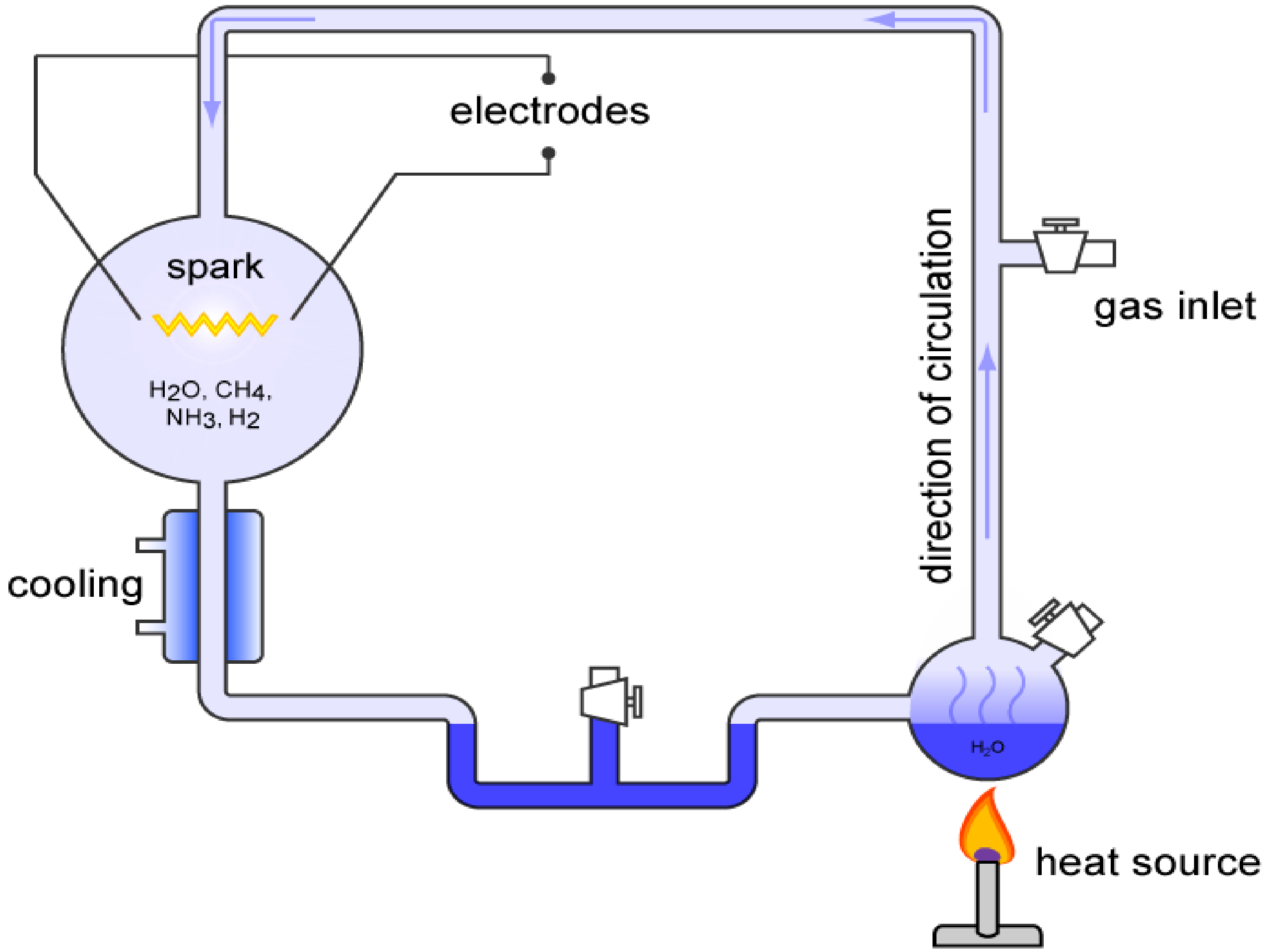
PLASMAS
AND THE ORIGIN OF LIFE

"A Production of Amino Acids Under Possible Primitive Earth Conditions

Stanley L. Miller
G. H. Jones Chemical Laboratory
University of Chicago, Chicago, Illinois"
Science, Vol. 117 p.528 (1953)

"Production of org Compounds under Primitive Earth Conditions"

Stanley L. Miller
G. H. Jones Chemical Laboratory
University of Chicago, Chicago, Illinois"
J. Am Chem Soc. Vol. 77,9 p.2351ff (1955)



plasma (spark) processing
of the pristine atmosphere on EARTH
a mixture of H_2O, CH_4, NH_3 and H_2

amino acids were produced in the discharge !

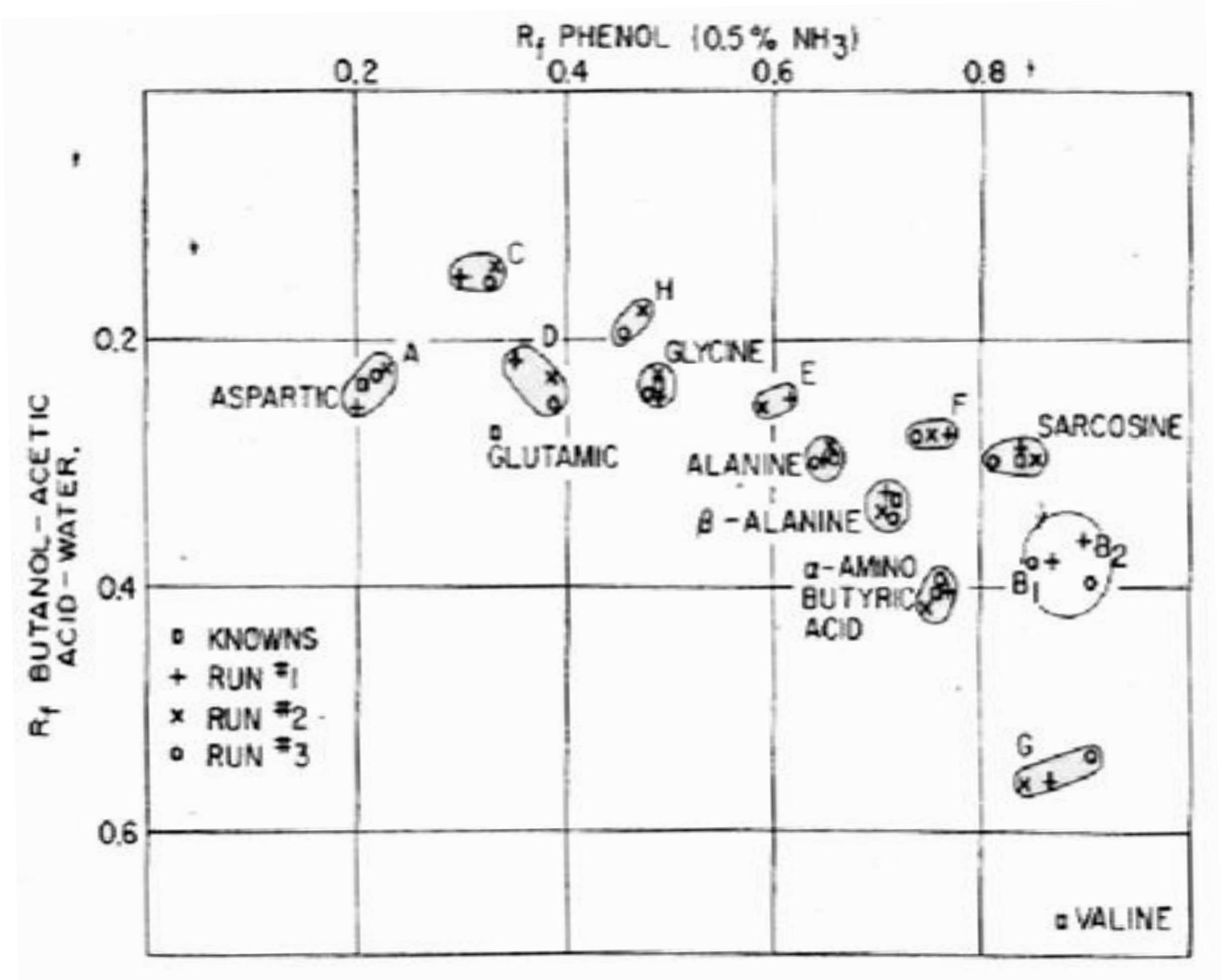
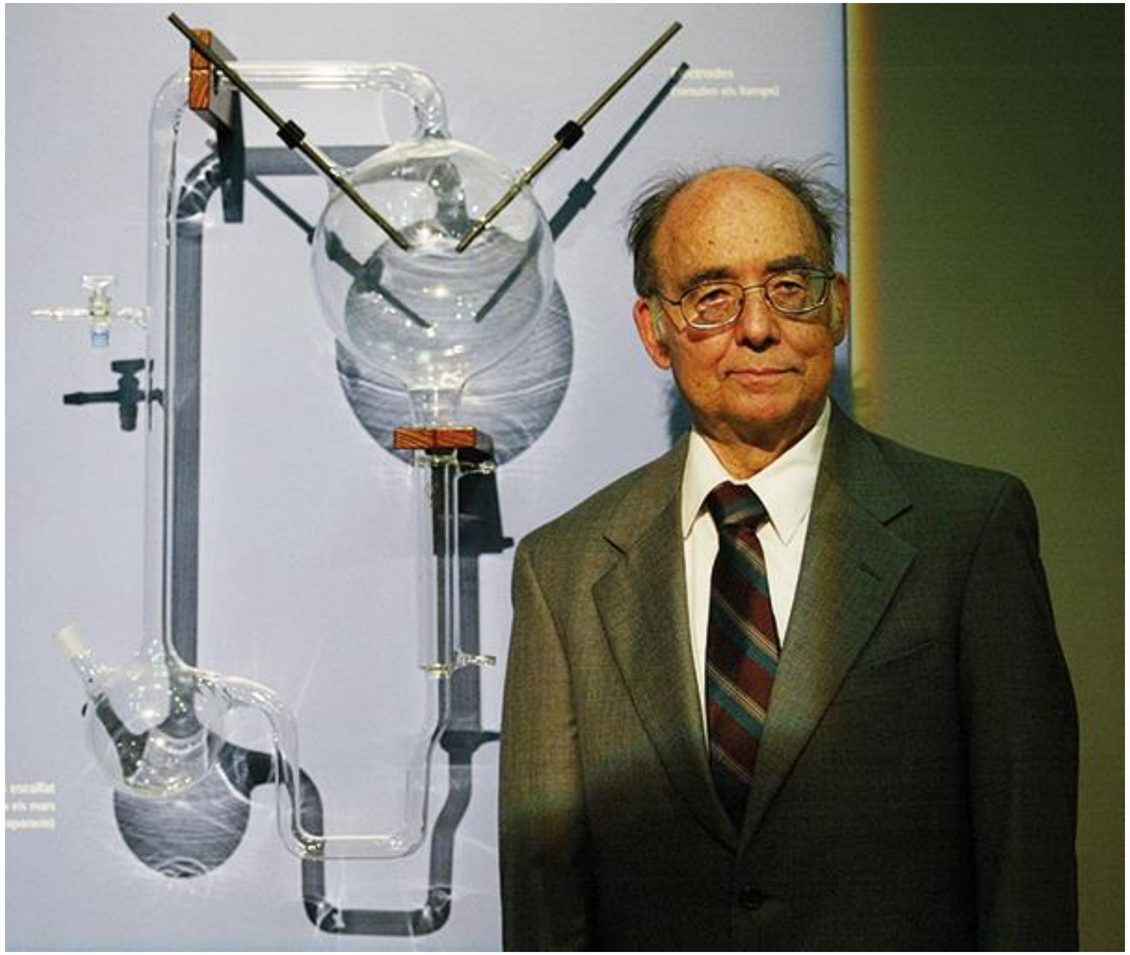
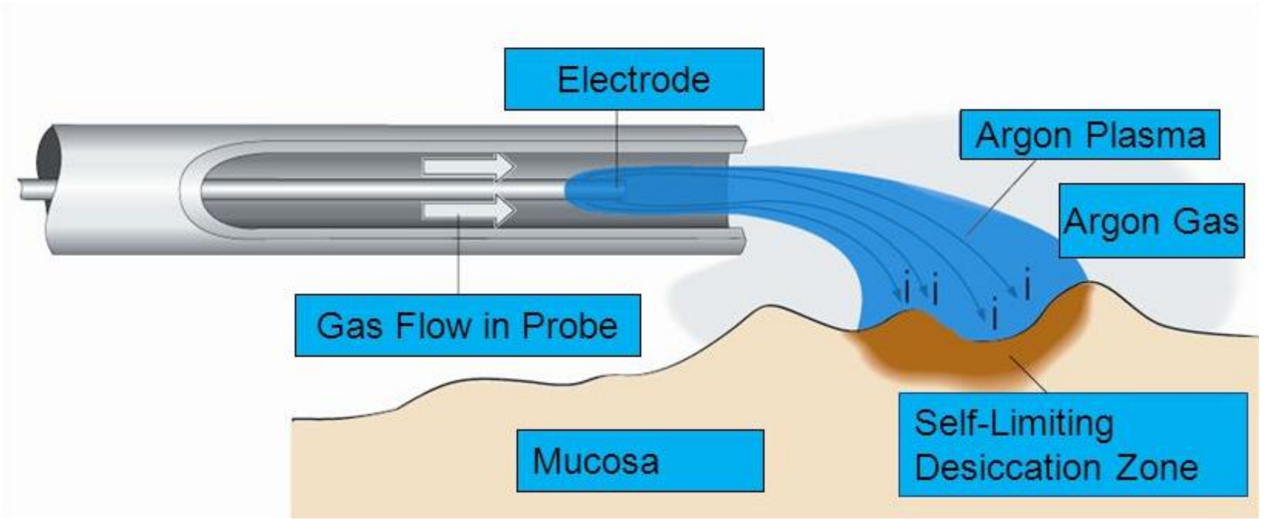


Fig. 8.—Paper chromatography of the amino acids.

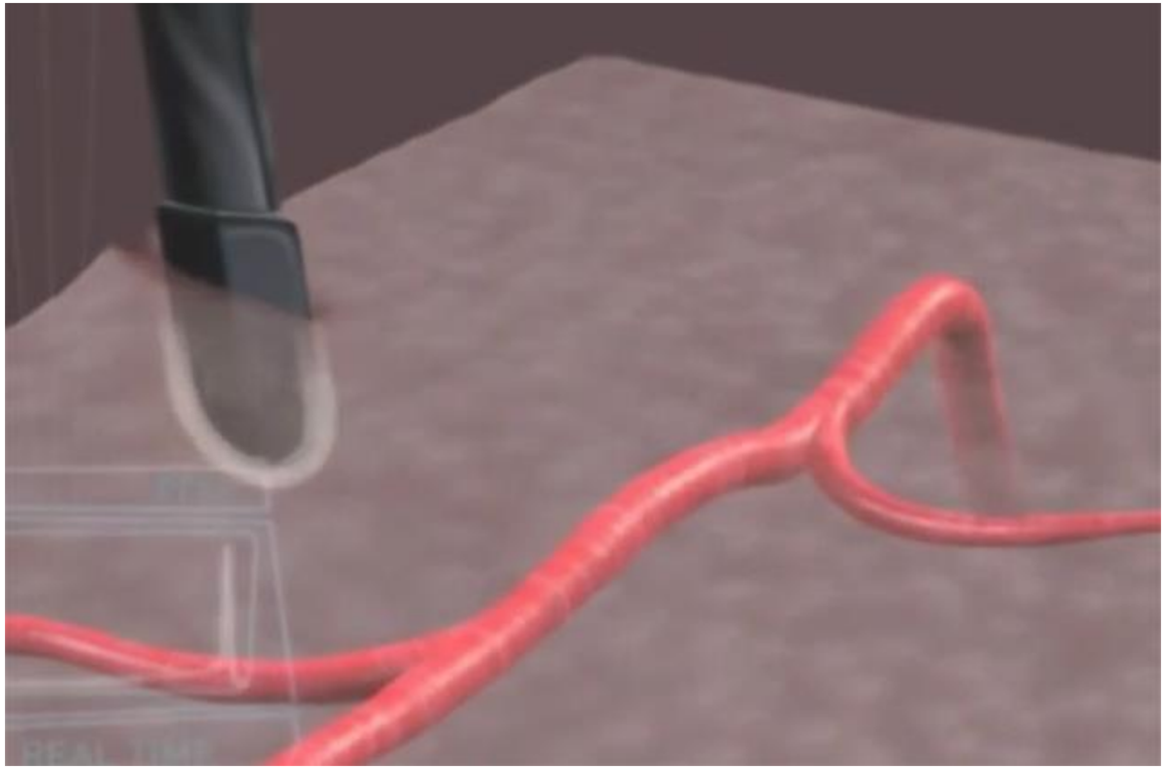


SURGICAL TOOLS
plasma coagulators, plasma scalpels

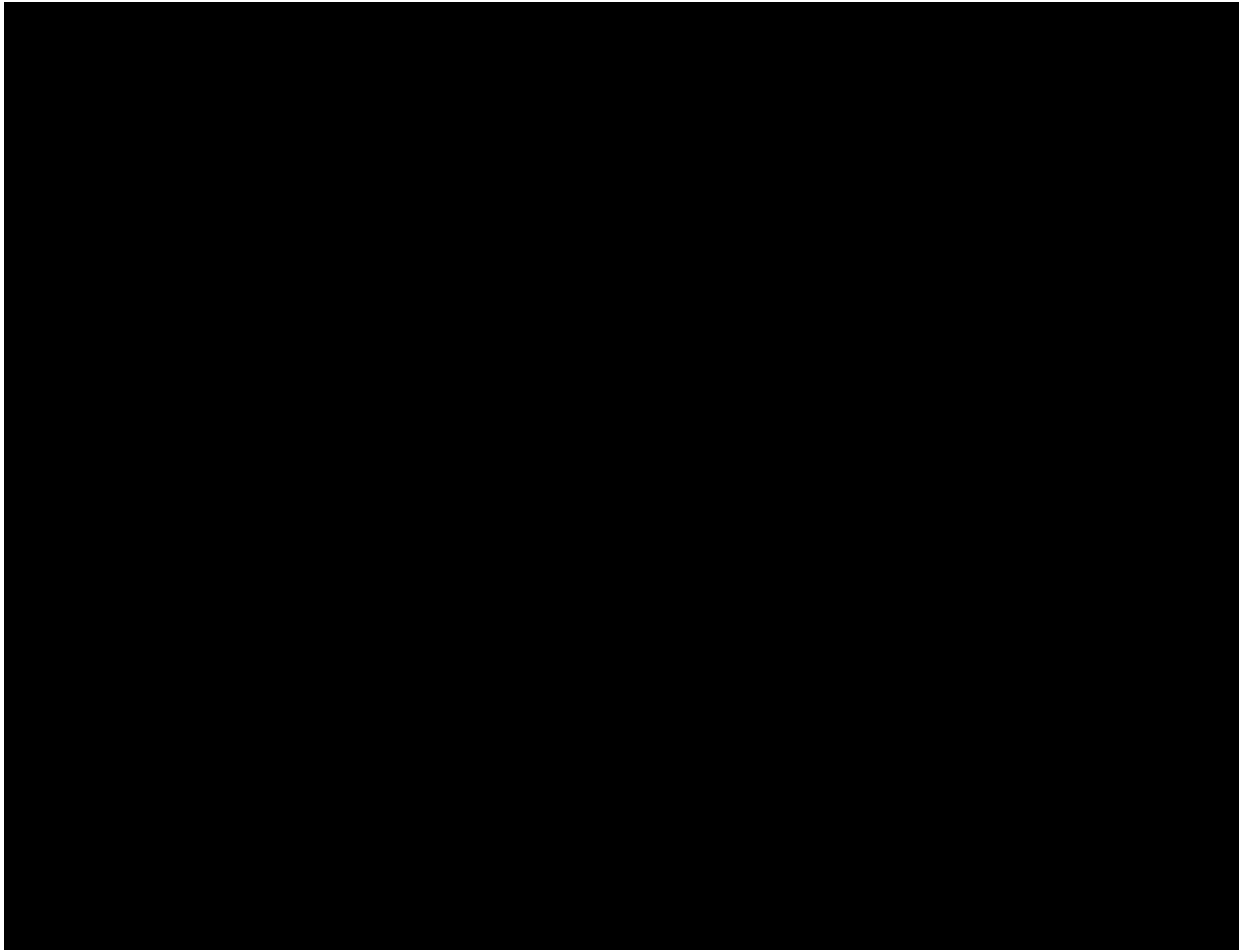


Argon Plasma Coagulation

Plasma Blade



Video
Plasma Scalpel



Ion Med

PLASMA STERILIZATION

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

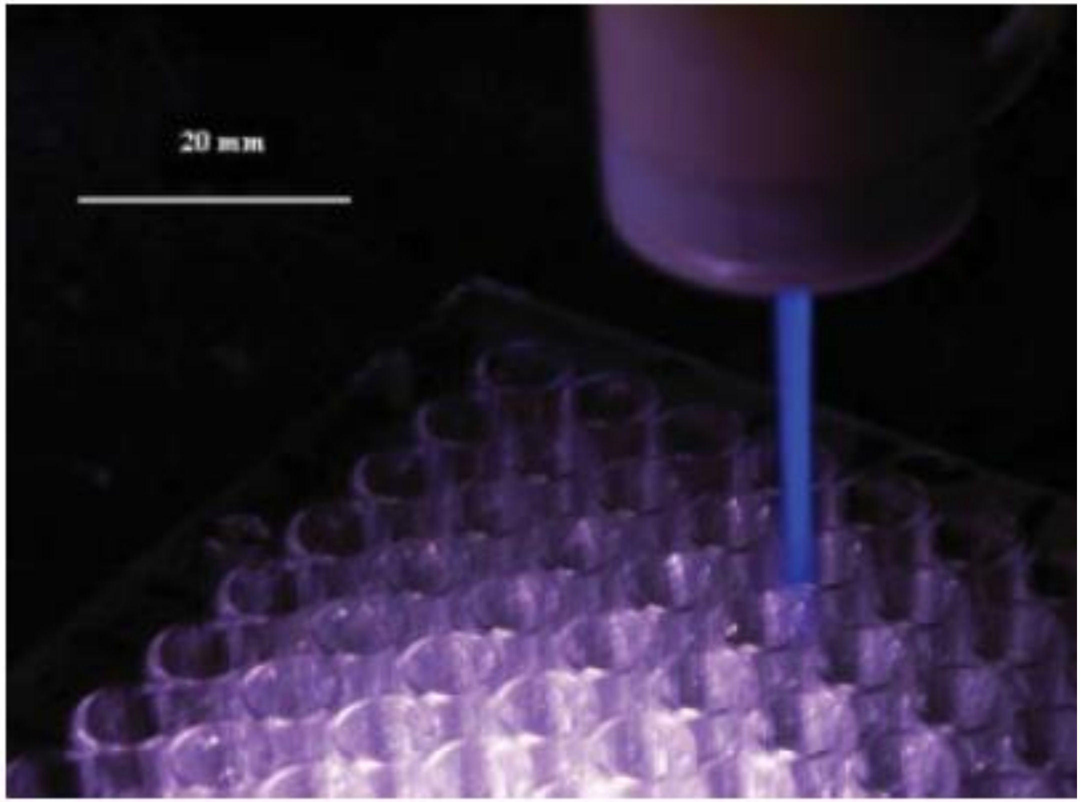


Figure 4. Photograph of the plasma pencil in operation

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

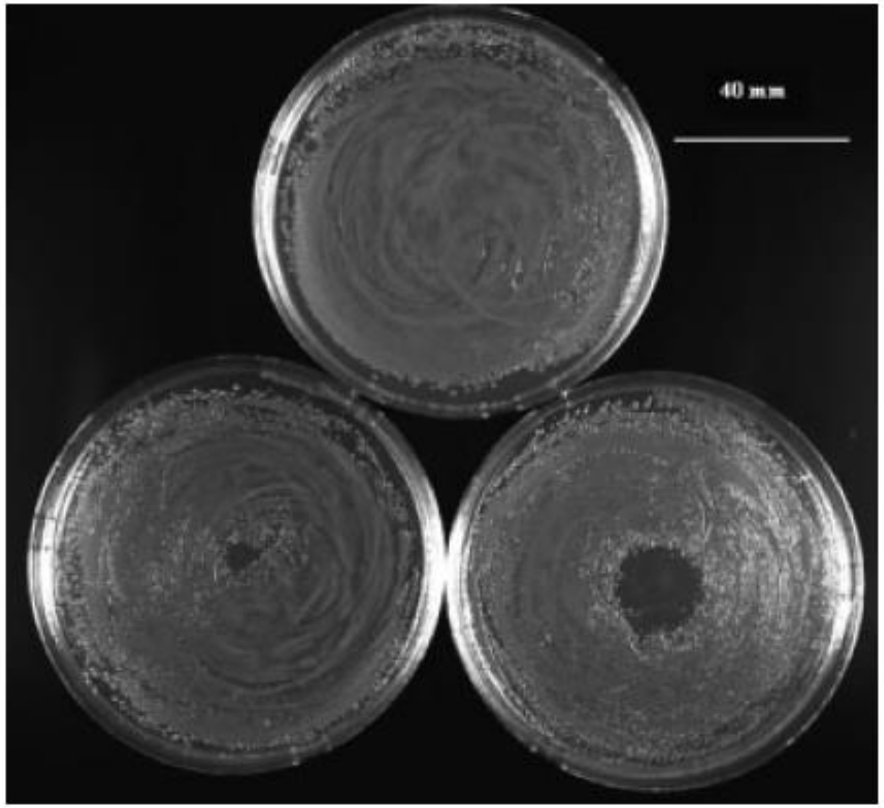


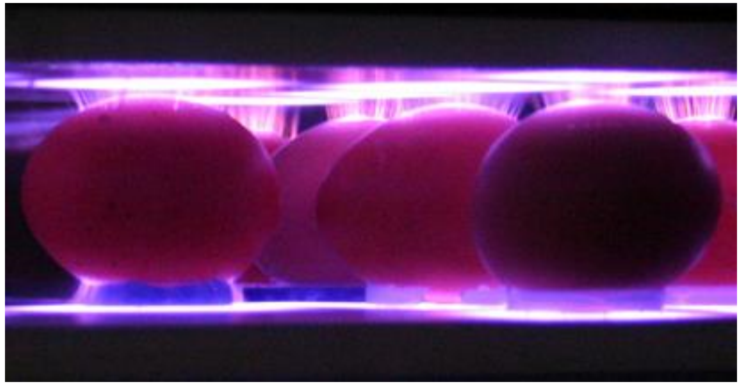
Figure 7. Localized inactivation of *E. coli* by the plasma pencil.[31] The top petri dish is the control, the left and right petri dishes represent 30 and 120 s plasma exposures, respectively. Helium is the operating gas.

plasma sterilization in hospitals

https://www.bing.com/videos/search?q=plasma+sterilization&&view=detail&mid=7602EAD157E5F21083177602EAD157E5F2108317&&FORM=VRDGAR&ru=%2Fvideos%2Fsearch%3Fq%3Dplasma%2Bsterilization%26%26FORM%3DVDVVXX

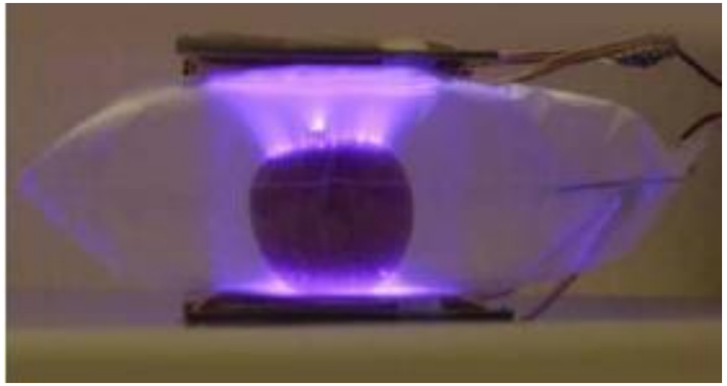
Decontamination / Sterilization

decontaminazione gusci d'uovo



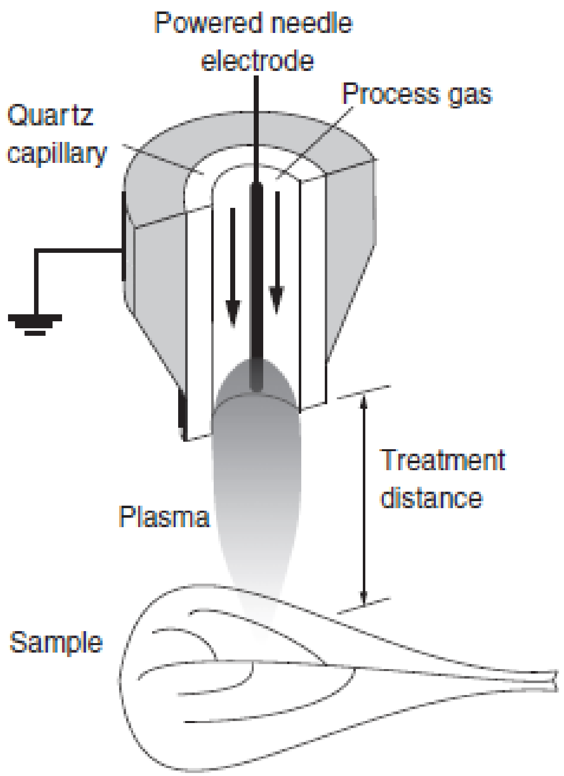
L. Ragni et al. / Journal of Food Engineering 100 (2010) 125–132

decontaminazione in-pack di mele



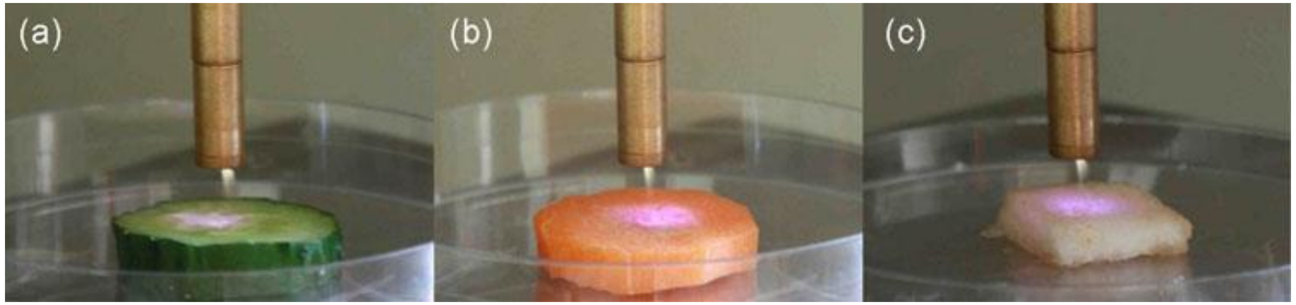
Safe-bag project

decontaminazione di foglie di valeriana



M. Baier et al., Innovative Food Science & Emerging Tech 22 (2014) 147–157

decontaminazione di fette di cetriolo, carota, pera



R.X. Wang et al./ Eur. Phys. J. D (2012) 66: 276

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, PPaP 5, 577, 2008
Isbary et al, Brit. J. Derm. 163, 78,2010
Isbary et al, Clin. Plasma Med. 1, 19, 2013
Laroussi, PPaP 11, 1138, 2014

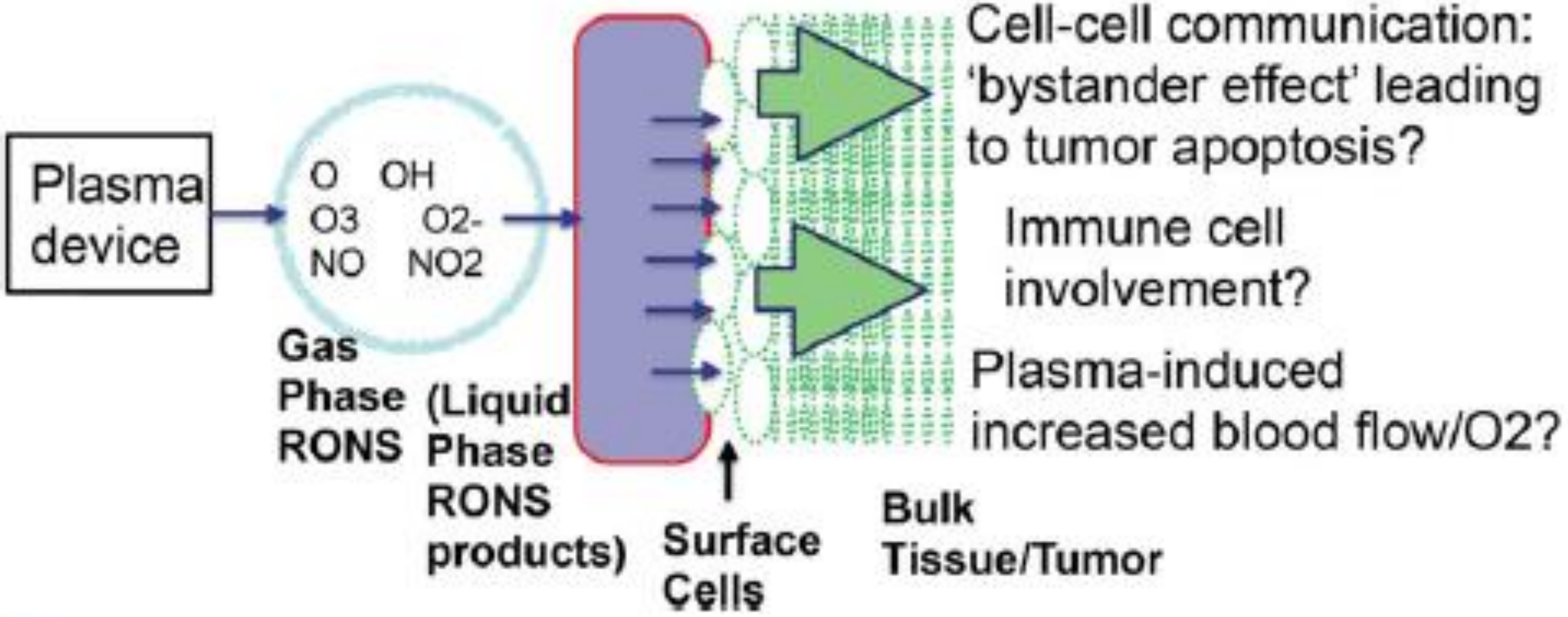


Figure 1. The plasma device operates to generate RONS that either enter a cell surface-covering liquid layer or enter the cells directly. Whatever the effects of the solvated RONS and their products are in the surface layer of cells that are exposed to them, the effects on deeper layers of tissue must involve some cell–cell communication. Some possibilities include mechanisms analogous to radiation-induced "bystander effects," the stimulation and involvement of the immune system, or possibly some effects associated with local blood flow and O_2 concentration.

Graves, PPaP 11, 1120, 2014

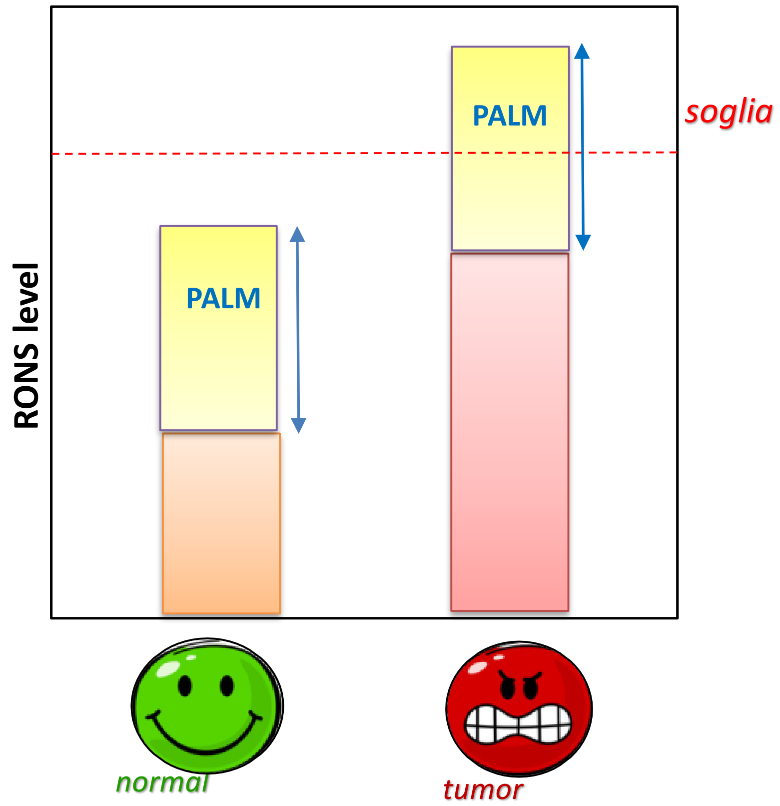
In Plasma Medicine the plasma is generally ignited in air, or in gases (He, Ar, …) in contact with air, to generate primary RONS such as O3, $^{\cdot}OH$, NO, NO_2, …). When these species come in contact with liquids (wound essudate, medium, water, PBS, …), secondary more stable RONS are generated (H_2O_2, NO_2^-, NO_3^-, …)

DIRECT TREATMENTS

the biological target is directly exposed to the plasma (Jet, FE-DBD)

INDIRECT TREATMENTS

a liquid medium (solution, PBS, cell culture media) is exposed to the plasma (Jet, DBD, etc), then the Plasma Activated Liquid Medium (PALM) is used to treat cells, wounds, tissues, etc)



From Killing Bacteria to Destroying Cancer Cells: 20 Years of Plasma Medicine

Mounir Laroussi

1. Preamble

With the advent of atmospheric pressure plasma discharges in the early 1990s various industrial and environmental applications that do not require low pressure operating conditions became possible. Among these the biomedical applications of low temperature plasmas took center stage. First, investigations of the efficacy of plasma to inactivate bacteria were conducted in the mid-1990s[1–6] (and references therein). The dielectric barrier discharge (DBD) was the plasma source used during the early studies. Later on, as plasma jets were developed, these were also used with equal success. The inactivation of bacteria on biotic and abiotic surfaces is useful for applications such as sterilization/decontamination[3,4] and wound healing.[5,7] By the early 2000s, investigations on mammalian cells which showed that under some conditions plasma can affect these types of cells without causing damage were conducted.[8,9] Some of the effects include cell detachment and apoptosis. The period between 2006 and 2013 witnessed two major quantum leaps in medical applications of low temperature plasma (LTP): (i) clinical trials on wound healing were conducted by Isbary et al.;[7] (ii) LTP was shown to be able to cause damage or even destroy cancer cells in vitro and, later, in vivo, by several investigators. First, Yonson et al. in 2006 tested a human hepatocellular carcinoma (HepG2),[10] then other adherent and non-adherent cells lines such as melanoma, glioblastoma, and leukemia cells were used by other investigators.[11–23] These crucial advances breathed great confidence and helped cement the idea that LTP could indeed one day revolutionize health care on several fronts.

In this essay, looking back at the last 20 years of efforts, the author's thoughts on the progress of plasma medicine, and especially on the use of LTP to kill cancer cells, are expressed. These thoughts and opinions include personal reflections and assessment of the field and its prospects for the next decade, especially in regards to the use of LTP in cancer therapy.

2. Historical Perspective: Thoughts and Impressions

It has been about 20 years since the biological and medical applications of low temperature atmospheric pressure plasmas, a field today known as "Plasma Medicine," had its first humble steps. This author's group was fortunate enough to take part and contribute to this exciting multidisciplinary field during its two-decade-long "formative" period. Our early work, mid- to late-1990s, focused on investigating the bacterial inactivation efficacy of LTP while in the last few years, 2010 to the present, we have been focusing more on cancer studies. In between these years, various other topics were entertained and experiments were conducted in our laboratory ranging from wound healing, to destruction of pathogenic proteins that cause neurodegenerative diseases, to dental applications. Each one of these lines of research presented its own set of challenges but also offered many rewarding experiences, the collaboration with biologists, biochemists, and dentists being one of these. During these two decades this author witnessed the incredible scientific progress that the field of plasma medicine had undergone as many groups around the world entered the field and achieved new research milestones. Most rewarding is seeing many colleagues who were somewhat skeptical early on (understandably hesitant) become some of the most ardent supporters of the field and many of them become some of the most productive. But regardless of when one enters a research discipline what is important is to positively contribute to the scientific knowledge that is necessary to carry the field forward and many of these colleagues did just that.

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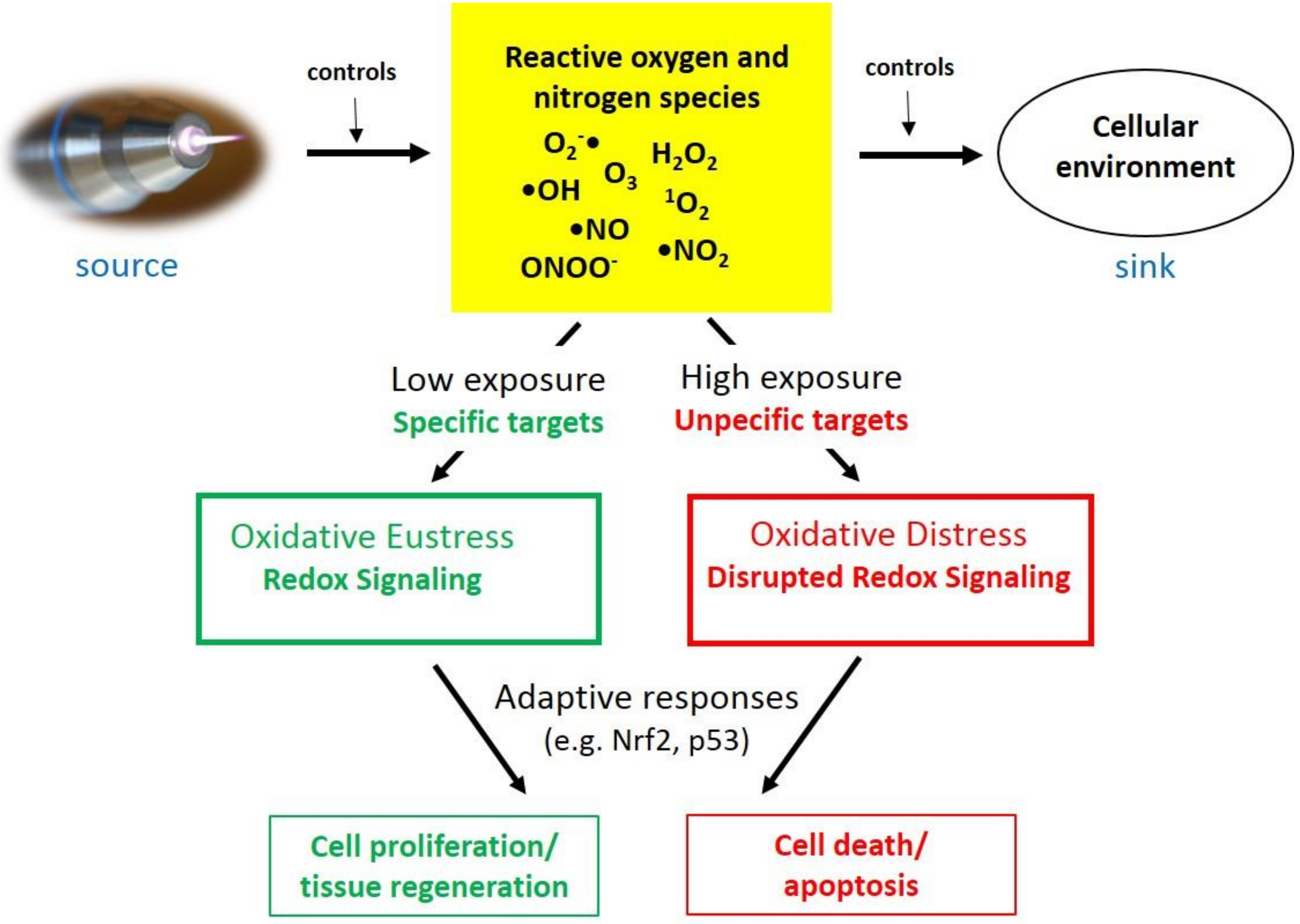
wileyonlinelibrary.com

DOI: 10.1002/ppap.201400152

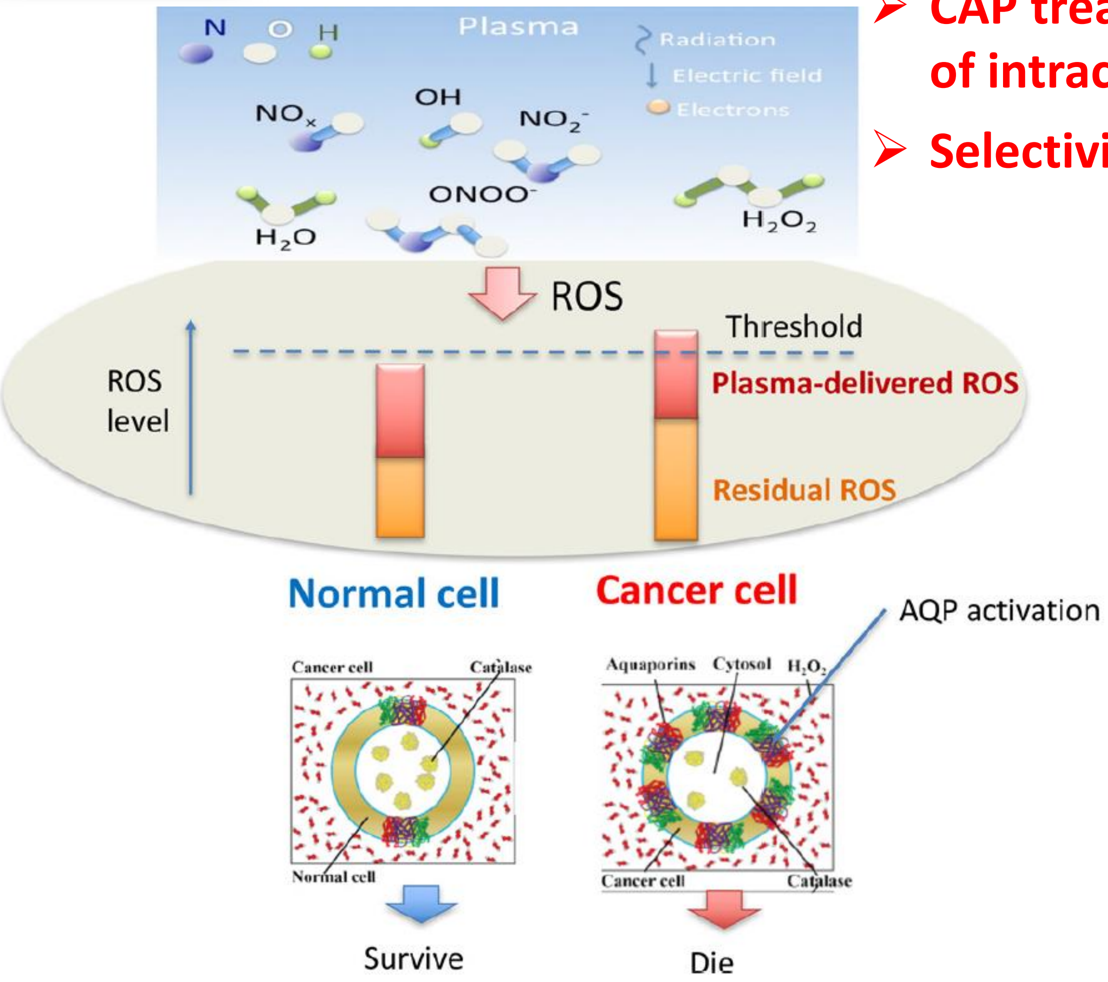
Plasma medicine: applied redox biology



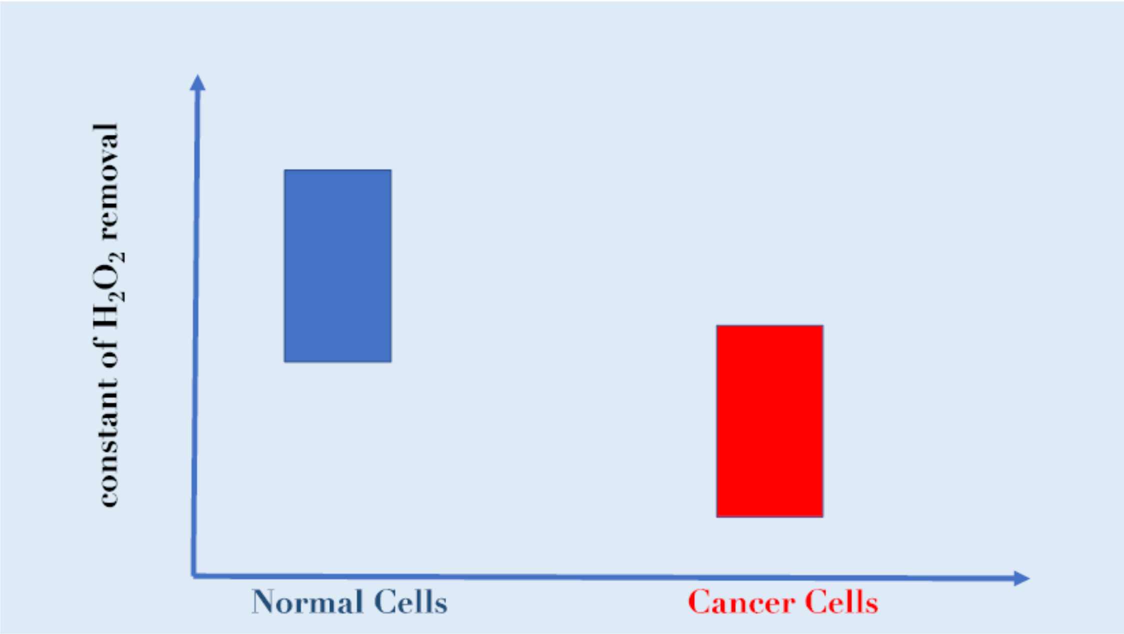
H. Sies, Curr. Opin. Toxicol. 7 (2018) 122-126; Th. von Woedtke, A. Schmidt, S. Bekeschus, K. Wende, K.-D. Weltmann. In Vivo 33 (2019) 1011-1026

Selective inactivation of cancer cells: hypotheses

- **CAP treatment causes apoptosis of cancer cells through a selective rise of intracellular ROS and corresponding ROS-based death pathways.**
- **Selectivity may be based on specific features of cancer cells**



- Enhanced (basic) ROS levels in cancer cells
- higher expression of aquaporins (AQP) by cancer cell membrane

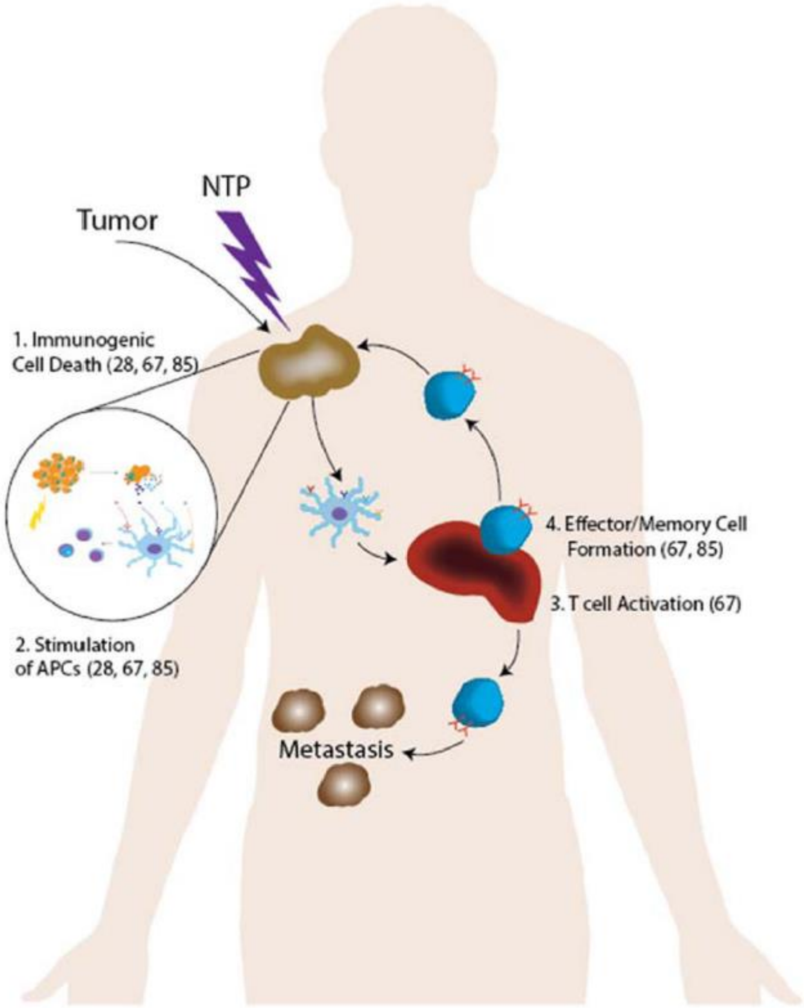


Normal cells have stronger capacity to remove extracellular H_2O_2 than tumor cells. Most cancer cells lacking the biochemical machinery needed to detoxify high fluxes of H_2O_2.

M. Keidar. Physics of Plasmas 25 (2018) 083504

plasma - induced immunogenic cell death (ICD) in cancer

Cold Atmospheric Plasma



M. Khalili, L. Daniels, A. Lin, F.C Krebs, A.E. Snook, S. Bekeschus, W.B Bowne, V. Miller. J. Phys. D: Appl. Phys. 52 (2019) 423001

Potential action of CAP in cancer immunotherapy: CAP both directly stimulates immune cells and induces ICD, resulting in in recruitment and stimulation of antigen presenting cells (APC), memory cell formation, and T cell development. These circulating cells can then target other non-CAP exposed metastatic tumors of the same origin.

www.nature.com/scientificreports

SCIENTIFIC REPORTS

OPEN

Plasma-activated medium triggers cell death and the presentation of immune activating danger signals in melanoma and pancreatic cancer cells

Received: 14 November 2018

Accepted: 18 February 2019

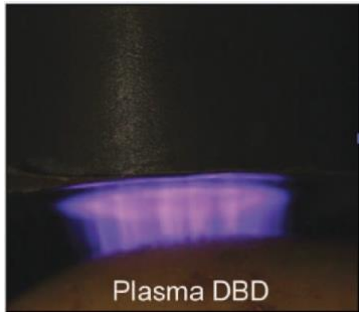
Published online: 11 March 2019

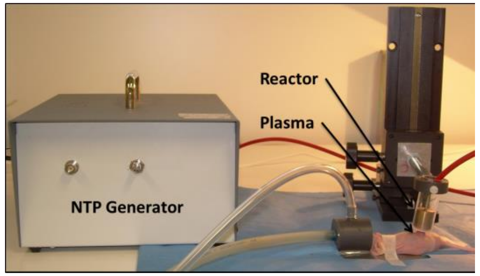
Amalia Azzariti[1], Rosa Maria Iacobazzi[1], Roberta Di Fonte[1], Letizia Porcelli[1], Roberto Gristina[2], Pietro Favia[2,3], Francesco Fracassi[2,4], Ilaria Trizio[4], Nicola Silvestris[5], Gabriella Guida[6], Stefania Tommasi[7] & Eloisa Sardella[2]

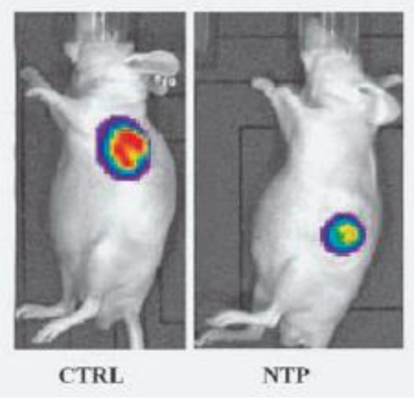
Over the past decade, cold atmospheric plasmas have shown promising application in cancer therapy. The therapeutic use of plasma-activated media is a topic addressed in an emerging field known as plasma pharmacy. In oncology, plasma-activated media are used to harness the therapeutic effects of oxidant species when they come in contact with cancer cells. Among several factors that contribute to the anticancer effect of plasma-activated liquid media (PALM), H_2O_2 and NO derivatives likely play a key role in the apoptotic pathway. Despite the significant amount of literature produced in recent years, a full understanding of the mechanisms by which PALM exert their activity against cancer cells is limited. In this paper, a sealed dielectric-barrier discharge was used to disentangle the effect of reactive nitrogen species (RNS) from that of reactive oxygen species (ROS) on cancer cells. Two cancers characterized by poor prognosis have been investigated: metastatic melanoma and pancreatic cancer. Both tumour models exposed to PALM rich in H_2O_2 showed a reduction in proliferation and an increase in calreticulin exposure and ATP release, suggesting the potential use of activated media as an inducer of immunogenic cell death via activation of the innate immune system.

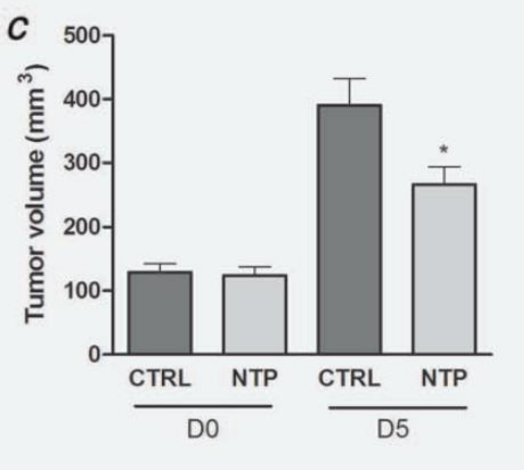
cancer treatment: *in vivo* experiments

- Animal model: Swiss nude mice; achievement of tumor xenografts by sc. injection of tumor cell suspension (glioblastoma U87MG)
- Plasma treatment by FE-DBD (GREMI), 6 min per day, 5 consecutive days (120 J/cm^2/day)



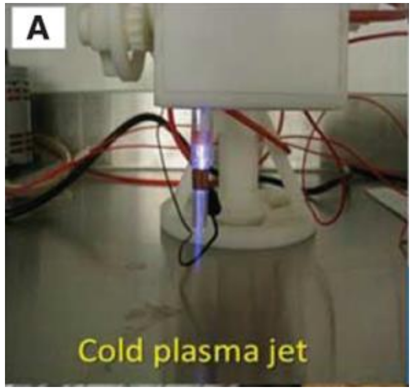






M. Vandamme et al., Int. J. Cancer 130 (2012) 3185-2194

- Murine melanoma model: sc. injection of B16-F10 melanoma cells, treatment once they were 5 mm dia
- Single treatment through intact skin: 5 min by He plasma jet (GWU)



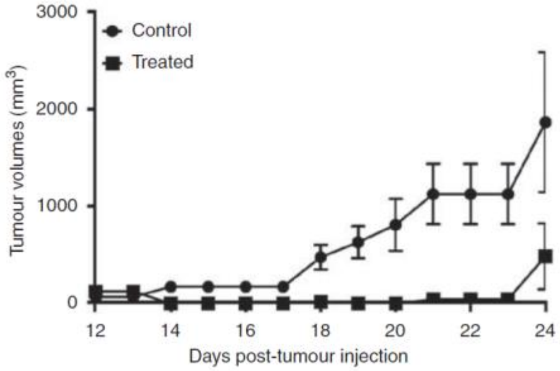


Figure 4 Cold plasma treatment effect on the growth of established tumour in a murine melanoma model.

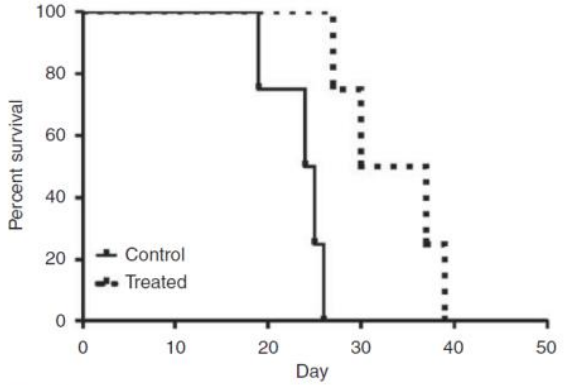
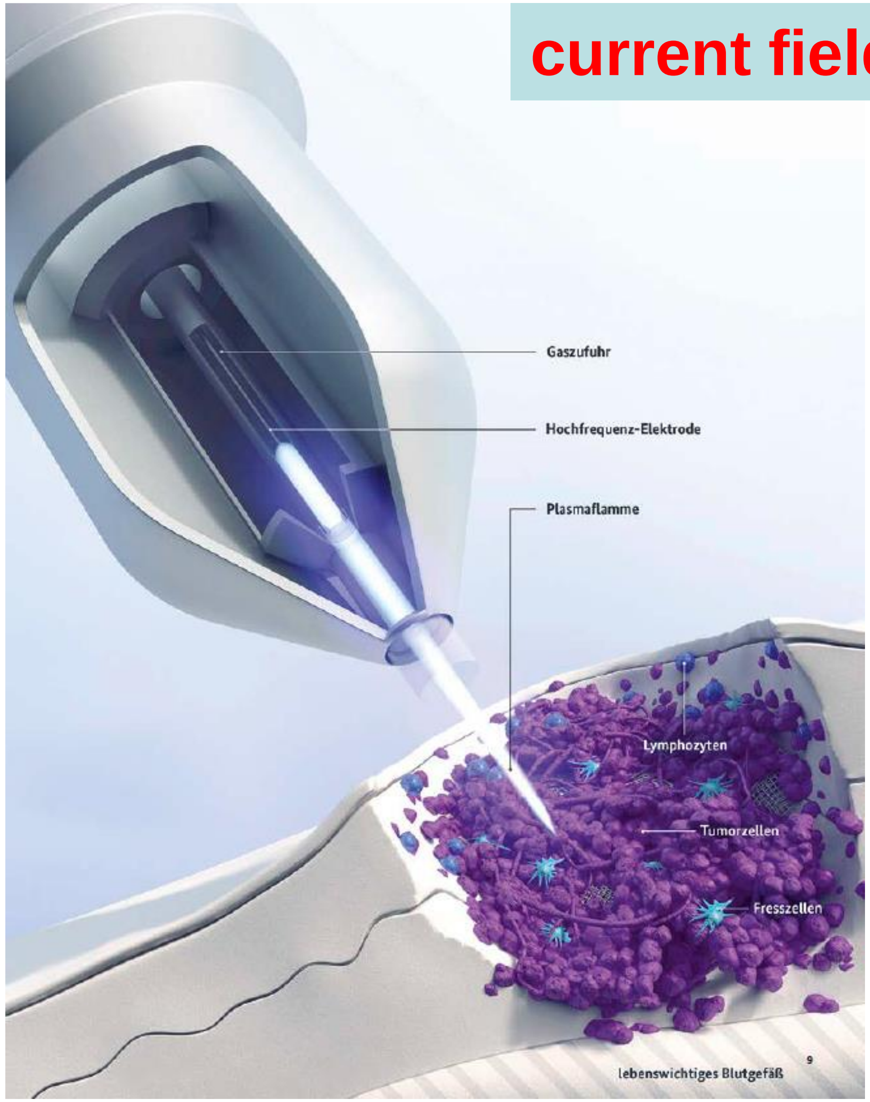


Figure 5 Cold plasma treatment effect on the mice survival in a murine melanoma model.

M. Keidar et al., Brit. J. Cancer 105 (2011) 1295-1301

Clinical plasma medicine: vision - cancer treatment



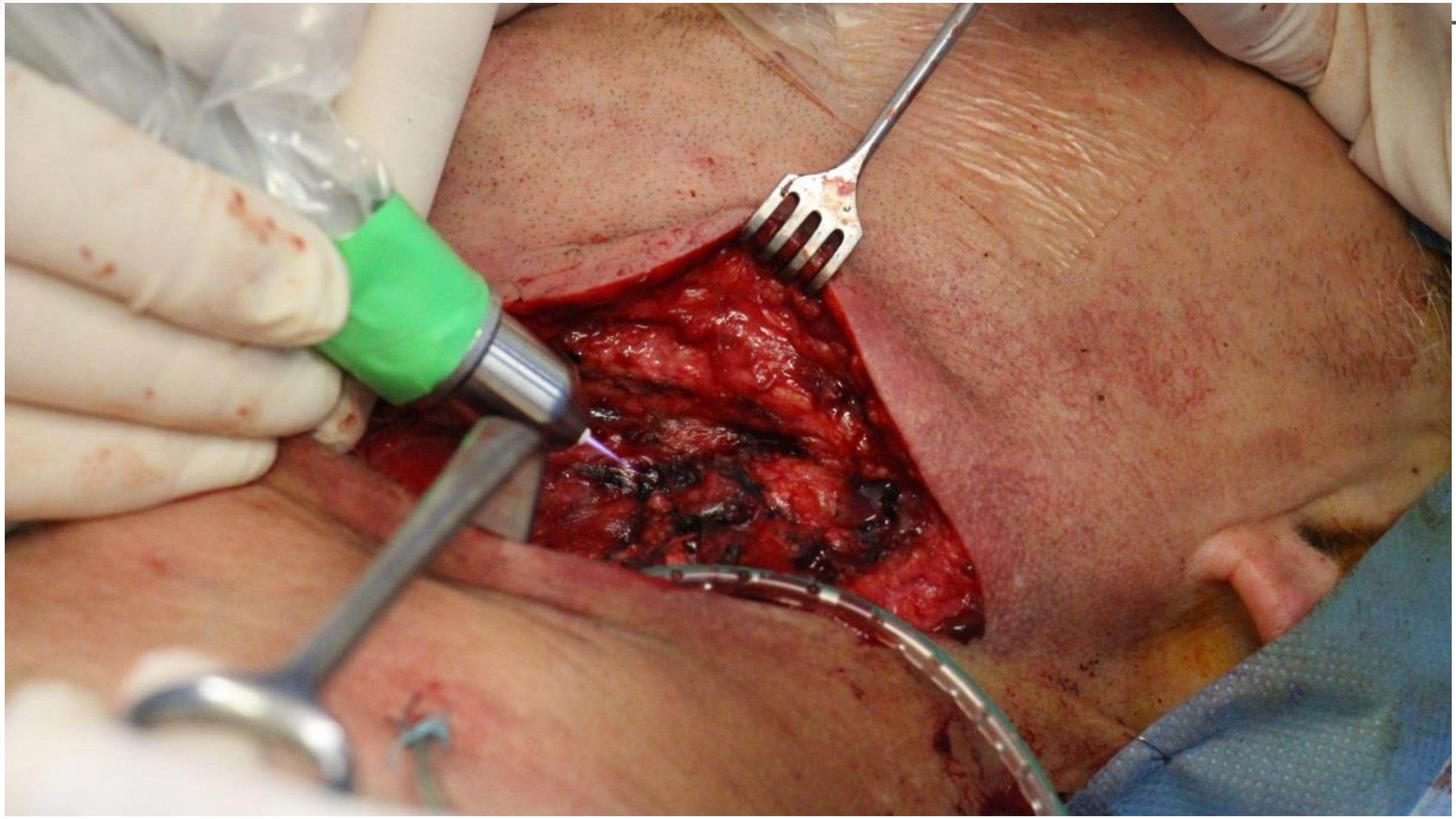
current field of basic and clinical research!

Inactivation of a broad spectrum of microorganisms including multidrug resistant pathogens

Inactivation of cells by initialization of programmed cell death (apoptosis)

Unternehmen Region 2/2016

Plasma Medicine: cancer treatment



KIN PEN plasma source intraoperatively scanning an infected neck lymph node metastasis

C. Seebauer, M. Schuster, R. Rutkowski, M. Mksoud, D.S. Nedrelow, P.H. Metelmann. Clin. Plasma Med. 3 (2015) 93-95

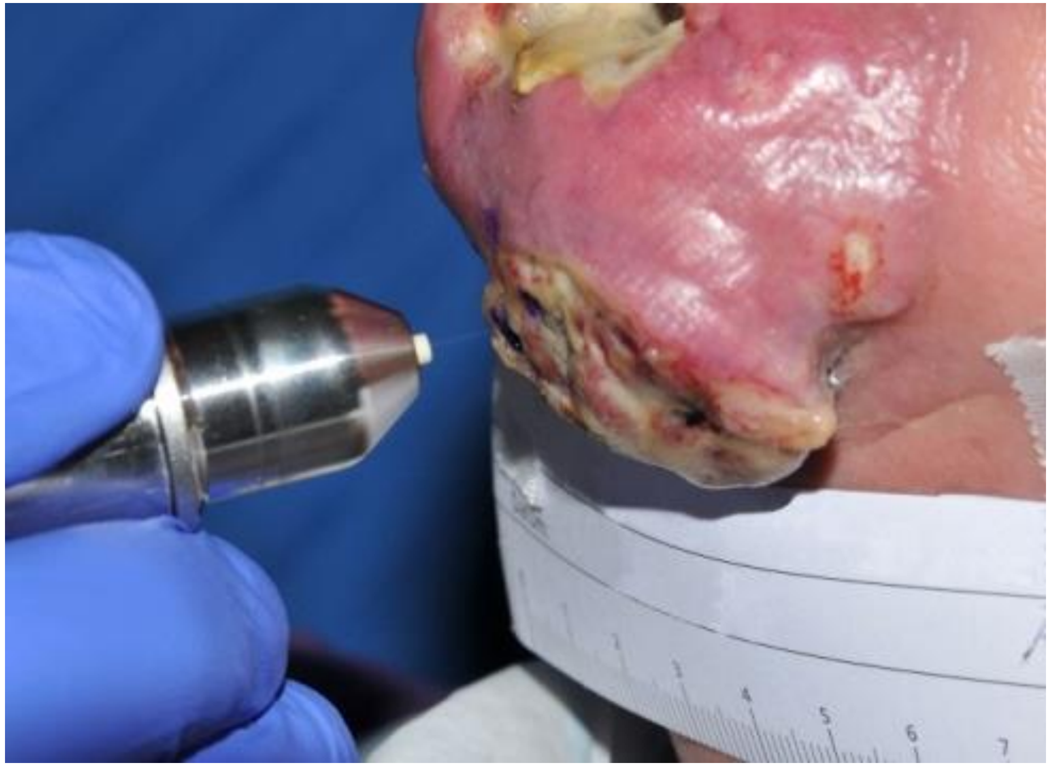
Vision:
CAP application in combination with surgery to inactivate remaining cancer cells in cases where large-scale excision is impossible

Cancer treatment: palliative plasma application

Antiseptic treatment of infected cancer ulcerations as part of palliative medicine program

12 patients; advanced squamous cell carcinoma of the head and neck, intraoral or extraoral ulcerations beyond reach of standard cancer therapies

- gently removal of biofilm covering with gauze
- repeated kinpen MED scanning over the area of the ulceration, 1 min/cm^2
- 1 cycle: 3 single treatments within 1 week, followed by 1 week intermittence
- 1-9 cycles per patient
- 3-18 months clinical follow-up



General results:

(1) **Reduction of microbial load**

(2) **Reduction of typical fetid odor**

(3) Decreased request for pain medication in some cases

(4) Superficial partial remission of tumor

(5) Wound healing of infected ulcerations

Metelmann, …, von Woedtke, Weltmann, et al. Clin. Plasma Med. 3 (2015) 17
von Woedtke & Metelmann, Clin. Plasma Med. 2 (2014) 37

Cancer treatment: palliative plasma application

Antiseptic treatment of infected cancer ulcerations as part of palliative medicine program

H5: 4/2016 6/2016 8/2016

General results:

(1) Reduction of microbial load

(2) Reduction of typical fetid odor

(3) Decreased request for pain medicationin some cases

(4) Superficial partial remission of tumor

(5) Wound healing of infected ulcerations

H.-R. Metelmann, C. Seebauer, V. Miller, A. Fridman, G. Bauer, D.B. Graves, J.-M. Pouvesle, R. Rutkowski, M. Schuster, S. Bekeschus, K. Wende, K. Masur, S. Hasse, T. Gerling, M. Hori, H. Tanaka, E.H. Choi, K.-D. Weltmann, P.H. Metelmann, D.D. Von Hoff, Th. von Woedtke. Clin. Plasma Med. 9 (2018) 6-13

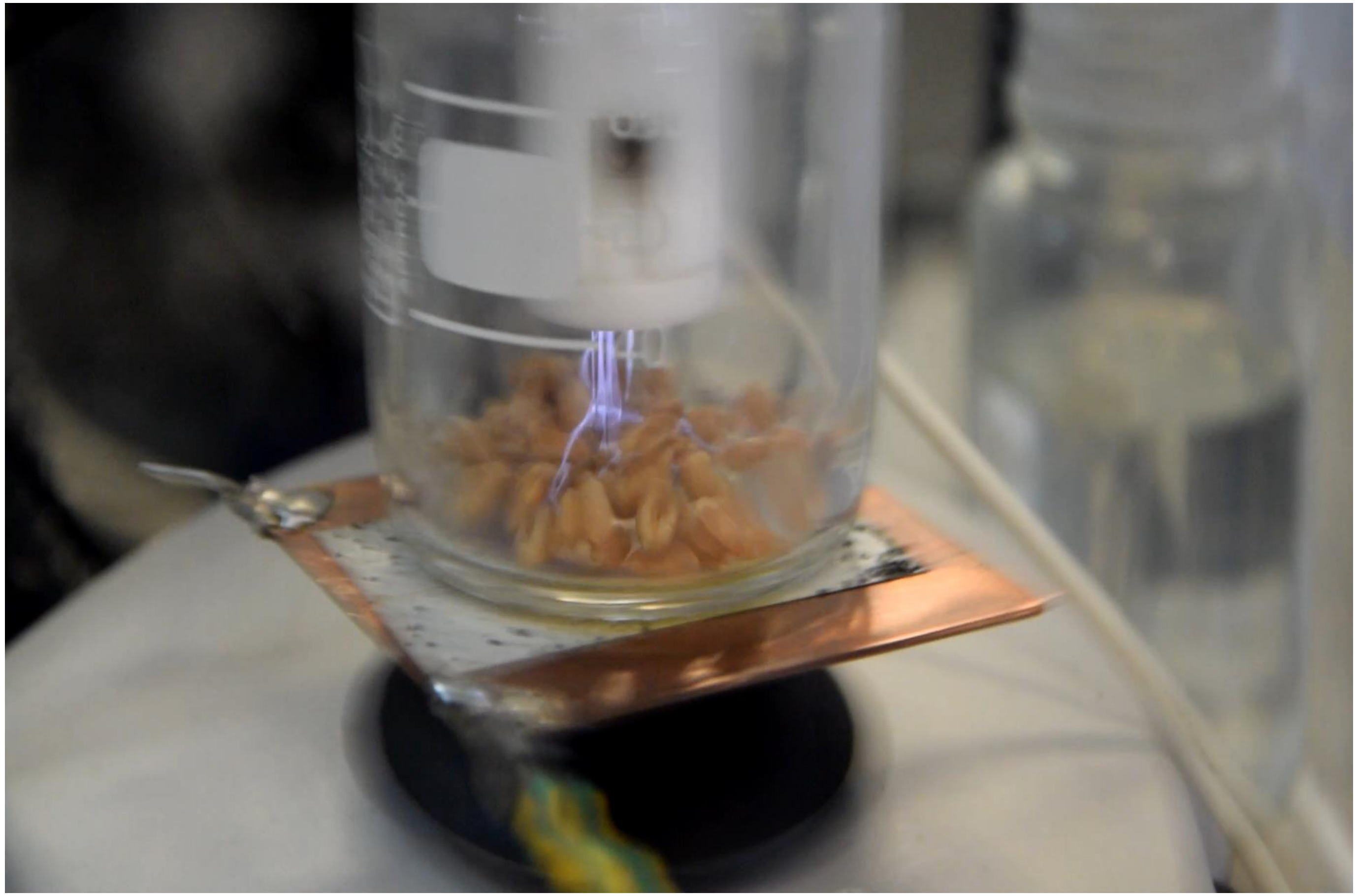
PLASMA AGRICOLTURE

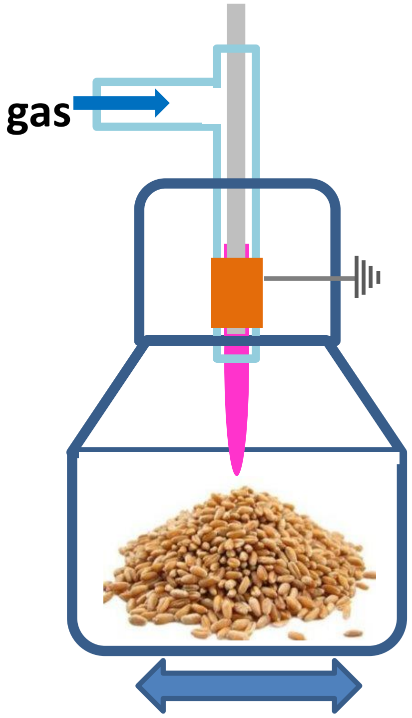
dopo 10 giorni dalla semina

innaffiate con acqua non trattata

… con acqua attivata via plasma in aria per 1 min

- Decontamination/ Sterilization of seeds/fruit/salad/…
- Coatings/treatments (active coatings) od seeds for prolonged shelf life





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

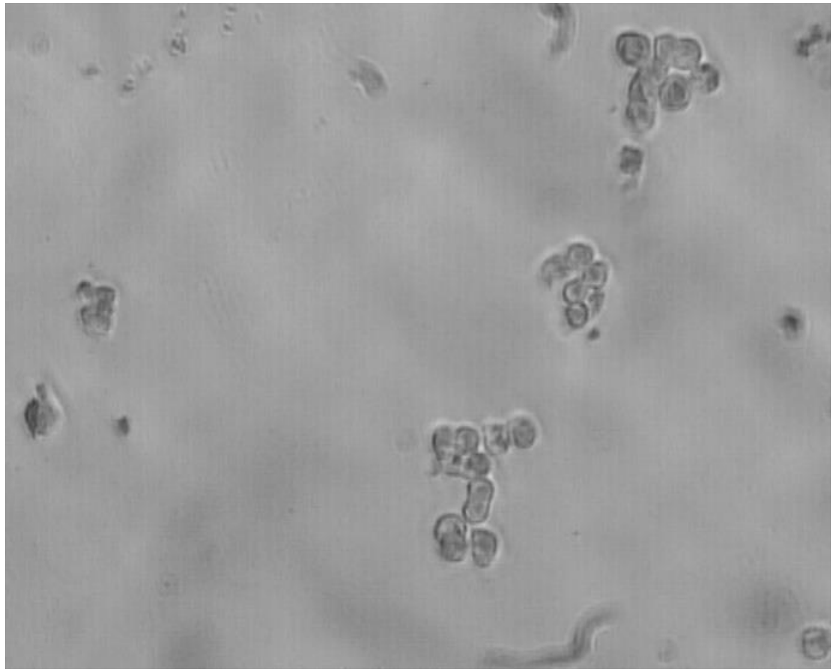
Human Fibroblasts on polystyrene substrates plasma-patterned with cell-adhesive tracks and PE-CVD non fouling domains. Cells grow confined within the 40 μm tracks and avoid the non fouling domains.

Polystyrene cell-culture plates plasma-hydrophilized with an Atmospheric Pressure corona discharge in air.

Sardella et al PPaP 3, 456, 2006

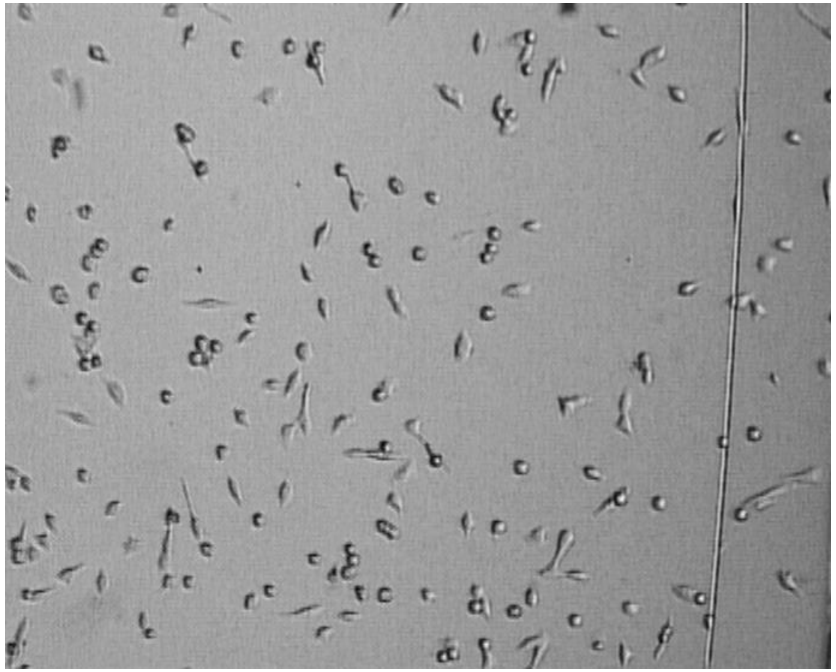
NCTC2544 human keratinocytes on PS and on plasma-treated PS (NH_3 RFGD, grafting of N-containing groups)

$WCA_{adv} \approx 95°$



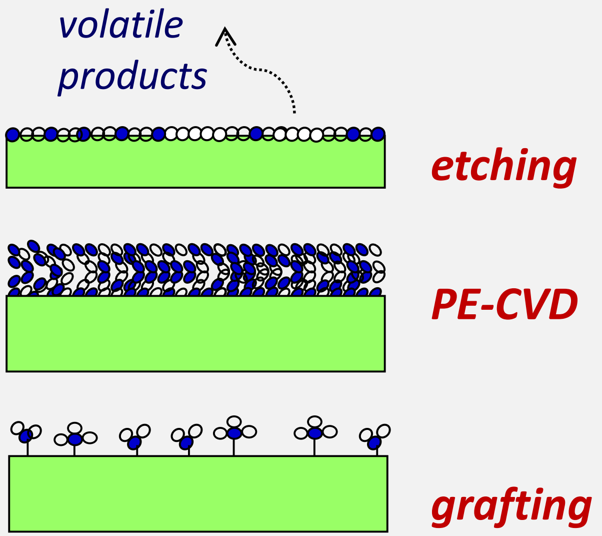
native polystyrene
bact. grade

$WCA_{adv} \approx 35°$

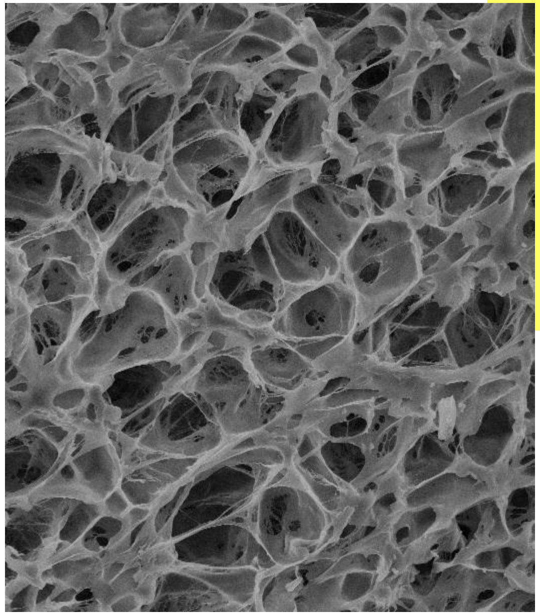


NH_3 plasma-treated polystyrene

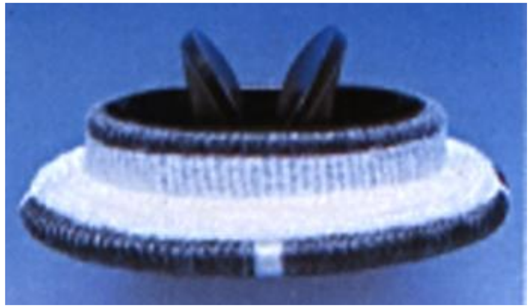
SURFACE MODIFICATION PLASMA PROCESSES

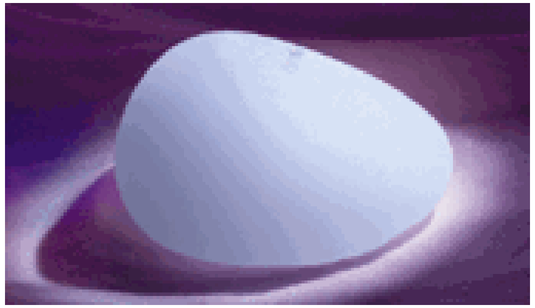


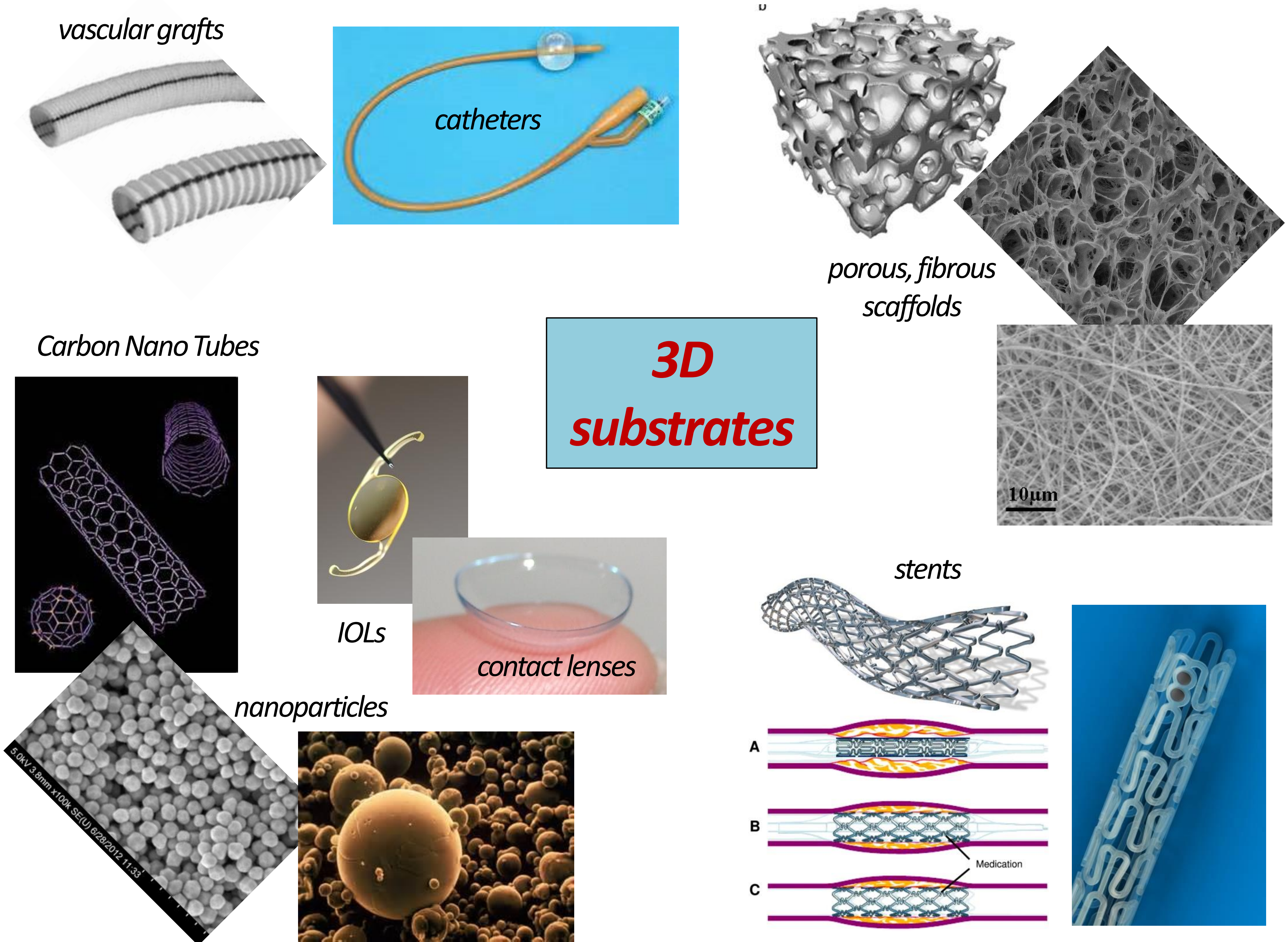
plasma processes are investigated since the 60's for adapting the surface of biomaterials



for
ex vivo in vitro in vivo
short medium long-term
contact with
biological entities



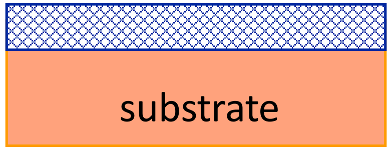



vascular grafts
catheters
porous, fibrous scaffolds
3D substrates
10μm
Carbon Nano Tubes
IOLs
contact lenses
stents
nanoparticles
5.0kV 3.8mm x100k SE(U) 6/28/2012 11:33
A
B
C
Medication

Functionalization by Plasma Enhanced Chemical Vapor Deposition (PE-CVD)

modified thickness 10 nm – 1 μm

dense, cross-linked

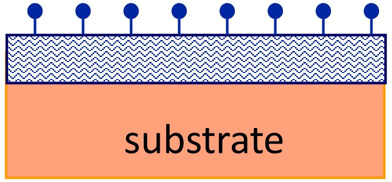


inorganic

DLC, SiOx, …

functionalized

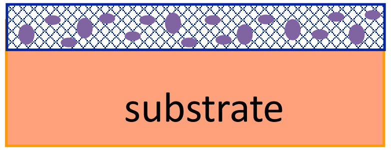
-COOH, $-NH_2$, -OH, >C=O …



organic

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

nano (bio) composite



(bio) organic/inorganic

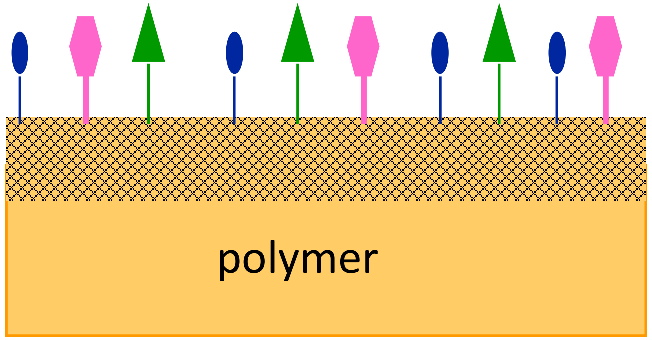
metal/ceramic clusters or biomolecules embedded in a matrix

hydrocarbon/H_2	**glycols**	**combined sputtering/CVD**
organosilicon/O_2	**acrylic acyd**	**AP aerosol**
	C_2H_4/CO_2	**nanoparticle suspension**
	fluorocarbon	**AP aerosol**
		biomolecule solution
	organosilicon	

functionalization by Plasma Treatments

grafting of (polar) functional groups

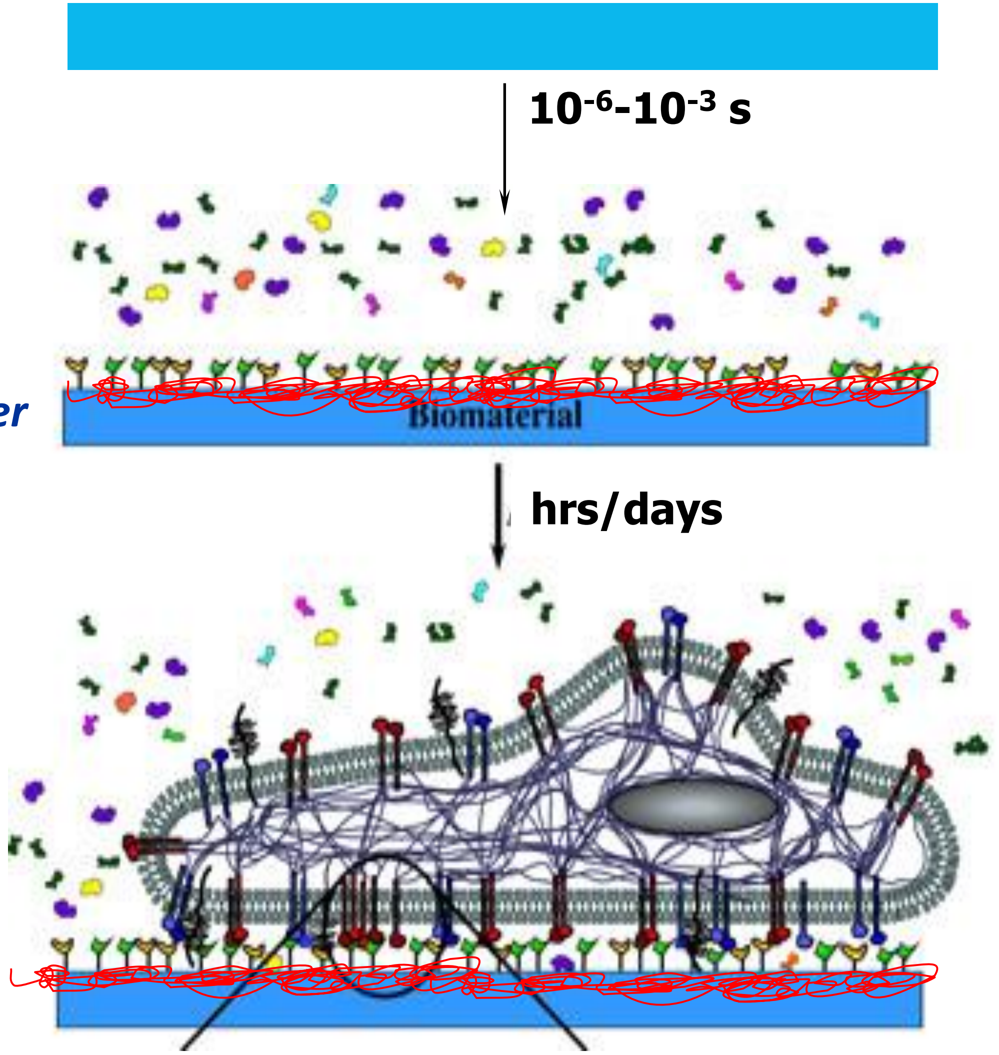
modified thickness 1 – 10 nm



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

surface modification (deposition, etching, grafting) plasma processes can be considered nanotechnologies for the z axis

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.

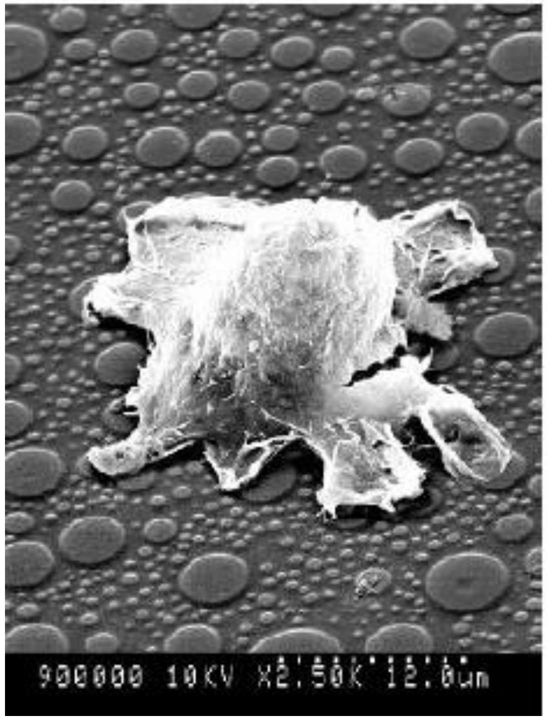
Density and conformation of proteins depend on the SURFACE CHEMISTRY of the substrate

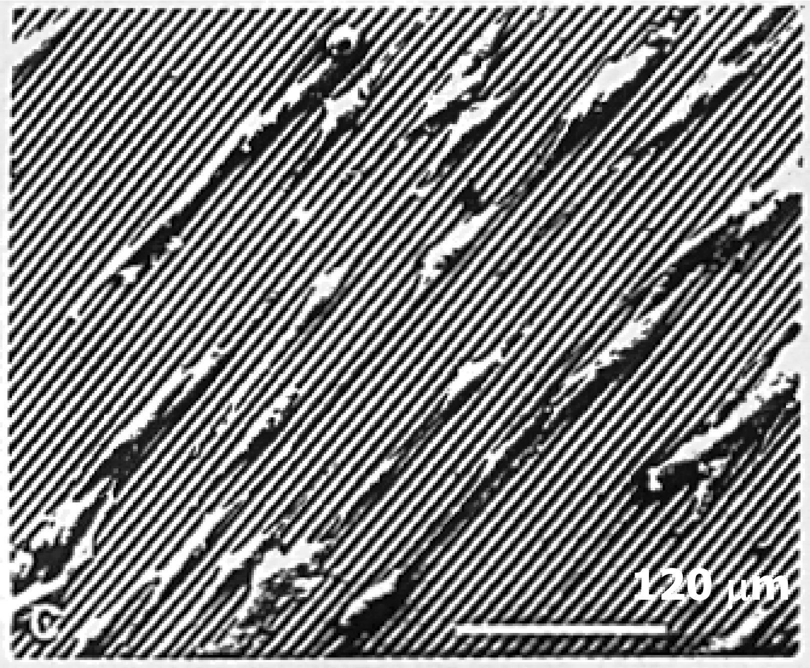
Within the FIRST DAY cells "interrogate" and "recognize" the protein layer through their FOCAL SITES (10-50 nm).

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.





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

CONTACT GUIDANCE

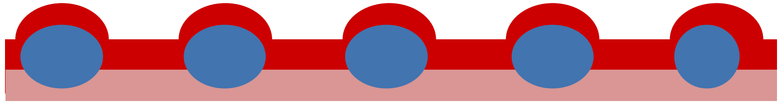
cell adhesion, growth and behaviour is mediated also by constrains induced in the cytoskeleton by MORPHOLOGY, ROUGHNESS, TEXTURE and surface PATTERNS of the substrate material

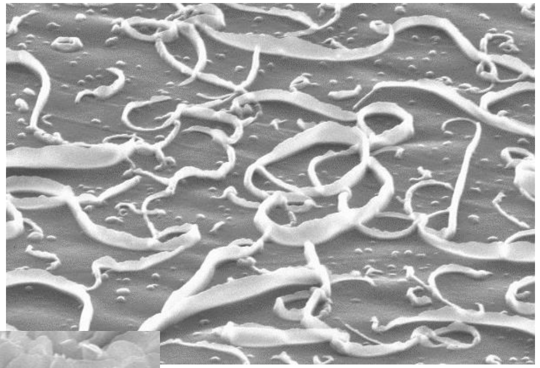
cold plasma can tailor INDEPENDENTLY
surface composition and surface morphology
of substrates

1- substrate

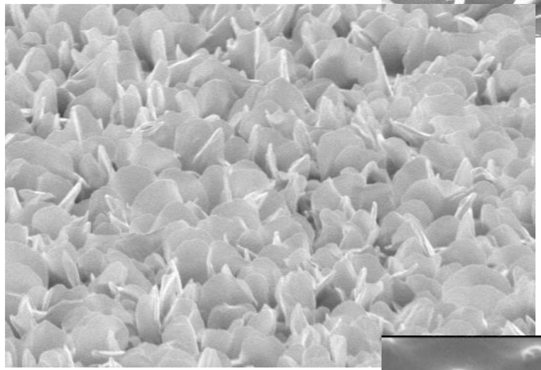
2- change surface morphology

3- change surface chemistry

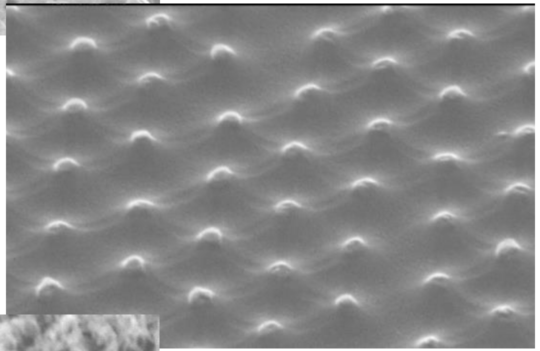




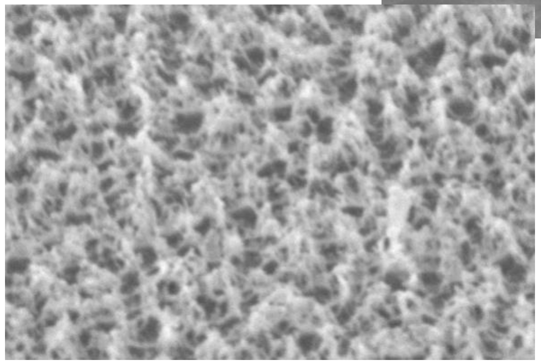
**PTFE-like
ribbon-like ctg**



**PTFE-like
desert rose ctg**



**PET nanostructured
by plasma-aided
colloidal lithography**



**PS nanotextured
CF_4/O_2 etch**

popular PE-CVD coatings for biomaterials research

coating	functional group	monomer	properties
teflon-like	CFx	TFE, HFP, HFE/H_2, HFPO several others	*hydrophobic, inert* *smooth-rough*
PEO-like	-(CH2CH2O)-	$CH_3O(CH_2CH_2O)_nCH_3$ n=2-4	*non fouling, stealth*
pdAA	-COOH	Acrylic Acid C_2H_4/CO_2 …	*cell-adhesion, cell transfer* *immobilization of biomolecules* *acid*
pdAAm	-NH2	allyl amine heptyl amine ….	*cell-adhesion* *immobilization of biomolecules* *basic*
DLC, SiOx, silicone-like, aldehyde, S-containing …			

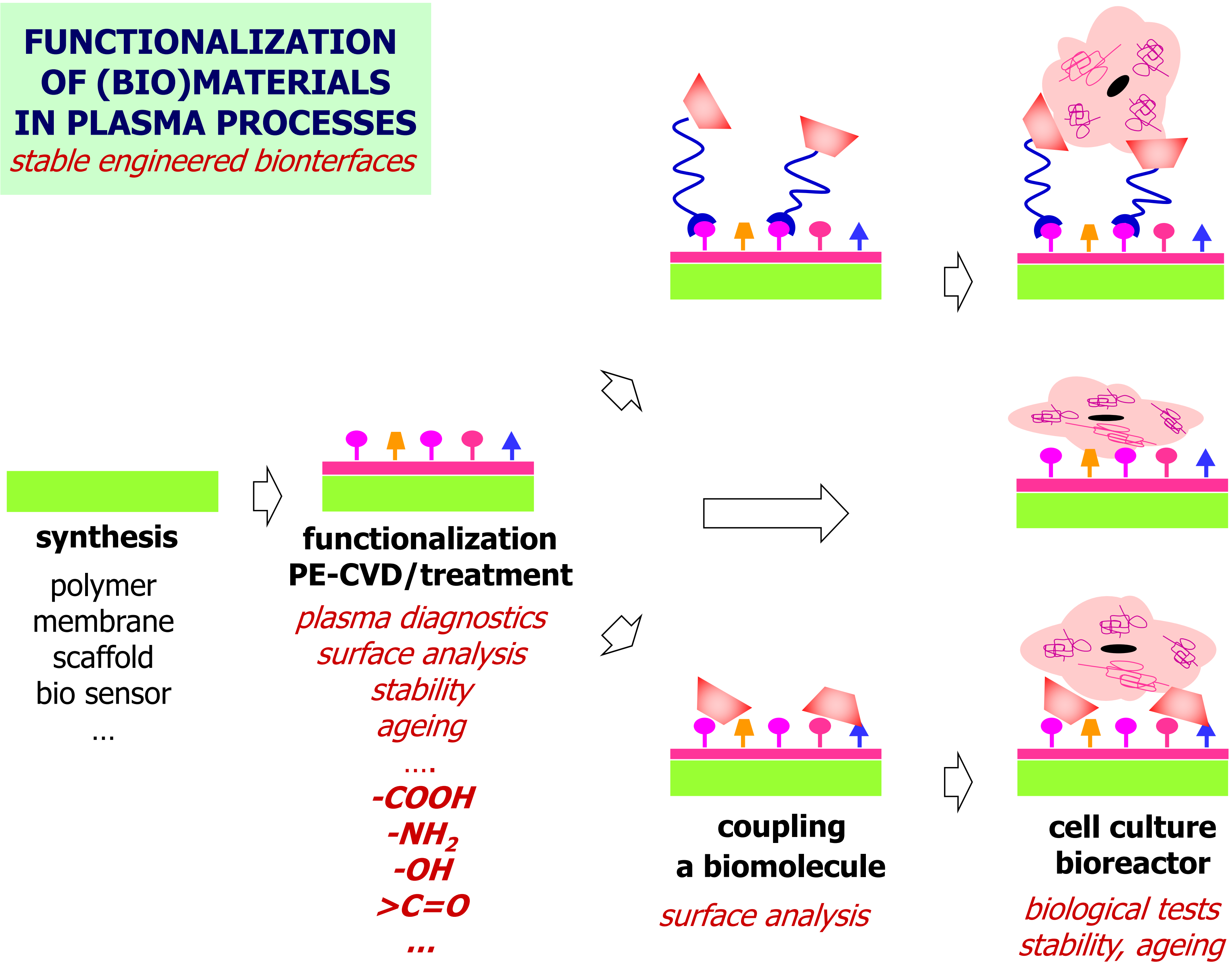
tuning plasma parameters allows to tune the fragmentation of the monomer in the plasma, that is related to the retention of functional groups in the coating

COMMERCIAL BIOMEDICAL SURFACES FROM GLOW DISCHARGES

cell/tissue culture PS plates	hydrophilic, cell-adhesive surfaces
contact lenses	hydrophilic gas-permeable contact lenses confortable for long wear
pacemakers	protective coating on wires
micro-fluidic devices	etched channels, hydrophilic/phobic surfaces at the microscale
lab-on-chip	non fouling coatings
wound healing bandages	transfer of autologous cells from culture to the skin of the patient
....	

SURFACE PROPERTIES AND APPLICATIONS OF BIOMEDICAL INTEREST THAT CAN BE TAILORED VIA PLASMA

- chemical composition
- roughness, morphology, texture, patterns
- hydrophobicity / hydrophilicity
- acid / basic character
- mechanical, elasticity
- cell adhesion/growth
- 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
- drug release


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

film cell-adhesive da acido acrilico (pdAA)

Film funzionali con gruppi acidi -COOH

Idrofili

Gruppi -COOH per immobilizzazione di biomolecole

Precursori

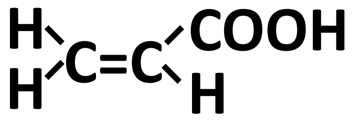
Acidi volatili R-COOH

Anidridi volatili

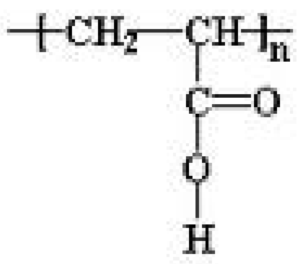
Parametri chiave

Ritenzione di gruppi –COOH nel film

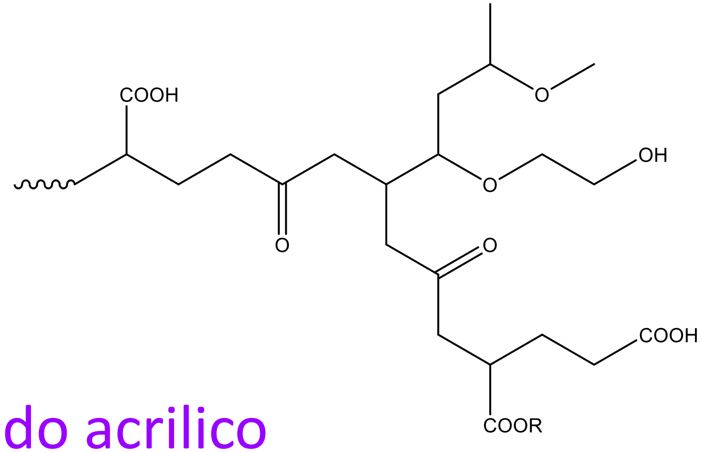
Stabilità in acqua



Acido Acrilico (AA)



Poli-Acido Acrilico (pAA)

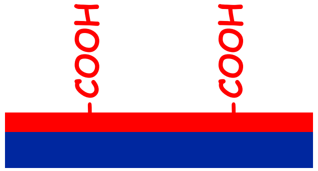


Acido acrilico depositato via plasma (pdAA)

film cell-adhesive da acido acrilico (pdAA)

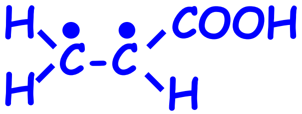
Alta
frammentazione

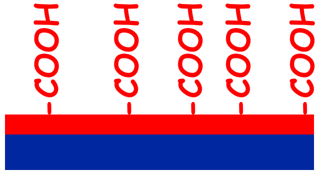
H, H_2, C, CO,…
O, CO_2, OH,…



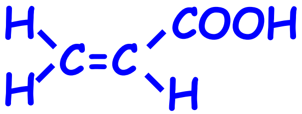
bassa (o assente) ritenzione di struttura del monomero

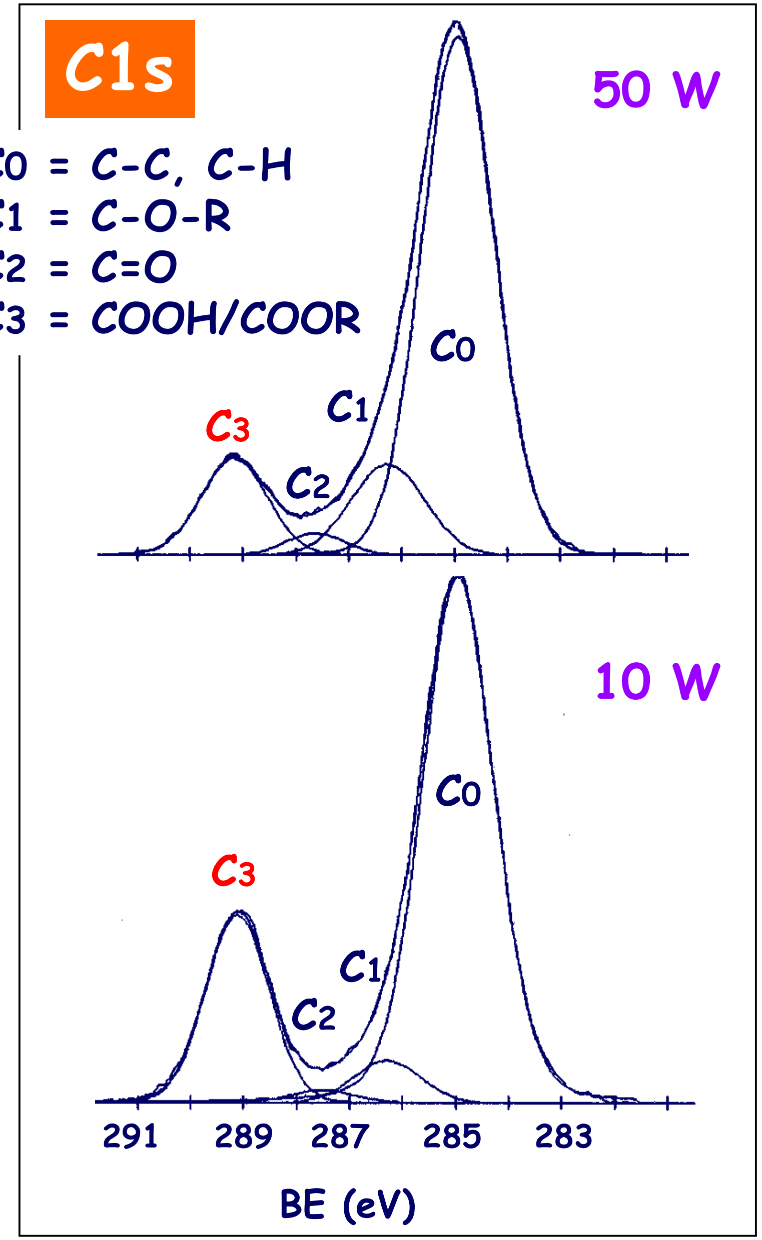
Bassa
frammentazione



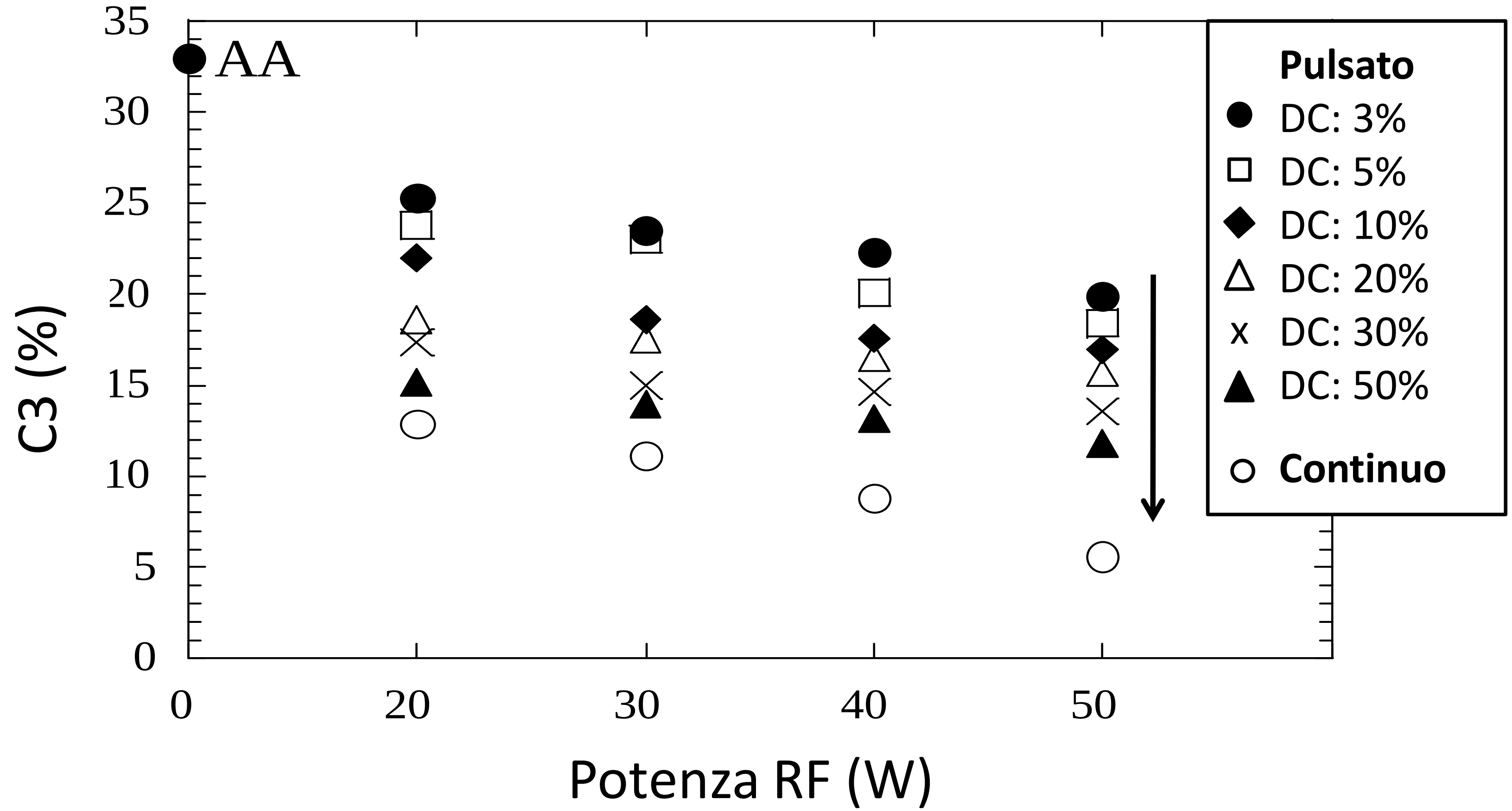


elevata ritenzione di struttura del monomero





film cell-adhesive da acido acrilico (pdAA)

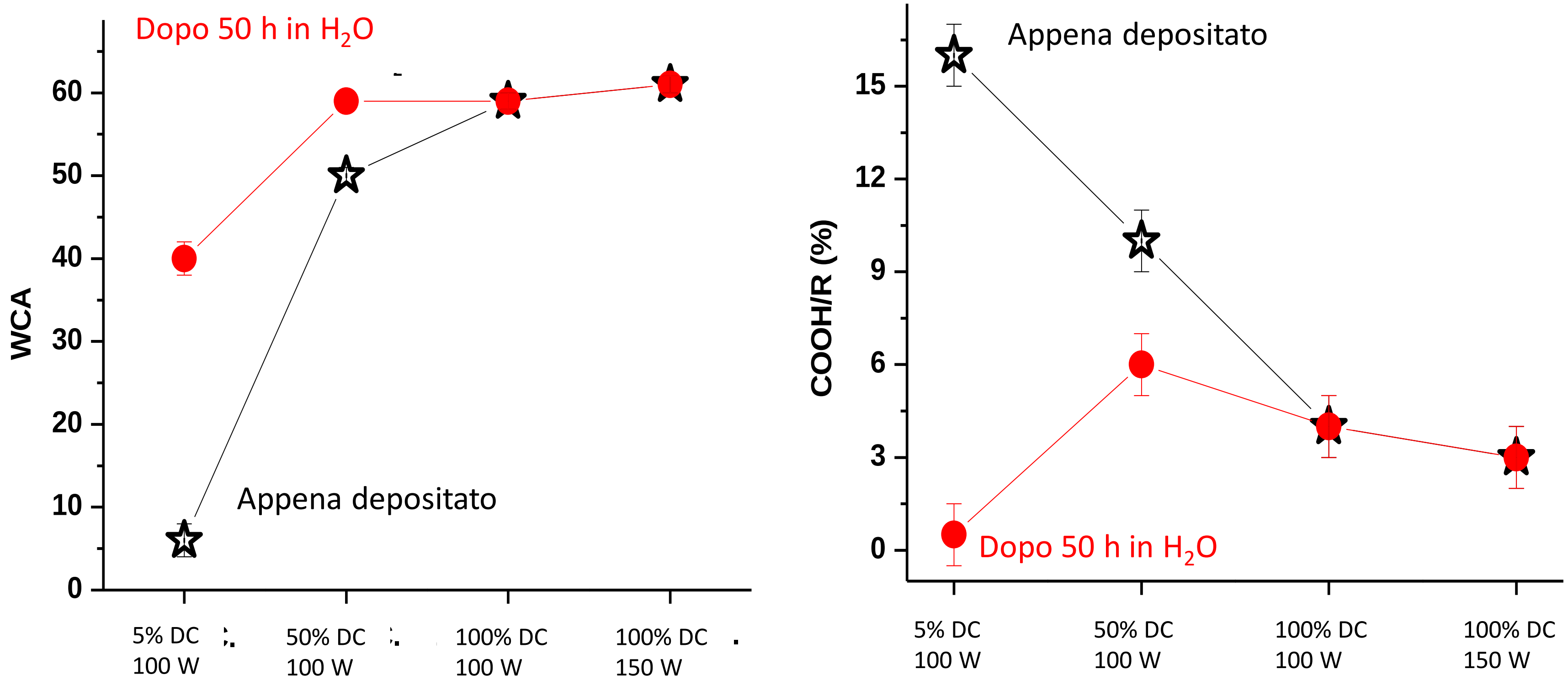


Duty Cycle (DC) = $(t_{ON}/period)*100$

$W_{pulsato} = DC * W_{continuo}/100$

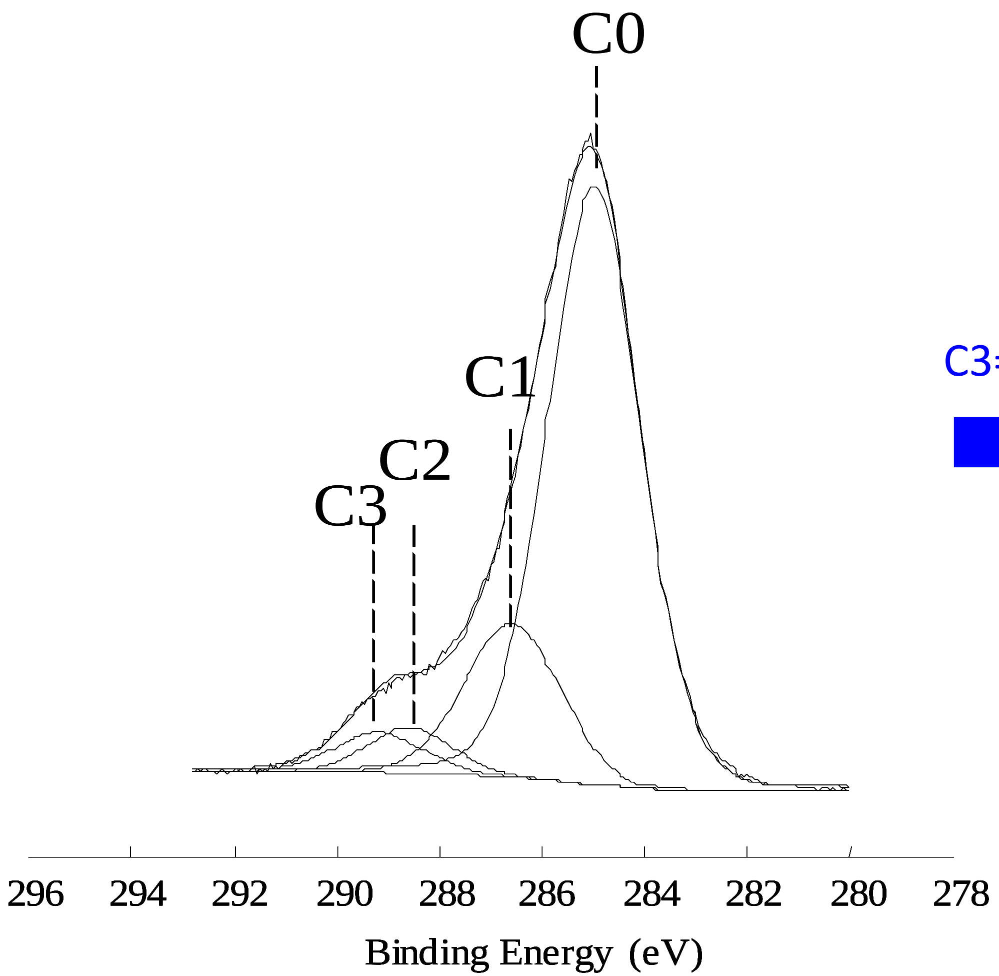
- PLASMI CONTINUI: Potenza ↑, % gruppi –COOH ↓
- PLASMI PULSATI: Duty Cycle (e Potenza)↑, % gruppi –COOH ↓

film cell-adhesive da acido acrilico (pdAA)

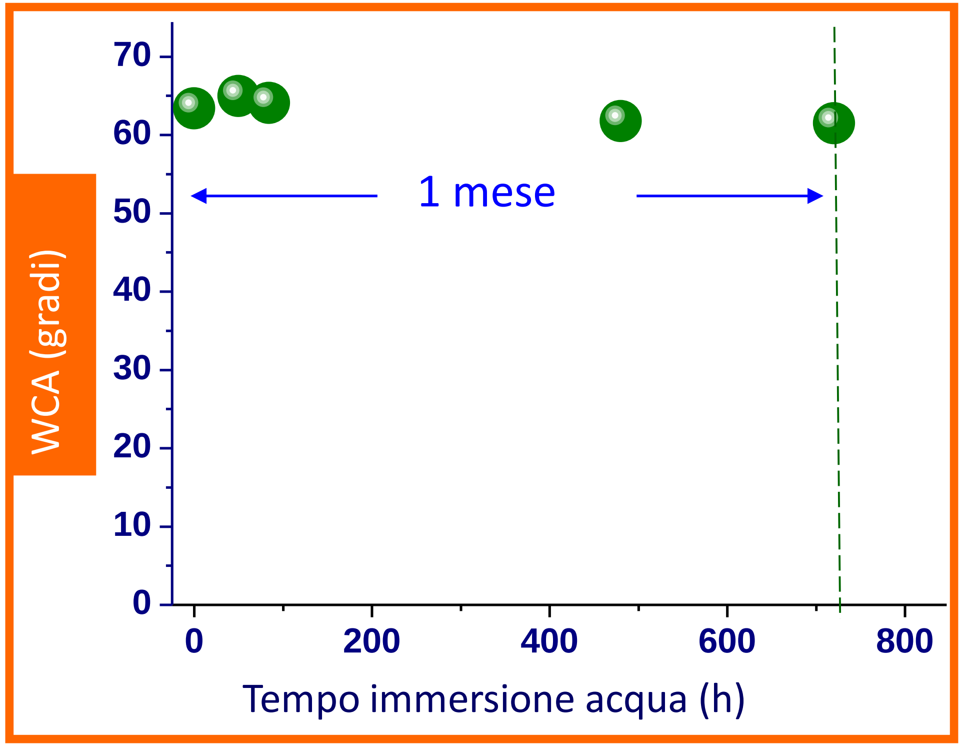


- I film più stabili nel tempo sono quelli con pochi gruppi –COOH
- I film con troppi gruppi –COOH si sciolgono e si delaminano

film cell-adhesive di acido acrilico (pdAA): stabilità in acqua

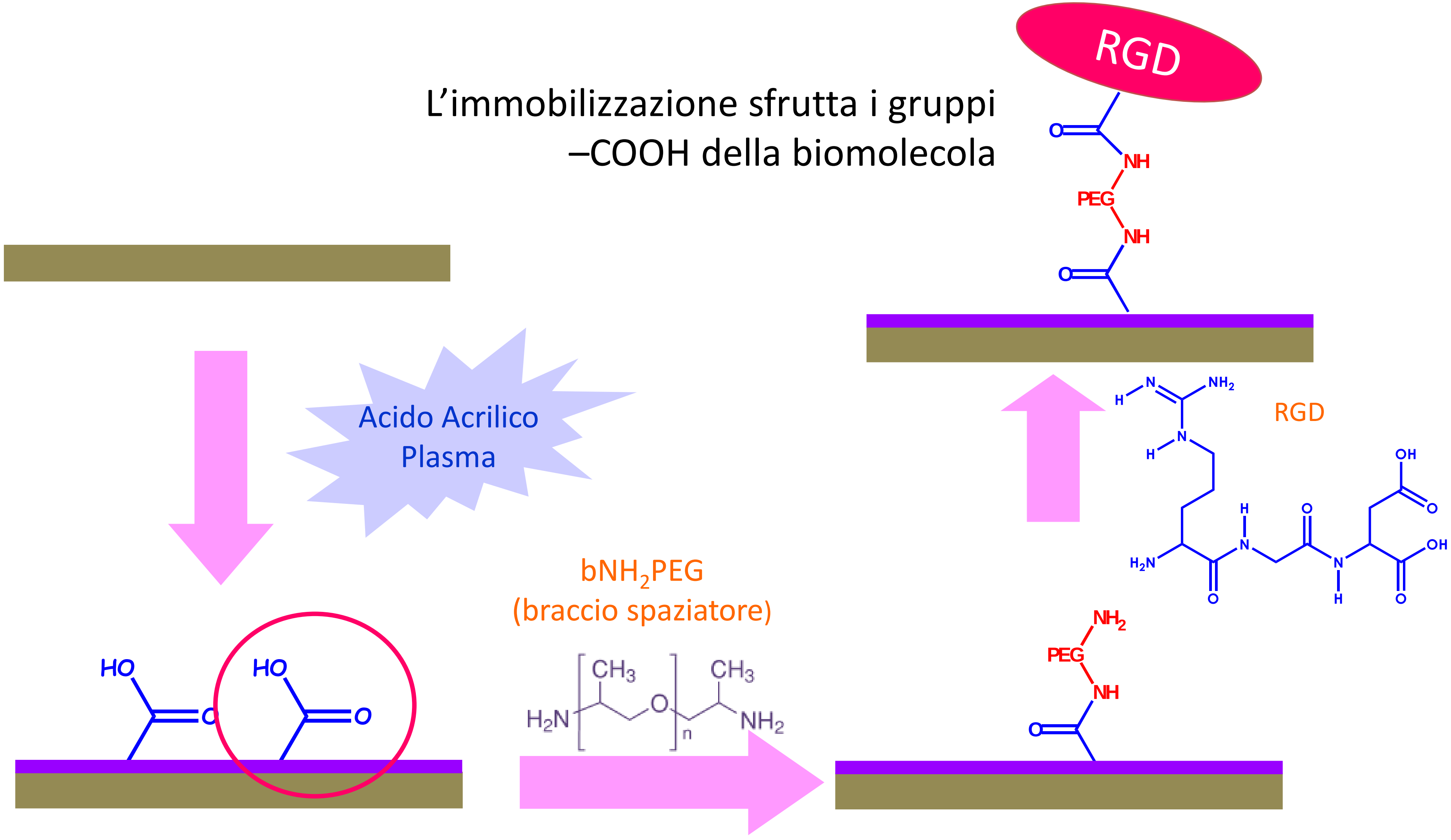


C3= 4%



film cell-adhesive di acido acrilico (pdAA): immobilizzazione biomolecole

L’immobilizzazione sfrutta i gruppi –COOH della biomolecola

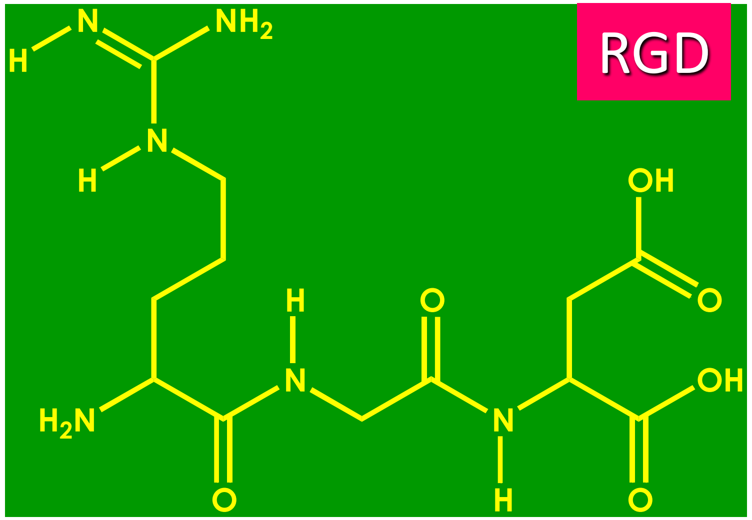


BIOMOLECULES → ECM

RGD (ARG-GLY-ASP) PEPTIDES

minimum adhesion domain in
Extra Cellular Matrix proteins
fibronectin, collagen, vitronectin

RGD

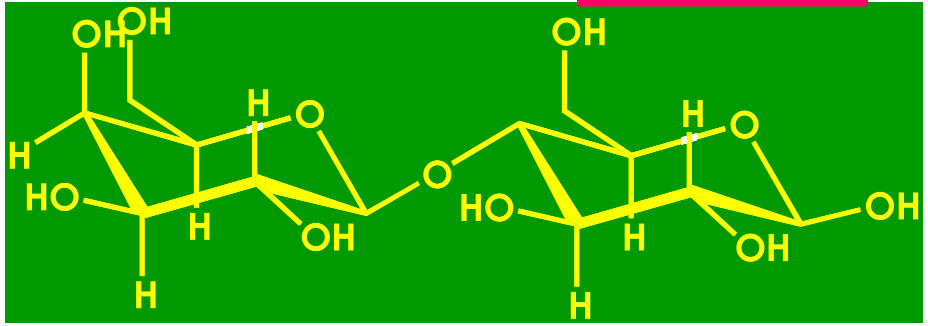


* Pierschbacher, Ruoslathi *Nature*, 309, 30, 1984
* Hersel, Dahmen, *Biomaterials*, 24, 4385, 2003

CARBOHYDRATES

galactose, galactonic acid,
gatactosamine, lactose, …
present in the Extra Cellular Matrix
are recognised by specific
cell membrane receptors

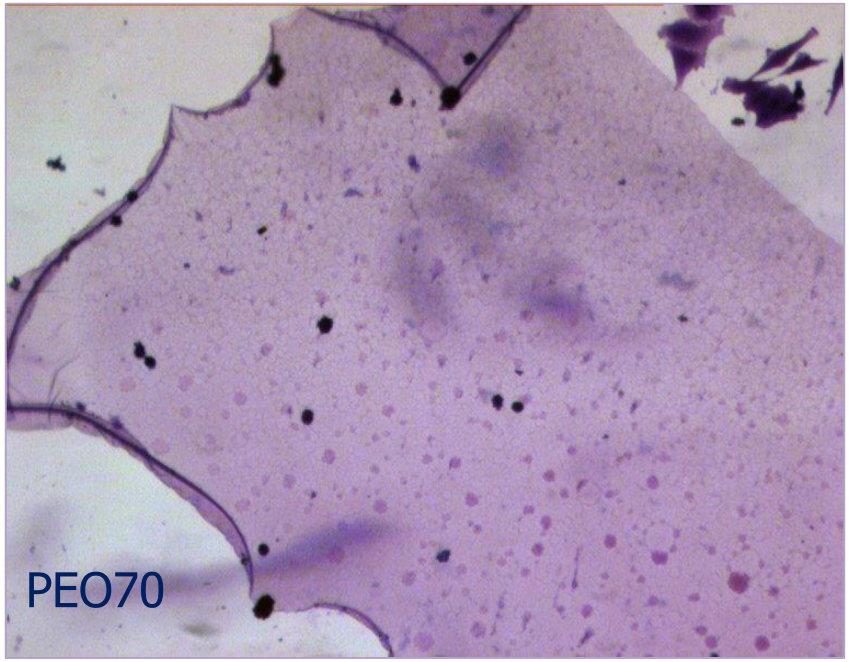
ß-Lactose

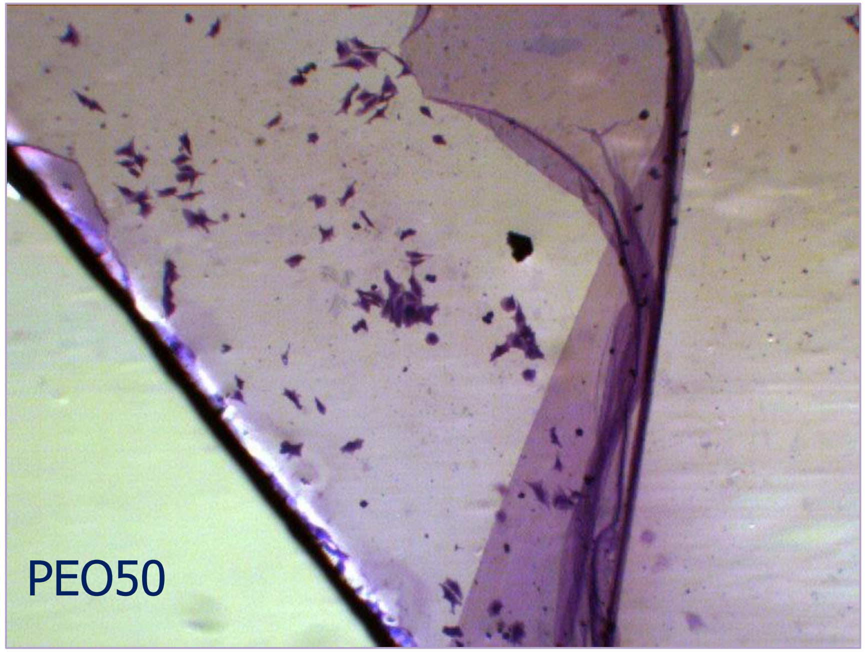


Park, J. Biomed. Mater. Res, 2002, 59 (1), 127-135

delamination of PE-CVD coatings in water … it should not happen !!

delamination of AP aerosol-assisted PE-CVD cell-adhesive PEO-like ctgs in cell-culture media (PS/glass substrate, SaoS2 cells)

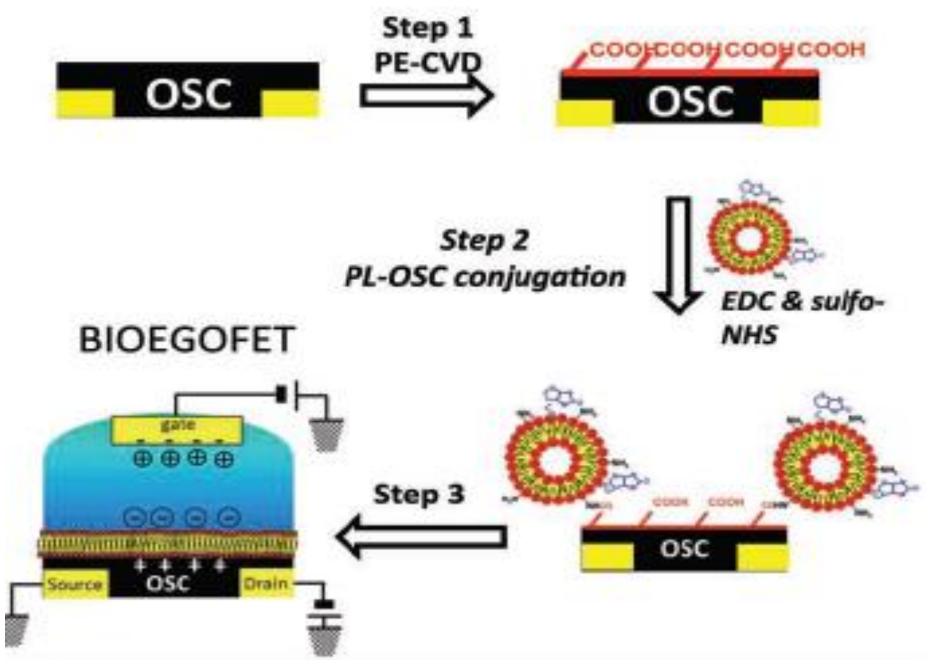


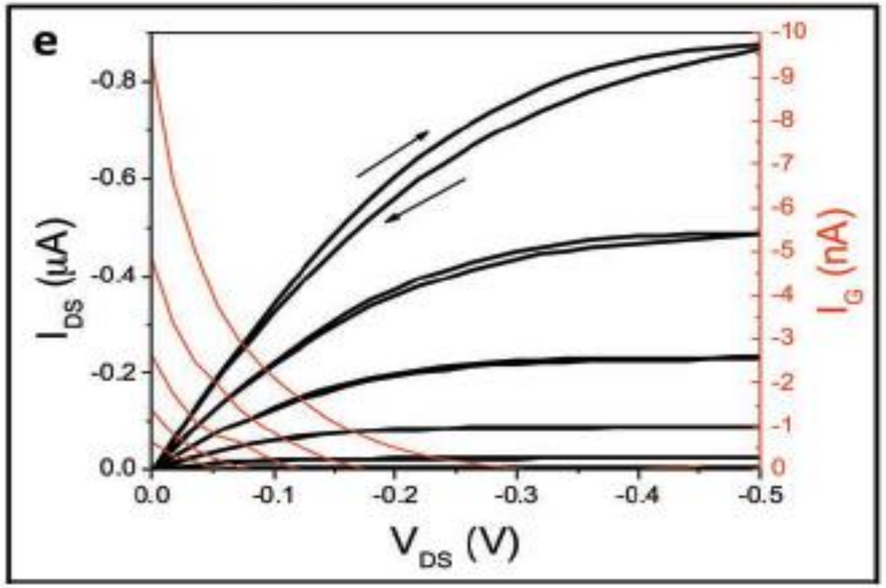


Da Ponte et al PPP 9, 1176, 2012
Da Ponte, Favia, Sardella, Gristina et al, in preparation

problem solved with a graded C_2H_4/TEGDME "primer" coating

scheme of phospholipid BIOEGOFET fabrication

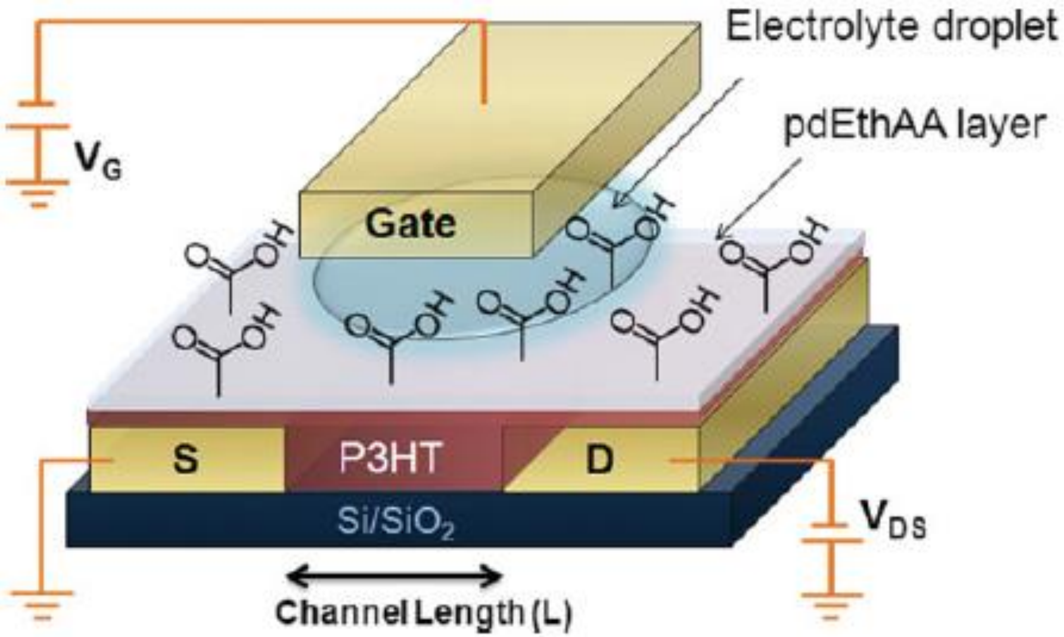


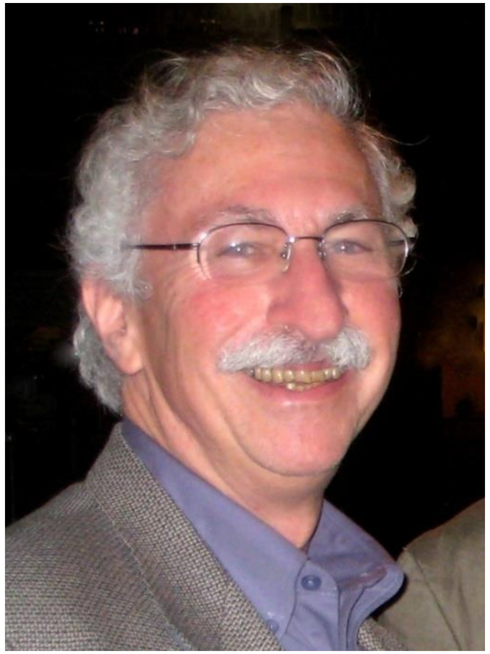


plasma functionalization of Organic SemiConductors in biosensors

immobilization of phospholipids on the OSC layer of EGOFET biosensors coated with a **very thin –COOH rich water stable** LP plasma-functionalized coating

Magliulo et al, PPP 10, 102, 2013
Magliulo et al, Adv. Mat. 25, 2090, 2013
Palazzo et al, Adv. Mat. 27, 911, 2015





Low Pressure PE-CVD of PEO-like coatings

B.D. Ratner *et al.*
J. Biom. Mat. Res., 26, 415, 1992

PEO-like COATINGS

non fouling, hydrophilic,
stealth, cell-repulsive

feed

glycols, crown ethers

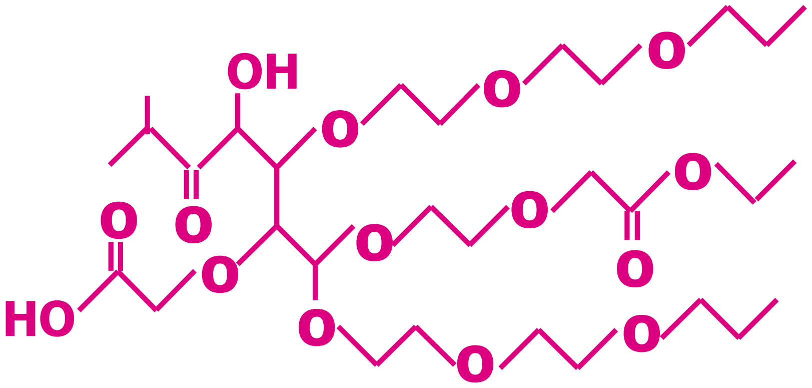
$CH_3O(CH_2CH_2O)_nCH_3$

Key Parameter

retention of the PEO
structure in the coating

PEO $-(CH_2CH_2O)_n-$

PEO-like

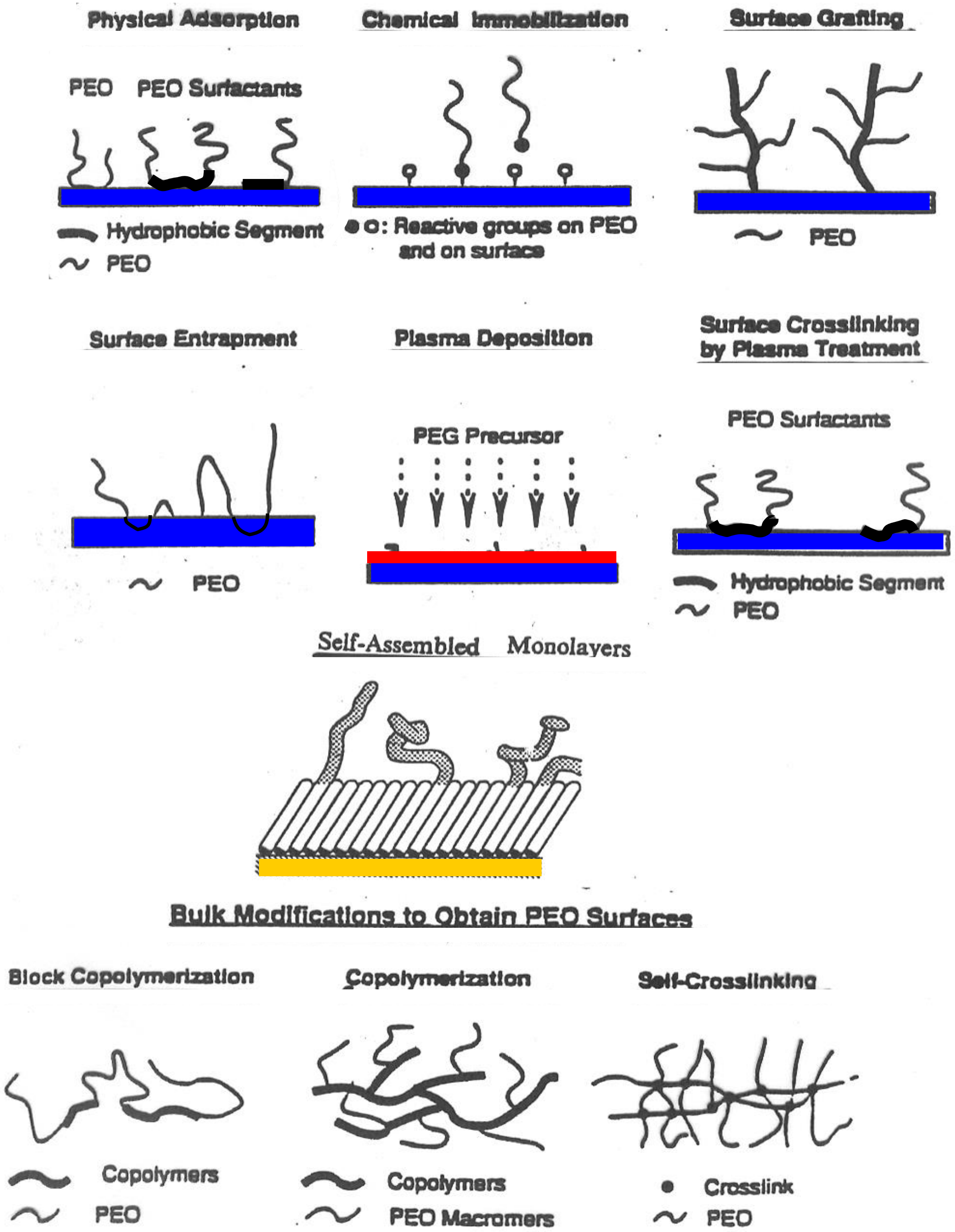


protein/cell repulsive surfaces

surface and bulk modification of polymers to yield PEO surfaces

courtesy of Allan Hoffman

$-CH_2CH_2O-$



PEO-like COATINGS: effect of RF input power

$CH_3O(CH_2CH_2O)_2CH_3$ DEGDME monomer

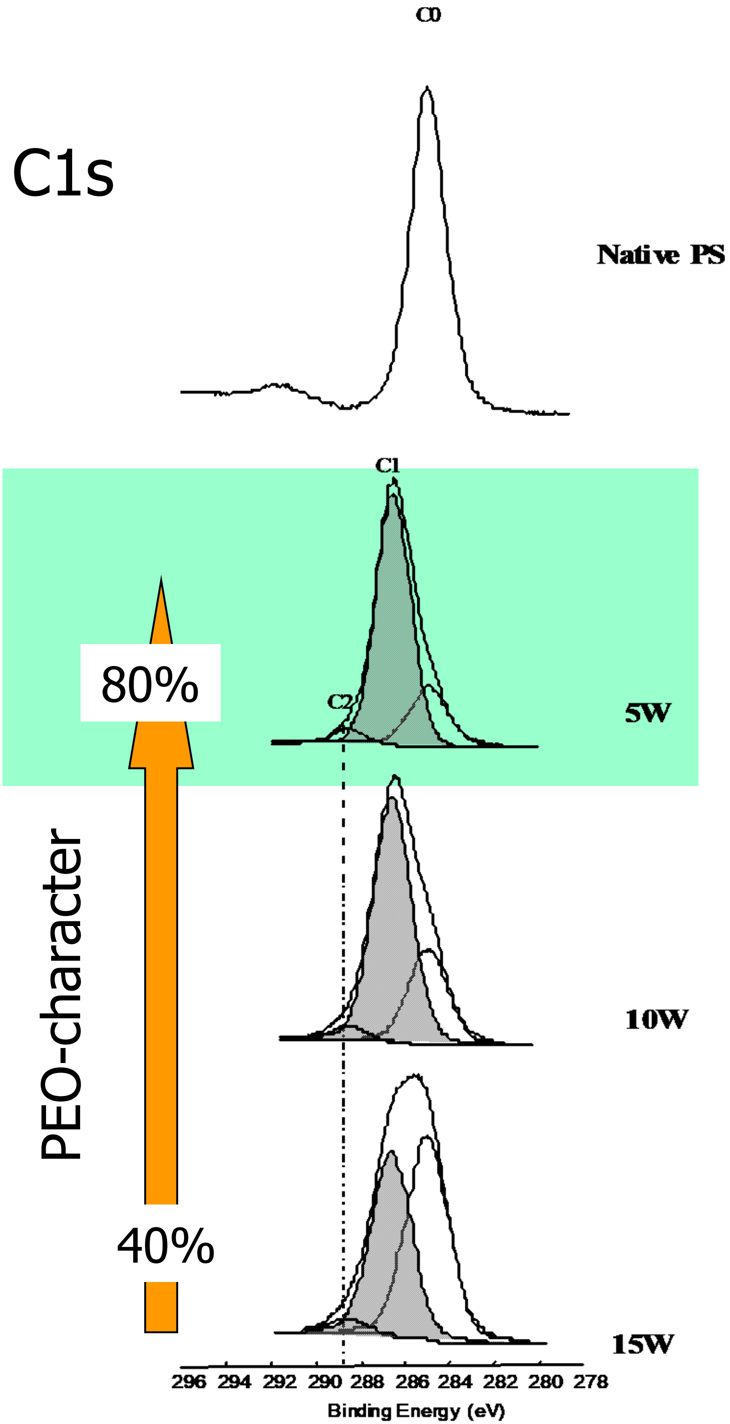
WCA

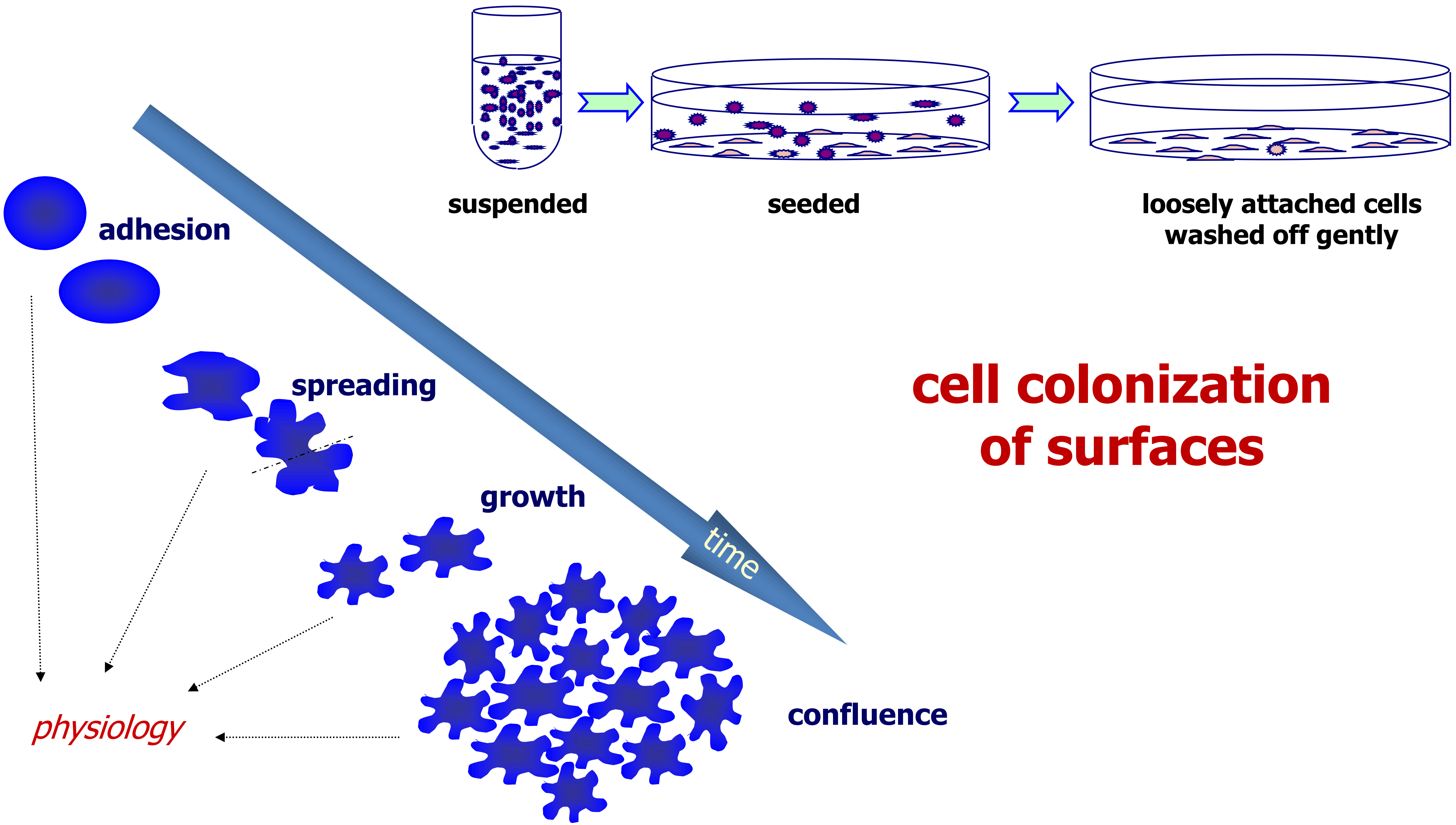
Power	Θ_{ad} (°)	Θ_{rc} (°)
5W	56±5	37±5
10W	67±5	46±5
15W	71±5	50±5

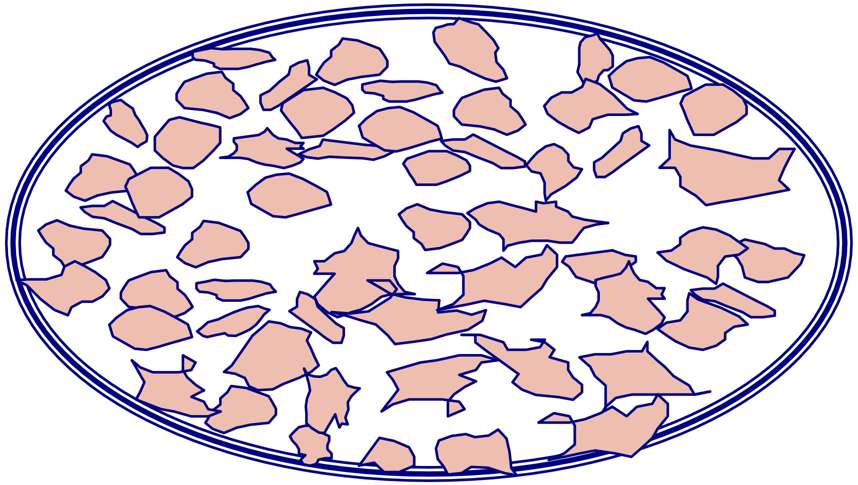
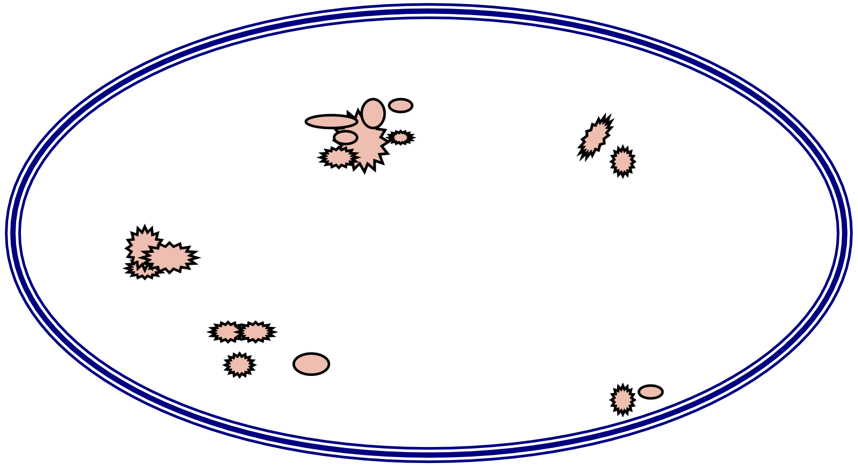
= protein/cell repulsive

Low Pressure
parallel plate plasma reactor

XPS C1s

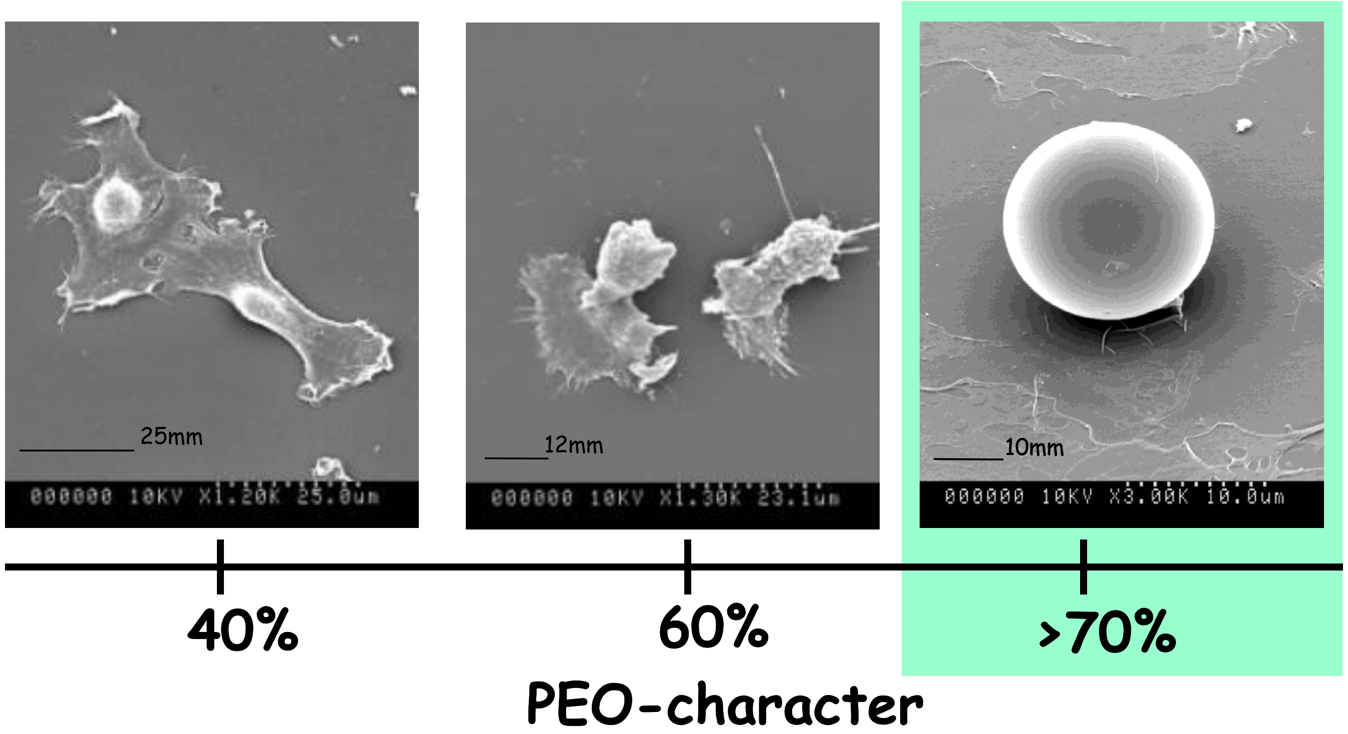


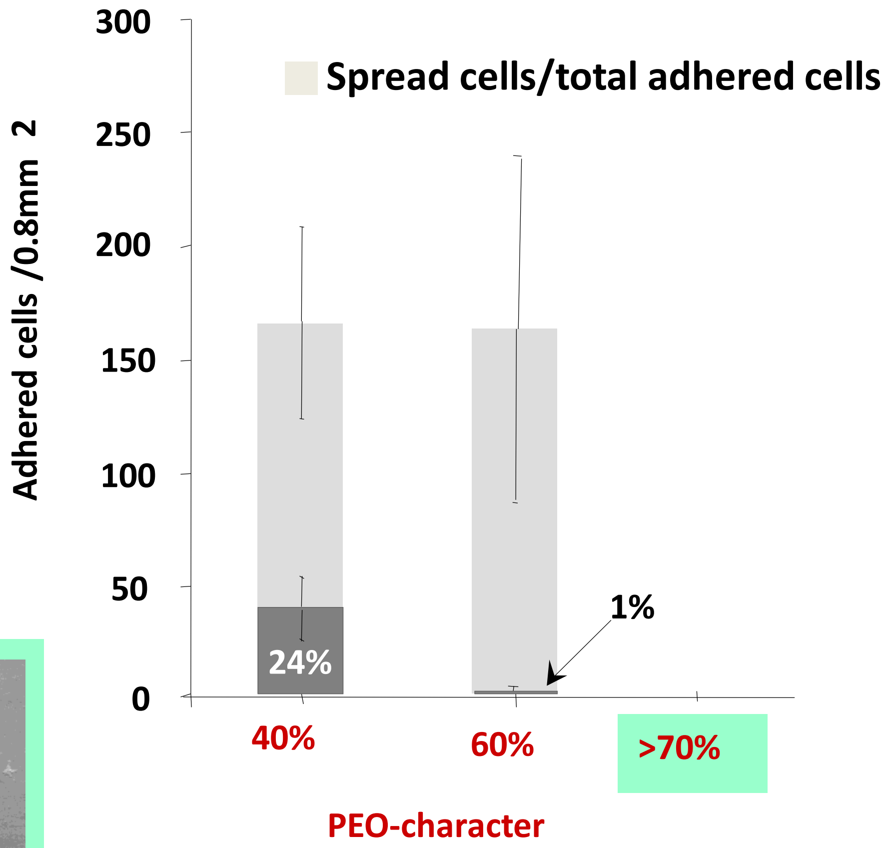

suspended
seeded
loosely attached cells washed off gently
adhesion
spreading
growth
time
confluence
physiology
cell colonization of surfaces

good cell culture	**poor cell culture**
	
many cells	sparse cells
well spread (flat)	rounded
difficult to distinguish between individual cells	easily distinguished
	clumped together

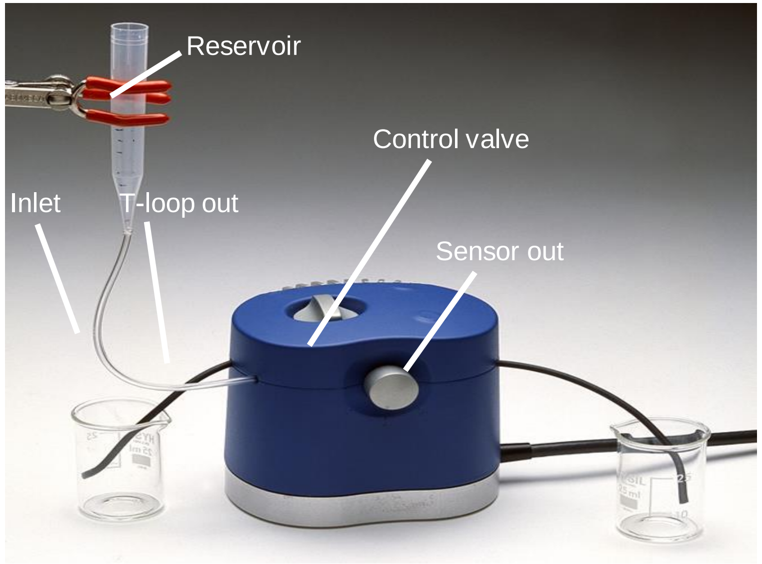
HTERTBJ1 fibroblasts
(24 hours)

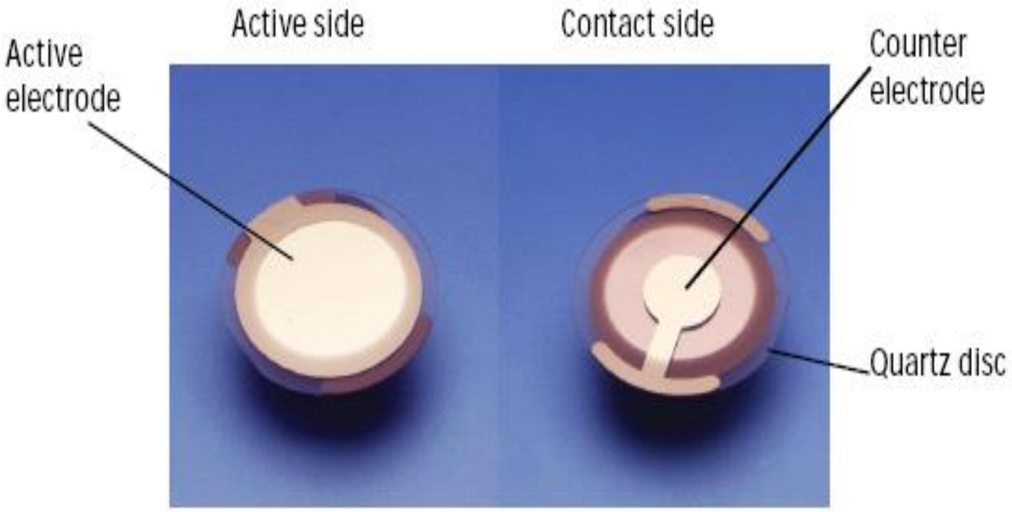
Sardella, Favia, d'Agostino et al;
***Plasma Processes and Polymers* 1, 63, 2004**

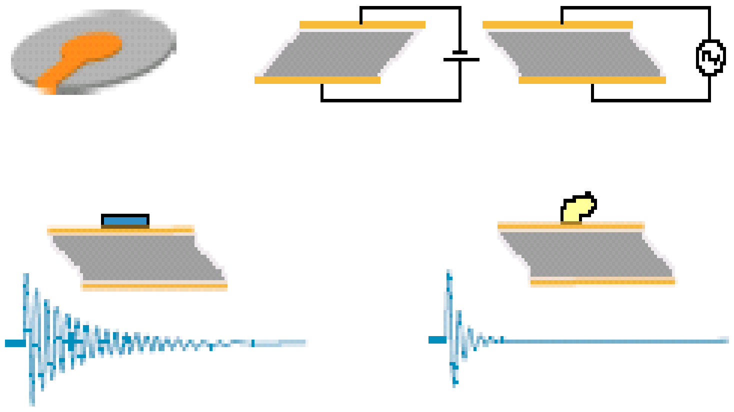




Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D)







Δf is related to the mass of the attached film

ΔD is related to the viscoelasticity

$$\Delta m = -\frac{C \cdot \Delta f}{n}$$

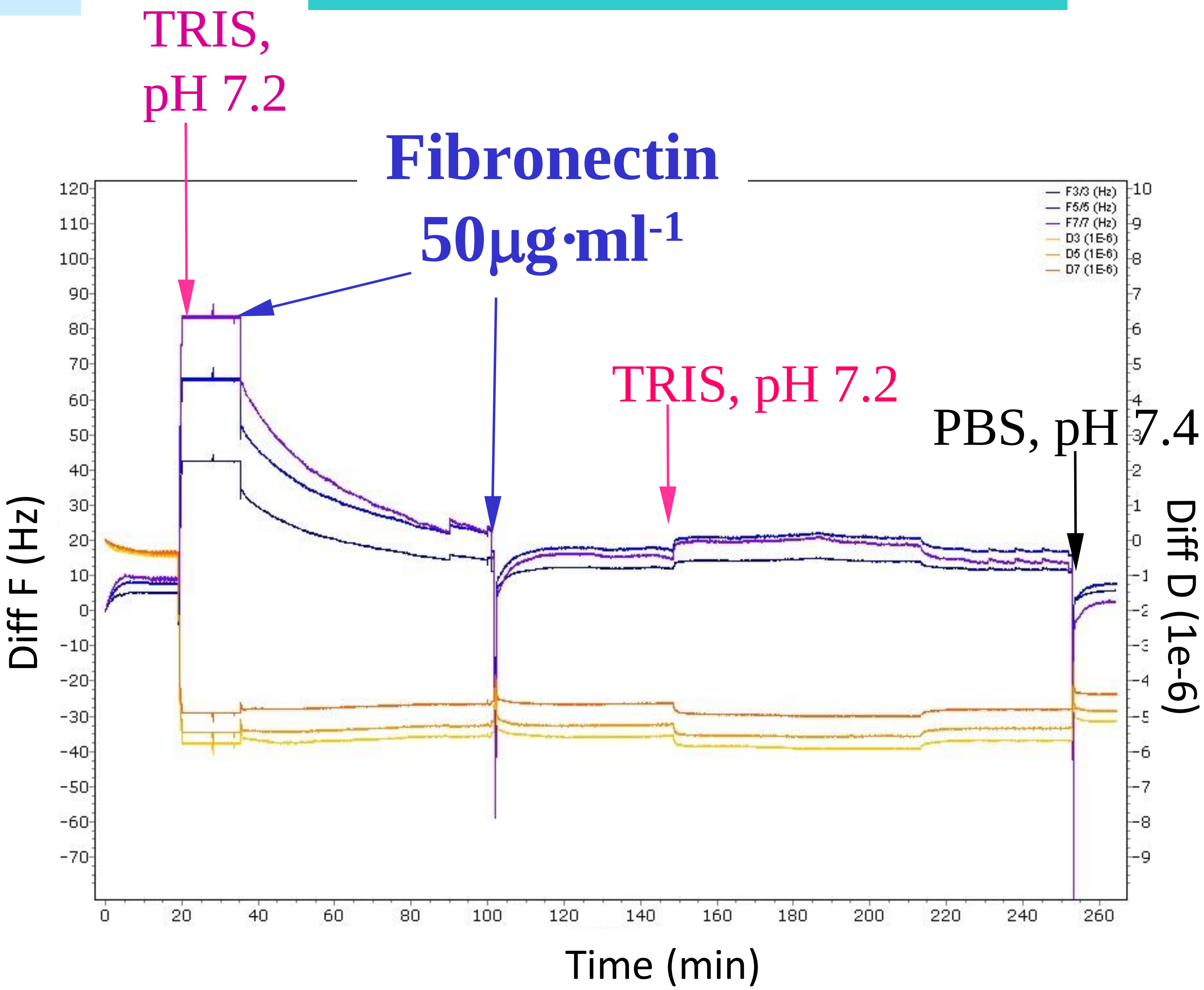
C = 17.7 ng Hz $^{-1}$ cm^{-2} for a 5 MHz quartz crystal
n = 1,3,5,7 is the overtone number

$$D = \frac{E_{lost}}{2\pi E_{stored}}$$

E_{lost} = energy lost (dissipated) during one oscillation cycle
E_{stored} = the total energy stored in the oscillator

QCM-D data

Results confirmed by XPS and ToF-SIMS



PEO character 30%

⇩

0.15 mg adsorbed fibronectin

Papadopoulos (JRC, Ispra) & Sardella (Univ. Bari)

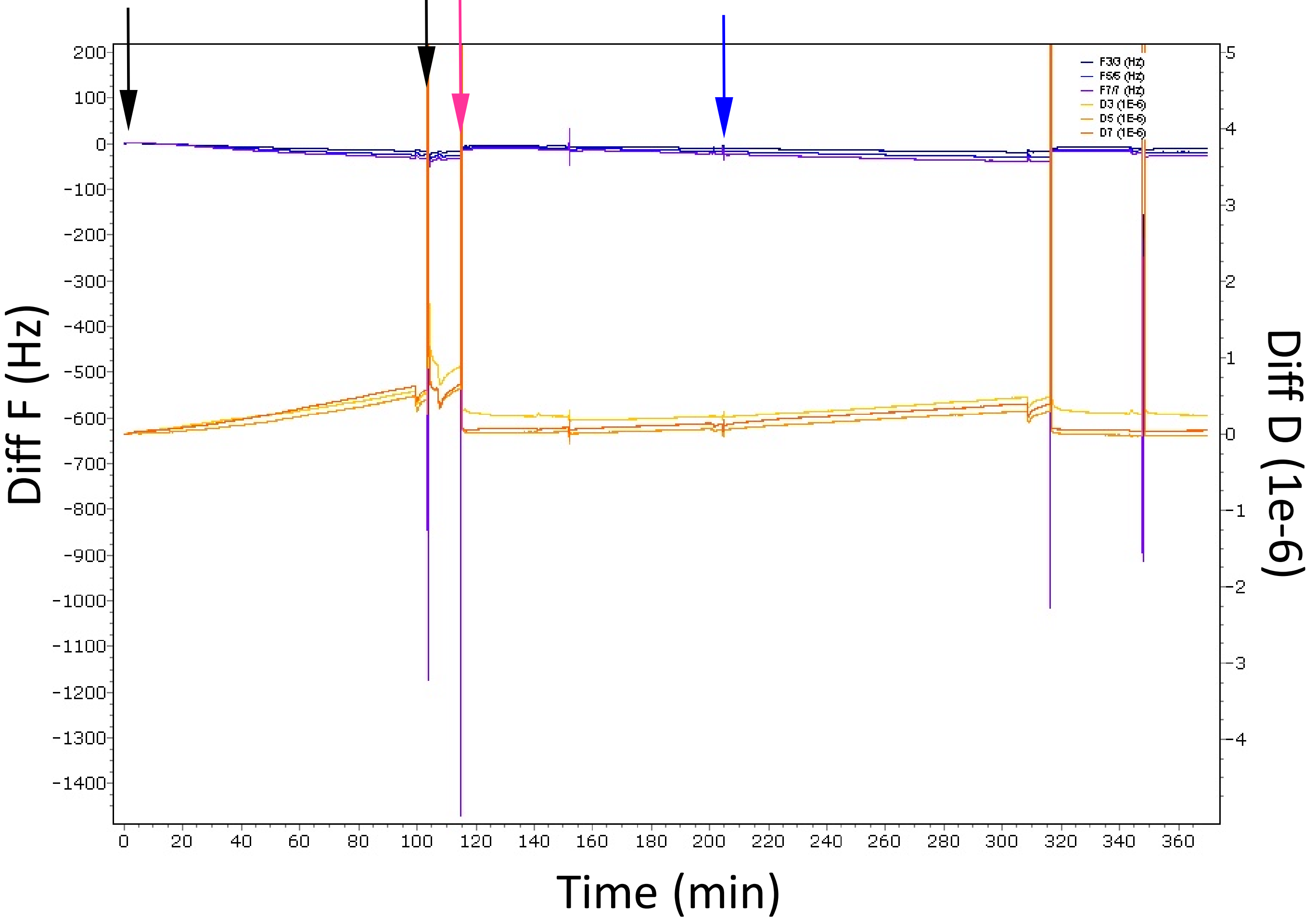
QCM-D data

PBS,
pH 7.4

PBS,
pH 7.4

TRIS,
pH 7.2

Fibronectin,
(50μg·ml^{-1})



PEO character >70%

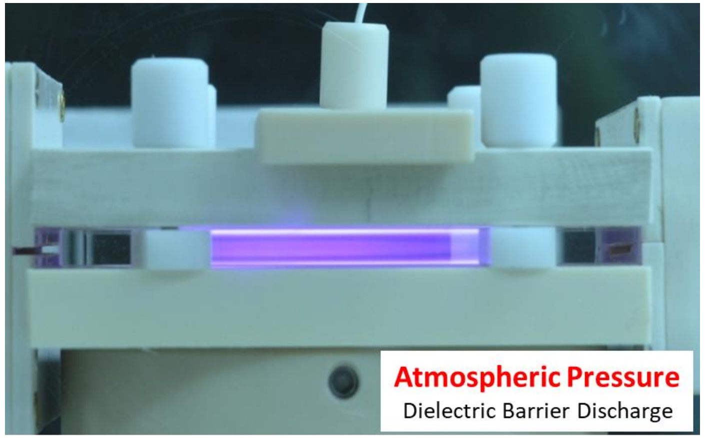
no adsorption

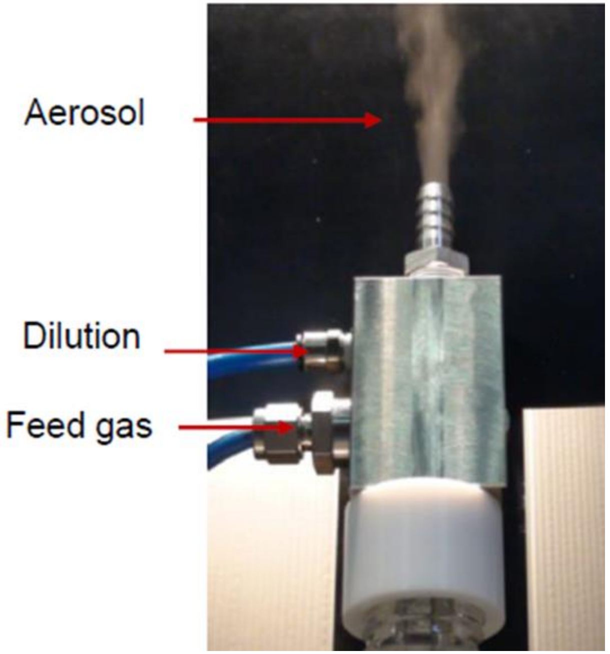
Papadopoulos (JRC, Ispra) & Sardella (Univ. Bari)

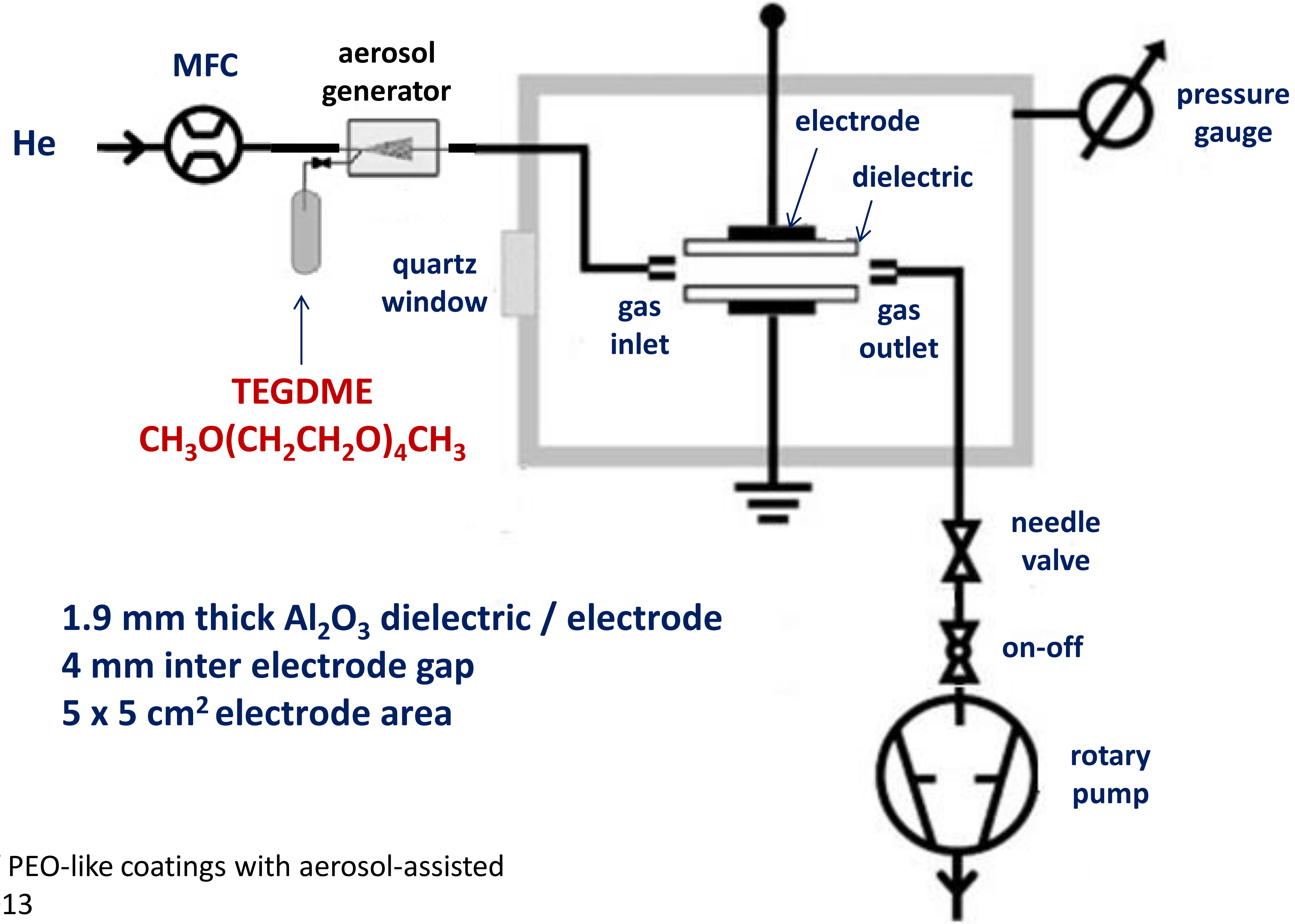
PEO character >70%

non fouling coating

PEO-like coatings – PE-CVD at ATMOSPHERIC PRESSURE







1.9 mm thick Al_2O_3 dielectric / electrode
4 mm inter electrode gap
5 x 5 cm^2 electrode area

G Da Ponte et al, Plasma deposition of PEO-like coatings with aerosol-assisted DBDs, Plasma Proc. Polym. 9, 1176, 2013

low P *vs* atm P plasmas for materials

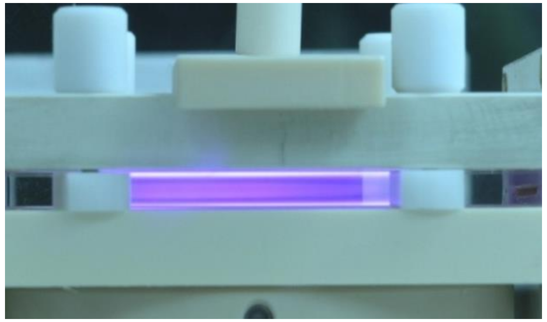
RF Glow Discharge system
Low Pressure

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

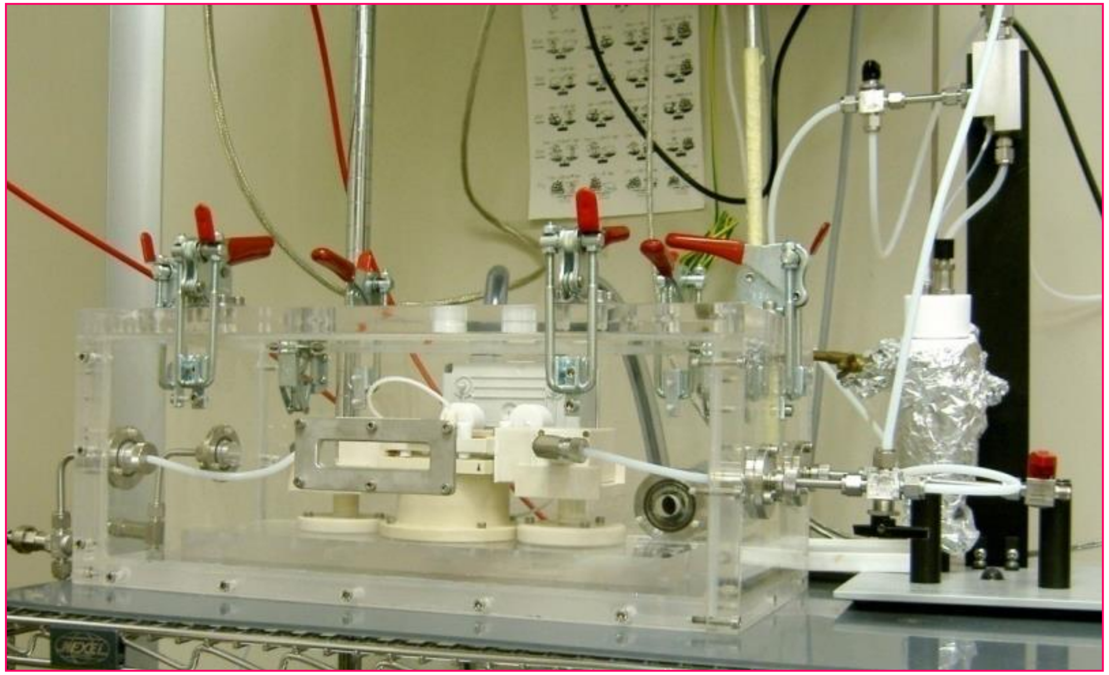
In recent years, however, ATMOSPHERIC PRESSURE PLASMAS started to produce surfaces formely synthesized only at low pressure



DBD system
Atmospheric Pressure

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

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, …

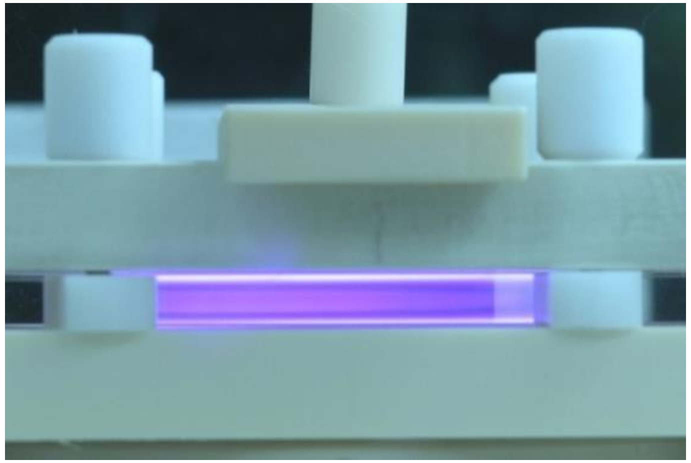
DBD (APP Jet)

no/reduced pumping system

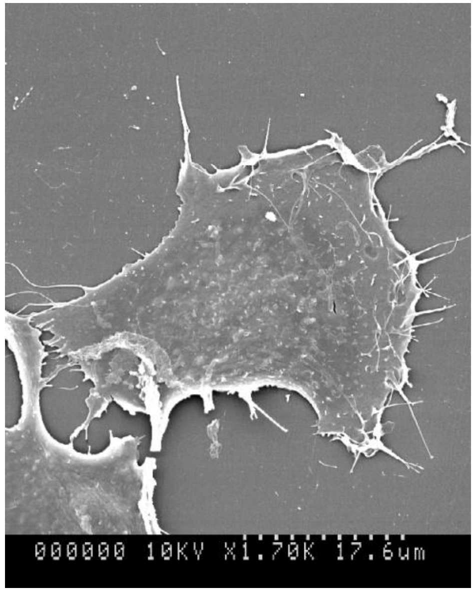
easy processing of highly degassing substrates

easier integration in on-line systems

possible use of precursors in aerosol



MICRO-STRUCTURED SURFACES OUT OF PLASMA PROCESSES



cell
ADHESIVE
PEO-like

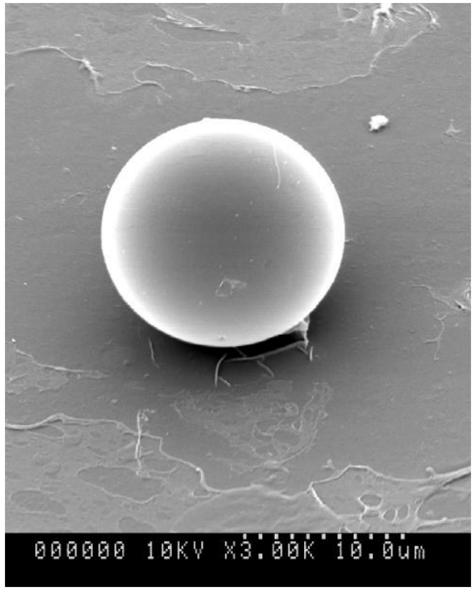
15W 40%

HIGH
momomer fragmentation in the plasma

cell
REPULSIVE
PEO-like

5W >70%

LOW
momomer fragmentation in the plasma



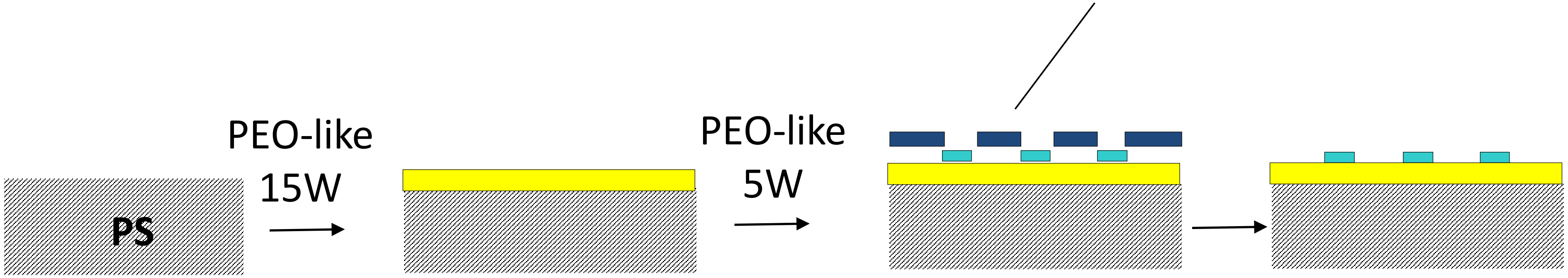
cell-adhesive *vs.* cell-repulsive μ-domains

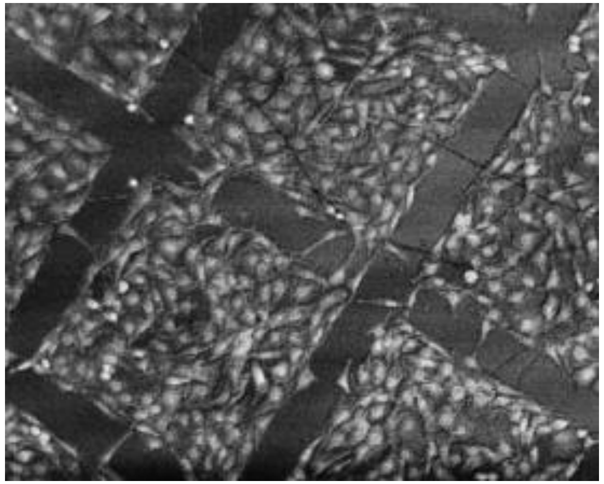
μ-patterning of polymers by PEO-like plasma deposition

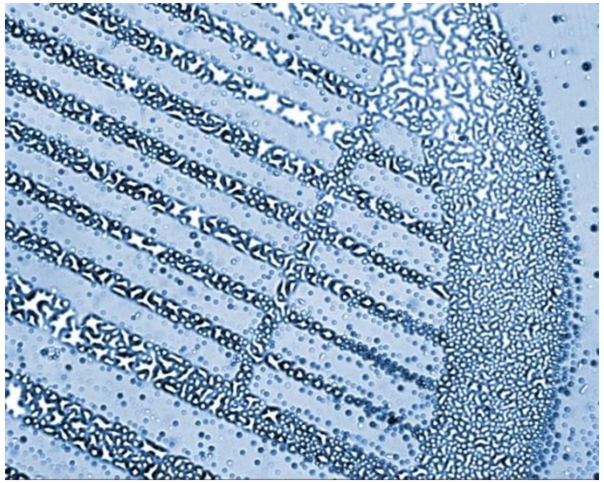
physical masks

G50P
spaces 416 μm
bars 84 μm

G50P
spaces 208 μm
bars 42 μm





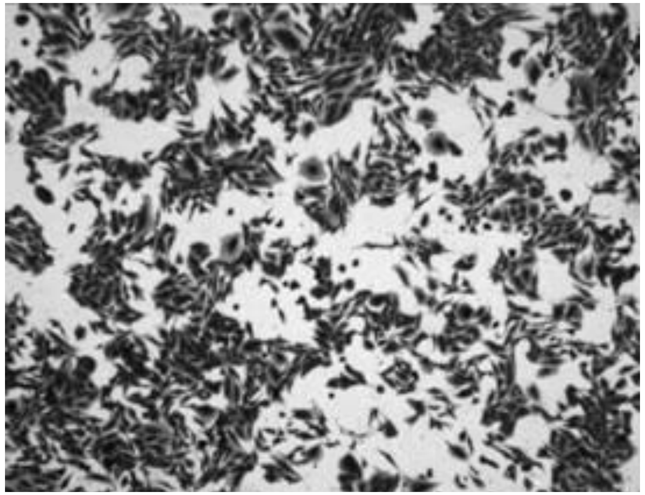


CELLS GROW AND MOVE INSIDE TRACKS

PEO-like STABLE > 4 WEEKS, NON-TOXIC, VIABLE FOR CELLS

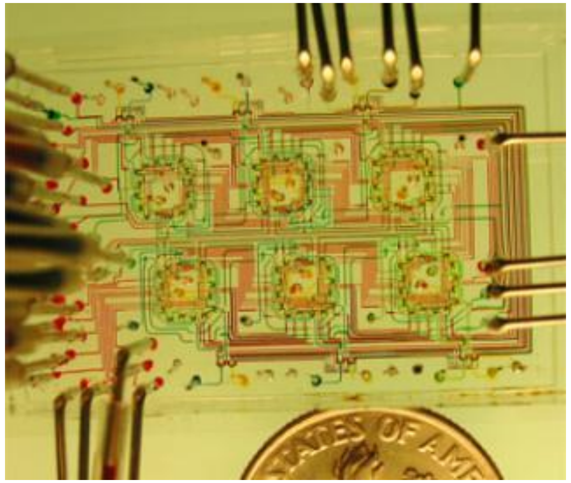
Sardella, Gristina, Senesi, d’Agostino, Favia
Homogeneous and micro-patterned plasma-deposited PEO-like coatings for biomedical surfaces
Plasma Processes & Polymers, 1, 63-72, 2004

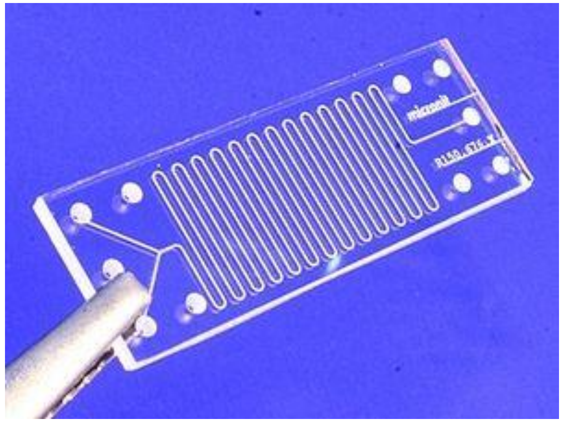
unfouling platform for lab-on-chip

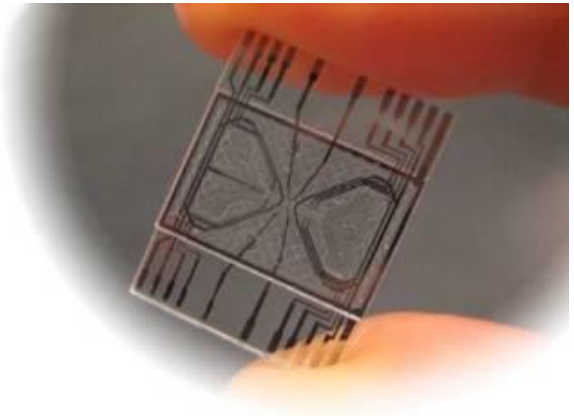


untreated glass: cell adhesive

plasma coated glass: unfouling







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materials

Review

Non-Equilibrium Plasma Processing for the Preparation of Antibacterial Surfaces

Eloisa Sardella [1,*], Fabio Palumbo [1], Giuseppe Camporeale [2,*] and Pietro Favia [1,2]

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Academic Editor: Mauro Pollini

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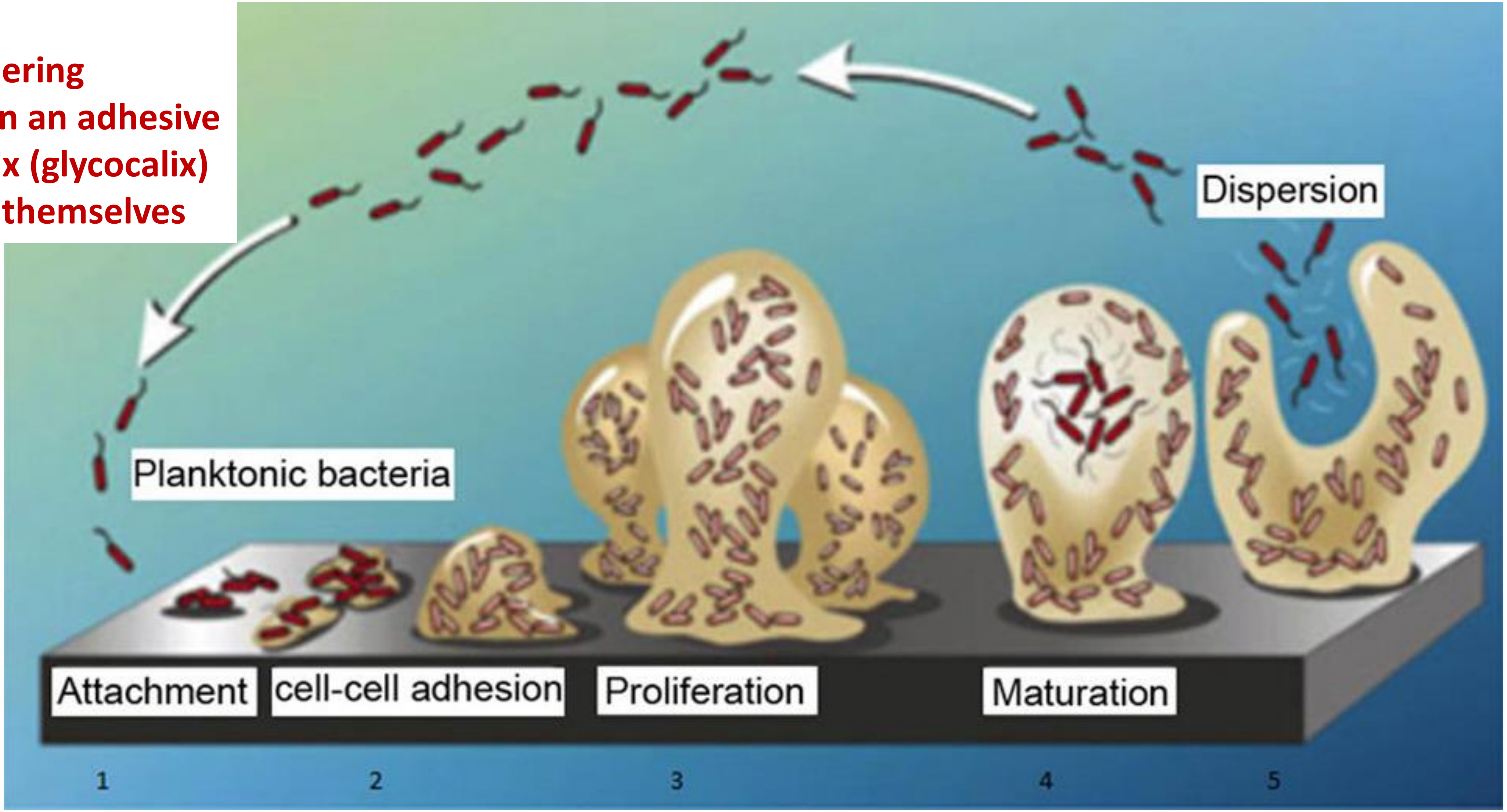
Abstract: Non-equilibrium plasmas offer several strategies for developing antibacterial surfaces that are able to repel and/or to kill bacteria. Due to the variety of devices, implants, and materials in general, as well as of bacteria and applications, plasma assisted antibacterial strategies need to be tailored to each specific surface. Nano-composite coatings containing inorganic (metals and metal oxides) or organic (drugs and biomolecules) compounds can be deposited in one step, and used as drug delivery systems. On the other hand, functional coatings can be plasma-deposited and used to bind antibacterial molecules, for synthesizing surfaces with long lasting antibacterial activity. In addition, non-fouling coatings can be produced to inhibit the adhesion of bacteria and reduce the formation of biofilm. This paper reviews plasma-based strategies aimed to reduce bacterial attachment and proliferation on biomedical materials and devices, but also onto materials used in other fields. Most of the activities described have been developed in the lab of the authors.

Keywords: antibacterial coatings; plasma processing; surface characterization

biofilms are made of microcolonies of adhering bacteria embedded in an adhesive polysaccharide matrix (glycocalix) secreted by the cells themselves



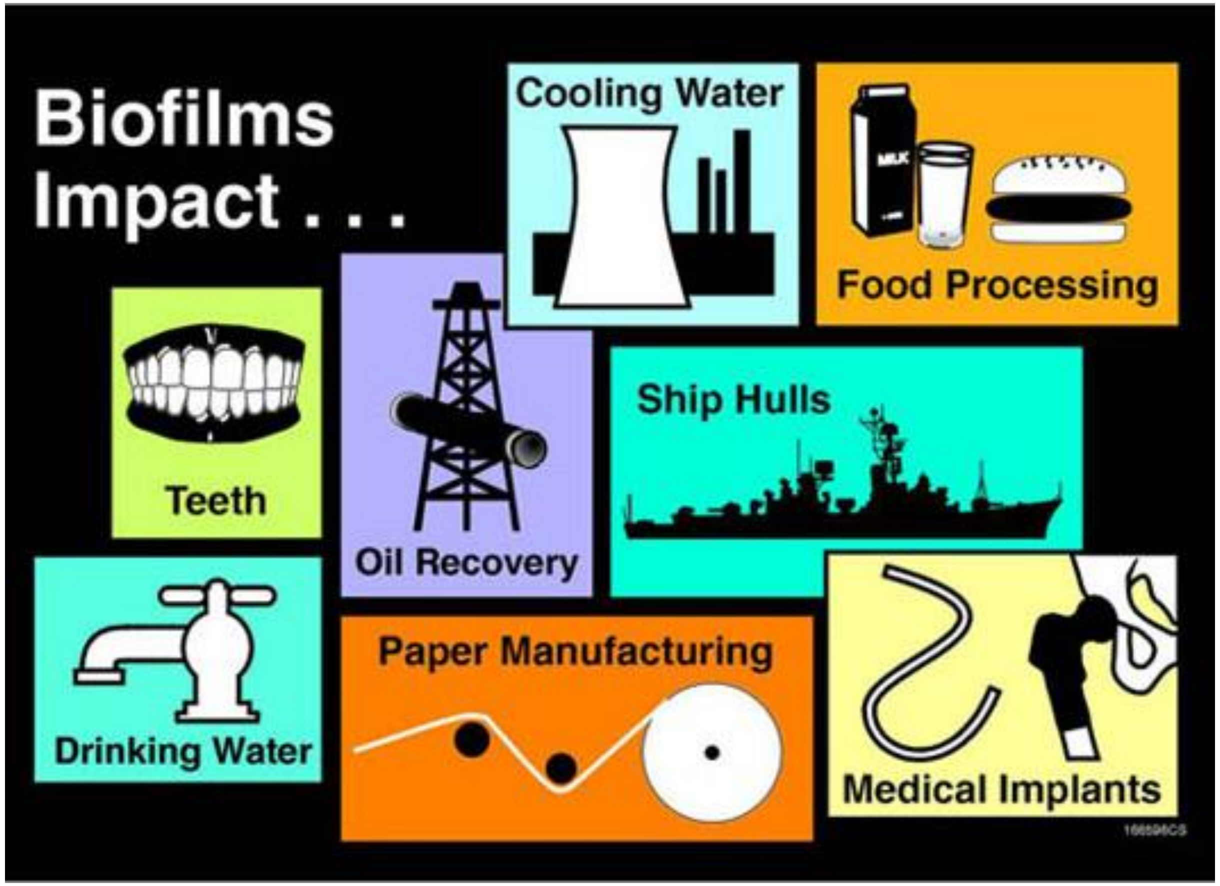
reversible adsorption (seconds)	irreversible attachment (minutes)	growth & division (hours)	biofilm formation (days)	and attachment of other microorganisms (months)

In campo medico l'infezione batterica è complicazione comune durante l'impianto di dispositivi medici.

Ogni anno negli USA vengono impiantati circa 3 milioni di cateteri, nel 30% dei casi si verifica infezione batterica.

Nell'industria alimentare, i batteri aderiscono alle superfici interne delle tubature e possono provocare infezioni.

I biofilm rallentano i flussi di acqua, olii e altri fluidi e promuovono la corrosione.



OCCORRONO AGENTI ANTIMICROBICI IN GRADO DI PENETRARE NEL GLICOCALICE

Variable	Microbes	Humans	Factor
No. on earth	5×10^{31}	8×10^{9}	$\sim 10^{22}$
Mass, metric tons	5×10^{16}	3×10^{8}	$\sim 10^{8}$
Generation time	30 min	30 years	$\sim 5 \times 10^{5}$
Time on earth, years	3.5×10^{9}	4×10^{6}	$\sim 10^{3}$

UMANI

vs

BATTERI

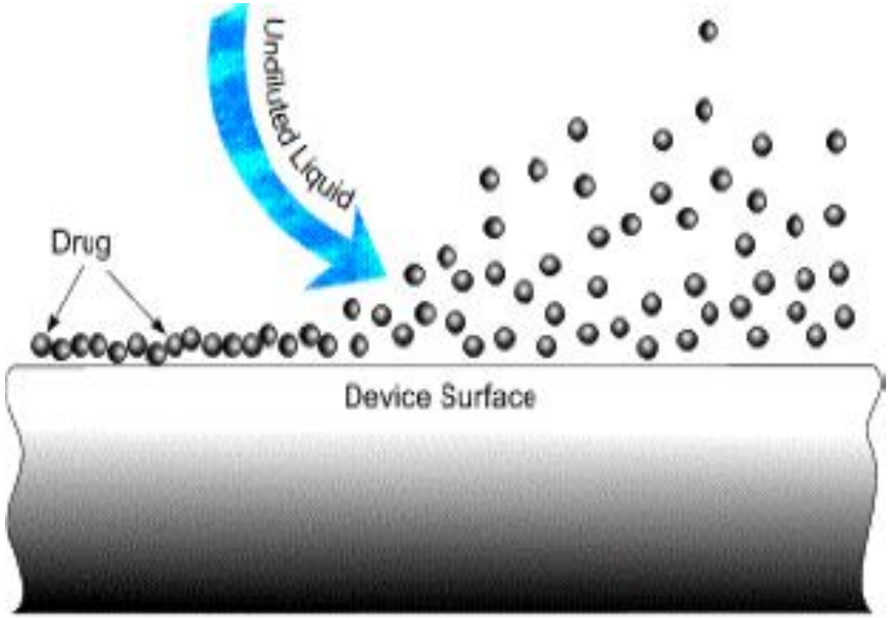
film nanocompositi antibatterici Ag/PEO-like

- Ag ha tradizione antica come antisettico
- Diverse forme attive: ioni, sali, …
- Ampio spettro:
  - Batteri Gram + and Gram –
  - Lieviti
  - Attivo sui biofilm
- Solo pochi casi di resistenza

L'**ARGENTO COLLOIDALE** è una sospensione di particelle di Ag in acqua o gelatina usato come disinfettante. Il suo uso indiscriminato porta all'argiria, una malattia responsabile della colorazione permanente blu-grigiastra della pelle e dei tessuti interni

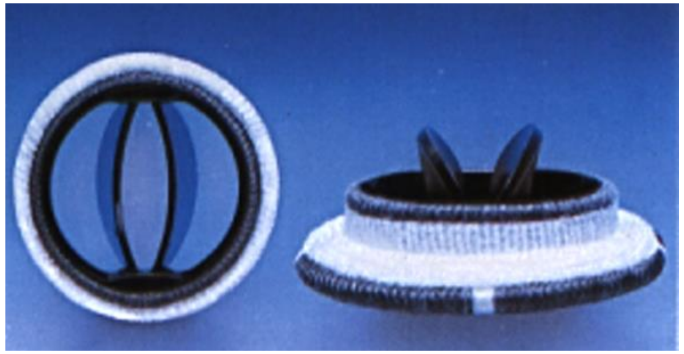
Concentrazione di ioni Ag^+ maggiori di 0.15 mg/ml possono essere dannose a cause delle interazioni con le proteine del sangue, e della precipitazione di AgCl, insolubile nel sangue

ACTICOAT®
Moisture Control
or SILVERLON ®



released silver (Ag^+): 100 µg/ml H_2O

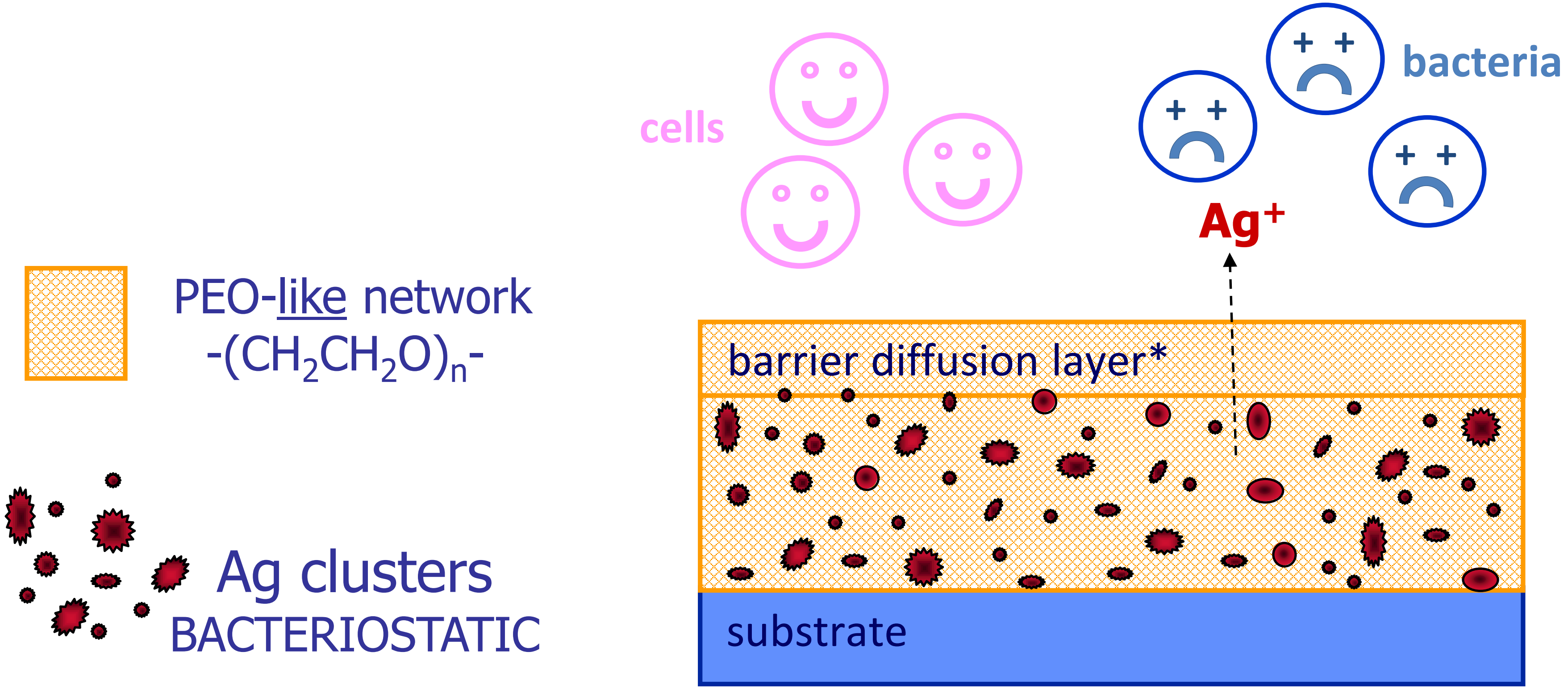
Minimum Inhibitory Concentration (MIC) of silver to *staphylococci* : 0.5-10 µg/ml.



Ag → Ag^+

nano-composite Ag/PEO-like bacterial resistant coatings

anti-infective coatings directly on biomaterials



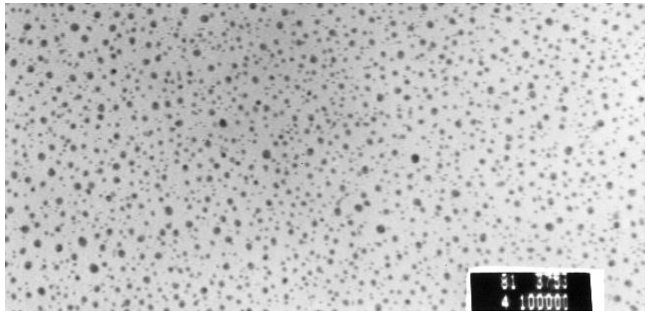
* the barrier diffusion layer can be created with cell/adhesive or cell/repulsive properties

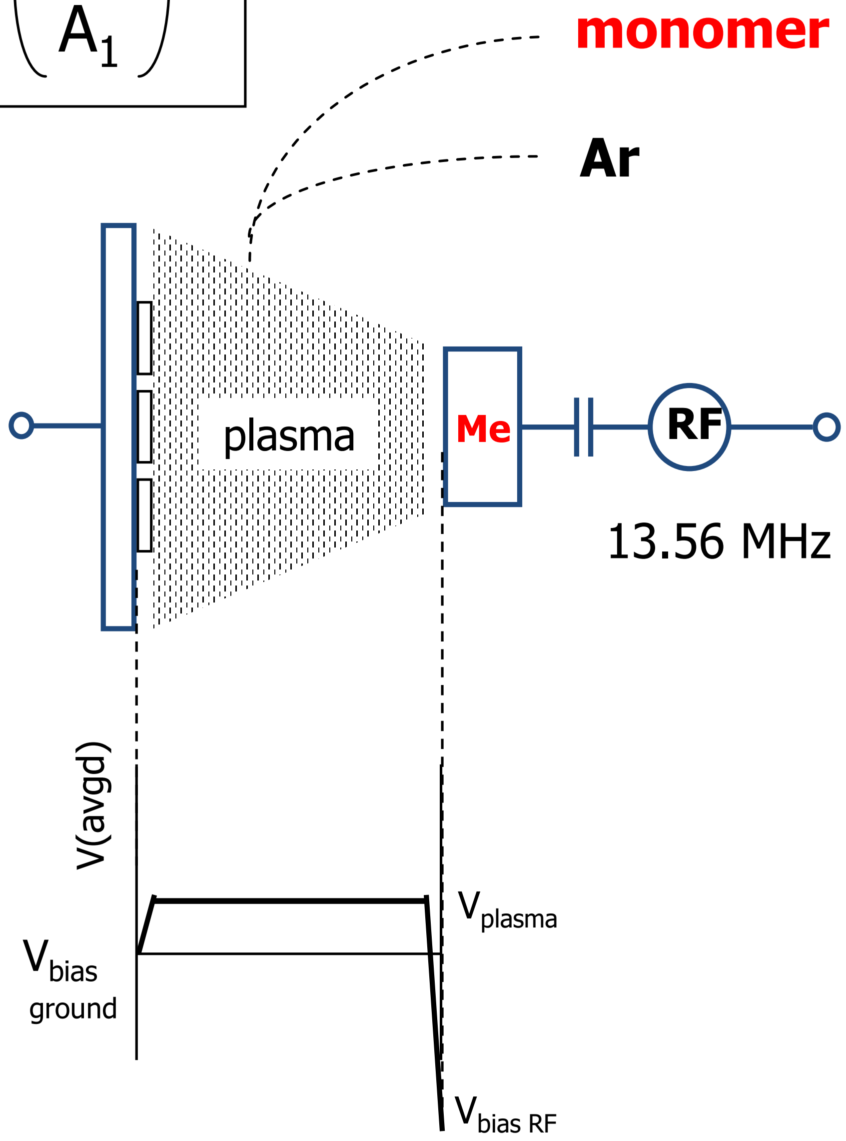
PLASMA REACTOR
for
nanocomposite coatings

$$\frac{V_1}{V_2} = \left(\frac{A_2}{A_1}\right)^n$$

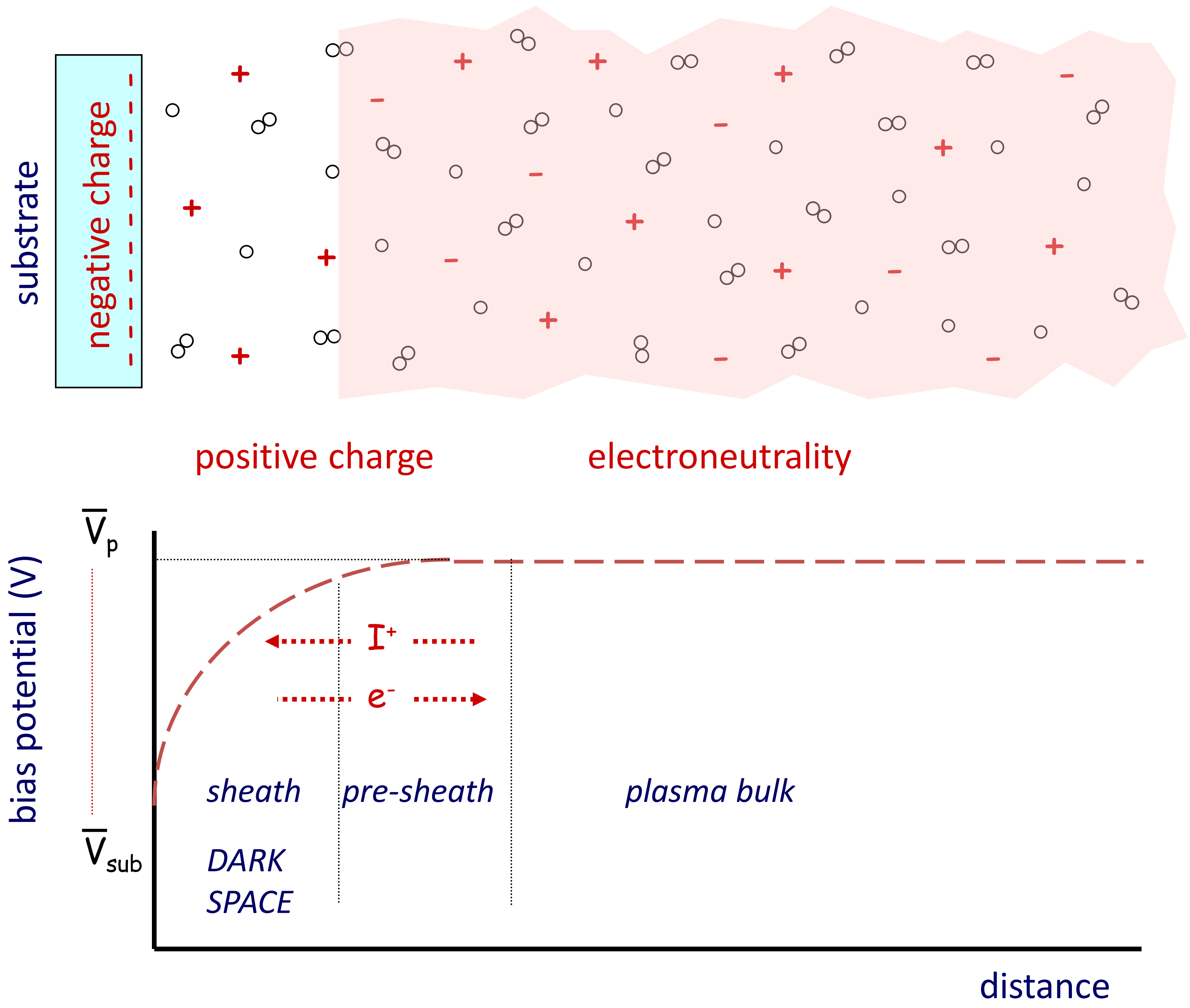
- mixed PE-CVD/sputtering process
- only at low P
- avoid poisoning of the metal
- electrode (source of the clusters)
  high Ar/monomer ratio
  high RF power
  low pressure

- several applications
  catalytic coatings (Pt)
  fuel cells
  antibacterial coatings (Ag, Zn, Cu)
  hard coatings

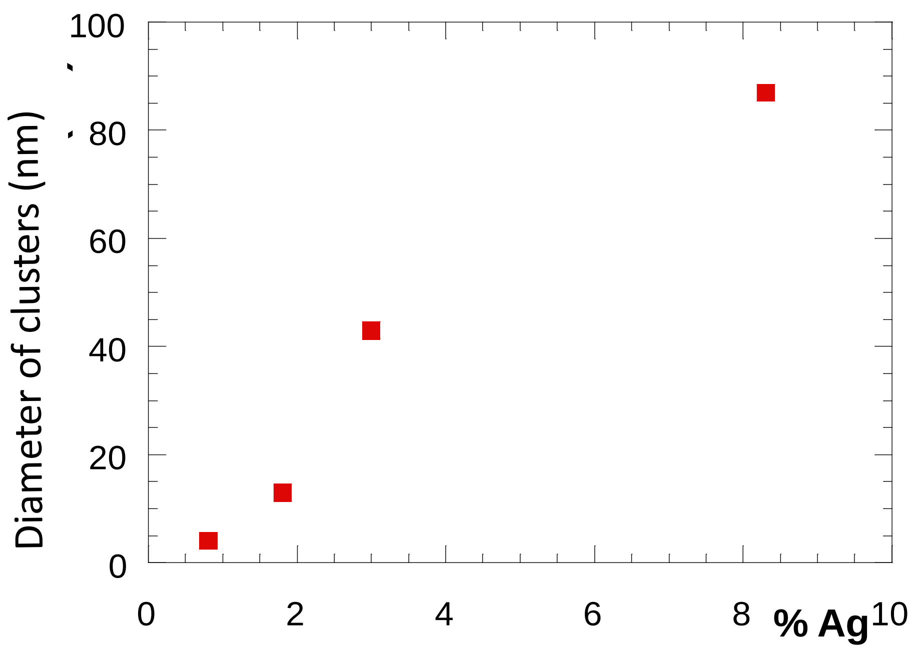




bias potential at Low P

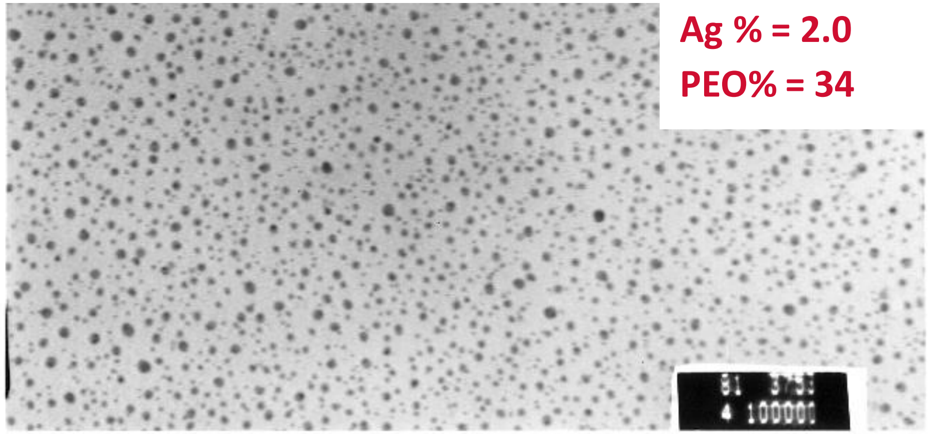


nano-composite Ag/PEO-like bacterial resistant coatings

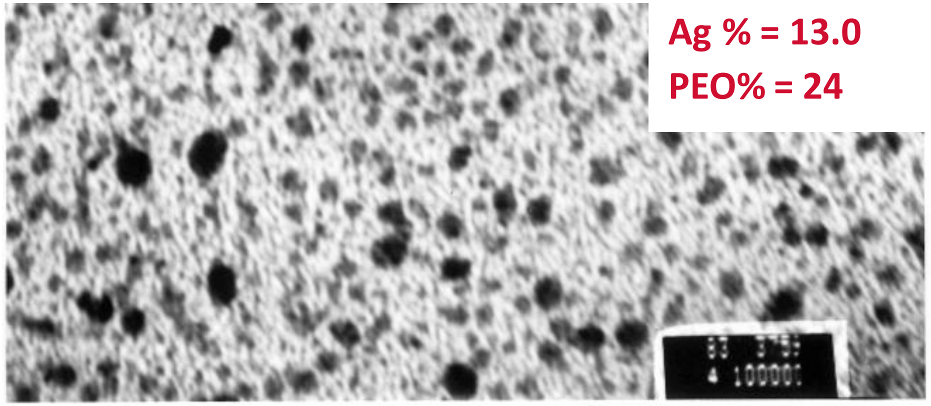


RF power input

Ag % = 2.0
PEO% = 34



Ag % = 13.0
PEO% = 24



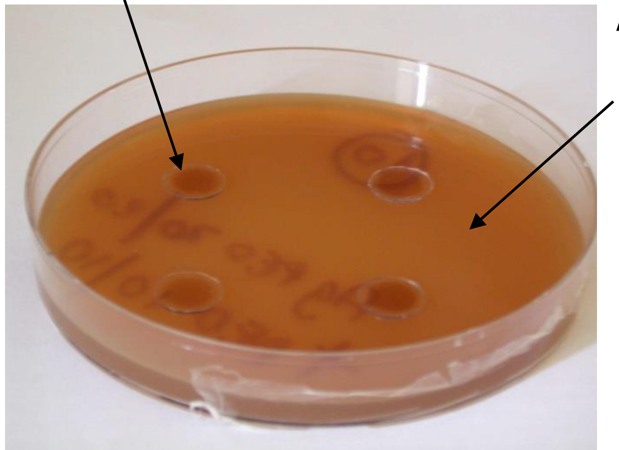
Ag/PEO-like coatings deposited onto polymers resist to the colonization of *Staphilococcus Aureus* and *Pseudomonas Aeruginosa* (6 logs)

DISK DIFFUSION SUSCEPTIBILITY TESTING

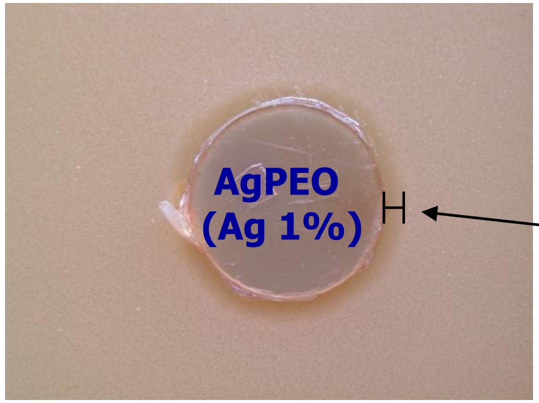
Staphylococcus epidermidis RP62A ATCC 35984
(slime producer, Gram +; $1x10^8$ cfu/mL into Tryptic Soy Agar)

Sample

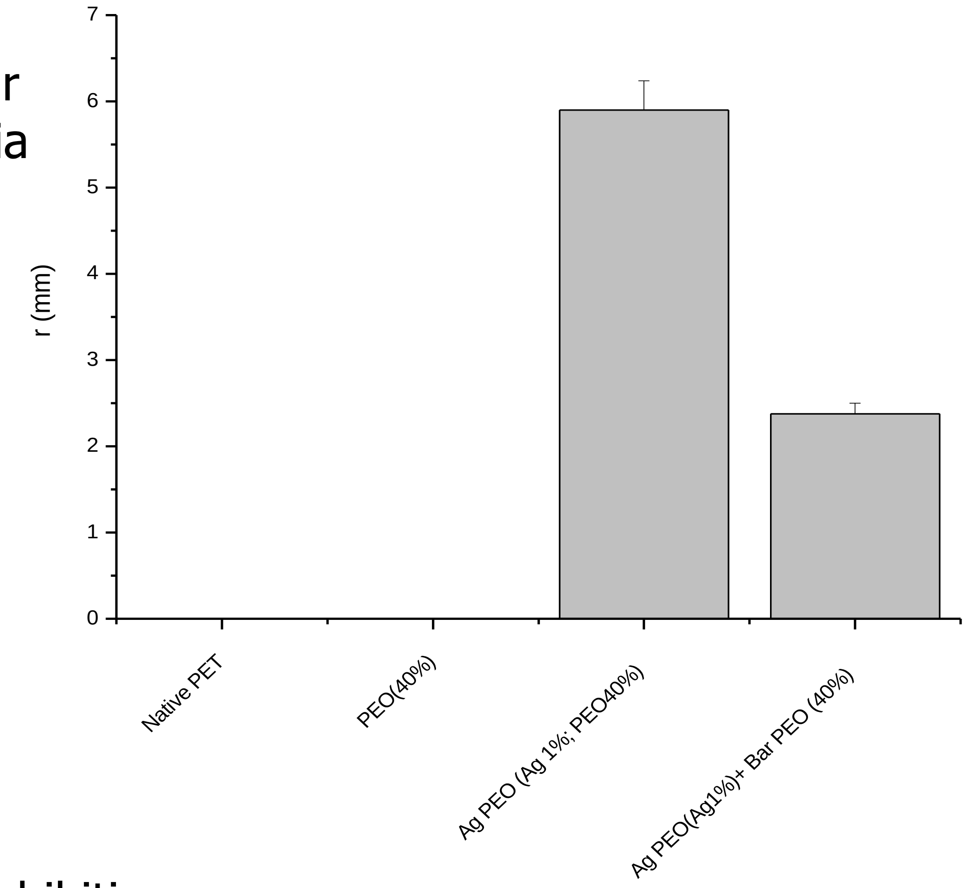
Agar Agar
+ Bacteria



72 hours
37°C

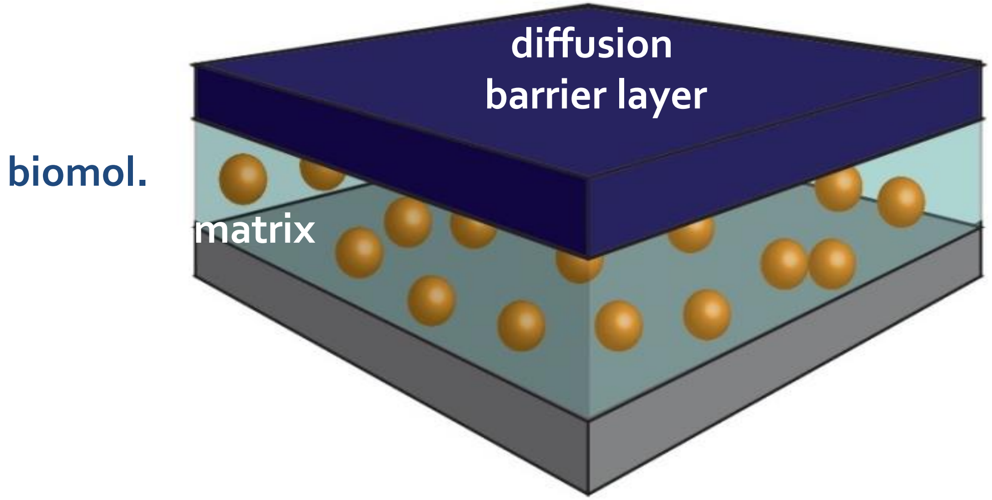


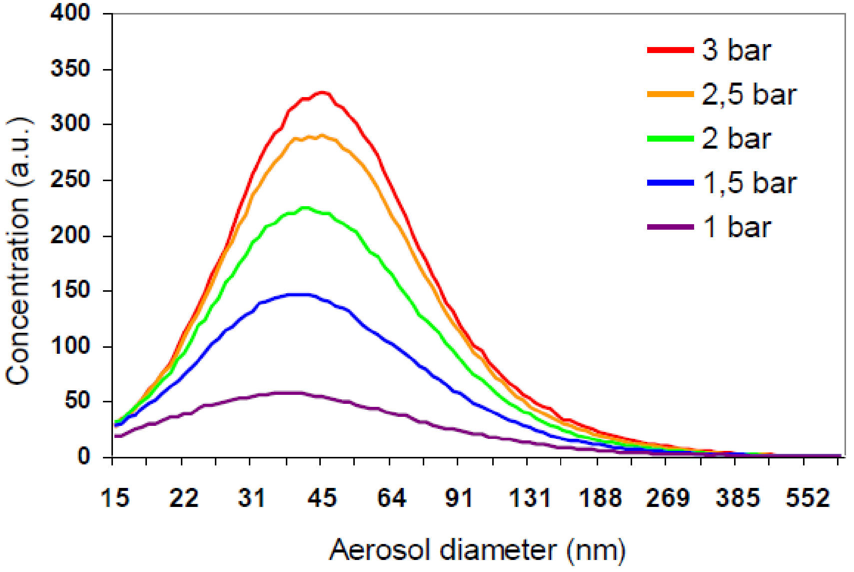
zone of inhibition



NANO/BIO COMPOSITE COATINGS

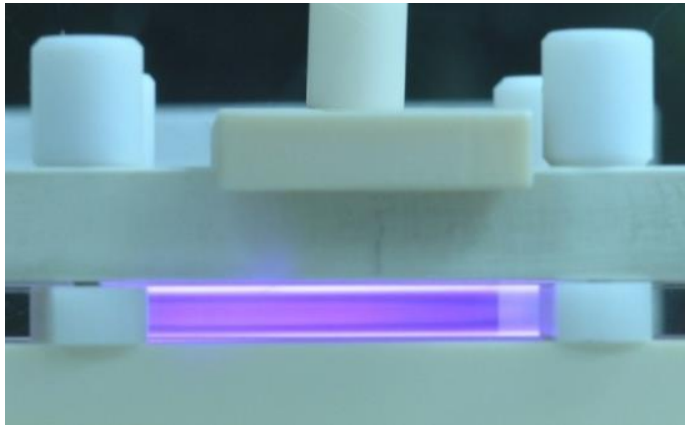
biomolecule-loaded **drug-release coatings**
deposited by **Aerosol-Assisted Atmospheric Pressure Plasma**

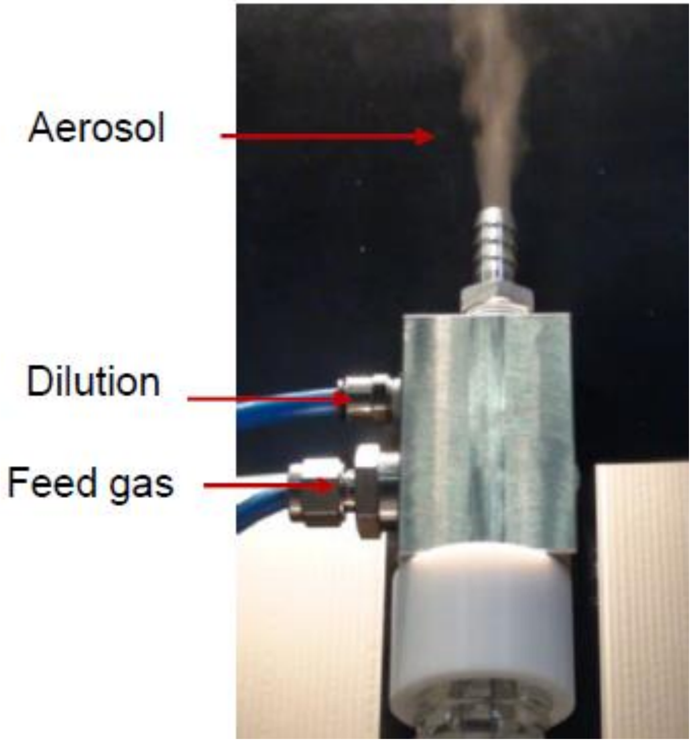




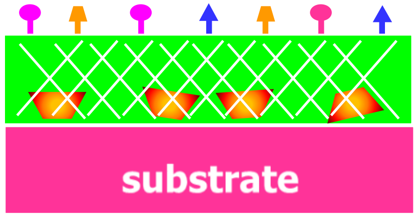
Aerosol is nano-sized, typical in range of 20-200 nm

DBD plasma source





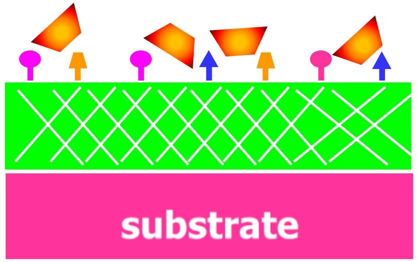
tipically 0-3 biomolecules are present per droplet



functionalized layer (coating, LP/AP)

adsorbed biomolecules
(non specific)

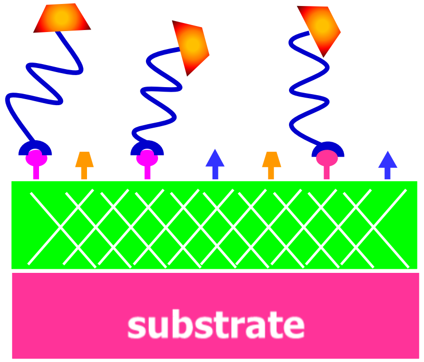
at least TWO steps



adsorbed biomolecules
(non specific)

functionalized layer
(grafted groups or coating, LP/AP)

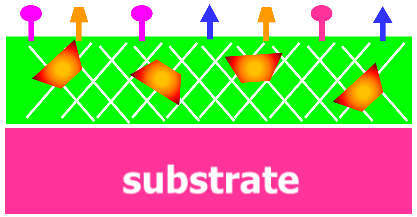
at least TWO steps



immobilized biomolecules
(specific, via molecular spacer arm)

functionalized layer
(grafted groups or coating, LP/AP)

MANY steps



functionalized layer (coating, AP)
embedded biomolecules

SINGLE step !

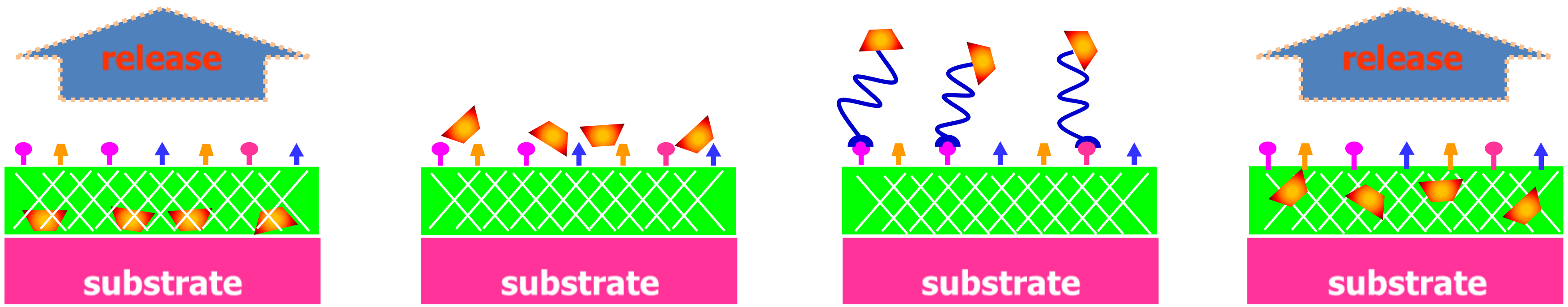
plasma processed
biomolecule-polymer nano composite surfaces

BIOMOLECULES
immobilized /embedded

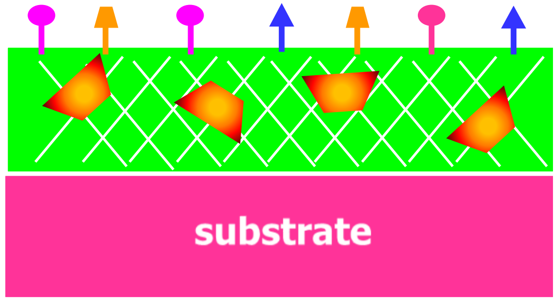
enzymes
proteins, peptides
DNA
anti oxidant molecules
anti thrombotic molecules
growth factors
anti bacterial
drugs
……
nanoparticles

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



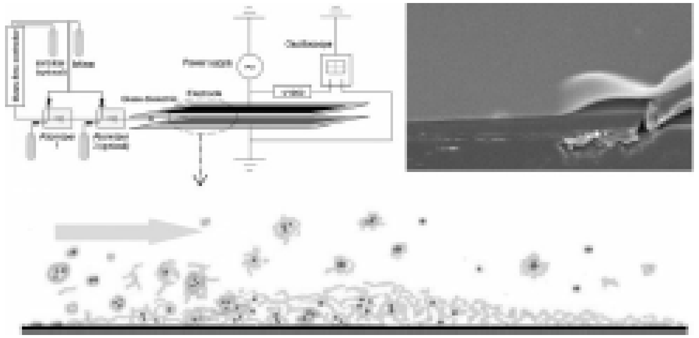
Plasma Process. Polym. **2011**, *8*, 965–974



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.

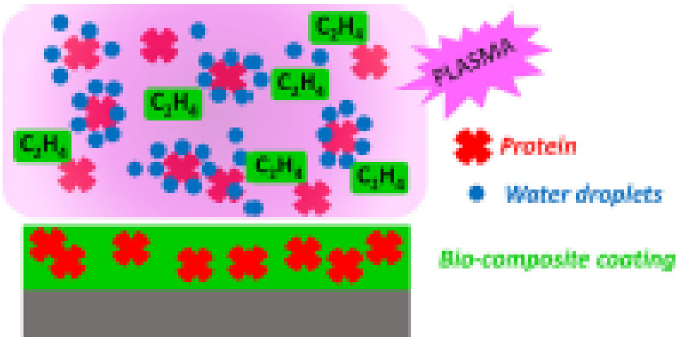


PPaP 12, 1302, 2015

Direct Plasma Deposition of Lysozyme-Embedded Bio-Composite Thin Films

Fabio Palumbo,* Giuseppe Camporeale, Yi-Wei Yang, Jong-Shinn Wu, Eloisa Sardella, Giorgio Dilecce, Cosima Damiana Calvano, Laura Quintieri, Leonardo Caputo, Federico Baruzzi, Pietro Favia*

Bio-composite coatings, consisting of an organic matrix embedding a bioactive molecule, have been deposited by means of atomizer-assisted atmospheric pressure plasma. Ethylene was chosen as the precursor of the matrix, while the atomizer was fed with a water solution of lysozyme. Coatings chemical composition was investigated by XPS, FTIR and MALDI-TOF spectroscopies, and it has been proved that the one-step inclusion of protein domains in the composite coatings is successful and lysozyme chemical structure is only slightly altered. The amount of embedded lysozyme is as high as 14 μg/cm^2 as evaluated from water release test. Finally, the activity of the plasma-embedded protein is close to that of pure lysozyme as verified against *Micrococcus lysodeikticus* ATCC 4698 through an agar plate diffusion test.



LYSOZYME

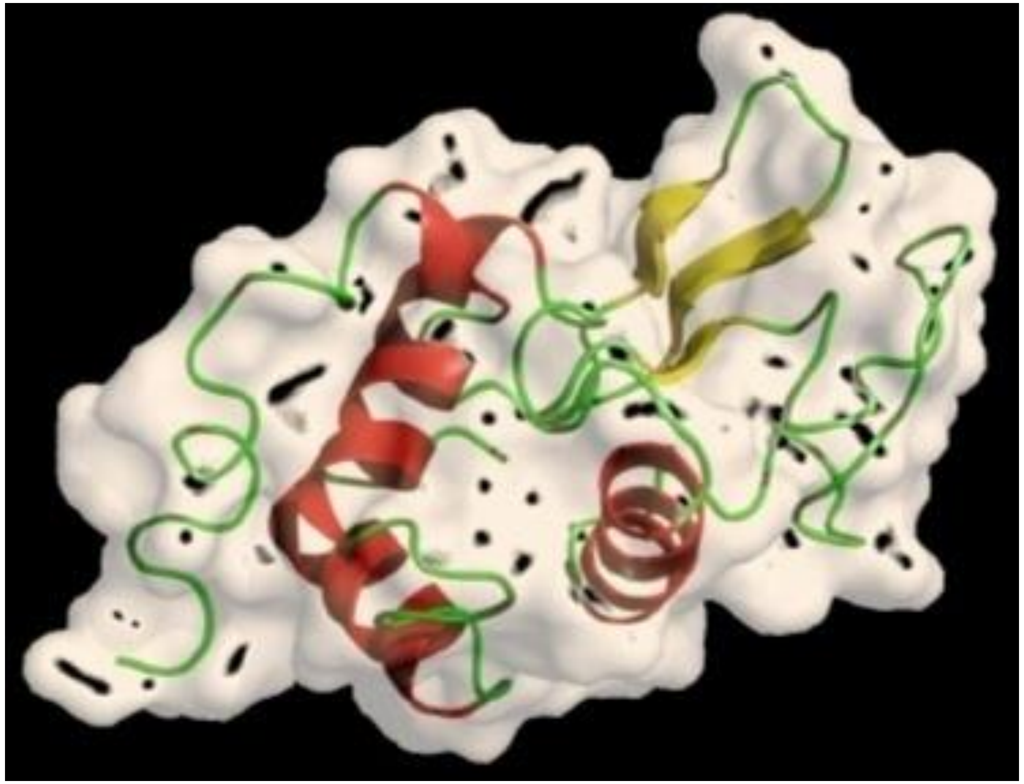
128 aminoacids; PM = 14.3 kDa; IP = 11.1.; 4-5 nm

Name coined by Alexander Fleming, discoverer of Penicellin.

Natural antibacterial molecules, active against gram positive patogens.

Abundant in tears, saliva, milk, mucus, egg white, neutrophils.

First enzyme to have a detailed, specific hydrolitic mechanism suggested for its catalytic action.



Second protein and first enzyme structure solved via X-ray diffraction.

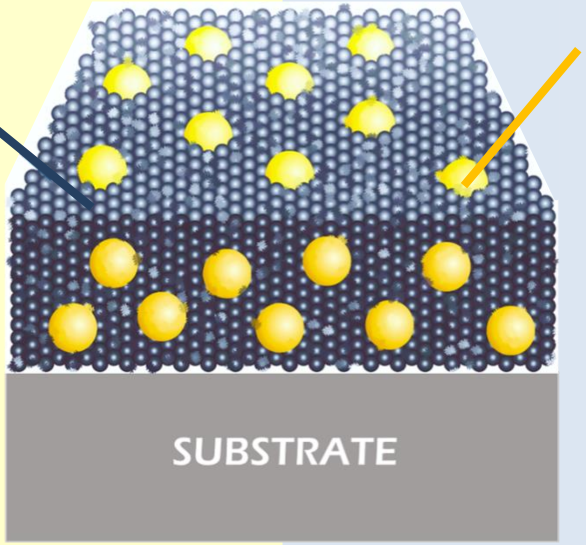
First enzyme to be fully sequenced

that contains all 20 common amino acids.

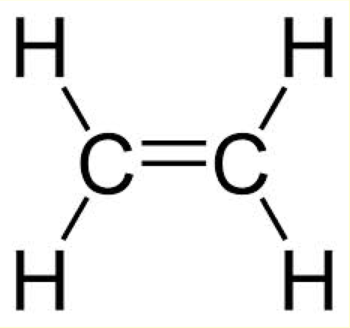
Cheap, FDA approved.

AIM OF THE WORK

AP-PLASMA DEPOSITION



ETHYLENE

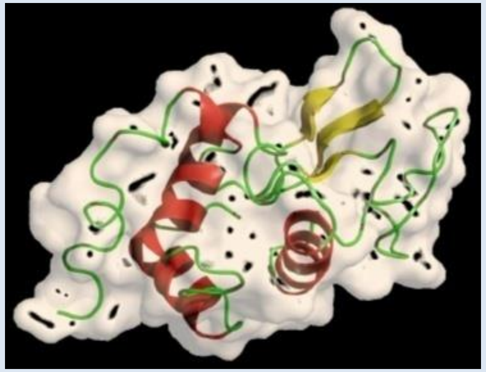


Reactive in plasma environment

Suitable source for CH_x matrix

H_2O addition for tuning coating properties

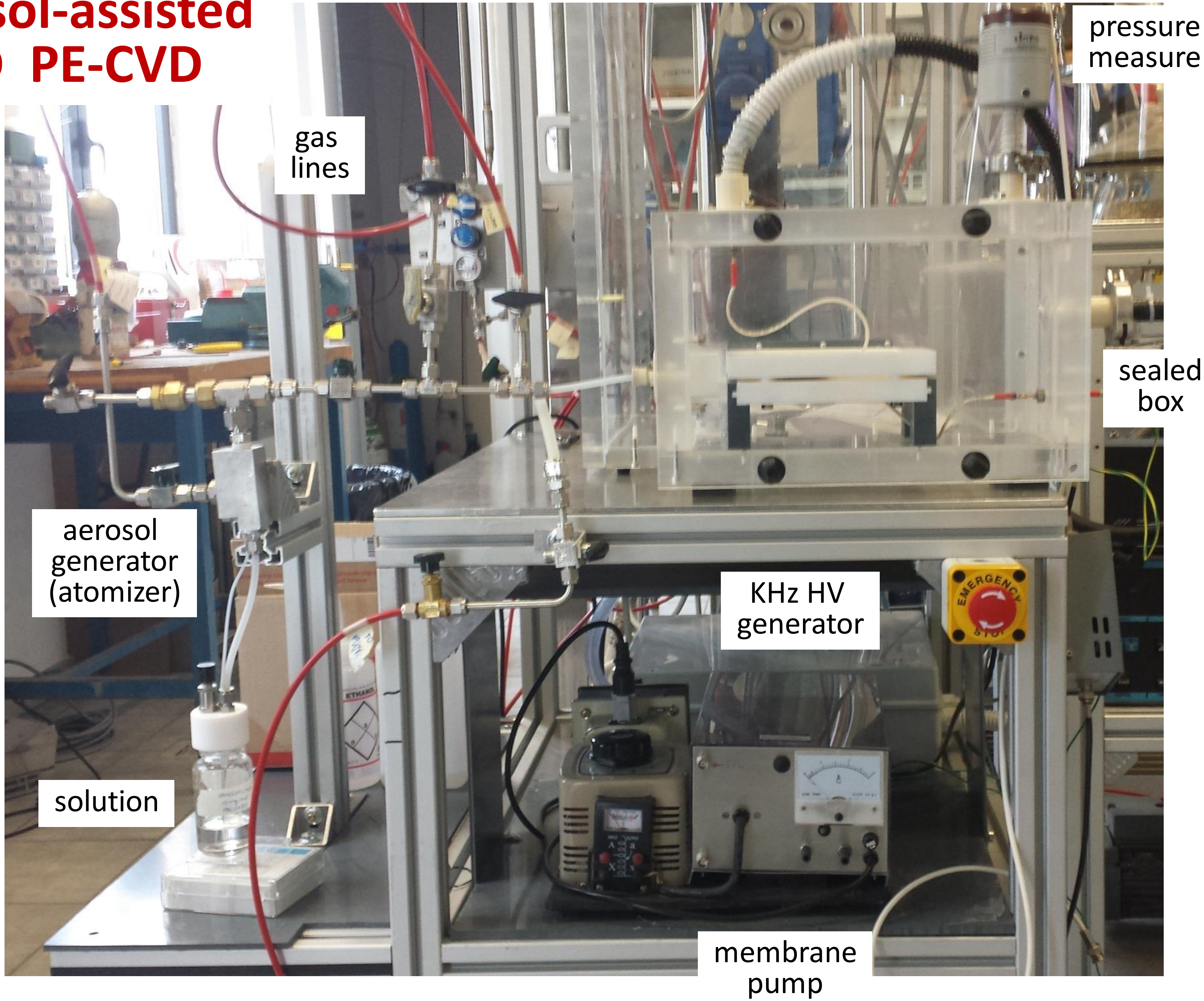
LYSOZYME

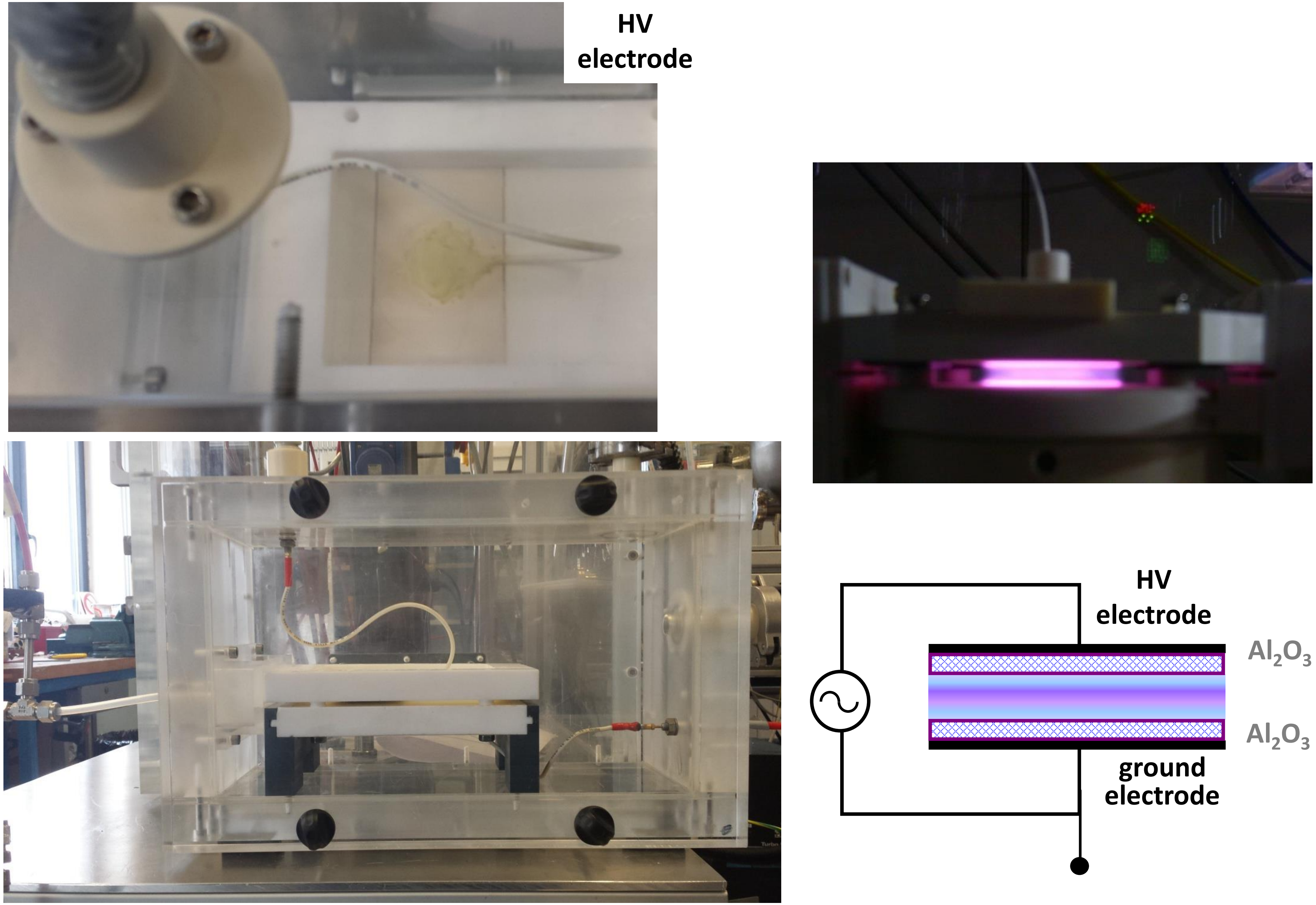


natural antibacterial molecule

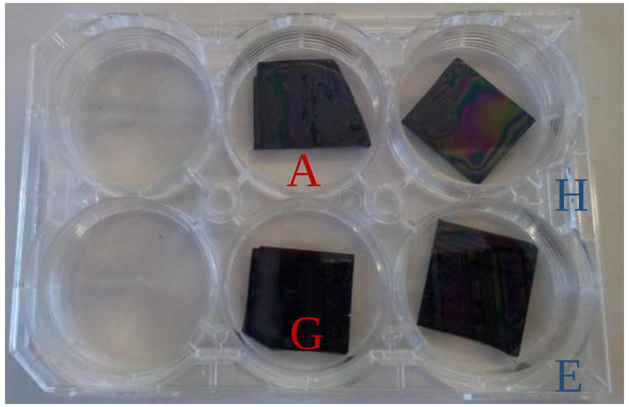
releasable

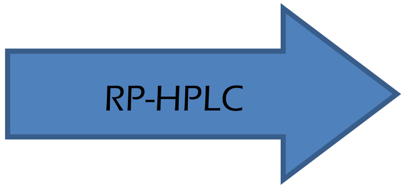
aereosol-assisted DBD PE-CVD



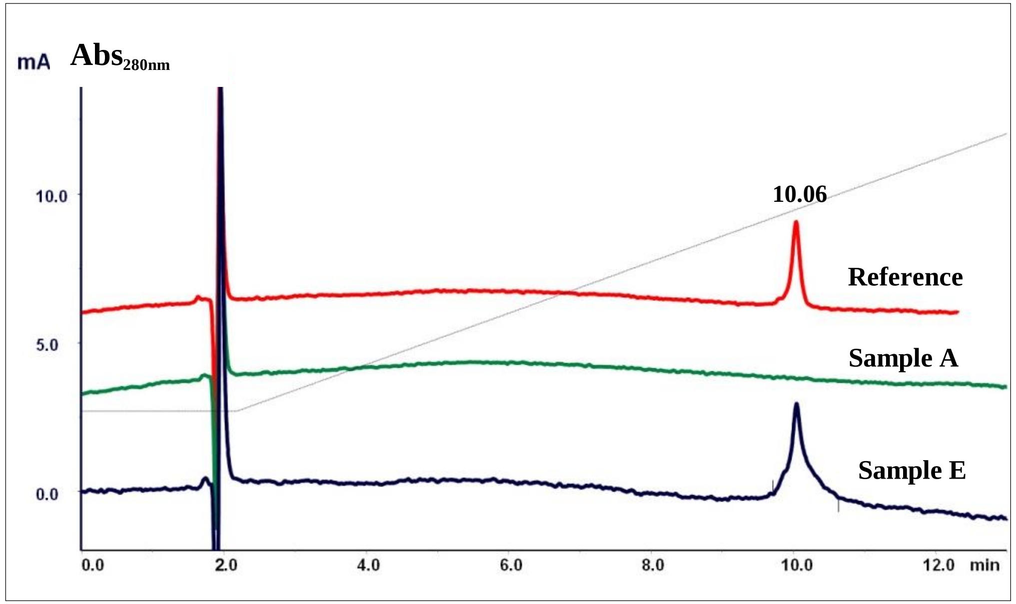

HV
electrode
HV
electrode
Al_2O_3
Al_2O_3
ground
electrode

$LYSOZYME_{sol}$ / C_2H_4 – release test 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

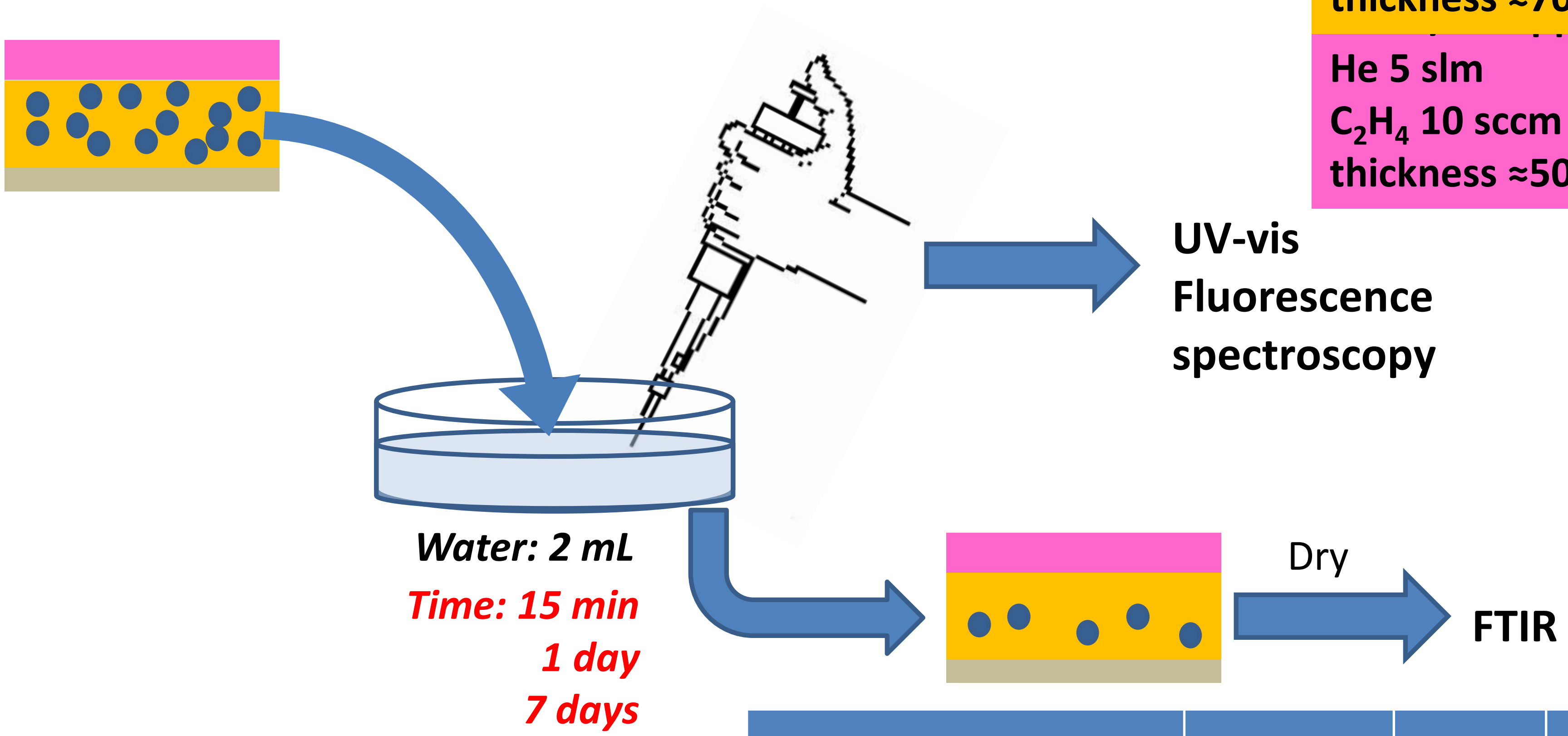
almost all Lysozyme embedded is released in 1h

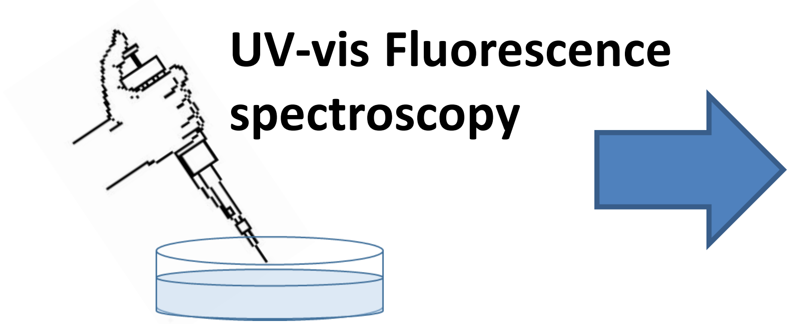
is Lysozyme still «alive»?

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

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

He 5 slm
C_2H_4 10 sccm
thickness ≈50 nm





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

Antimicrobial assay against *Micrococcus lysodeiktikus*

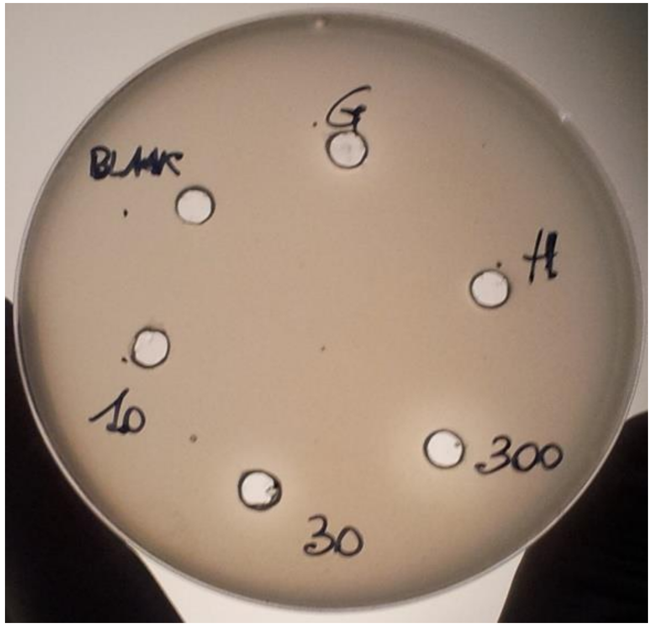
(Lie et al., Acta Veterinaria Scandinavica, 27(1): 23-32, 1986)

- 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.

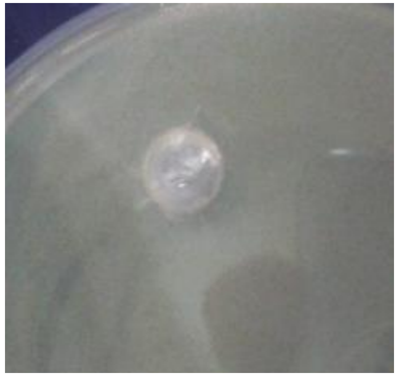
Well content	**Inhibition halo diameter [mm]**
C_2H_4/Lyz_{sol} HiLyz coating	8 ± 1
C_2H_4/H_2O plasma deposited coating (control)	0
Lyz standard solution *(10 μg/mL)*	0
Lyz standard solution *(30 μg/mL)*	6 ± 1
Lyz standard solution *(300 μg/mL)*	12 ± 1
Blank (negative control)	0

Palumbo et al, PPaP 12, 1310, 2015

Antibacterial effect of C_2H_4/Vancomycin coatings

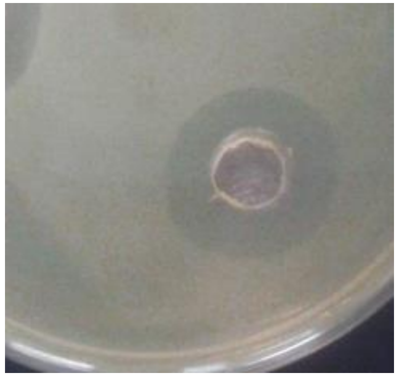
Agar diffusion test against Staphylococcus Aureus: Preliminary results

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



Uncoated Ti

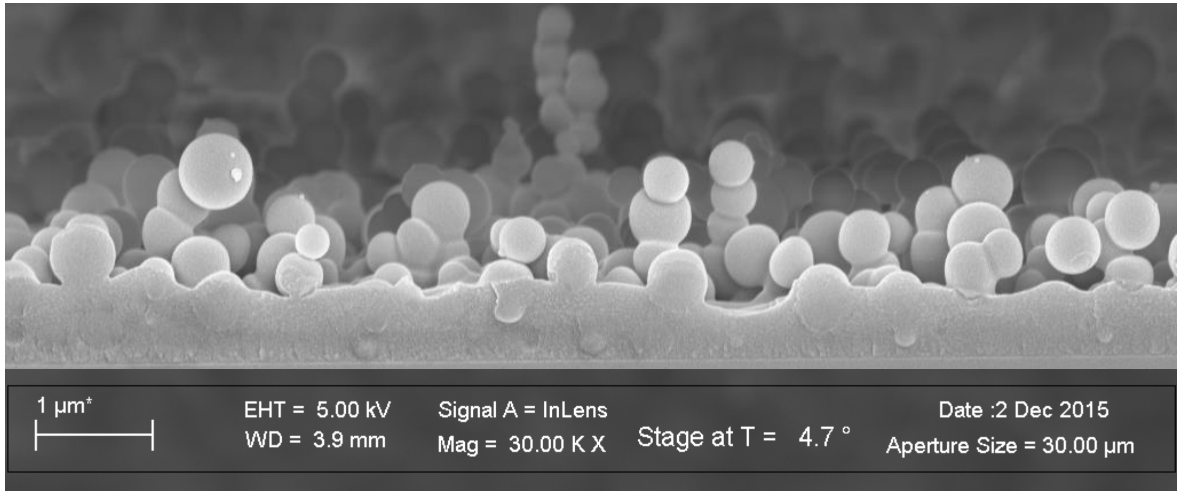
Ti coated with C2H4/H2O

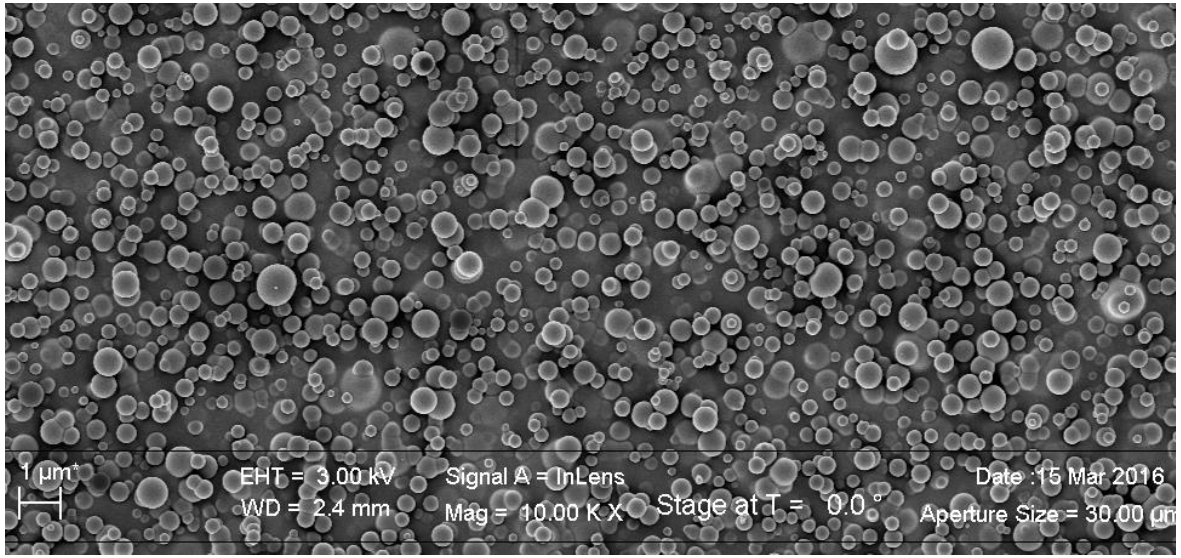


Ti coated with C2H4/Vancomycin$_{(aerosol)}$

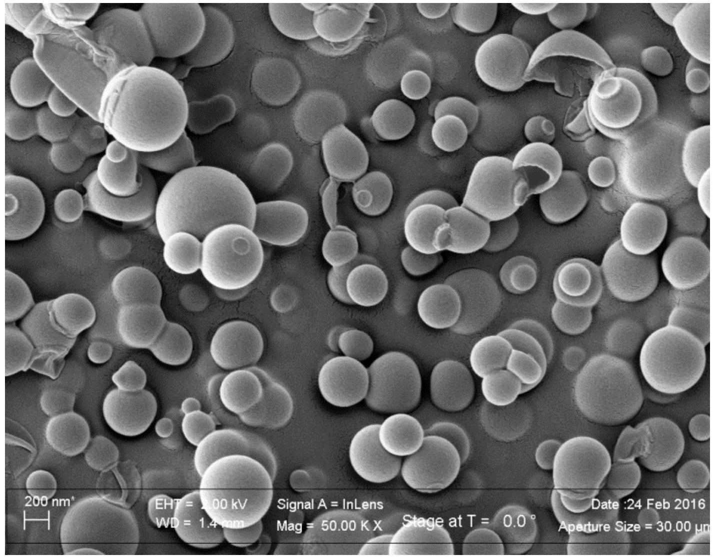
In collaboration with:
Biomaterials, Biomechanics and Tissue Engineering group
Dept. de Ciència dels Materials i Enginyeria Metallúrgica
Technical University of Catalonia (UPC)

Lo Porto, Palazzo, Palumbo, Favia
direct plasma synthesis of nano-capsules loaded with antibiotics
Polymer Chemistry 8, 1746, 2017



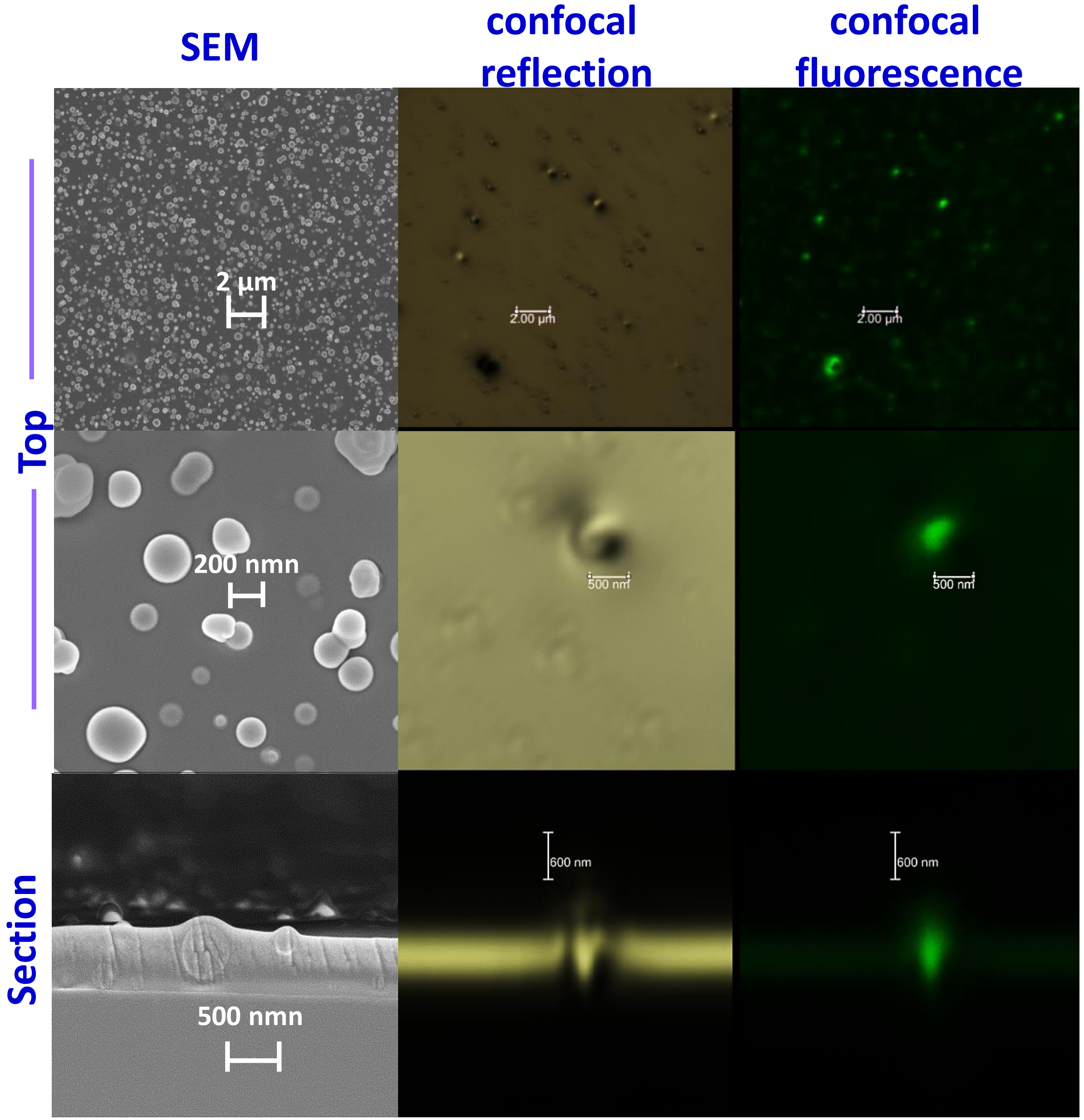


after immersion in water

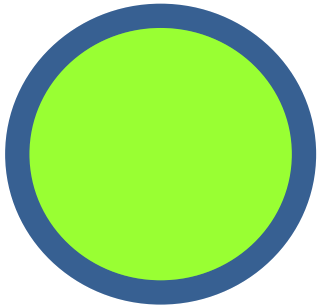


$Vancomycin_{sol}$ / C_2H_4

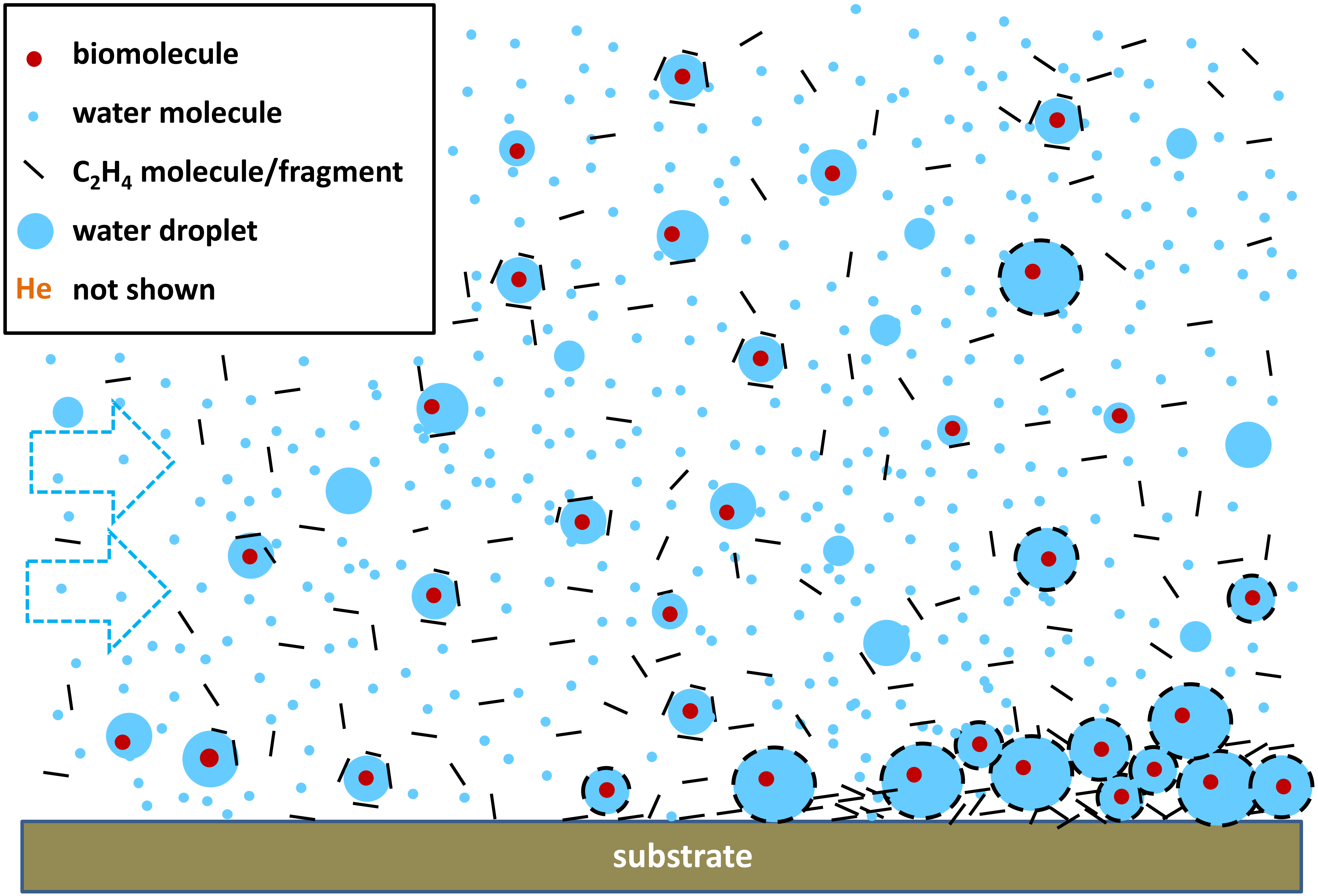
confocal microscopy (with fluorescein)



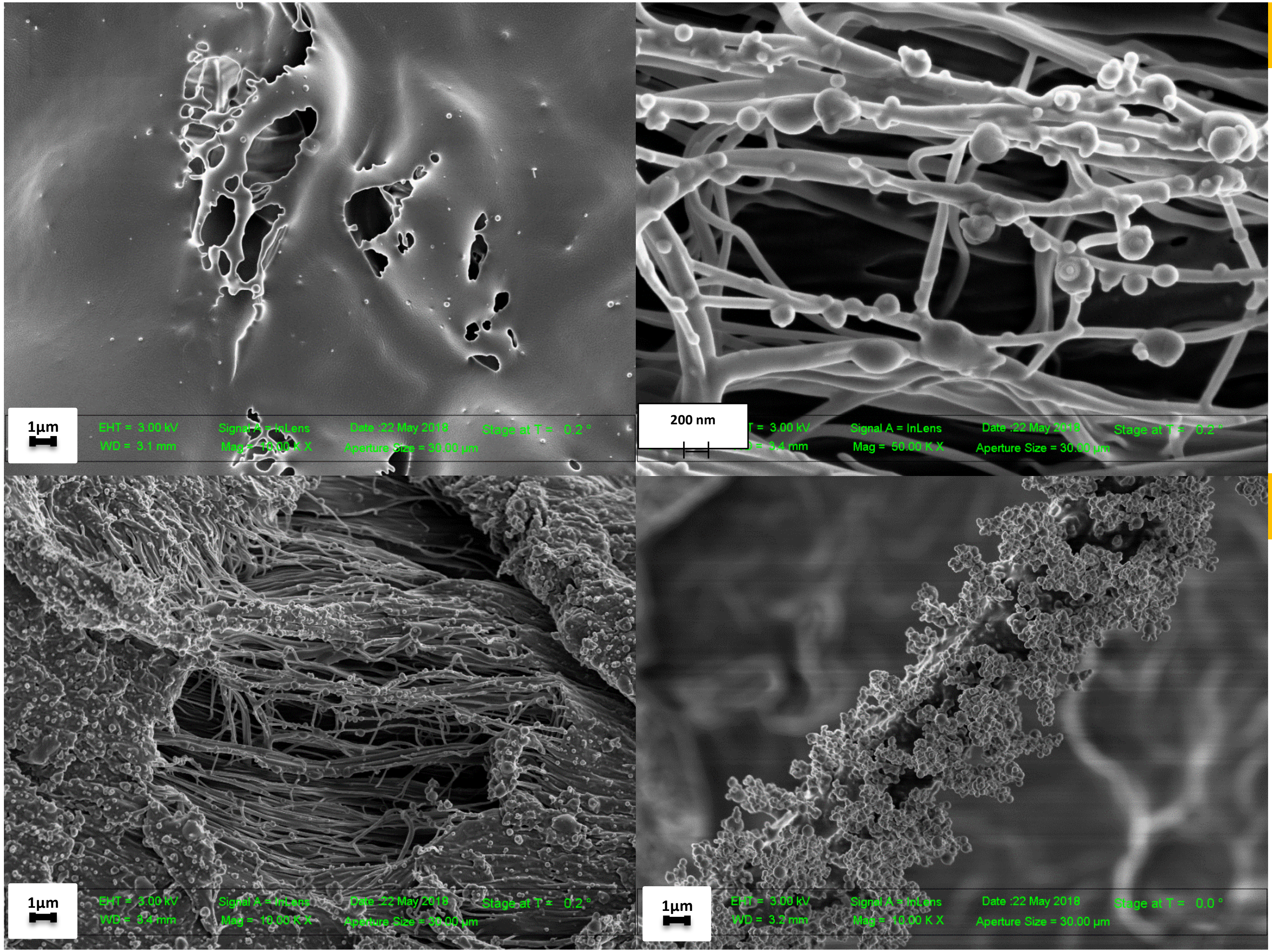
$[FLUO]_{aerosol}$ 1 mg/mL
He: 5 sLm
C_2H_4: 20 sccm
600 nm
continuous mode



we are producing IN THE PLASMA nanometric capsules loaded with vancomycin (fluorescein)


biomolecule
water molecule
C_2H_4 molecule/fragment
water droplet
He not shown
substrate

PLASMA DEPOSITION ON DECELLULARIZED SCAFFOLDS



CONTINUOUS MODE

PULSED MODE

nanocapsules loaded with gentamicyn deposited on collagen decellularized tissue

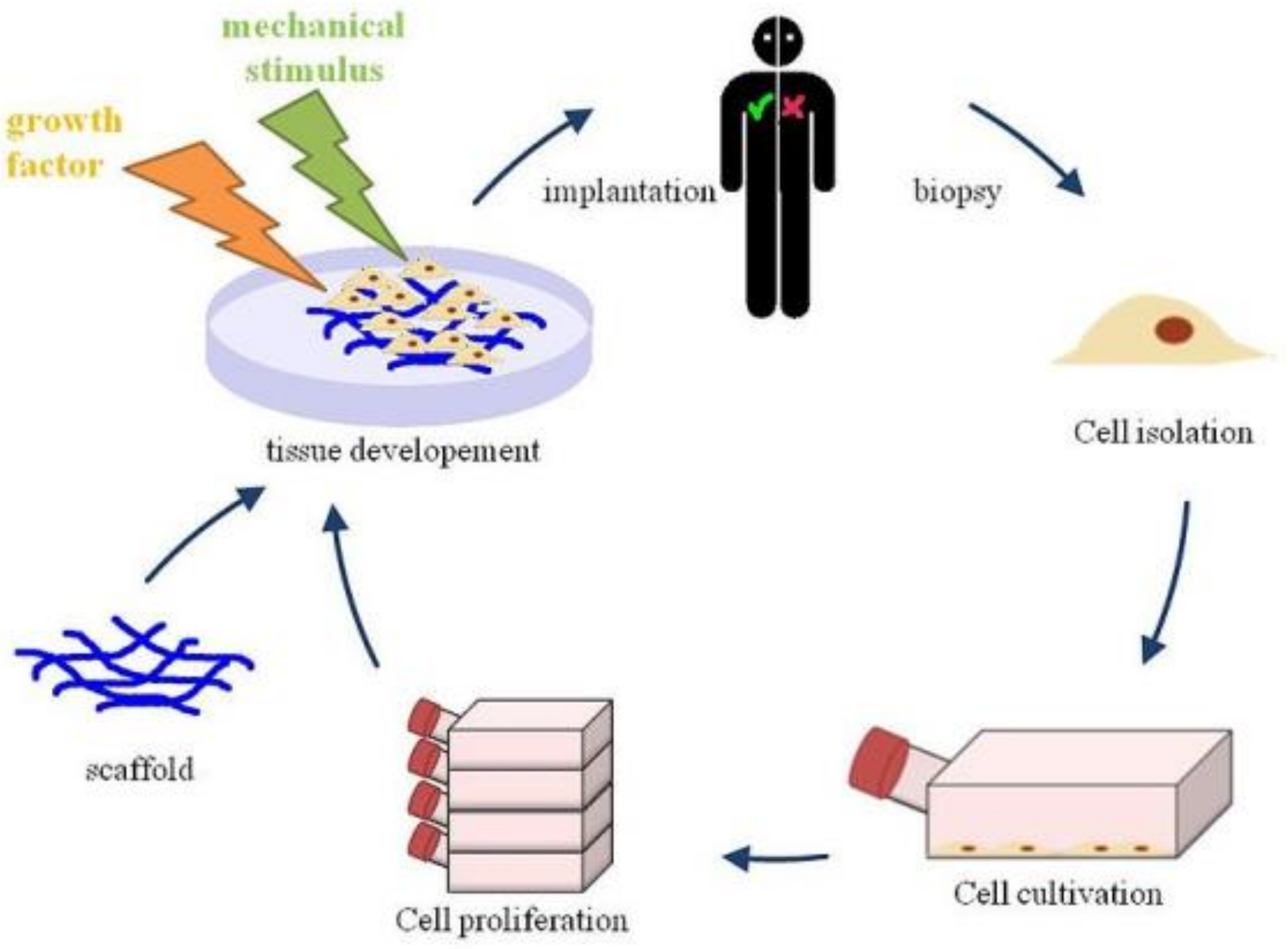
TissueGraft

INGEGNERIA TISSUTALE. – MEDICINA RIGENERATIVA
Procedure di rigenerazione di tessuti del corpo umano.

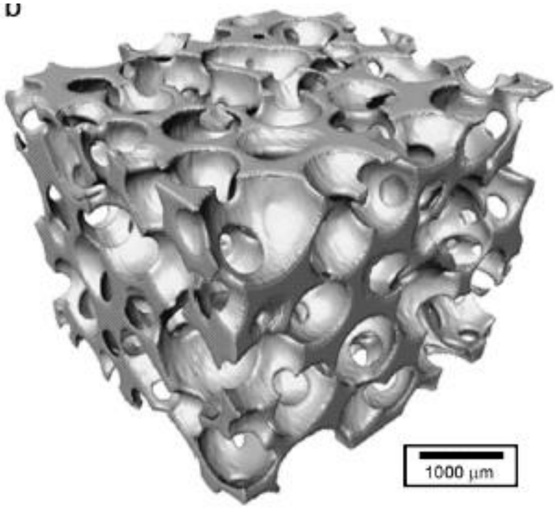
Materiali bioattivi e riassorbibili, per stimolare risposte cellulari specifiche a livello molecolare

In vitro

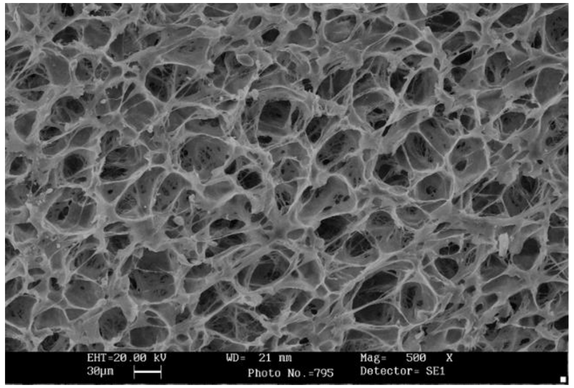
1. Prelievo cellule dal paziente
2. Proliferazione extracorporea
3. Semina delle cellule su strutture (scaffold) riassorbibili
4. Coltivazione in appositi reattori (bioreattori) → produzione nuovo tessuto
5. Impianto del nuovo tessuto nel paziente
6. Il tessuto vivente progettato si adatta all'ambiente fisiologico. Stabilità (?)

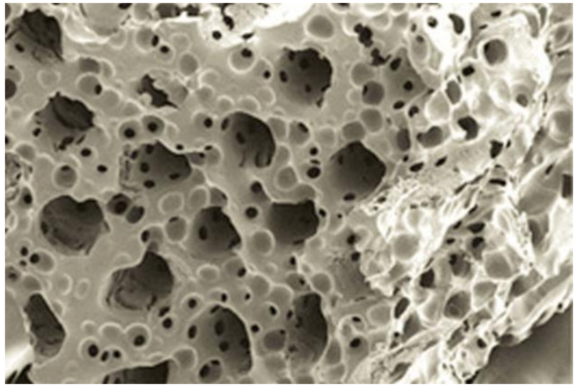


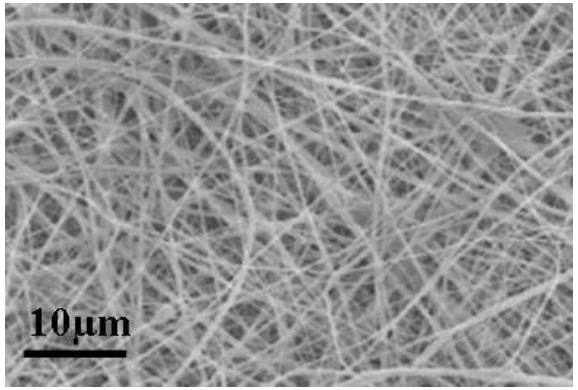
scaffolds for TISSUE ENGINEERING and REGENERATIVE MEDICINE



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

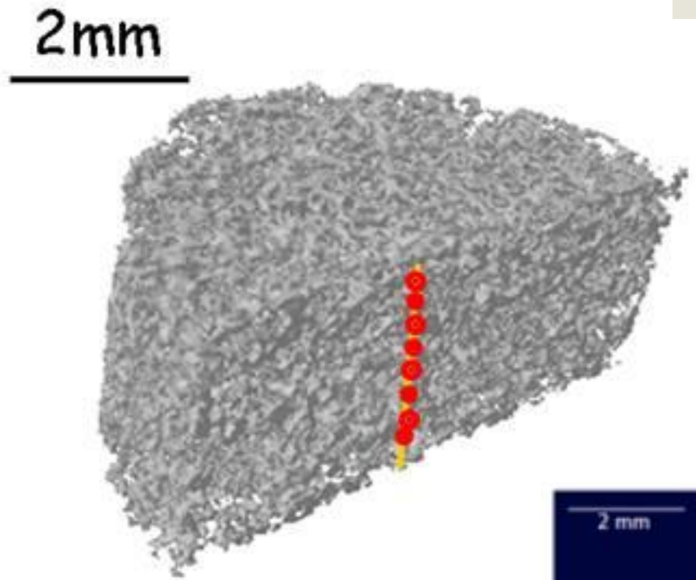
proper surface composition

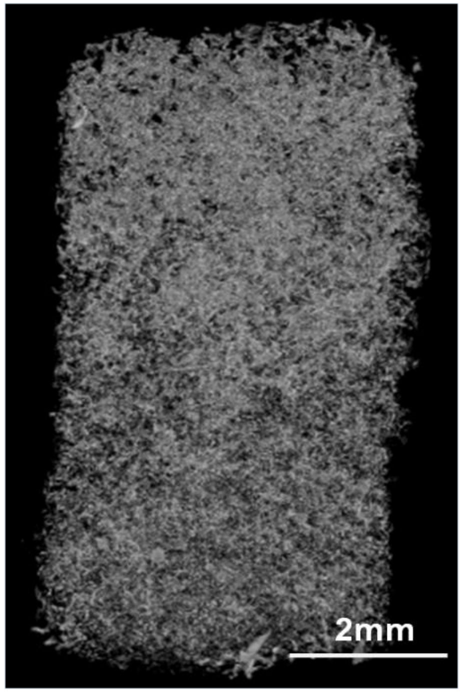
hydrophobic --> hydrophilic

cell-repulsive --> cell-adhesive

PCL porous scaffolds prepared in our lab with the Solvent Casting – Particulate Leaching technique

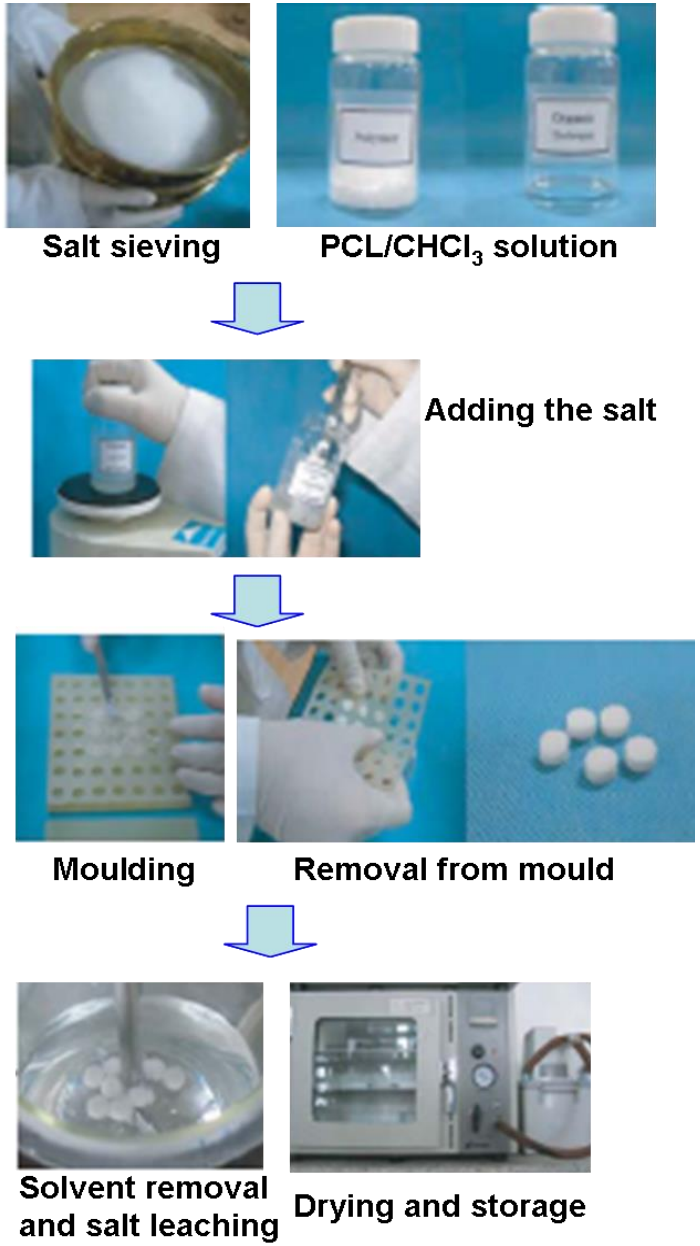
Micro-CT 3D rendering of different scaffolds





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

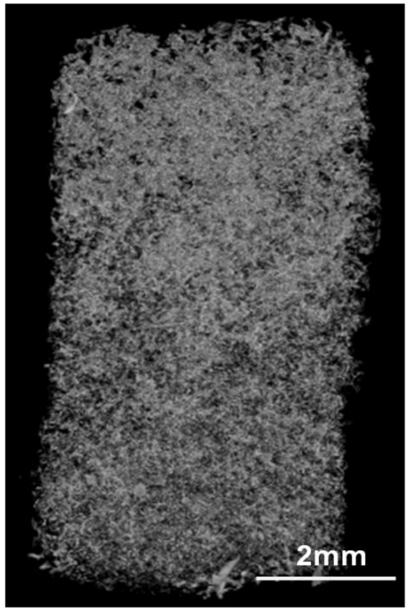
PCL/NaCl <u>5/95</u> - 8/92 - 10/90 wt/wt

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



Trizio, Intranuovo, Gristina, Dilecce, Favia
He/O_2 Atmospheric Pressure plasma jet treatments of PCL scaffolds for Tissue Engineering and Regenerative Medicine
Plasma Proc. Polym., 12, 1451, 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. 12, 786, 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

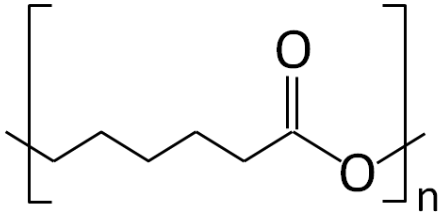
Domingos, Intranuovo, Gloria, Gristina, Ambrosio, Favia, Bartolo
Improved osteoblast cell affinity on plasma-modified 3D extruded PCL scaffolds
Acta Biomaterialia 9, 5997, 2013

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

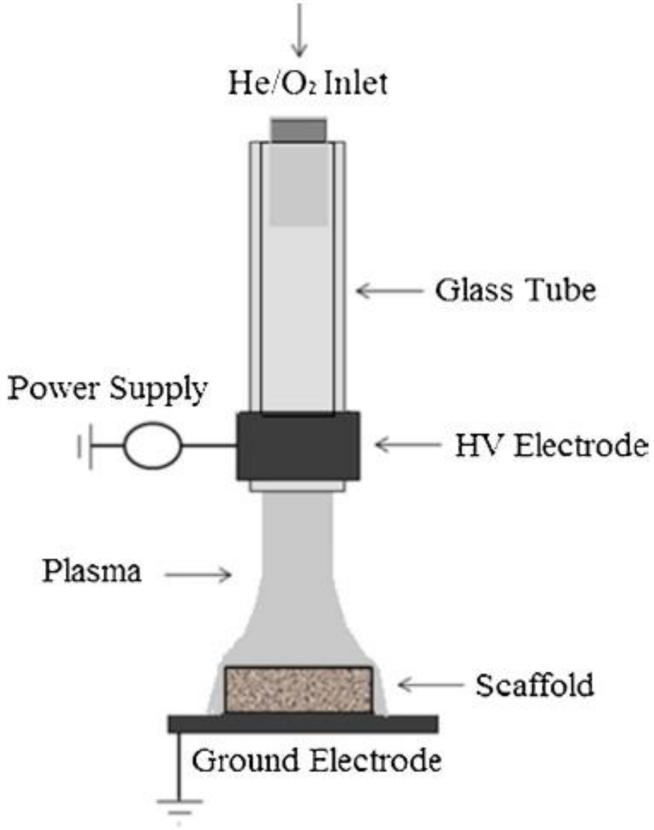
LOW PRESSURE

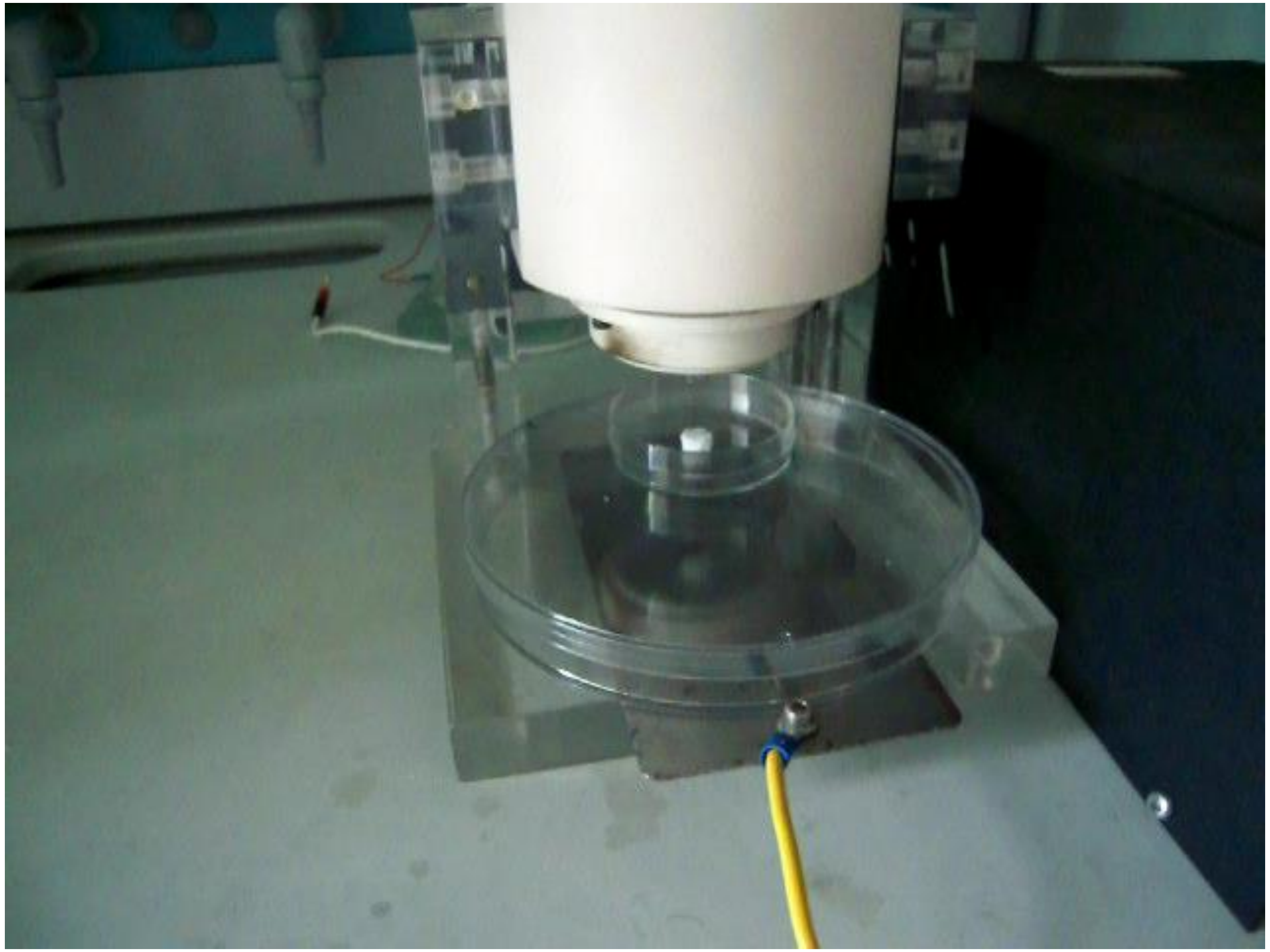
many configurations
13.56 MHz
N_2/H_2O
$C_2H_4/N_2 + H_2$
$C_2H_4/N_2 + C_2H_4$
C_2H_4/AA
O_2 + DEGDME



Trizio et al, Plasma Proc. Polym. 12, 1451, 2015

He/O_2 Atm P Plasma Jet Treatments of Poly Capro Lactone (PCL) Scaffolds for Tissue Engineering and Regenerative Medicine





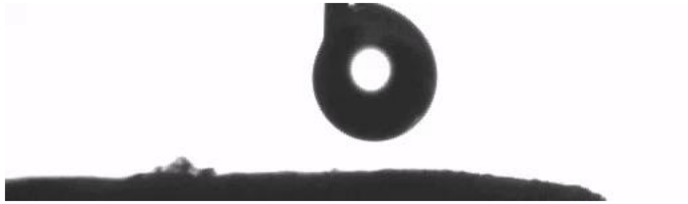
He 3000 slm

O_2 0-100 sccm

WCA 122 ± 7°
non water absorbant

PCL scaffold **before**

WCA very low
highly water absorbant

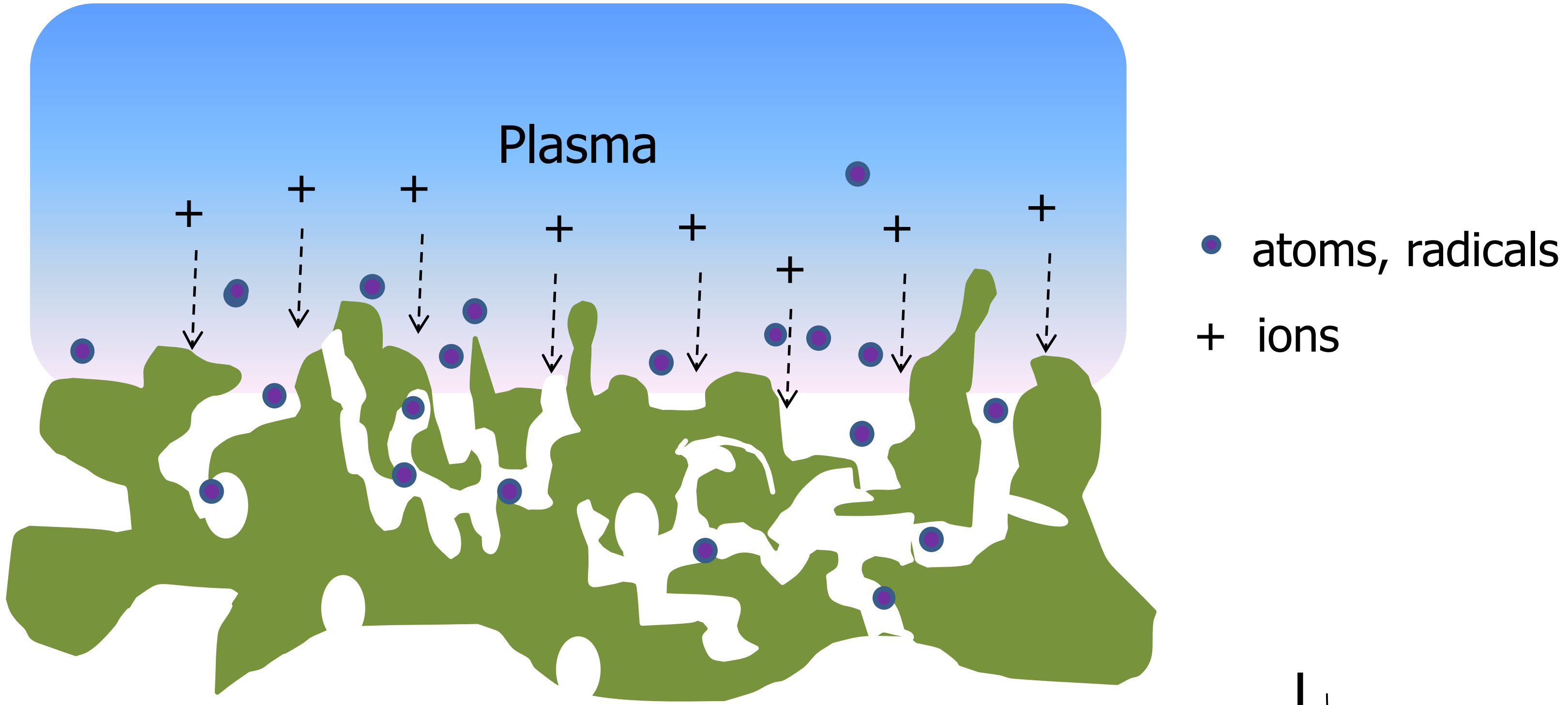


and **after** the plasma treatment

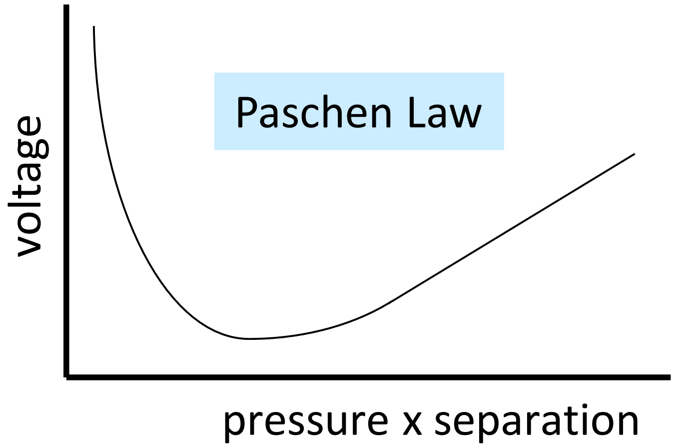
plasma functionalization of the internal walls and pores of polymer scaffolds (treatments, coatings) allows:

1- **hydrophilic and cyto-compatible surfaces for a better and rapid cell colonization**
2- **better perfusion of nutrients**
3- **better elution of metabolytes and degradation products**

plasma processing of porous substrates



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



APPJ He/O_2 modification of PCL scaffolds

effect of the O_2 content in the He/O_2 feed on the water absorption rate

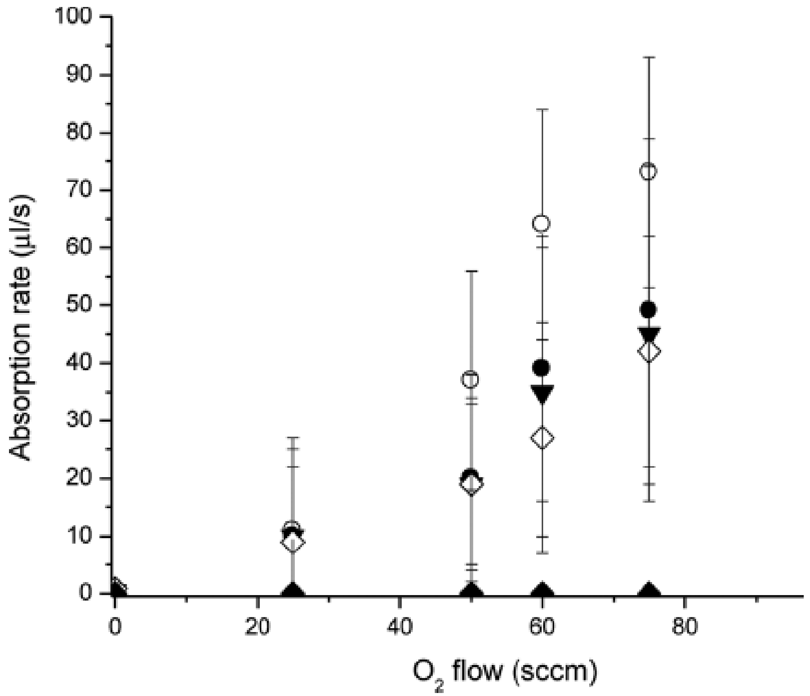


Figure 4. Water absoption rate of untreated (▲) and APPJ treated (He/O2, 60 s) scaffolds after 0 (○), 1 (●), 2 (▼), and 9 days (◇) of ageing, as a function of O2 flow rate. No significant differences were found between means calculated with Two-way ANOVA followed by Bonferroni post-test.

water absorption rate vs pH: presence of acid surface sites

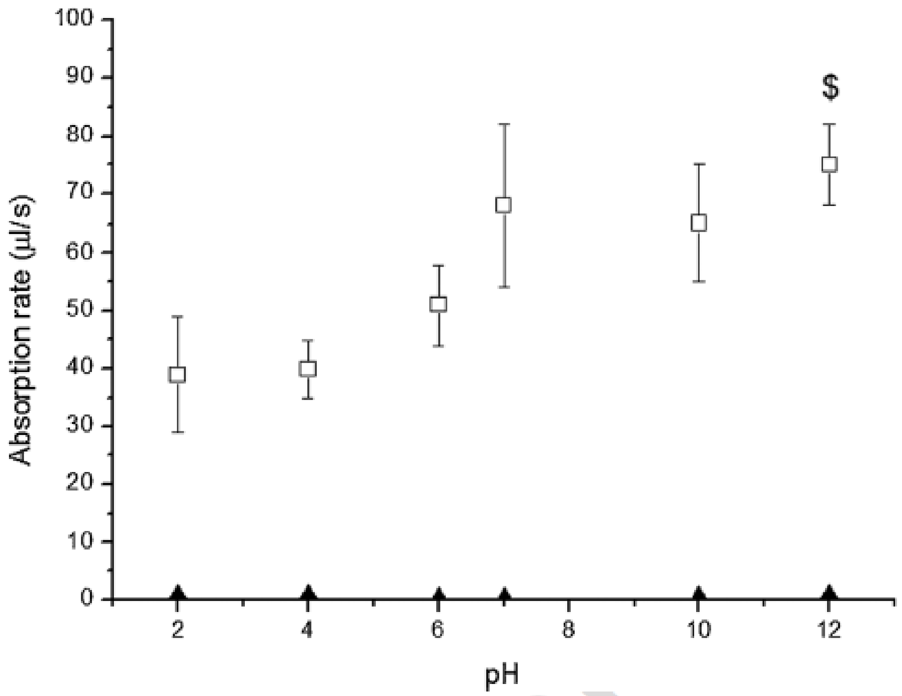


Figure 5. Absorption rate for untreated (▲) and APPJ treated (He/O_2, 60 s) scaffolds (□) as a function of the pH of the test water solutions. Significant differences between means were calculated with Two-way ANOVA followed by Bonferroni post-test ($^{\$}p < 0.05$ vs. pH 2).

Trizio et al, PPaP12, 1451, 2015
He/O_2 APPJ treatments of PCL scaffolds for Tiss. Eng. & Reg. Medicine

Grafting of O-containing groups at the surface of PCL scaffolds

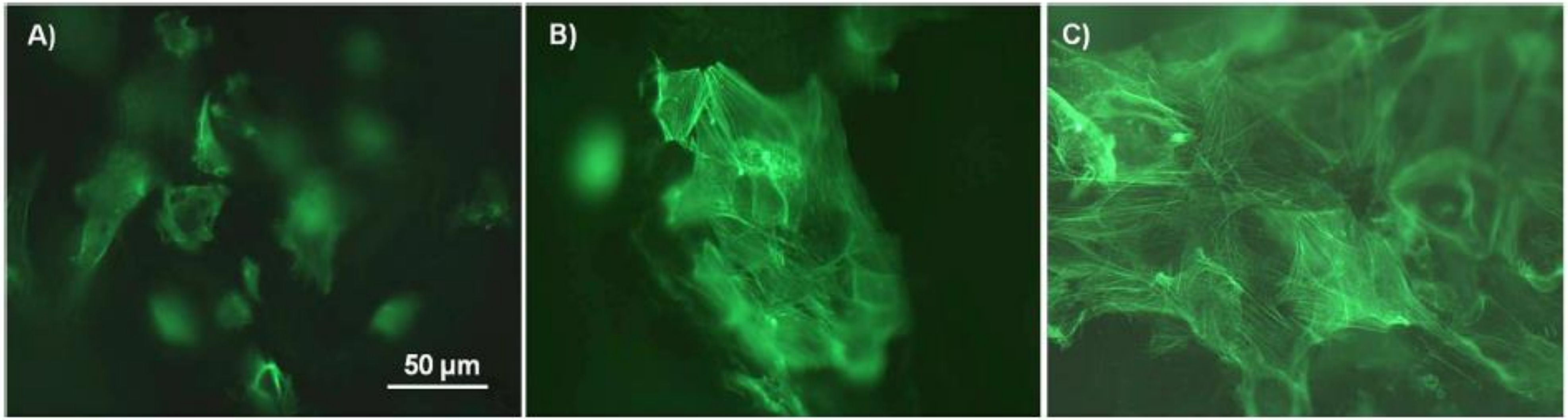
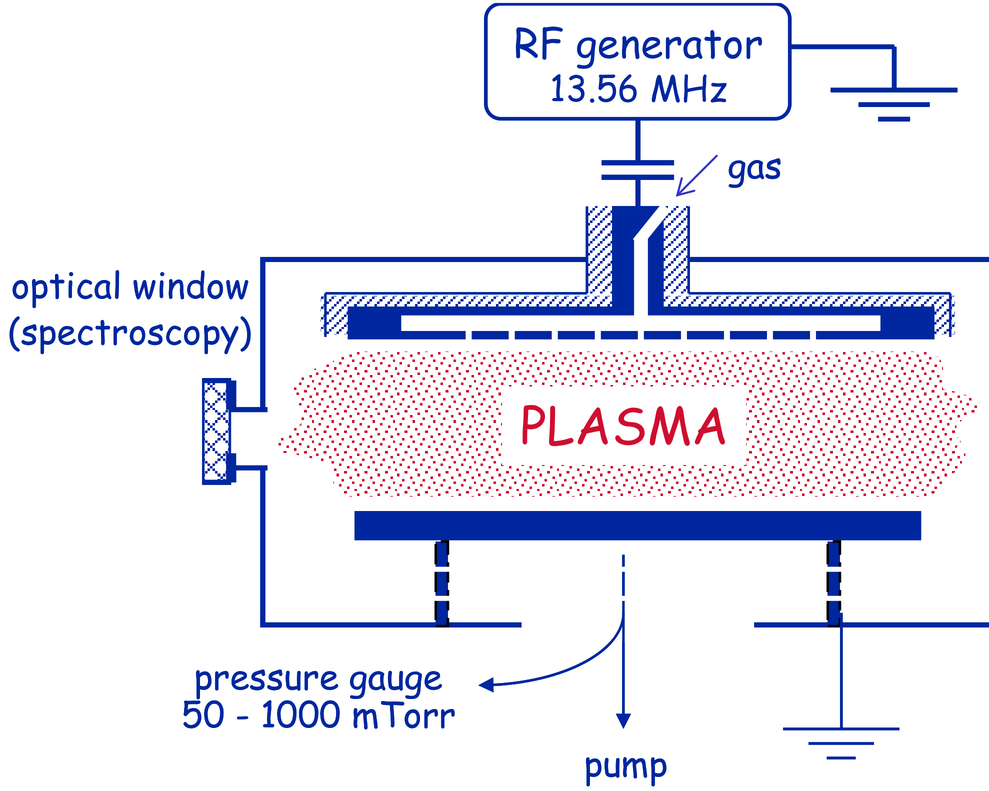


Figure 9. Fluorescence images of Saos-2 cells cultured for 72 h on PCL scaffold: untreated A); APPJ He (60 s) treated B); and APPJ He/O_2 (100 sccm O_2, 60 s) treated C).

PE-CVD of functional coatings on/within PCL scaffolds



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

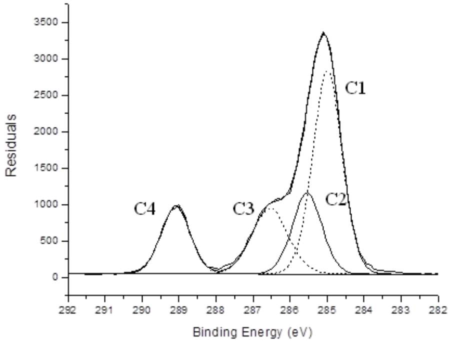
N_2/ethylene 5/1; 47 Pa; 50 W; 30 min; followed by H_2; 20 W; 3 min

2) pdE:AA coating with oxygen containing functional groups

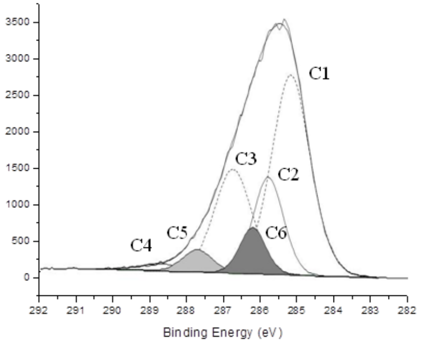
acrylic acid/ethylene/Ar 3/1/2; 33 Pa; 30 W; 20 min

chemical composition on the top

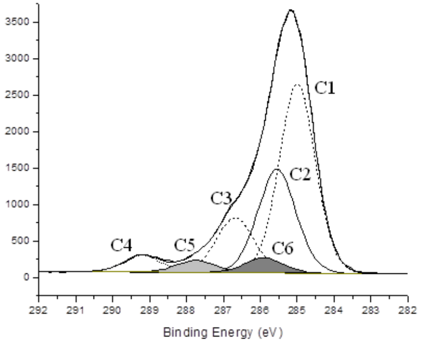
PCL



PdE:N



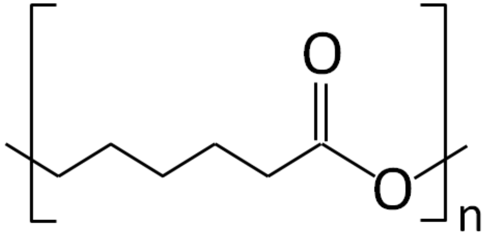
$PdE:N/H_2$

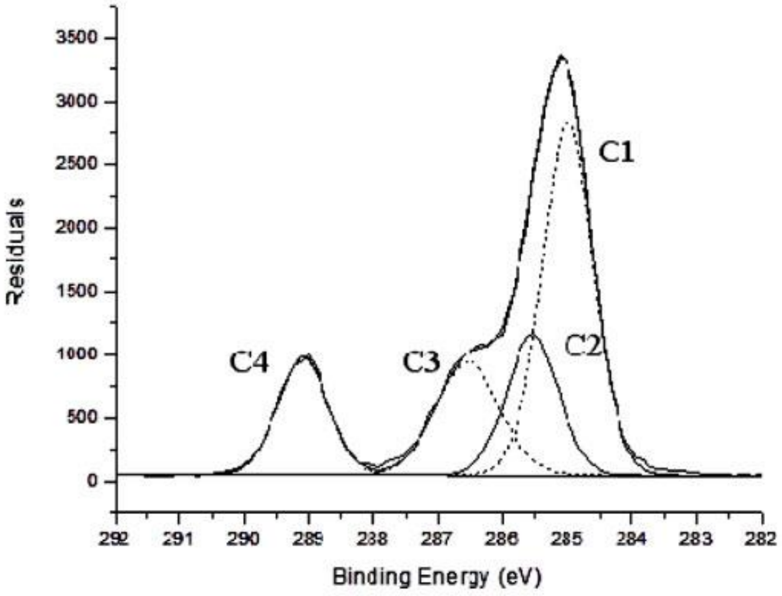


C1s

	C%	O%	N%	C1%	C2%	C3%	C4%	C5%	C6%
BE (eV)				285.0	285.6	286.5	289.1	288.0	286.0
group				C-H/C-C	C-C-C=O	C-C_2-C=O	C(O)=O	N-C(=O)	C-N
PCL	77±1	23±1	0	48±1	18±1	19±1	15±1	/	/
PdE:N	85±2	5±1	10±1	47±4	17±1	23±1	2.0±0.5	4±1	7±1
$PdE:N/H_2$	82±2	12±1	6±1	53±6	22±5	14±1	5±1	3±1	3±1

PCL scaffold

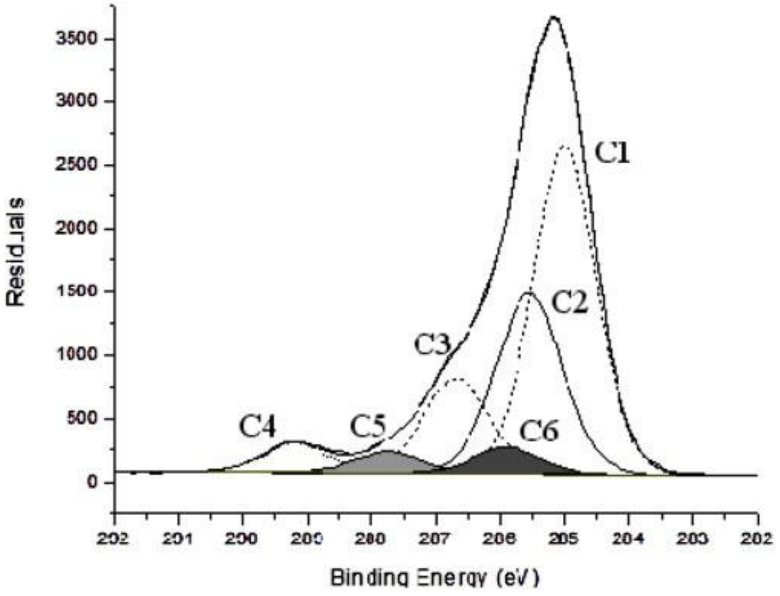




	PCL
WCA$_{stat}$	$115 \pm 1°$
WCA$_{adv}$	$129 \pm 7°$
WCA$_{rec}$	$103 \pm 11°$
WCA$_{hysteresis}$	$26 \pm 3°$

no water absorption

PCL scaffold PdE:N/H_2 processed



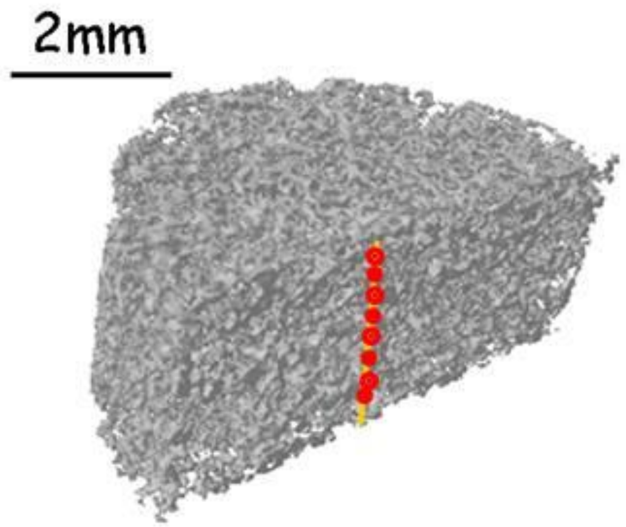
	PdE:N/H_2-PCL
WCA$_{stat}$	$77 \pm 1°$
WCA$_{adv}$	$108 \pm 13°$
WCA$_{rec}$	$75 \pm 2°$
WCA$_{hysteresis}$	$33 \pm 1°$

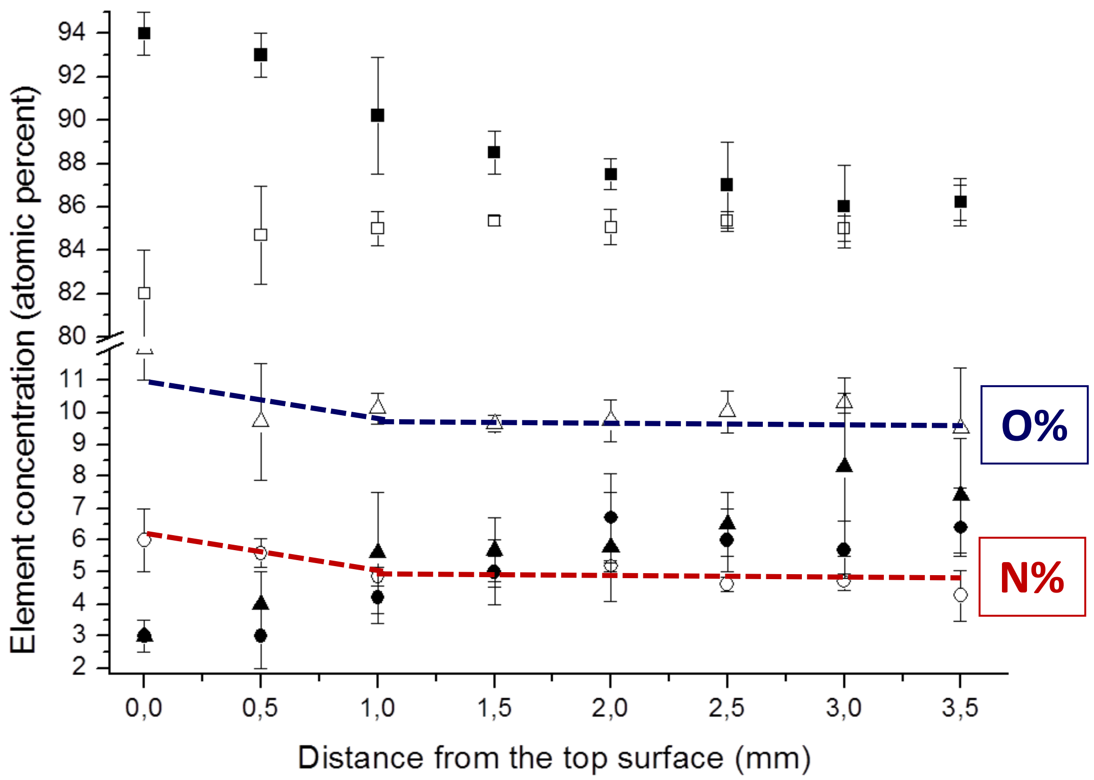
slow water absorption

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

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 of PdE:N/C_2H_4 (C%: ■, O%: ▲, N%: ●) treated scaffolds.

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

PdE:N/H_2 treated PCL scaffolds become:

- functionalized with polar -N and O containing groups outside and inside the 3D porous structure

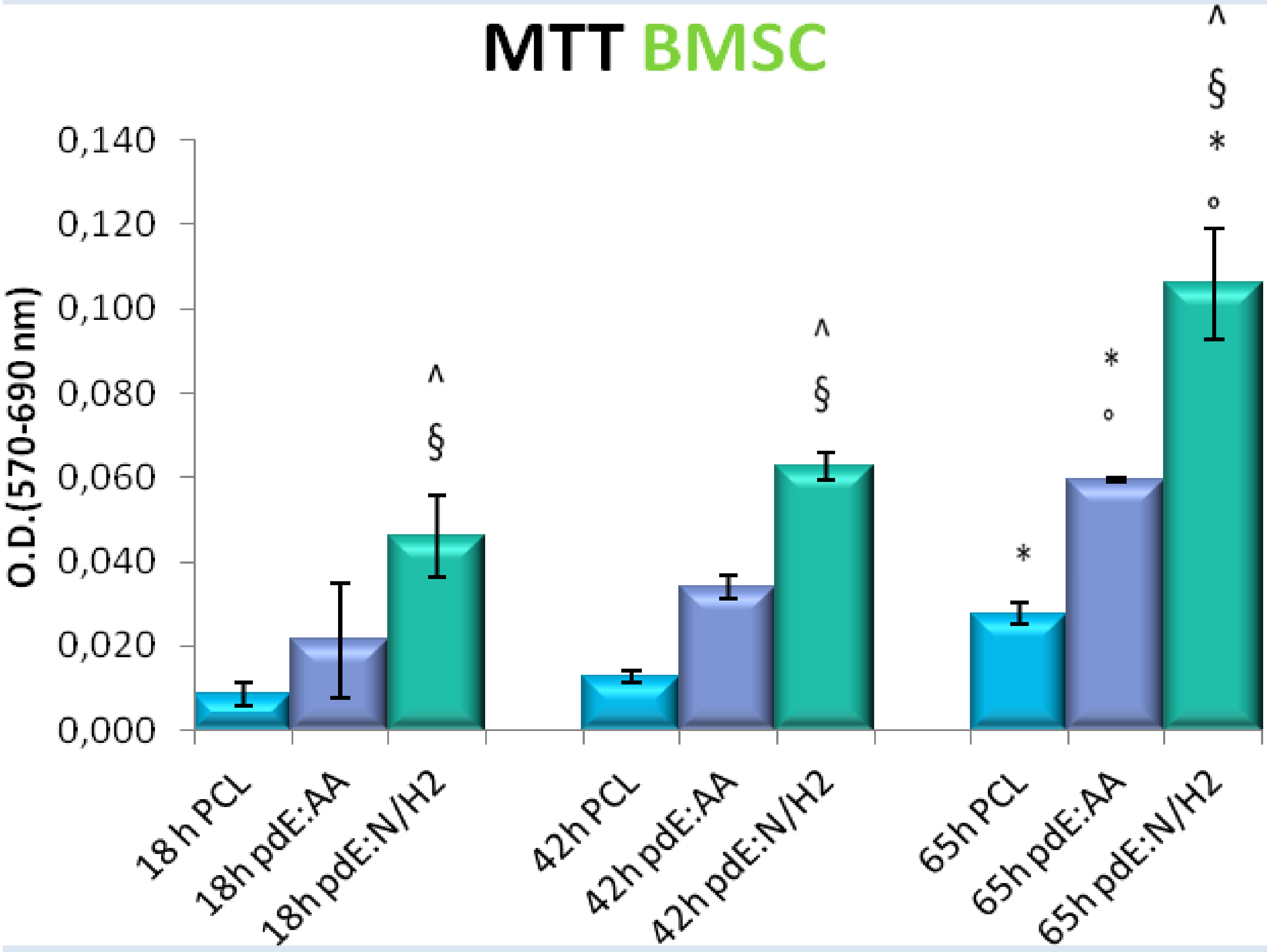
  → **BETTER CELL ADHESION & PROLIFERATION**

- wettable and water absorbing

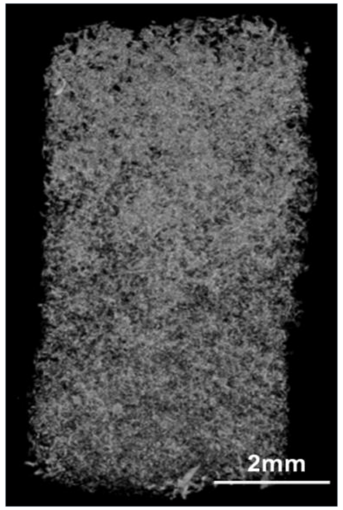
  → **IMPROVED PENETRATION OF WATER & MEDIUM**
  IMPROVED PENETRATION OF NUTRIENTS *in vivo*

in vitro experiments

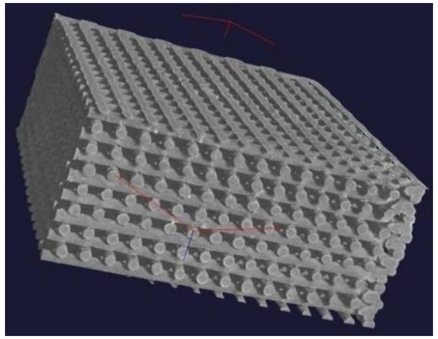
1x10^4 **BMSCs** were seeded on each 2D/3D PCL sample. Cell viability (**MTT**) and morphology (**actin cytoskeleton** fluorescence microscopy) were studied at 18, 42 and 65 h of culture.



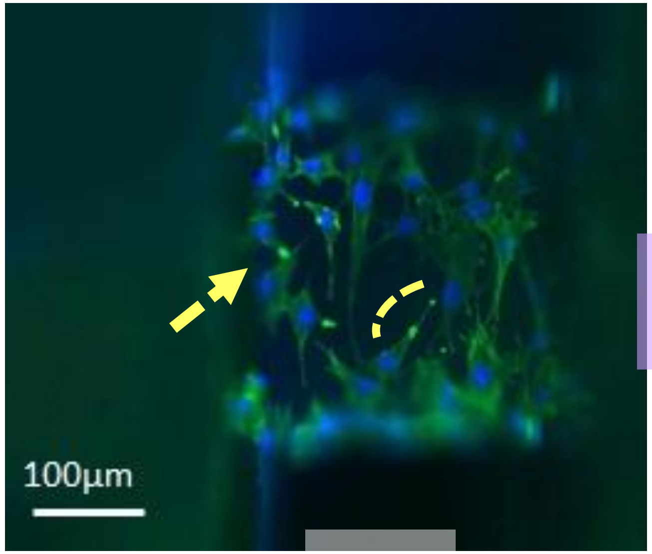
RIGENERA Regional Project



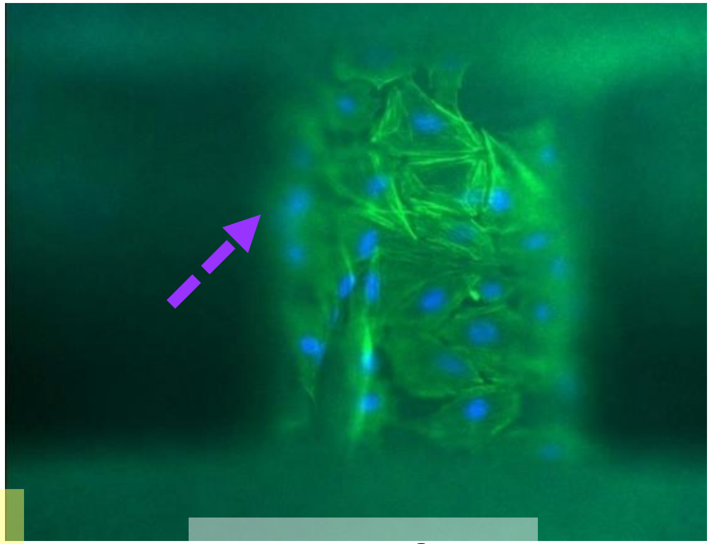
Intranuovo et al, Acta Biomaterialia 9, 5997, 2013
Improved osteoblast cell affinity on plasma-modified 3D extruded PCL scaffolds



N-containing PE-CVD coating on PCL scaffolds from C_2H_4/ N_2



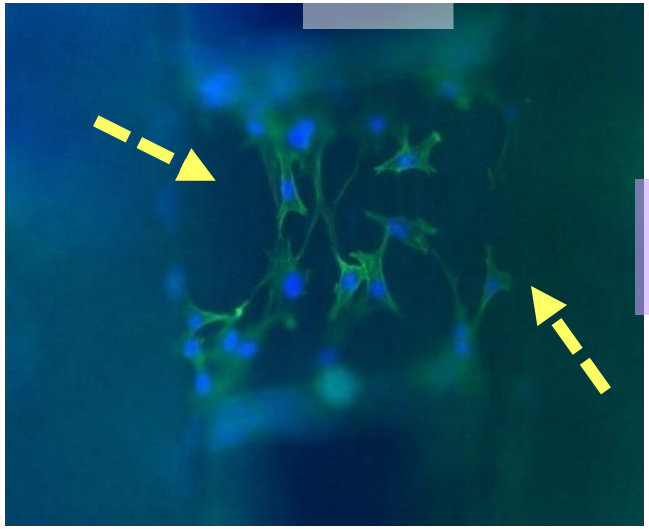
spots of actin at cell periphery



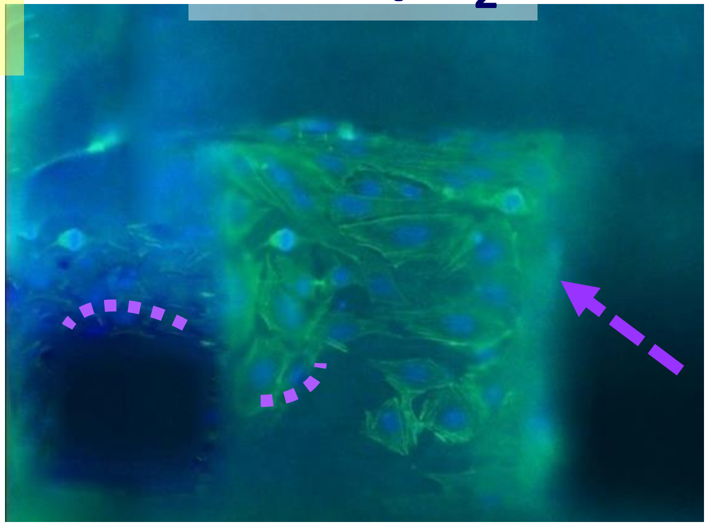
PCL

clustered and polygonal-shaped body

PdE:N/H_2



spindle-shaped cell body



in vivo experiments

pdE:N/H_2 coated PCL scaffolds were implanted in ovine knees (9-10 yo, 40-50 Kg, left lateral decubitus, limb abducted).

An osteochondral defect (4 mm dia) was sculpted in the medial condyle of the right femur and replaced with the scaffold.

Prof. A. Crovace, Sez. Veterinaria, Dipartimento Emergenza e Trapianto Organi, D.E.T.O. Univ. Bari

TIMELINE

2 weeks
to get used to the place

time 0
surgery

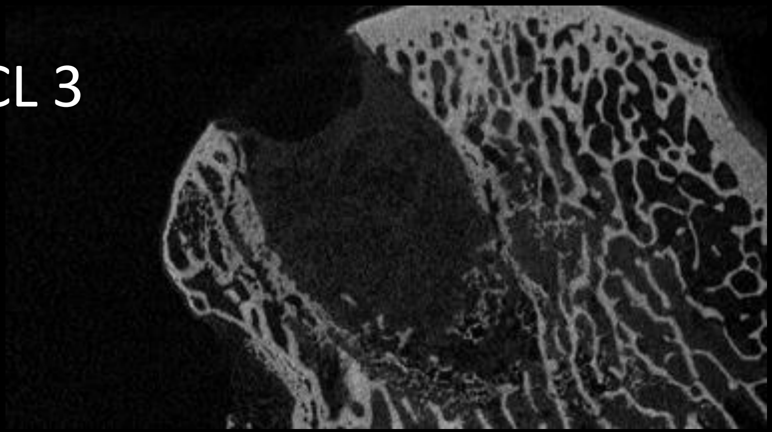
time 3 months
sacrifice GROUP 1

time 6 months
sacrifice GROUP 2

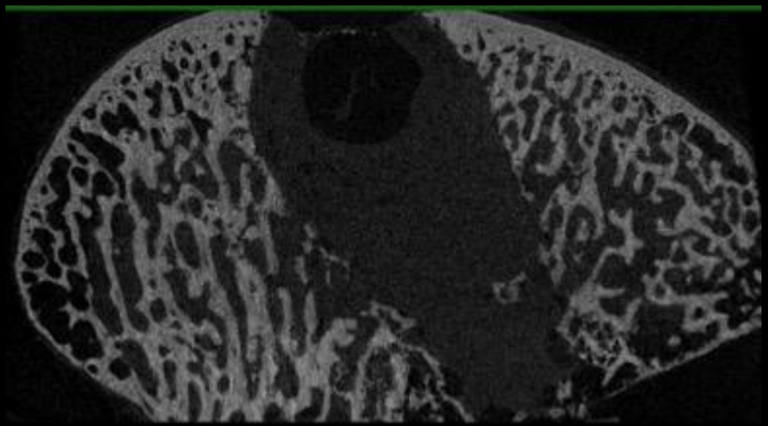
PCL 3 (2)
PCL + plasma 3 (4)
PCL + plasma + BMS cells 3 (4)

PCL 6 (2)
PCL + plasma 6 (4)
PCL + plasma + BMS cells 6 (4)

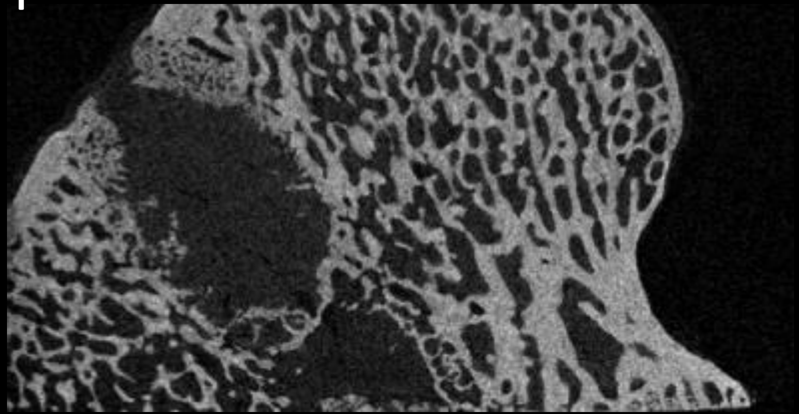
PCL 3



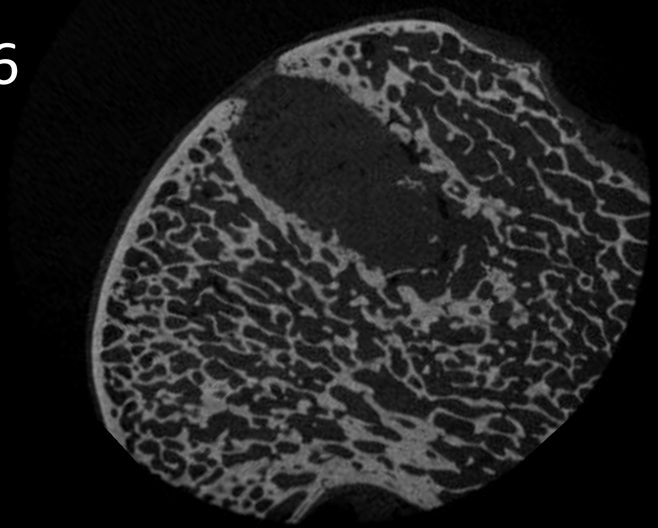
PCL 6



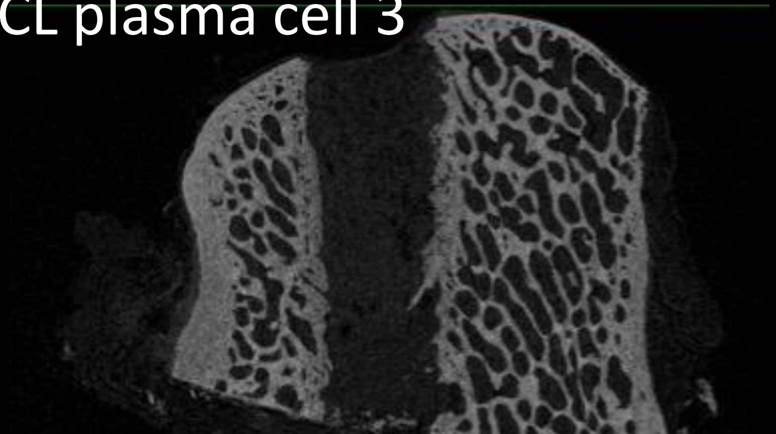
PCL plasma 3



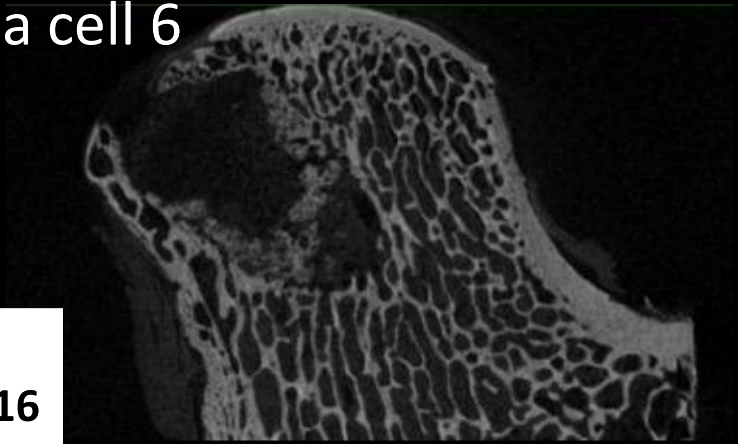
PCL plasma 6



PCL plasma cell 3



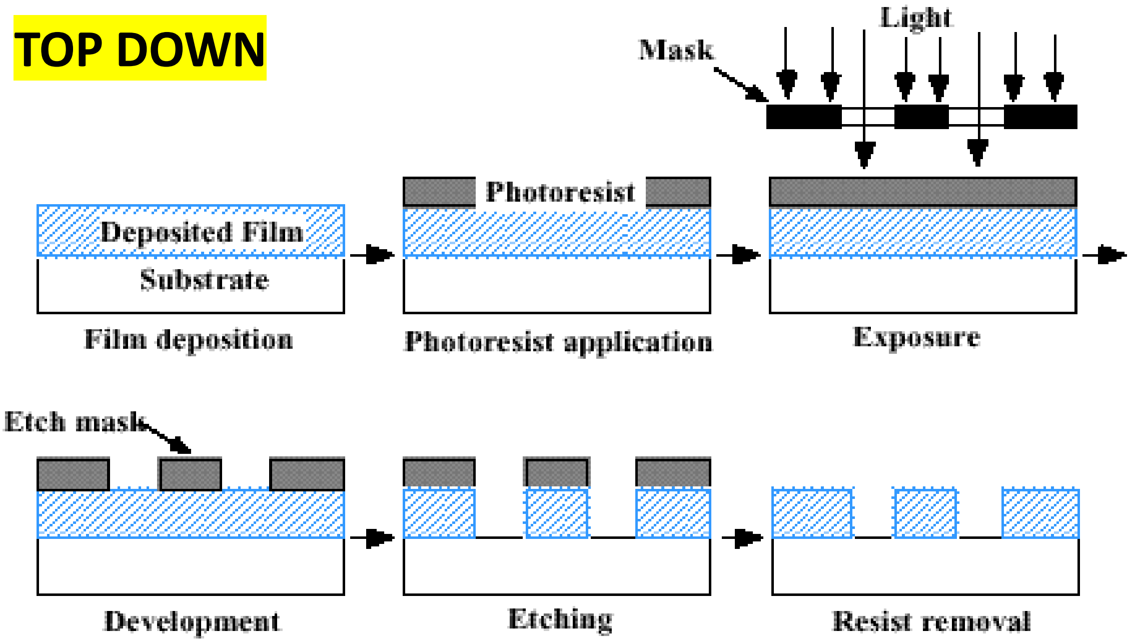
PCL plasma cell 6



Intranuovo et al
PCPP 36,369, 2016

TOP DOWN

Superfici patternate: fotolitografia



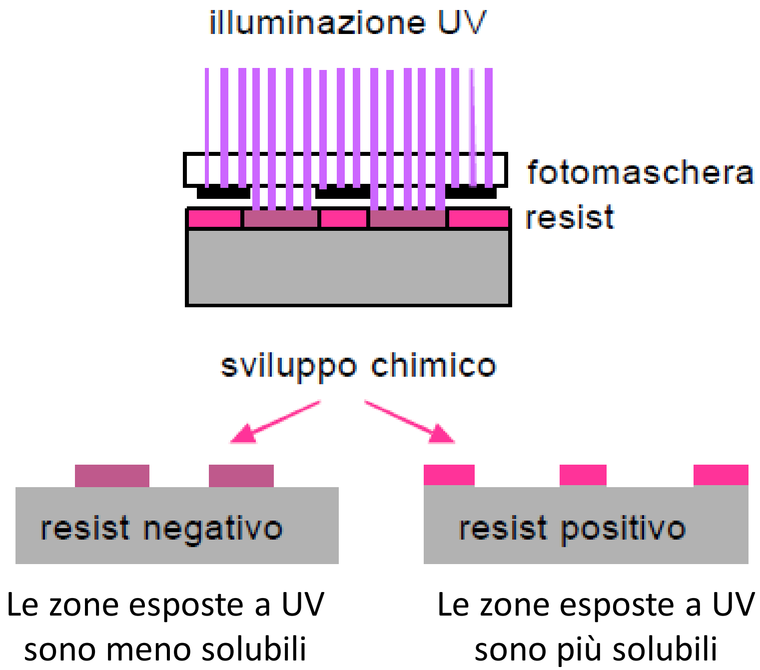
La fotolitografia prevede il trasferimento di un disegno da una maschera alla superficie di film/substrati

Permette di ottenere strutture estremamente ordinate

La risoluzione max. è 100 nm (separazione tra strutture vicine)

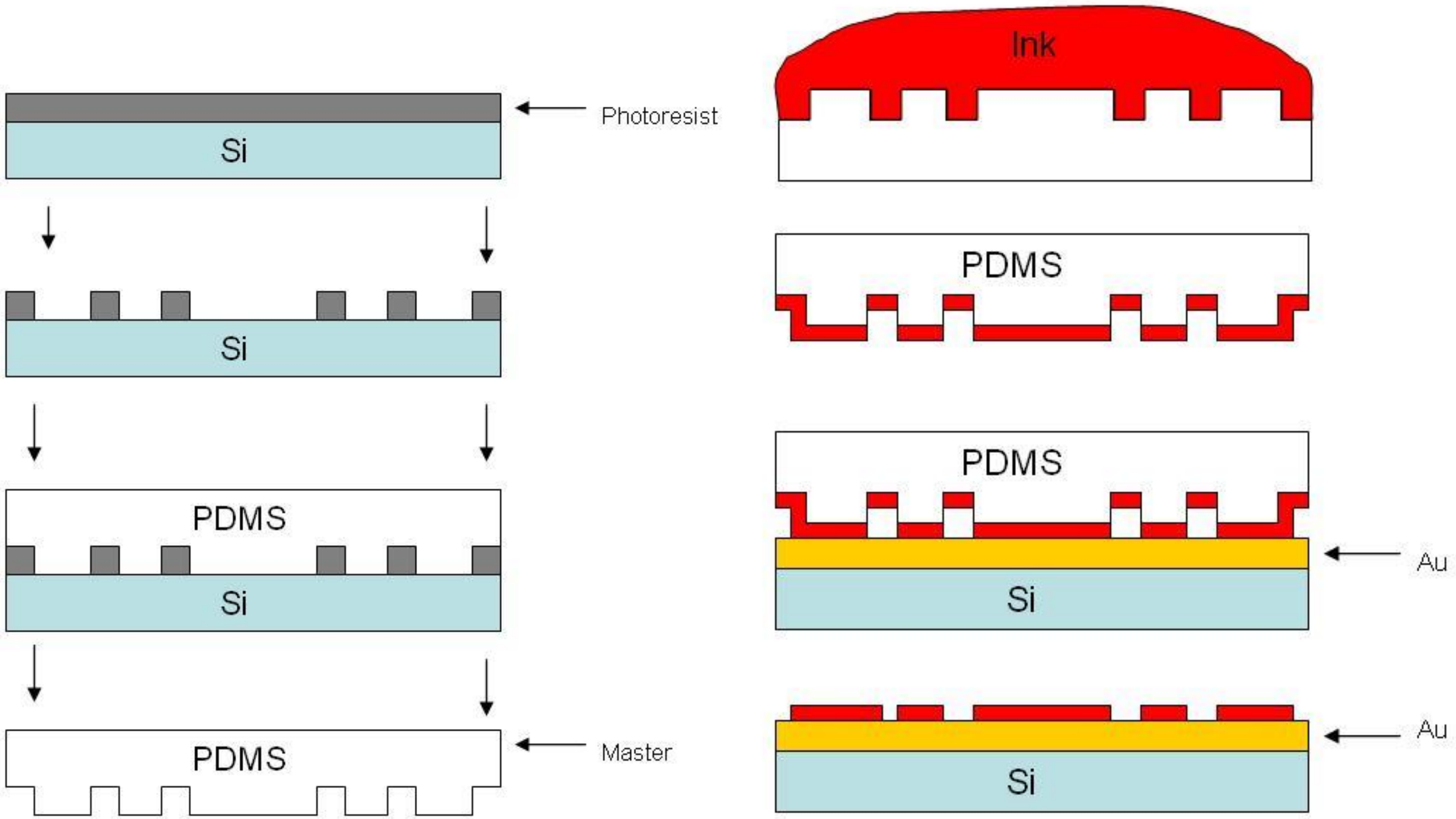
Un processo di fotolitografia consta di 4 fasi:

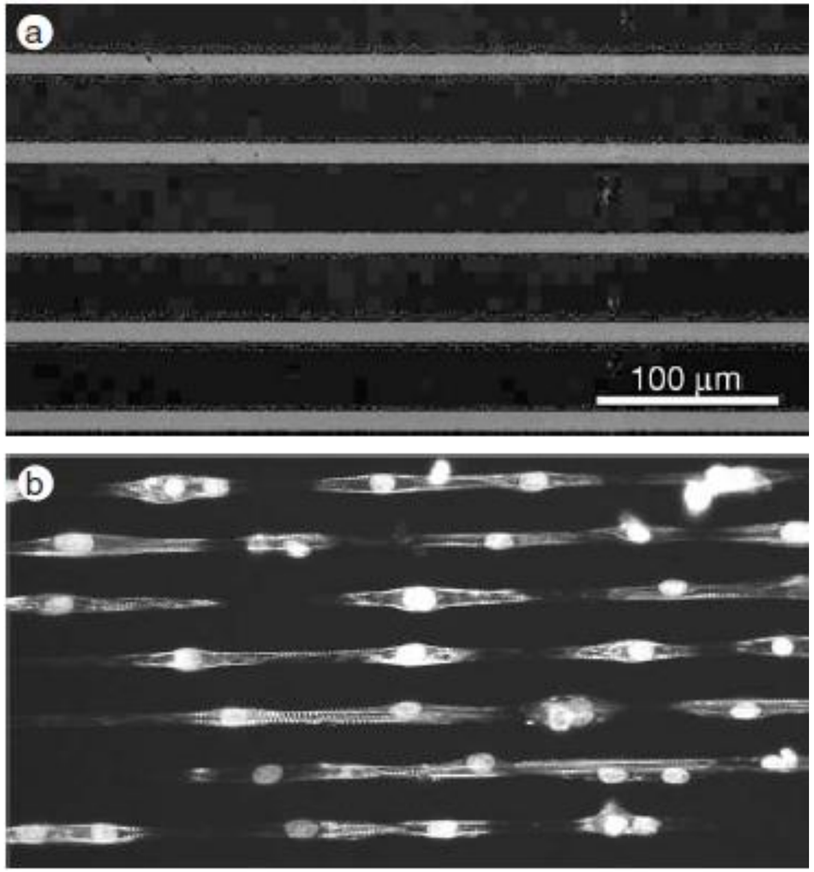
1. Deposizione del resist (polimero fotosensibile) sul substrato
2. Esposizione a luce UV
3. Sviluppo: rimozione selettiva di parti di resist, a seconda che siano state esposte o no agli UV
4. La maschera di resist serve come protezione, nei processi successivi, delle zone di substrato che non vanno attaccate.



Superfici patternate: Microcontact Printing (μCP)

La tecnica sfrutta uno stampo (master) di polidimetilsilossano (PDMS) per formare geometrie di SAMs, proteine, polimero (usati come inchiostro) sul substrato di interesse. Il trasferimento del pattern avviene per contatto intimo tra master e substrato





(a) Linee di laminina stampate per μCP su substrato nonfouling
(b) I cardiomiociti si dispongono lungo le linee di laminina

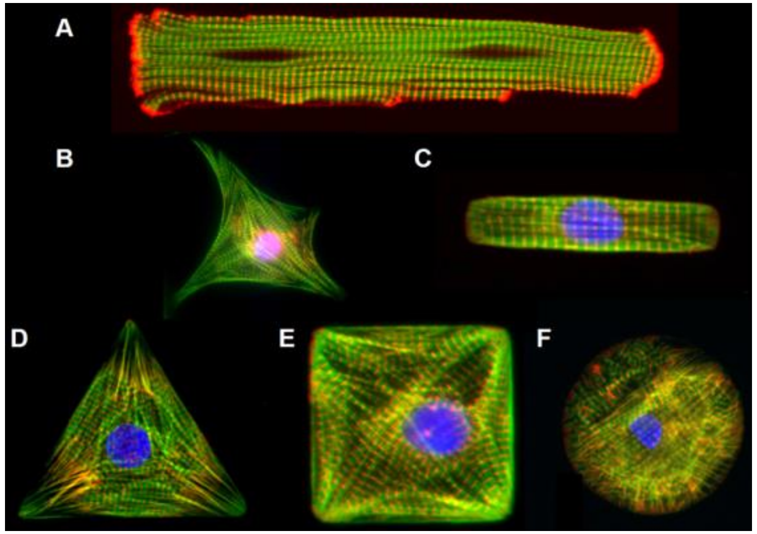
Superfici patternate: Microcontact Printing (µCP)

Mechanical Stress, Cell Shape, and Cell Architecture in Mechanotransduction

Studying the role of cytoskeleton in organization and regulation of cell physiology implies using many enabling technologies, including µcontact printing, epifluorescence and confocal microscopy, electrophysiological conduction mapping, SEM, and AFM.
Adult myocytes have a characteristic rectangular structure that does not change even when extracted from the whole heart. This structure enhances contractile function of the heart, as the cell generates contractile force along the axis of the sarcomeric actin perpendicular to that of the sarcomere Z-line, which together compose the myofibril. In contrast, neonatal rat cardiac myocytes have a malleable myofibrillar architecture after extraction. It is supposed that structure and organization of the cardiac myocyte cytoskeleton can be influenced by geometrical cues in the extracellular environment. Neonatal rat myocytes were cultured onto geometrically controlled ECM islands. In the absence of defined geometrical cues, myofibrils in neonatal cells assemble randomly. However, in geometries with defined boundary conditions myofibrils assemble based on the edges and corners of their environment. In circular patterns, with no edges and corners, the cells lack a regular myofibrillar pattern based on imposed cell geometry.

Cardiomiciti di topo neonatale

Verde: actina
Rosso: α-actinina
Blu: nucleo



µCP usato per controllare la geometria dei cardiomiciti e l'architettura cellulare.
A: In assenza di modificazione strutturale
B: In assenza di modificazione strutturale, su strato di proteine ECM
C: … su un'isola rettangolare di proteine ECM
D: … su un'isola triangolare di proteine ECM
E: … su un'isola quadrata di proteine ECM
F: … su un'isola circolare di proteine ECM

http://diseasebiophysics.seas.harvard.edu/research/mechanotransduction/

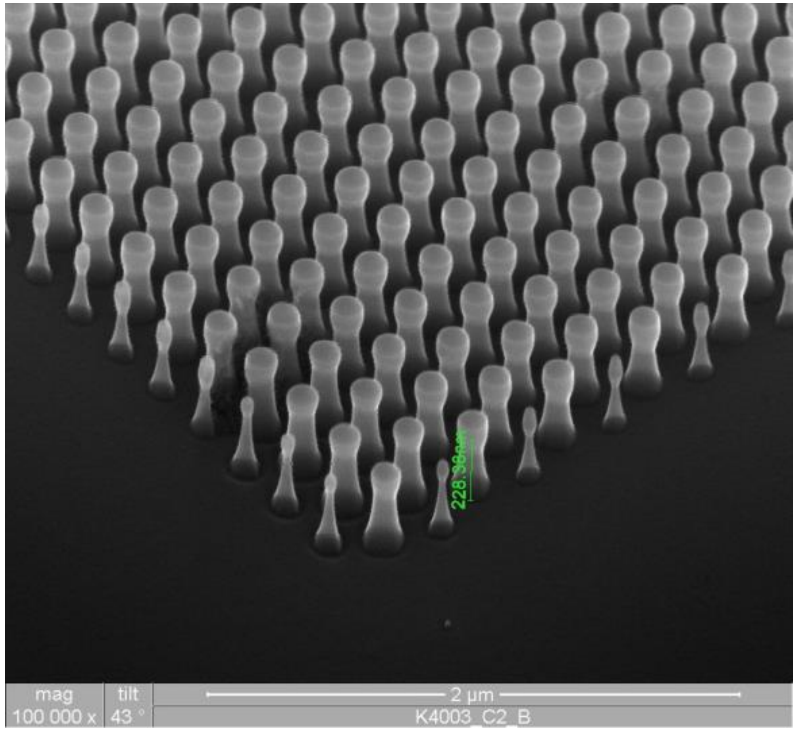
Superfici patternate:
litografia a fascio elettronico (EBL)

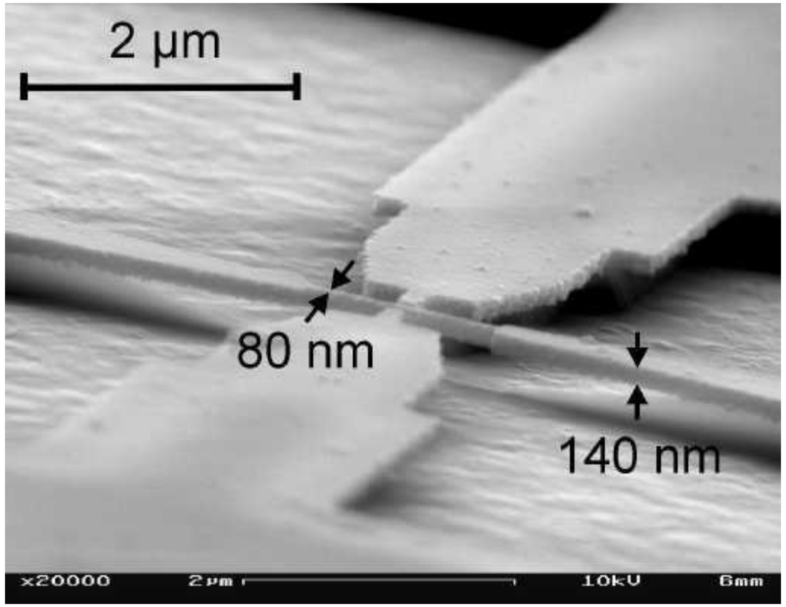
Un fascio di elettroni collimati viene indirizzato su un substrato coperto di resist elettronico (sensibile agli elettroni, es. PMMA)

Il fascio viene deflesso per scrivere direttamente il disegno voluto sul substrato

La deflessione è limitata ad aree di grandezza specifica

Per scritture su aree maggiori, il campione viene mosso; il movimento è controllato, con precisione di una decina di nm, da un interferometro laser



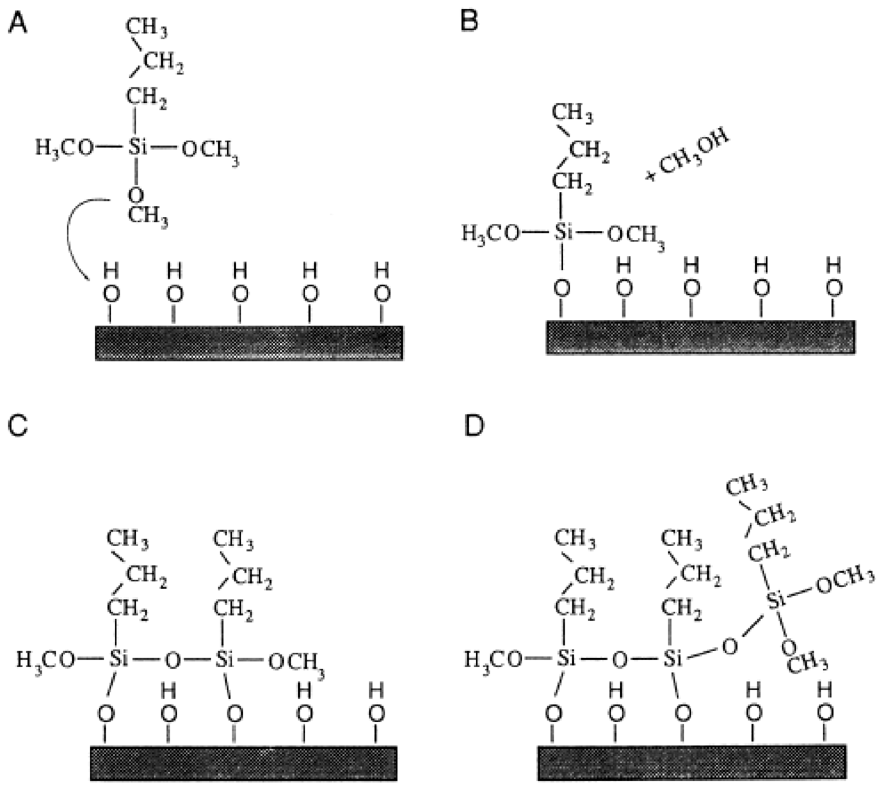


reazioni chimiche

silanizzazione

Coinvolgono una fase liquida

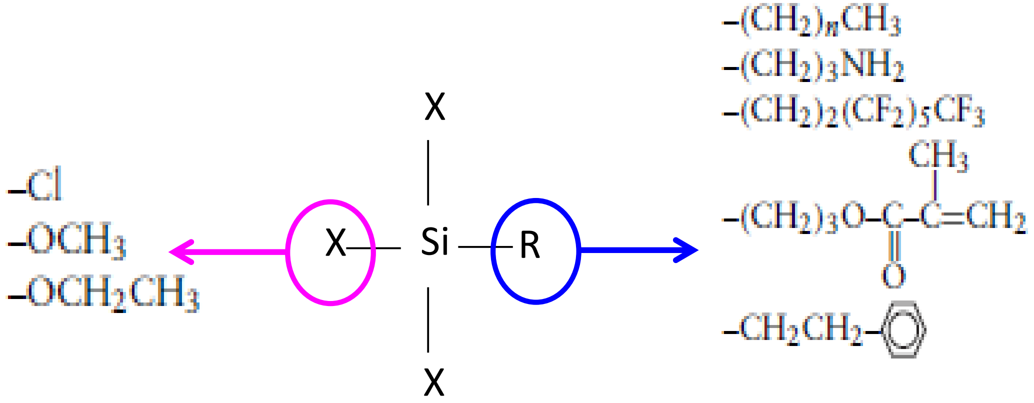
Usate per modificare superfici con gruppi –OH
- Vetro
- Silicio
- Allumina
- Metalli ossidati



Semplici, stabili (crosslinking covalente)

Svantaggio: il legame Si-O si idrolizza (OH^-)

Biomateriali: adesione cellulare, immobilizzazione di biomolecole, superfici nonfouling, superfici modello per studi di biointerazione

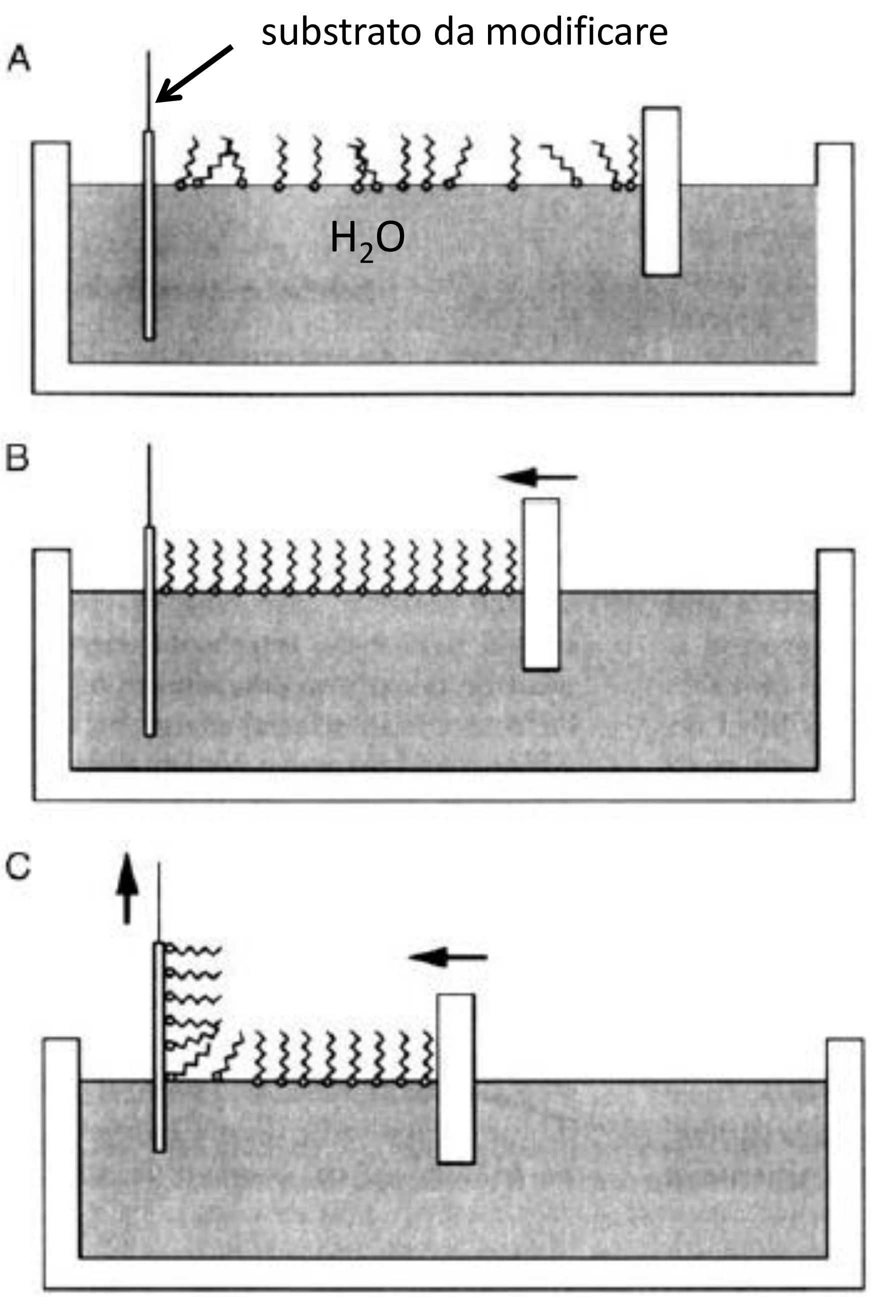


film di Langmuir-Blodgett (LB)

- Le superfici vengono rivestite con uno o più strati di molecole anfifiliche (testa polare e coda apolare).
- Meccanismo di deposizione film LB (es. strato lipidico)

A. Il film lipidico galleggia sulla superficie dell'acqua
B. … e viene compresso con una barriera mobile.
C. Contemporaneamente viene gradualmente estratto il substrato. Si ottiene così la migrazione delle molecole lipidiche alla superficie del substrato

- Vantaggi: elevato ordine e uniformità dei film depositati, grande varietà di precursori
- Svantaggio: stabilità dei film. Può essere aumentata con reazioni di reticolazione tra le code alifatiche



Self-Assembled Monolayers (SAMs)

BOTTOM UP

- Film di molecole anfifiliche capaci di autoassemblarsi spontaneamente su determinate superfici, con struttura altamente ordinata (cristalli bidimensionali)

- Le molecole anfifiliche presentano ad una estremità un gruppo funzionale con elevata affinità per il substrato.

- Esempi: n-alchil-silani su substrati con gruppi –OH; alcantioli su Au, Ag e Cu; ammine e alcoli su Pt; acidi carbossilici su Al_2O_3 e Ag; fosfati (PO_4^{3-} o gruppi fosfonici) su Ti e Ta.

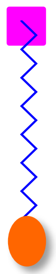
- Il chemiadsorbimento dei gruppi di ancoraggio sul substrato è esotermico e ne abbassa l'energia superficiale. Quando tutti i siti superficiali sono occupati le forze di Van der Waals tra le catene alchiliche portano alla cristallizzazione.

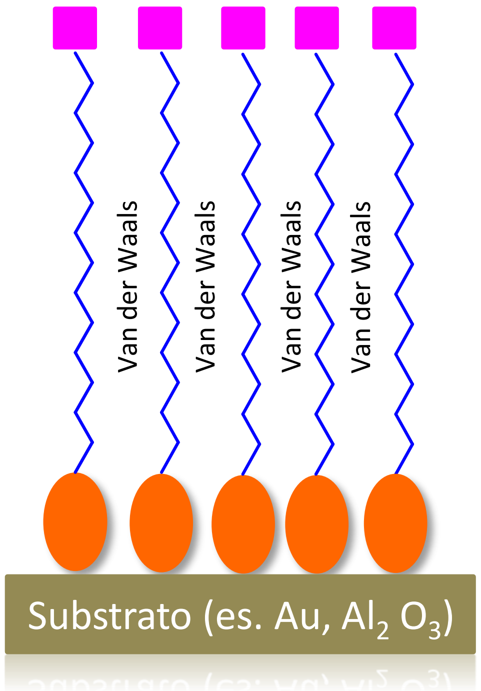
- Lunghezza catena: 24 < CH_2 < 9 (<9 forze di interazione insufficienti; >24 assemblaggio difficile, molti difetti

GRUPPO FUNZIONALE SUPERFICIALE
(es. –OH, $-CF_3$, -CH=O)

STRUTTURA DI ASSEMBLAGGIO
(es. catene alchiliche)

GRUPPO DI ANCORAGGIO
(es. –OH, -SH, -silani)





CONCLUSIONS

Cold Plasma Processeses

are nowadays an established tool for biomedical applications,

a fascinating interdisciplinary field

able to provide newer useful approaches for Biomedical Materials

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