MEETING

ITA FIRB (2001-2007) RBNE014589_006,

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M. Nardulli[1], P. Favia[1]; R. d'Agostino[1]

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UNIBA

- PLASMA DEPOSITION OF TEFLON-like COATINGS
- PLASMA TREATMENT TO IMPROVE Hyal/material interactions
- PLASMA DEPOSITION OF NANO-COMPOSITE COATINGS
- PLASMA-AIDED NANO-PATTERNING

Other Units

- UNINA (Barbarisi);
  UNISI (Ziche)
  UNIBO (Bigi) *
- UNISI (Barbucci)
- NOBILBIO RICERCHE (Morra)
- ...

Abstracts

- **E. Sardella**, R. Gristina, D. Gilliland, G. Ceccone, G.S. Senesi, F. Rossi, R. d'Agostino, P. Favia; Plasmadeposited nanopatterned and nanocomposite coatings for biomedical applications; **17th International Symposium on Plasma Chemistry (ISPC-17)** Toronto, Canada, **Aug 2005**
- **E. Sardella**, R. Gristina, G. Ceccone, D. Gilliland, A. Papadopoulou-Bouraoui, F. Rossi, P. Favia; R. d'Agostino; Bactericidal Silver containing nanocomposites deposited by Means of RF (13.56 MHz) Glow Discharges; **19th European Conference on Biomaterials (ESB-19)**, Sorrento, **Sett 2005**
- **P. Favia**, E. Sardella, M. Nardulli, R. Gristina; R. d'Agostino; Plasma-deposited Silver Containing Nanocomposite coatings with Bactericidal properties; **52nd AVS Meeting**, Boston, US, Nov 2005.
- **E. Sardella**, R. Gristina, P. Favia, R. d'Agostino, bactericidal silver containing composites deposited by means of RF (13.56MHz) Glow Discharges; **National Congress on Biomaterials GIB-SIB**, Milano, **Ott 2005**

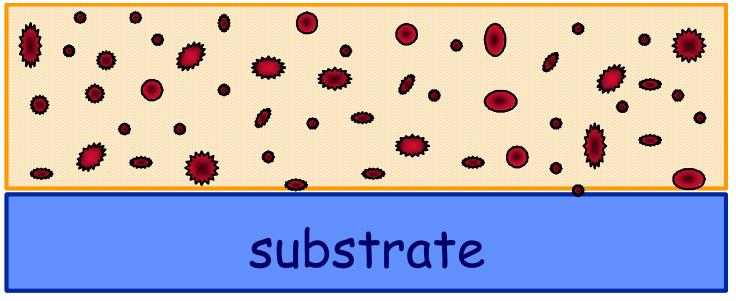
PAPERS

- **E. Sardella**; R. Gristina; A. Mangone, M. Casavola; P.Favia; R. d'Agostino; Plasma Deposition of Ag containing Nano-composite coatings; **Journal of Applied Biomaterials & Biomechanics**, submitted 2005
- **F. Rosso**, G. Marino, L. Muscariello, G. Cafiero, A. Barbarisi, P. Favia, E. D'Aloia, R. d'Agostino, Adhesion and proliferation of fibroblasts on plasma-deposited nanostructured fluorocarbon coatings: evidence of FAK activation. **Journal of Cellular Physiology**. Accettato 2005.

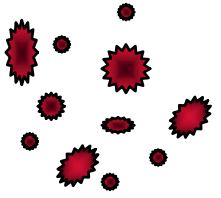
PLASMA DEPOSITION OF NANO-COMPOSITE COATINGS

FIRB : E. Sardella, M. Casavola, R. Gristina; M. Nardulli, M. Morra, C. Cassinelli, P.Favia, R. d'Agostino;

Collaboration: A. Mangone (ICP-MS), G. Ceccone (XPS); D. Gilliland (ToF SIMS); A. Papadopoulou-Bouraoui (QCM-D)



Bactericidal effect



Clusters of Ag

Hydrophilic matrix

PEO-like $-(CH_2CH_2O)_n-$

…Phoenicians used silver to keep water from spoiling when sailing on long voyages…

NOWDAYS:

Colloidal Silver

SYSTEMIC THERAPY **OR/AND** **TOPICAL THERAPY**

PROBLEMS !

Toxicity related to excess

Ag ions released in the blood *

Ag films on biomedical devices

Catheters

Local treatments partially substitute systemic therapies:

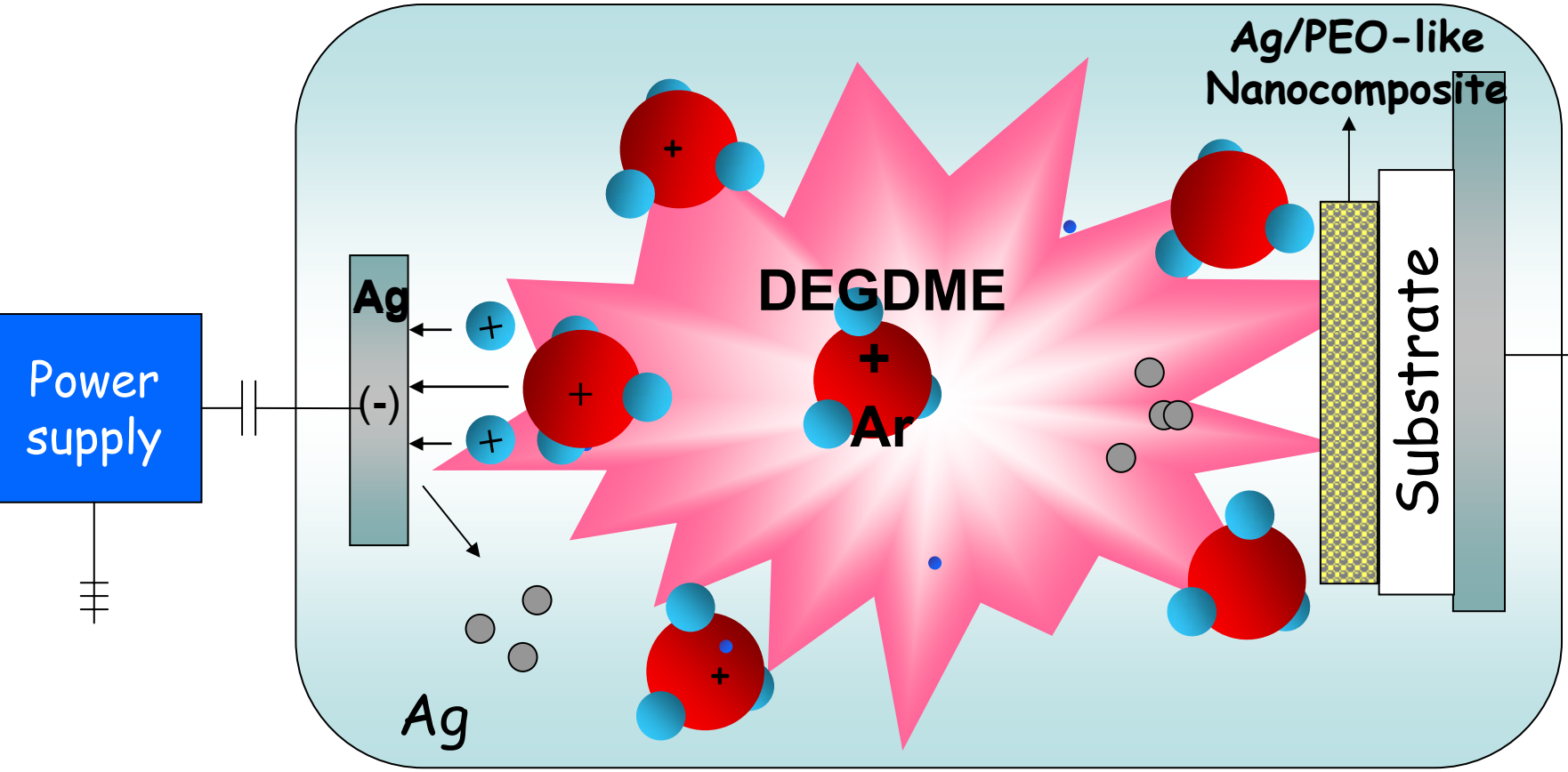
anti-infective coatings directly on biomaterials

IT IS DIFFICULT TO CONTROL
THE AMOUNT OF RELEASED SILVER

NANO-COMPOSITE MATERIALS:
CLUSTERS OF SILVER
EMBEDDED INTO A SWELLABLE MATRIX

RF (13.56MHz) Glow discharges
"cold plasmas"

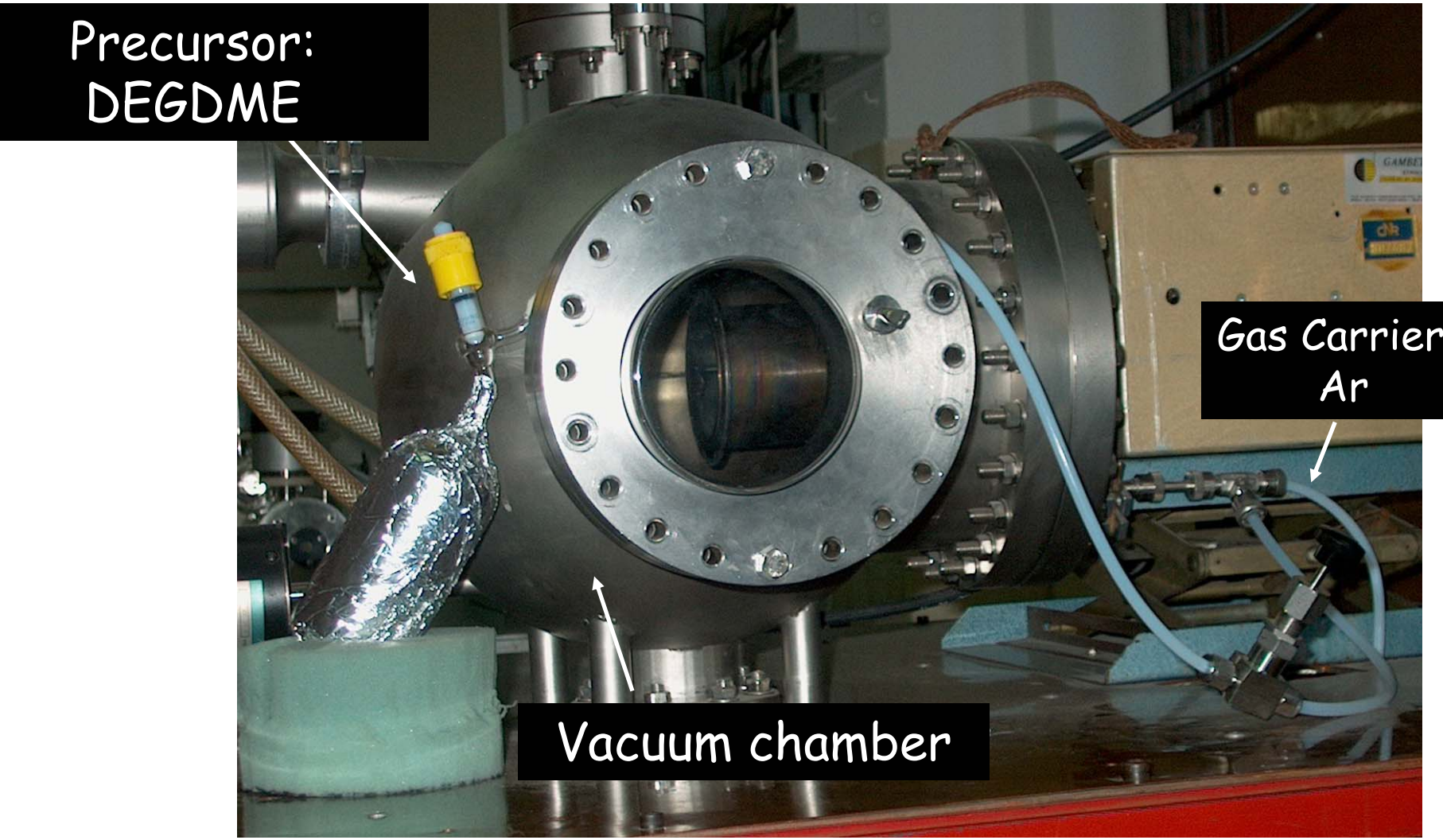
PLASMA DEPOSITION OF Ag NANOCOMPOSITE COATINGS



DEGDME: di-ethylene glycole di-methyl ether, $CH_3O(CH_2CH_2O)_2CH_3$

. Favia, *et al.* Plasmas Polym. (2000), 5(1), 1-14

PLASMA DEPOSITION OF Ag NANOCOMPOSITE COATINGS

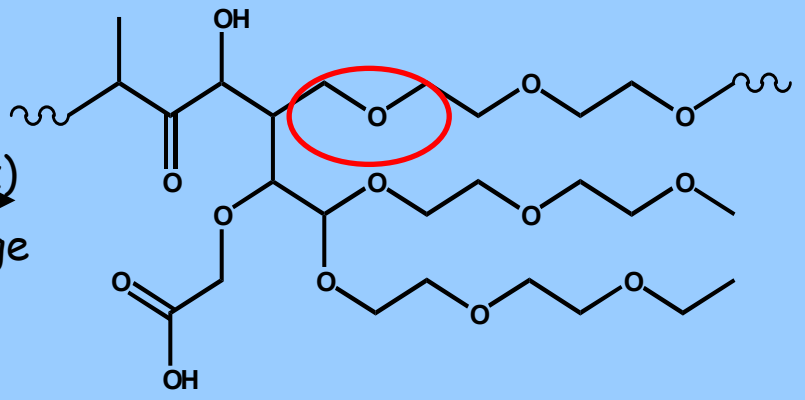


DEGDME: di-ethylene glycole di-methyl ether; $CH_3O(CH_2CH_2O)CH_3$

Plasma deposition of PEO-like coatings

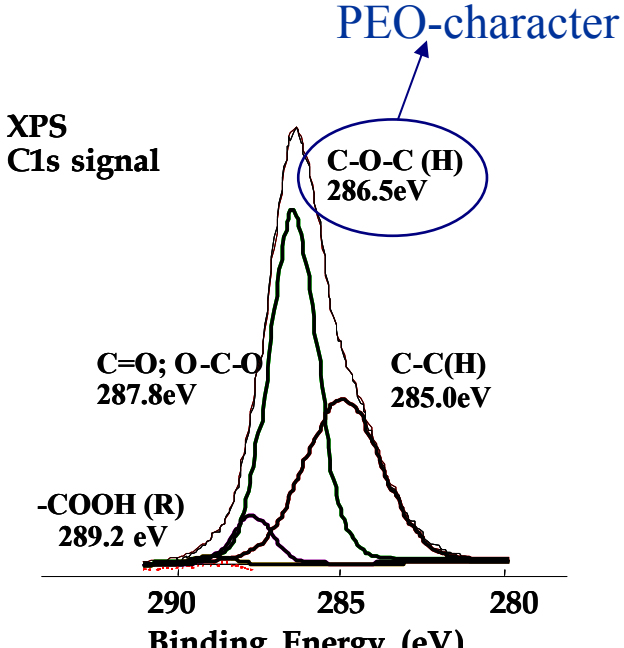
$CH_3O(CH_2CH_2O)_2CH_3$ + Ar $\xrightarrow[\text{Glow discharge}]{\text{RF(13,56MHz)}}$

Diethylene glycol dimethyl ether
DEGDME



Polyethyleneoxide-like (PEO-like)

Key parameter:
retention of the EO, $-CH_2CH_2O-$ structure in the coating



* Andrade *et al. Biomaterials* 1988, 9, 76
Lee *et al.* Prog. Polym. Sci. 1995, 20, 1043
Many papers from Hoffman and Ratner
Many papers from Timmons et al

EXPERIMENTAL CONDITIONS

RF electrode	DEGDME (sccm)	Ar (sccm)	Power (W)	Pressure (mTorr)	Depth
Ag	0.25	10	10	50	60nm
Ag	0.25	20	10	50	60nm
Ag	0.25	20	20	50	60nm
SS	0.25	10	10	50	60nm
SS	0.25	20	10	50	60nm
SS	0.25	20	20	50	60nm

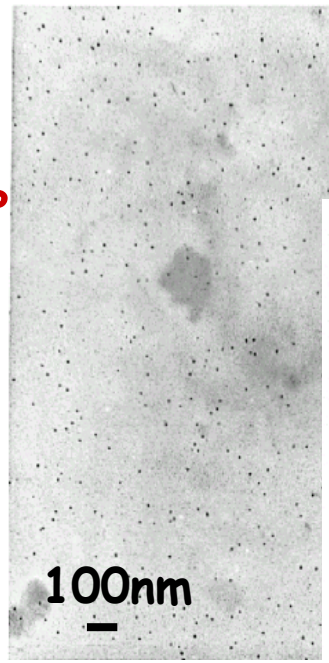
SS: Stainless Steel

CHEMICAL-PHYSICAL-BIOLOGICAL CHARACTERIZATION

- X-ray Photoelectron Spectroscopy (XPS)
- Water Contact Angle (WCA)
- Trasmission Electron Microscopy (TEM)
- Atomic Force Microscopy, AFM (non contact and lateral force modes)
- Z-Potential
- Quartz Crystal Microbalance with dissipation monitoring (QCM-D)
- Inductively Coupled Plasma- Mass Spectrometry (ICP-MS)
- Biological *in vitro* tests with 3T3 Murine Fibroblasts
- Disk diffusion Susceptibility testing with *S. epidermis*

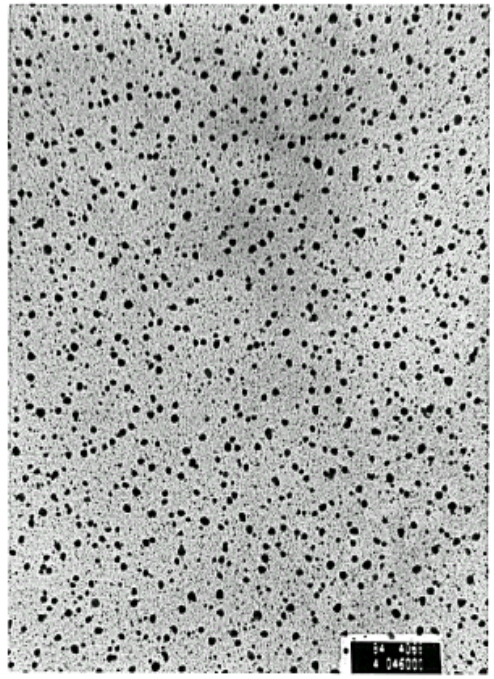
OBJECTIVE 1: Synthesize different Ag/PEO-like coatings with different amounts of embedded silver;

Ag:1%
EO-character: 40%



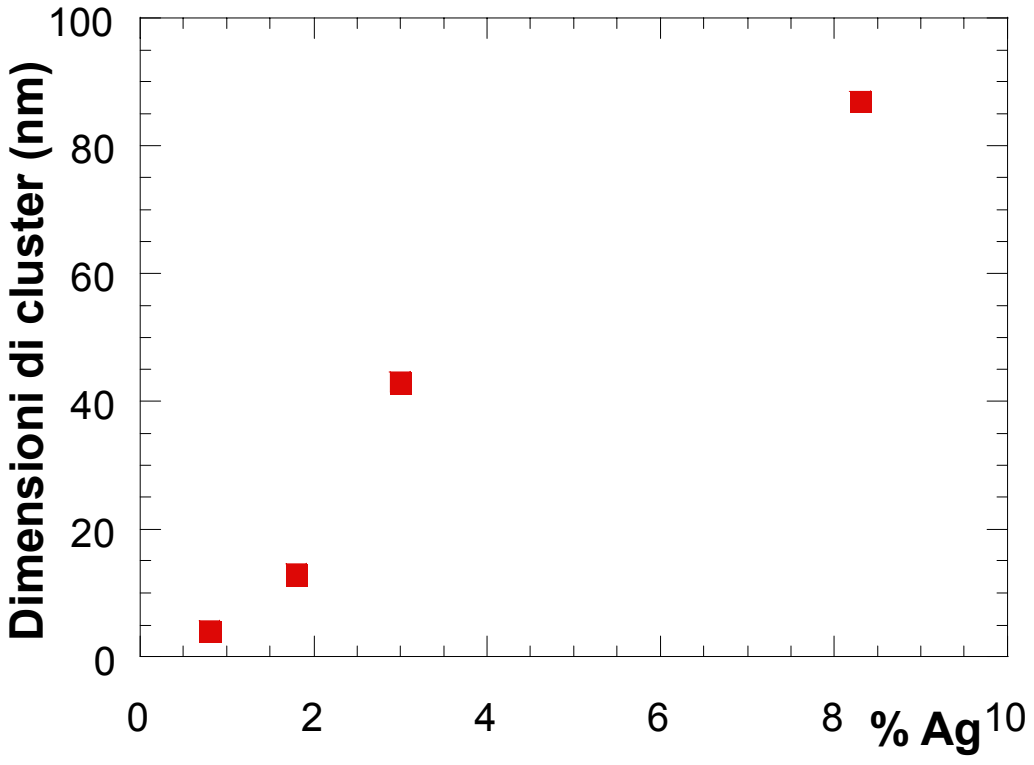
Ag:3%
PEO-character: 30%

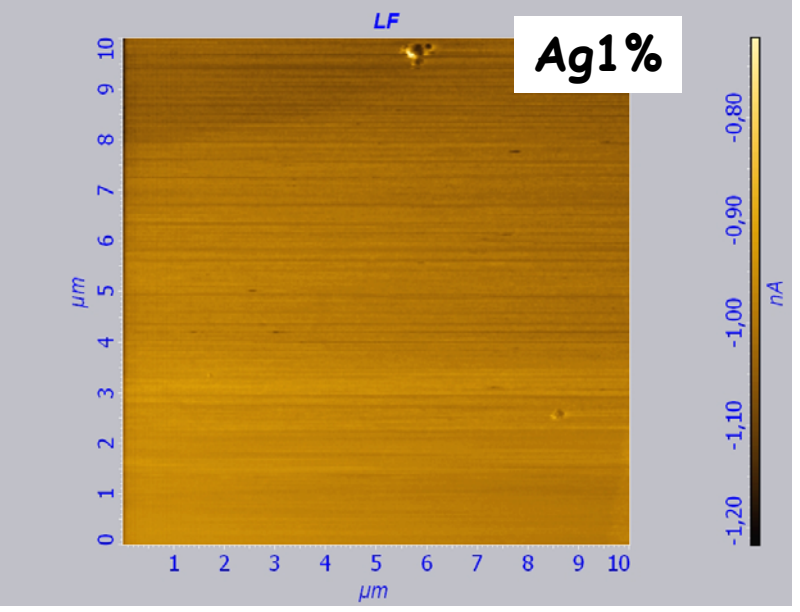
Ag:5%
PEO-character: 20%

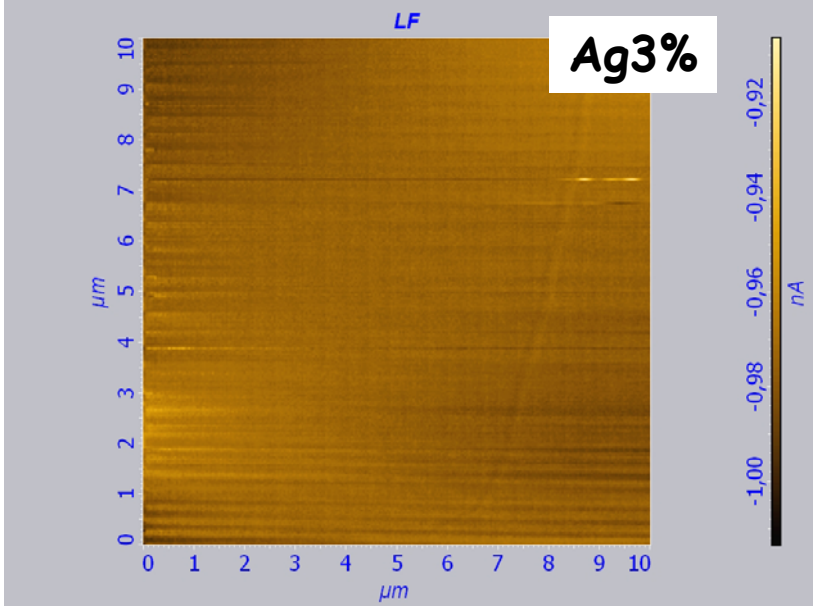


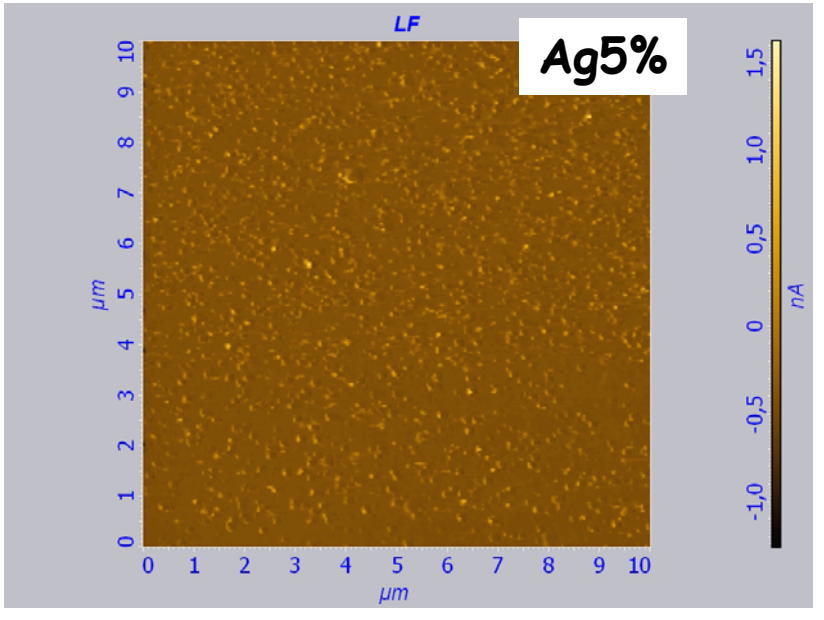
TEM IMAGES OF Ag/PEO-like COATINGS

CORRELAZIONE TRA LA DIMENSIONE DEI CLUSTER DI AG CON LA PERCENTUALE DI AG CONTENUTO NEL COATING





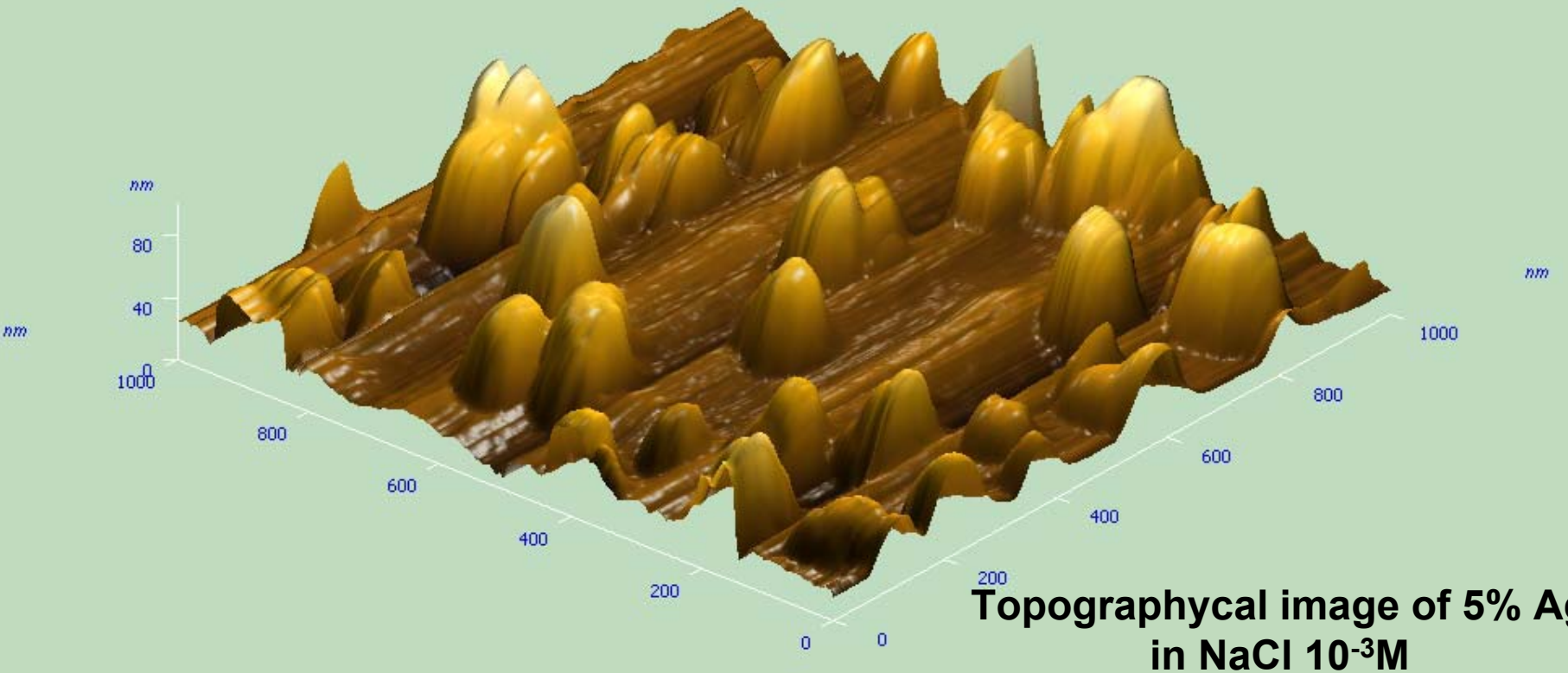




Lateral Force mode in NaCl 10^{-3}M

ONLY 5% Ag coatings are ROUGH (nano-patterned)!

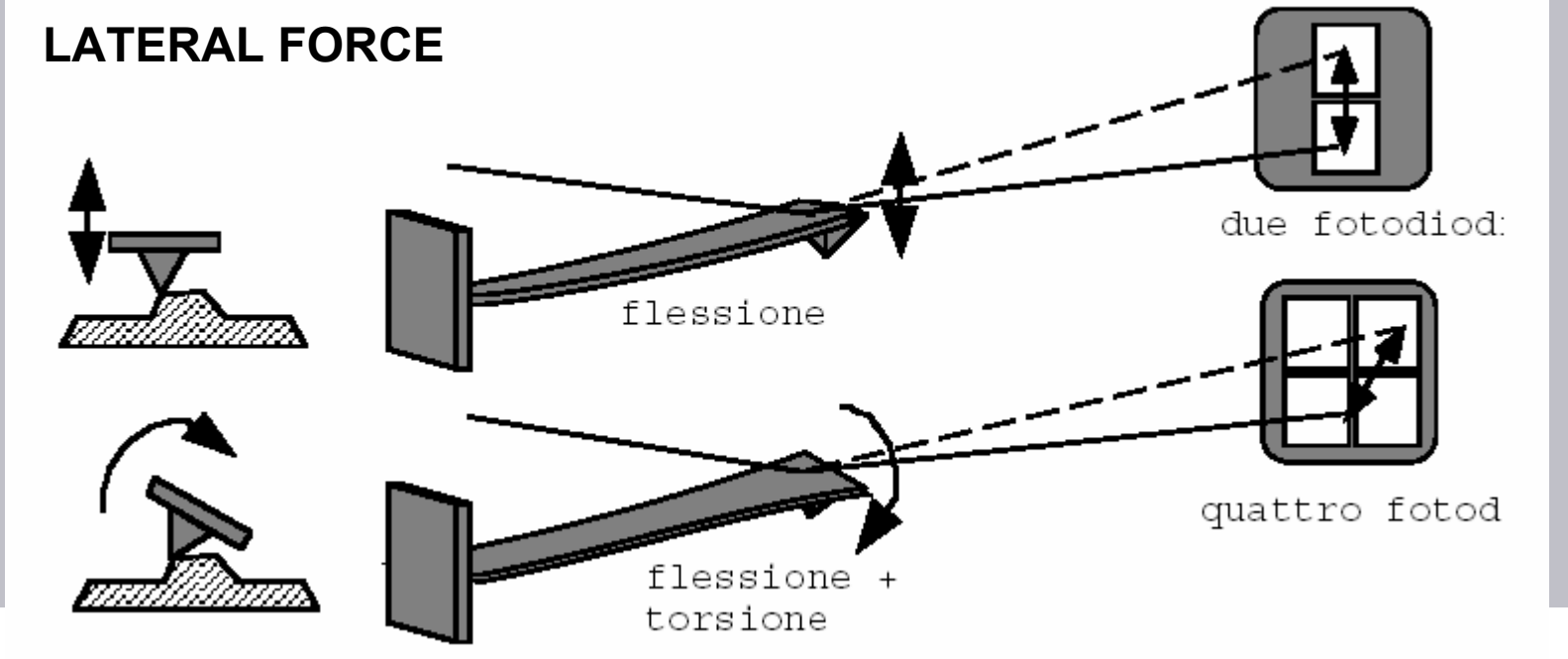
ARE THE CLUSTERS RESPONSIBLE OF TOPOGRAPHYCAL DIFFERENCES AMONG SAMPLES?



Topographycal image of 5% Ag in NaCl 10^{-3}M

ONLY 5% Ag coatings are ROUGH (nano-patterned)!

•OBJECTIVE 2: Understand the mechanism of SILVER DELIVERY

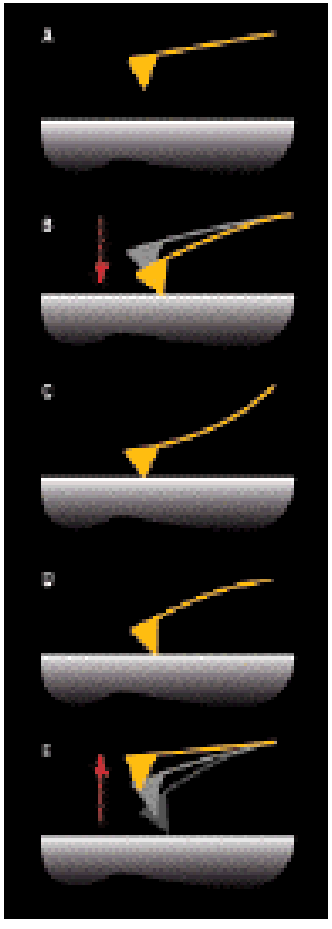


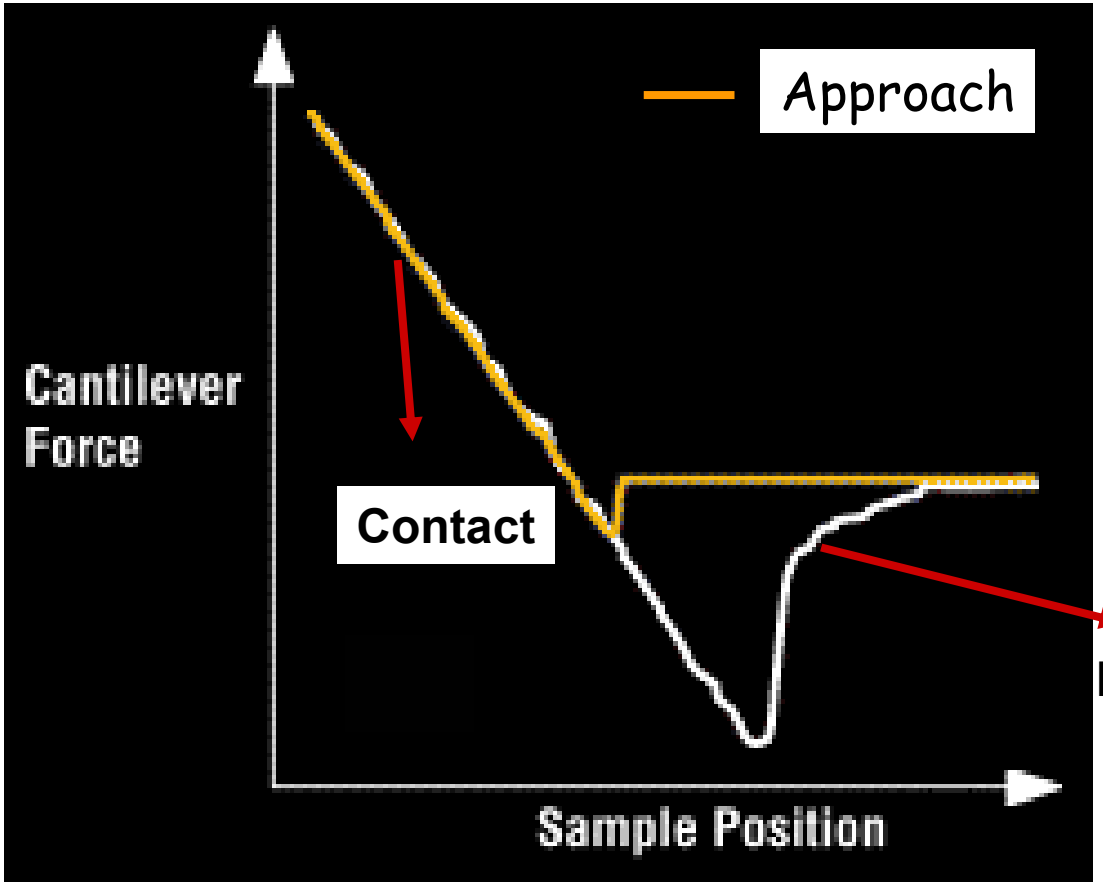
Topographical picture Lateral Force picture

AFM in 10^{-3} M NaCl

Are the clusters embedded in the PEO-like matrix?

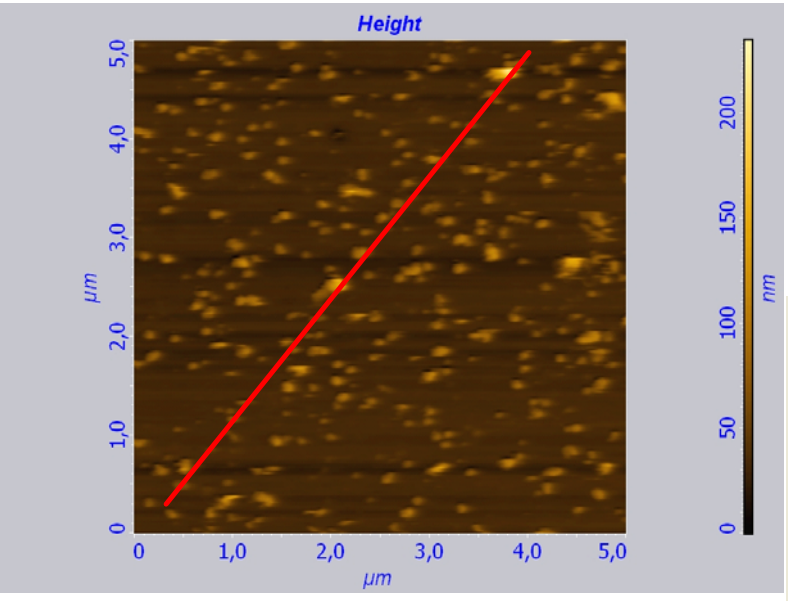
SEPARATION CURVES in 10^{-3}M NaCl





Non-contac

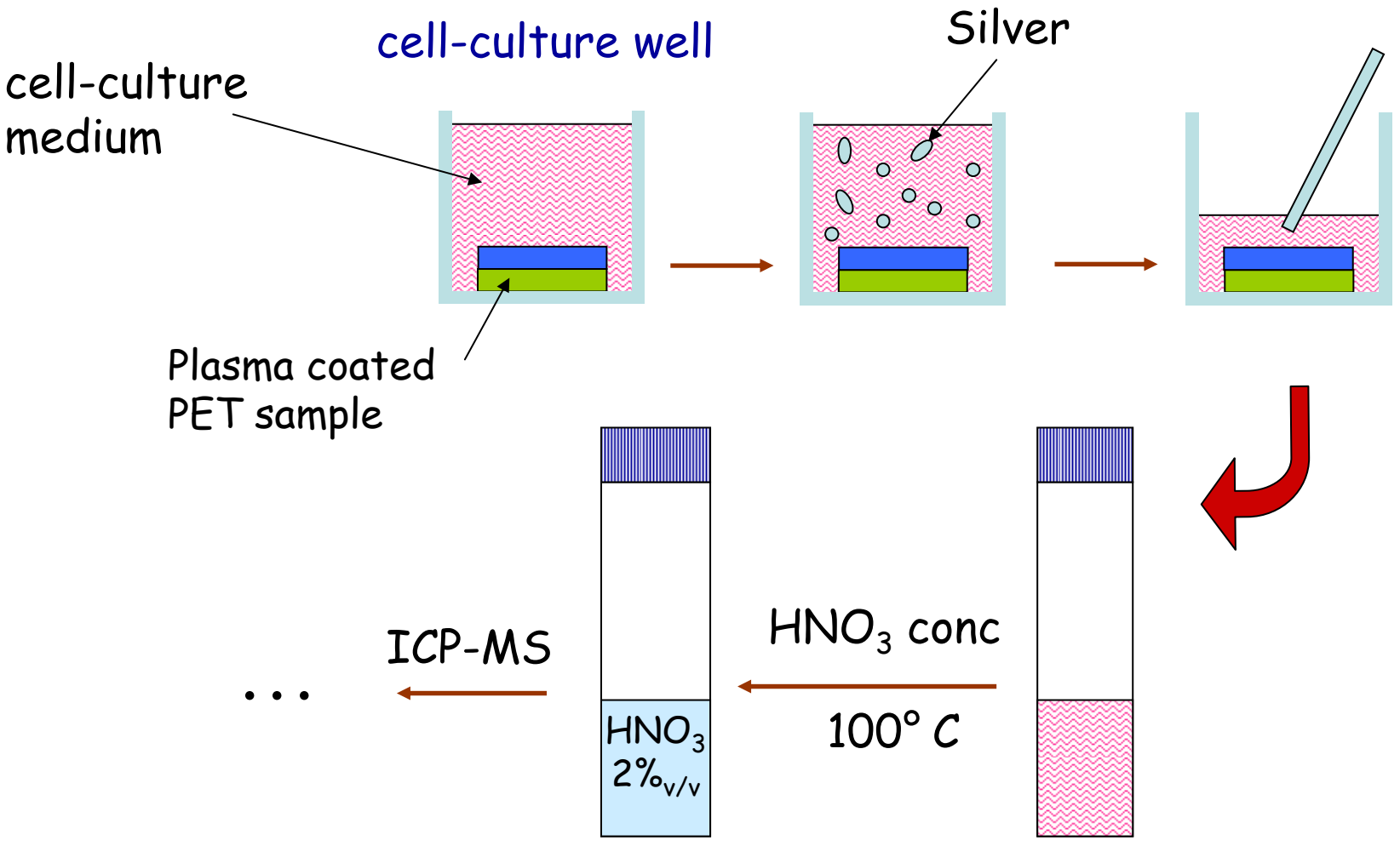
5% Ag coatings



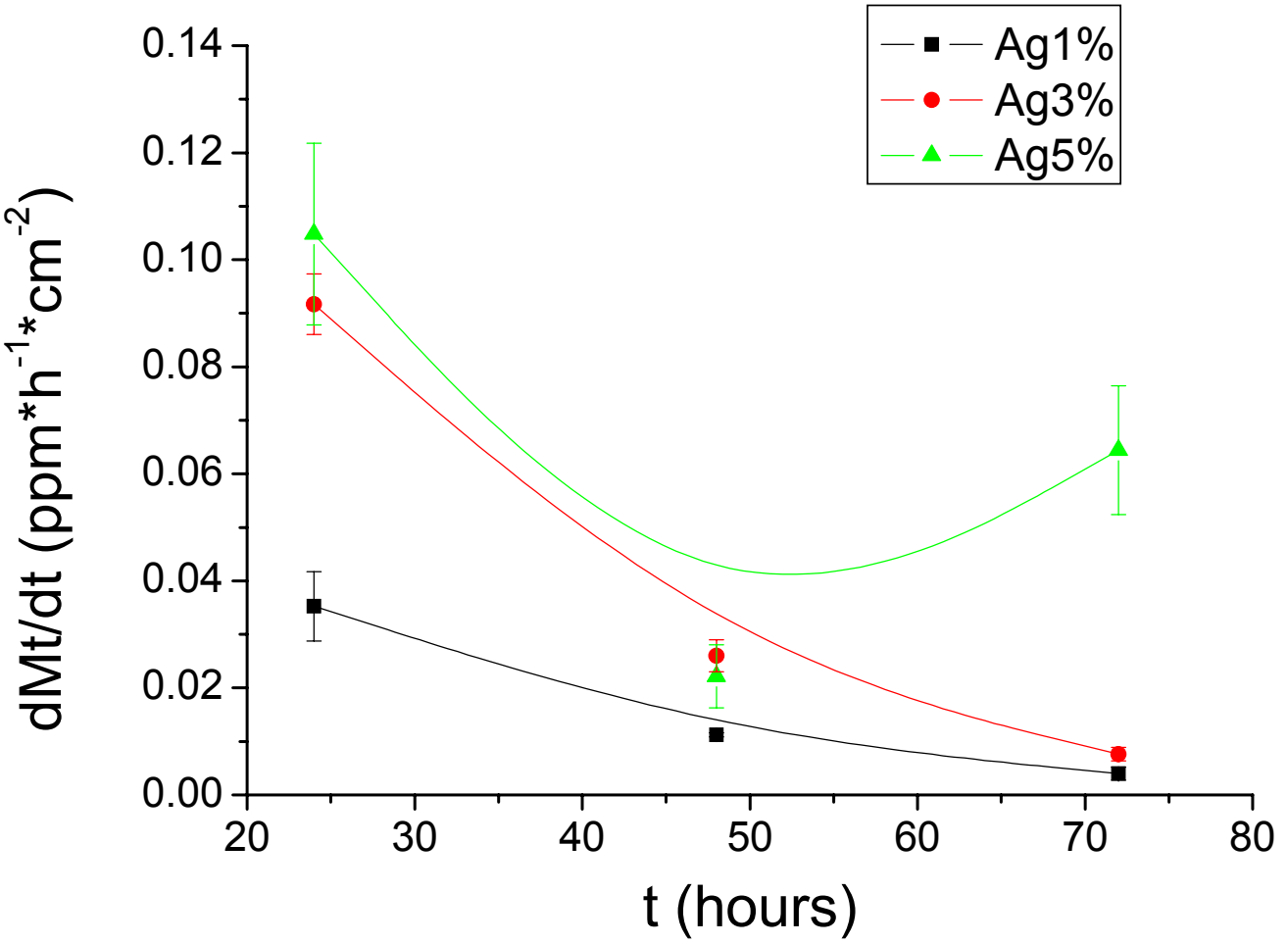
SIMILAR CURVES for each point of analysis attesting for the presence of an hydrophilic (PEO-like) coating also on silver clusters

Ag clusters appear to be embedded in the PEO-like matrix

Silver release in the cell-culture medium



Silver release in the cell-culture medium

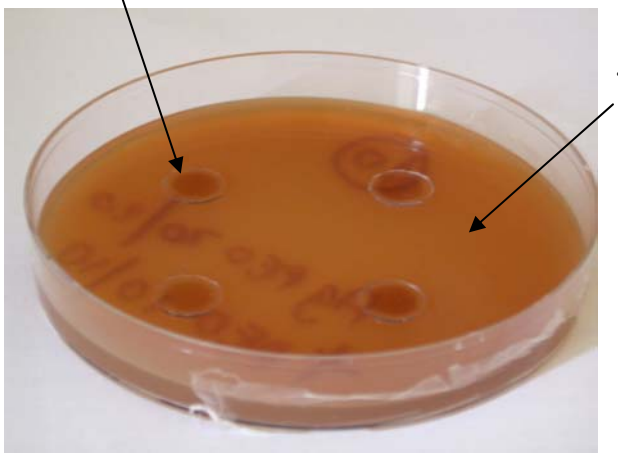


OBJECTIVE 3: Correlate cell (prokaryotic and eukaryotic) behaviours with chemical-physical characteristics of the coatings;

DISK DIFFUSION SUSCEPTIBILITY TESTING

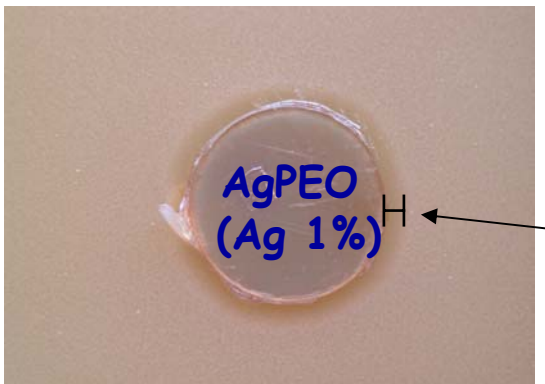
Staphylococcus epidermidis RP62A ATCC 35984
(slime producer, Gram +; $1x10^8$ cfu/mL into Tryptic Soy Agar)

Sample



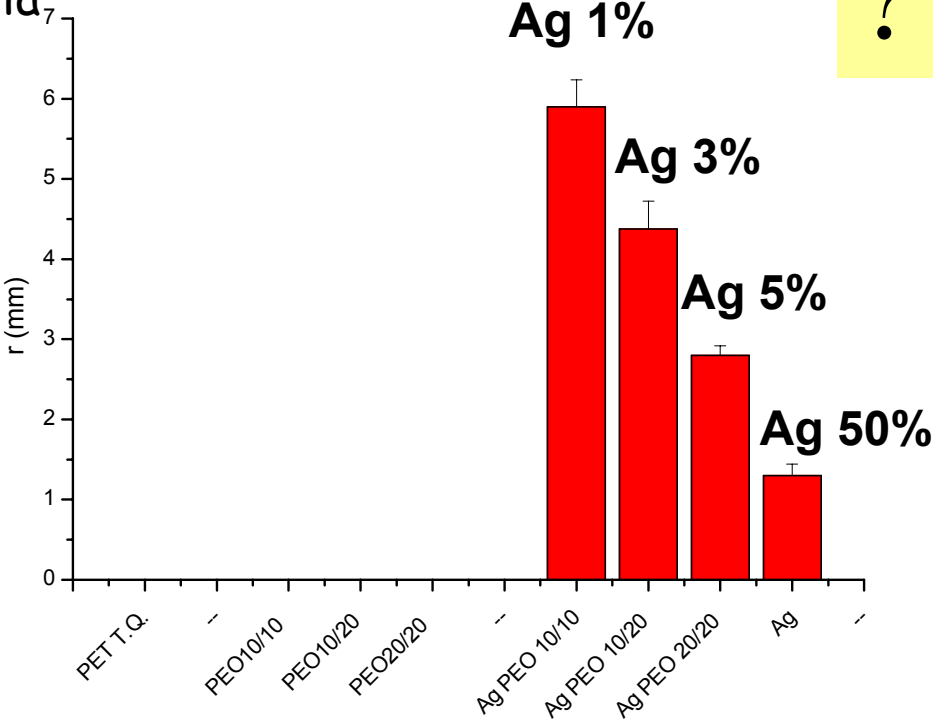
Agar Agar
+ Bacteria

72 hours
37°C



Zone of inhibition

?



3T3 MURINE FIBROBLASTS ($1x10^4$ cells/ml)

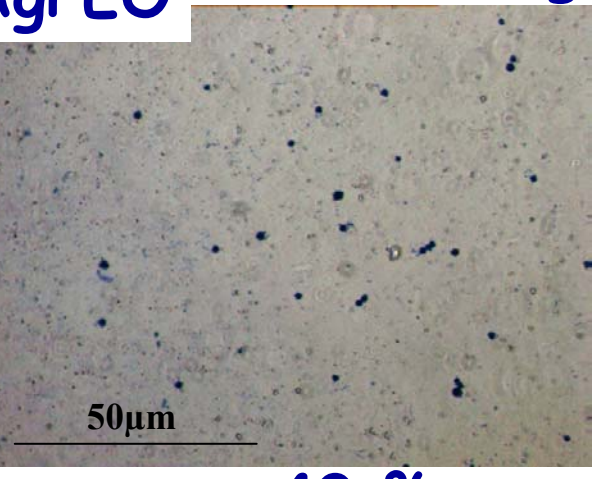
24hr

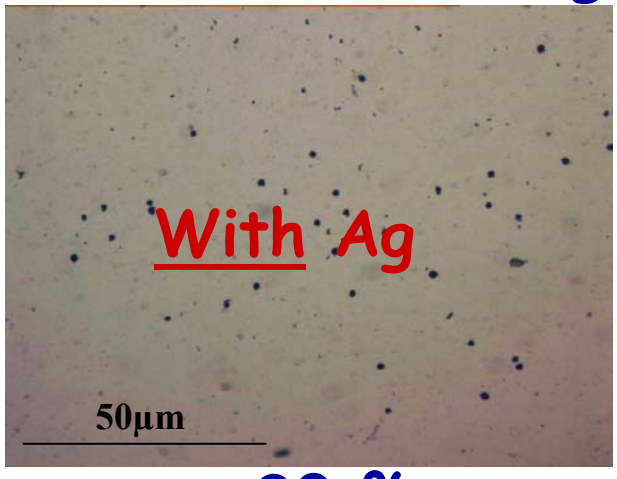
AgPEO

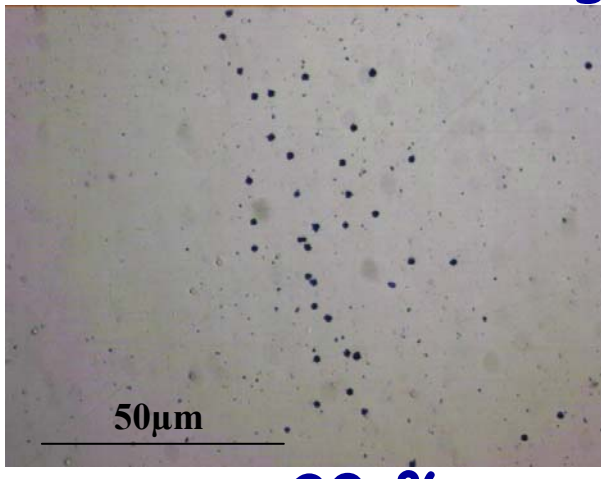
1% Ag

3% Ag

5% Ag





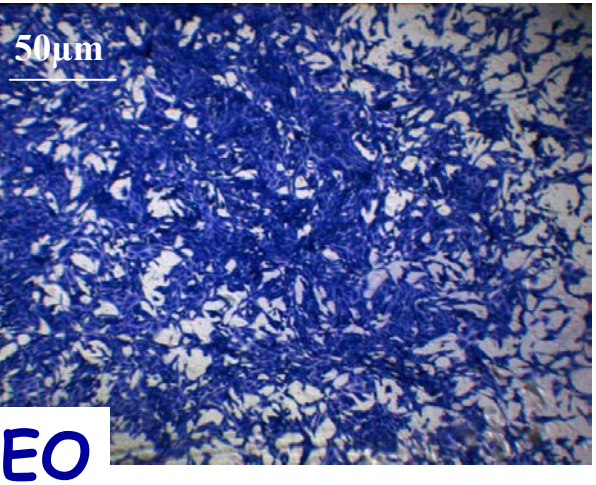


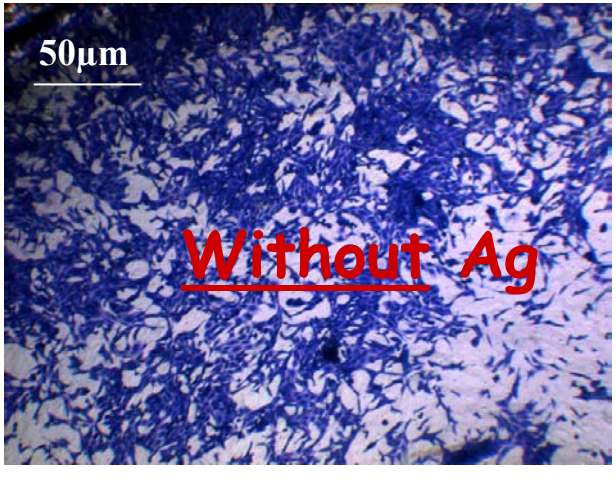
40 %

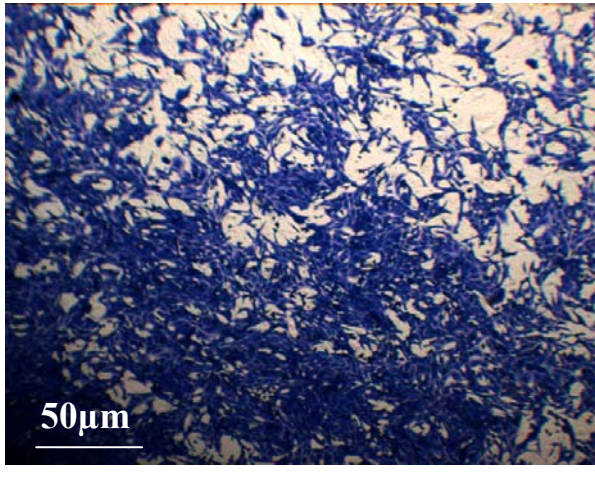
30 %

20 %

PEO-character



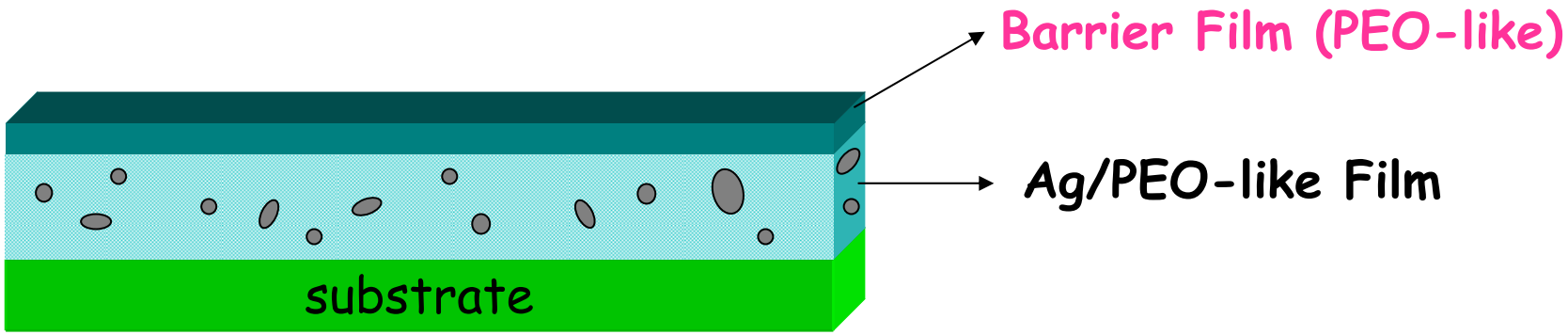




EO

OBJECTIVE 4: Improve medical effectiveness of Ag/PEO-like coatings: obtain an HIGH anti-bacterial effect and LOW eukaryotic cytotoxicity.

…controlled diffusion of Silver through a barrier film*…



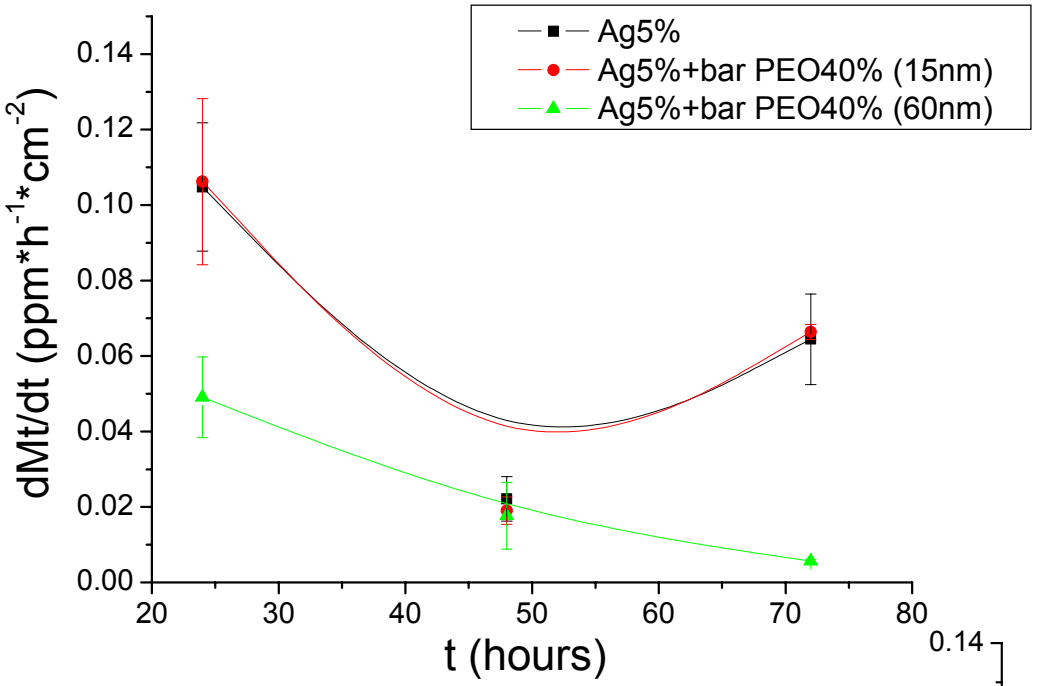
THE RATIONALE:

-CHEMICAL COMPOSITION (of the barrier) vs. amount of RELEASED SILVER

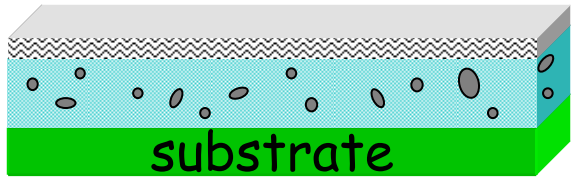
-THICKNESS (of the barrier) vs. amount of RELEASED SILVER

* B.D. Ratner, A.S. Hoffman,F.J.Schoen, J.E. Lemons editors *Biomaterials Science* ElsevierScience; second edition

Silver release through the barrier

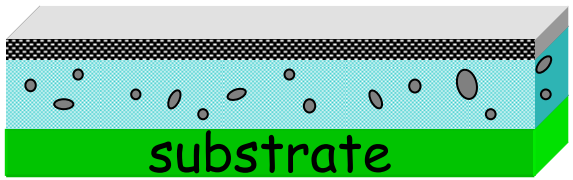


PEO-character 40%

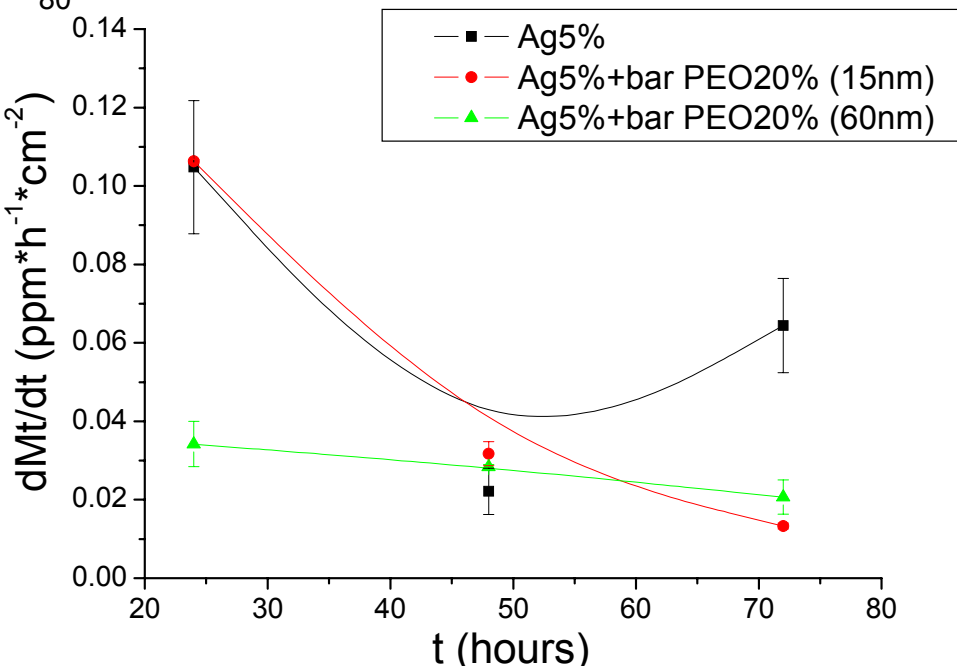


Ether groups ↑

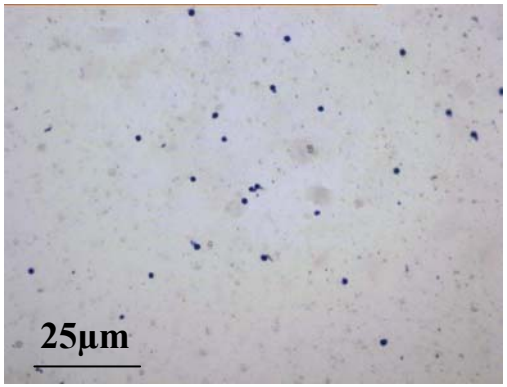
PEO-character 20%



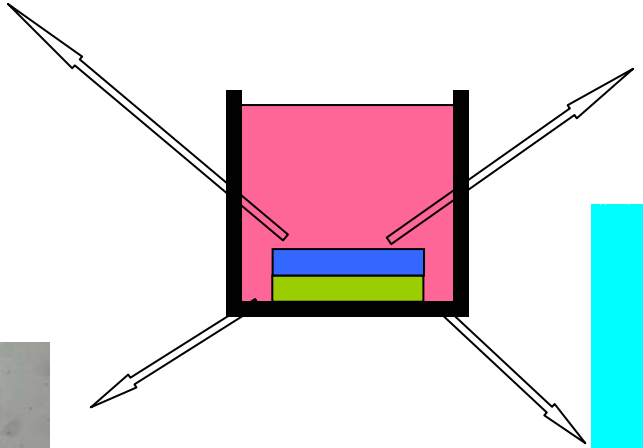
Ether groups ↓



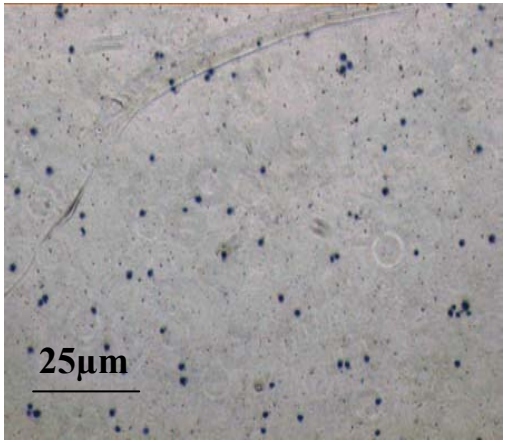
Ag5% films



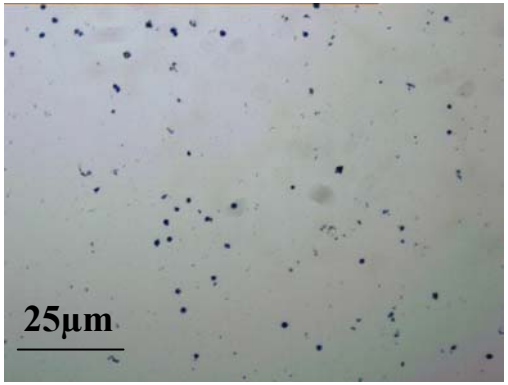
...on the coating



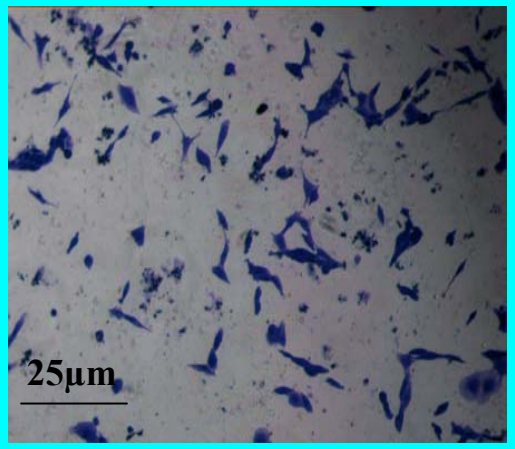
Ag5% films + Barrier (PEO40%) 15nm



...on the coating



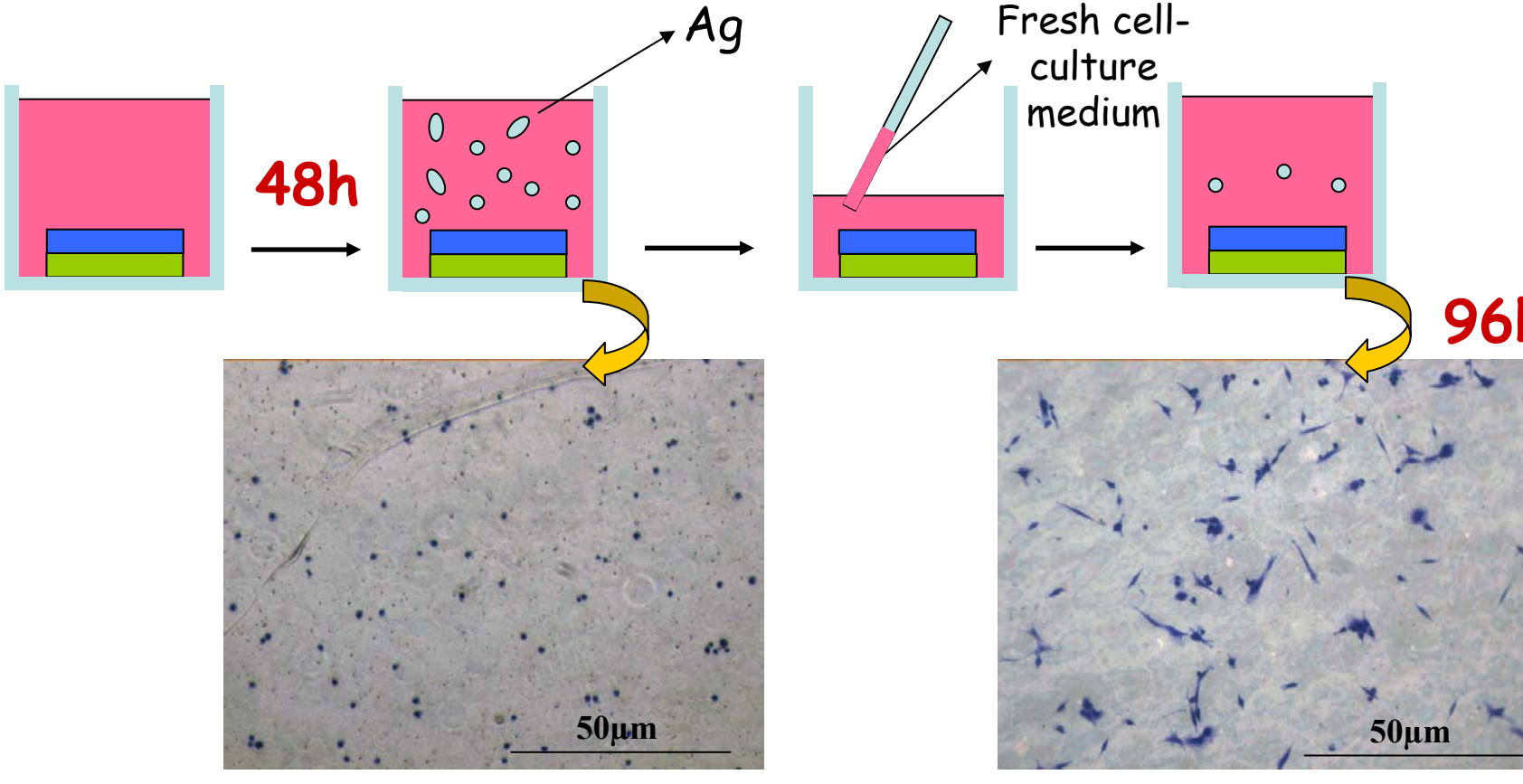
...on the bottom of the well (TCPS)



...on the bottom of the well (TCPS)

NO CYTOTOXIC EFFECT AROUND THE MATERIAL !

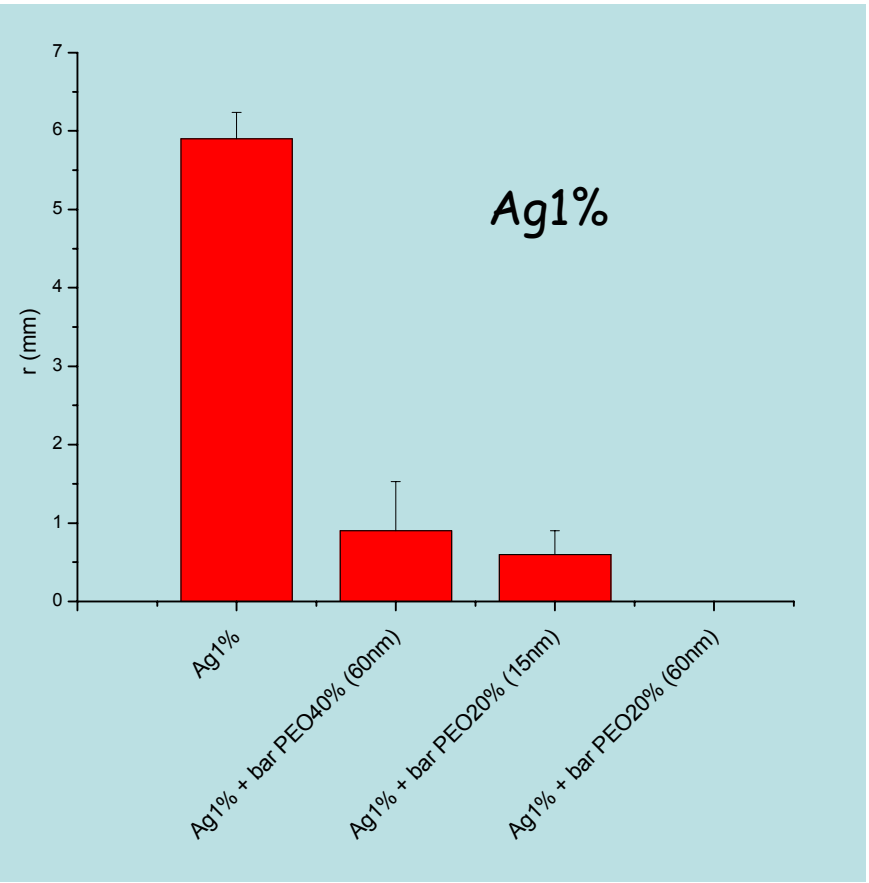
Substitution of cell-culture medium after 48hrs

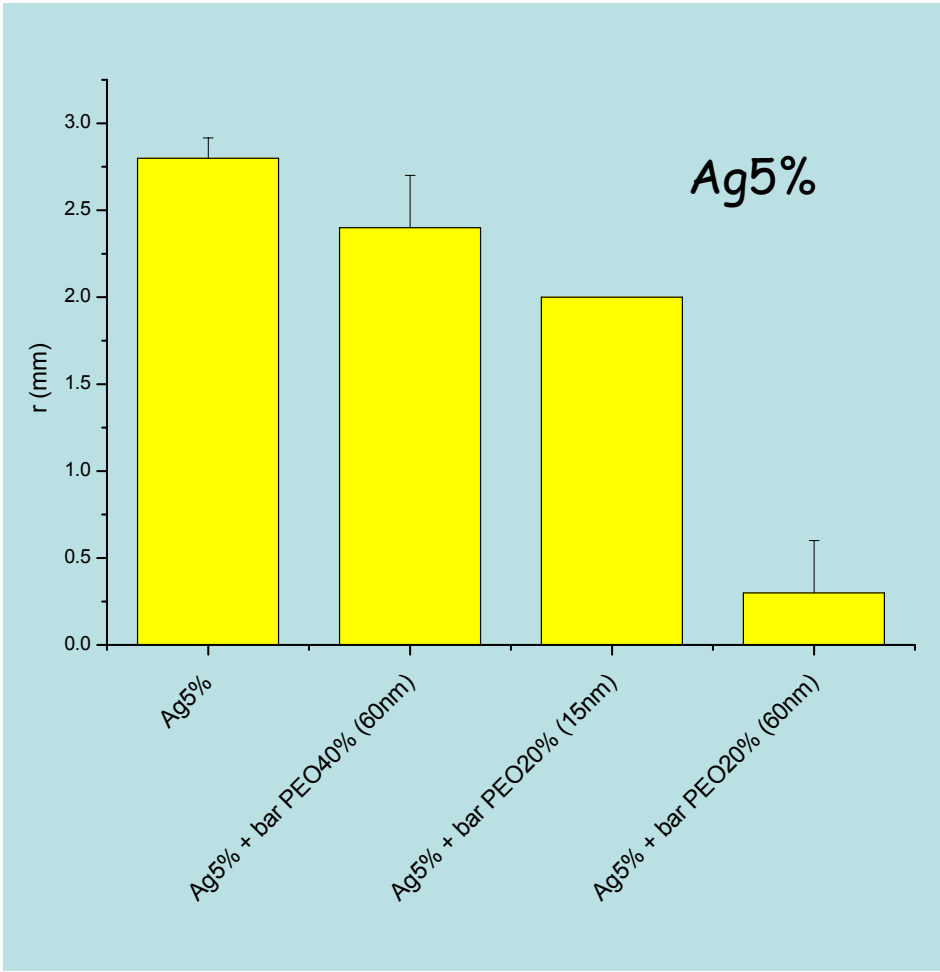


… only an inhibition on cell-growth and not a cytotoxic effect

DISK DIFFUSION SUSCEPTIBILITY TESTING

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





THE BARRIER:

- IMPROVES EUKARYOTIC CELLS BIOCOMPATIBILITY
- STORES THE BACTERIOSTATIC EFFECT

NEXT

-TEM characterization (diffusion of the clusters through the coating)

-Cell culture tests

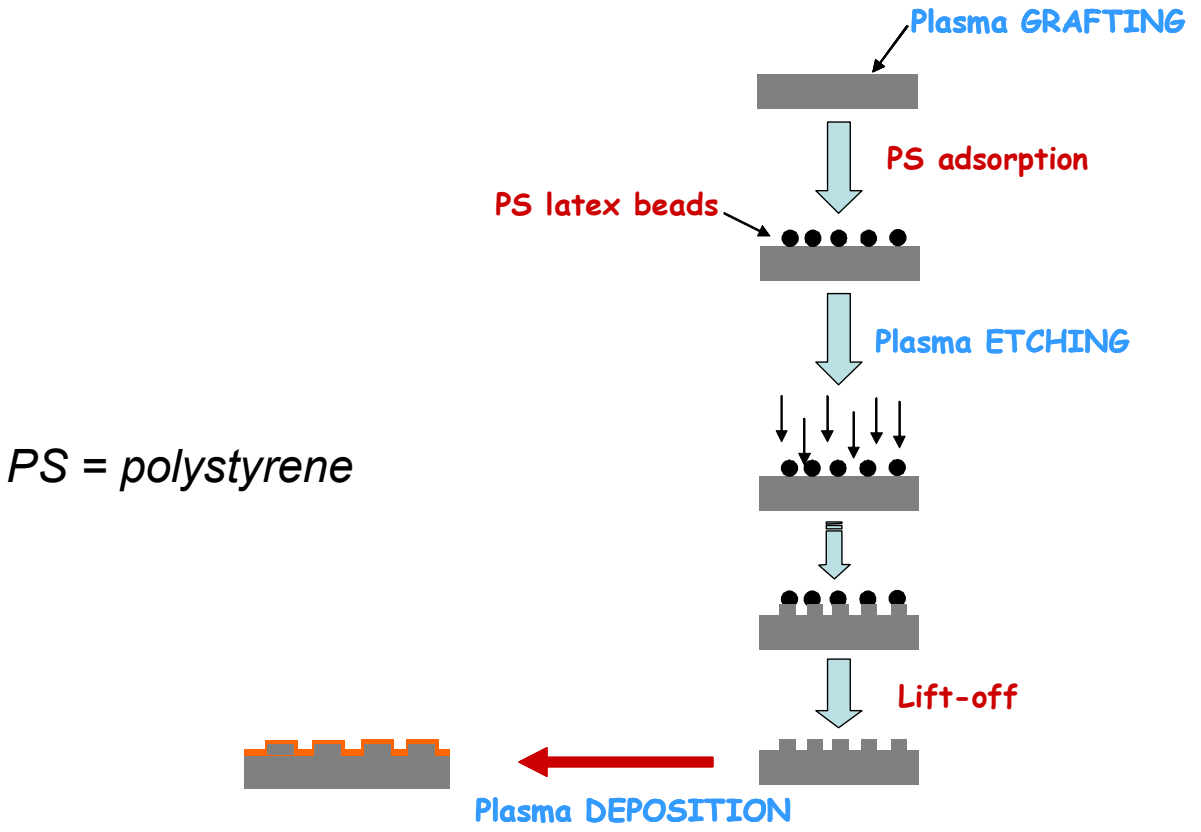
-Possible implantation on living systems

NANO-PATTERNING by means of COLD PLASMAS

FIRB : E. Sardella, S. Lovascio, R. Gristina; M. Nardulli, G. S. Senesi

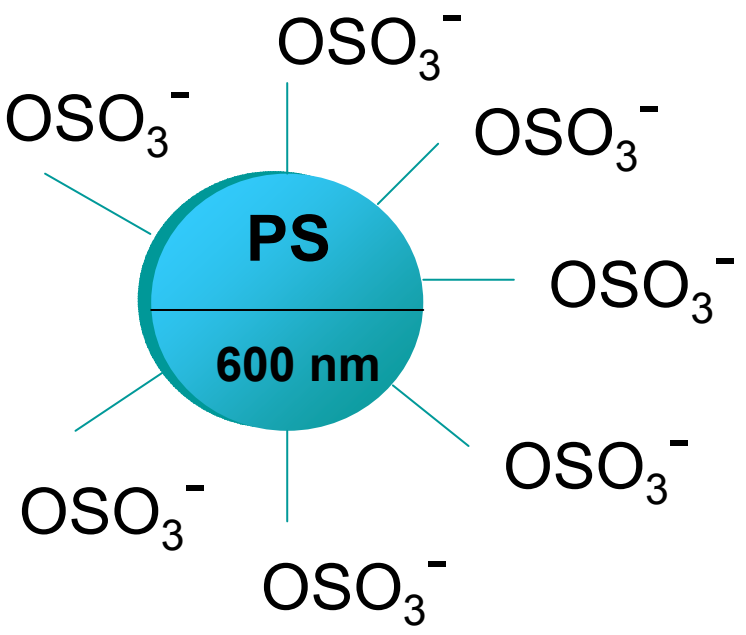
P.Favia, R. d'Agostino

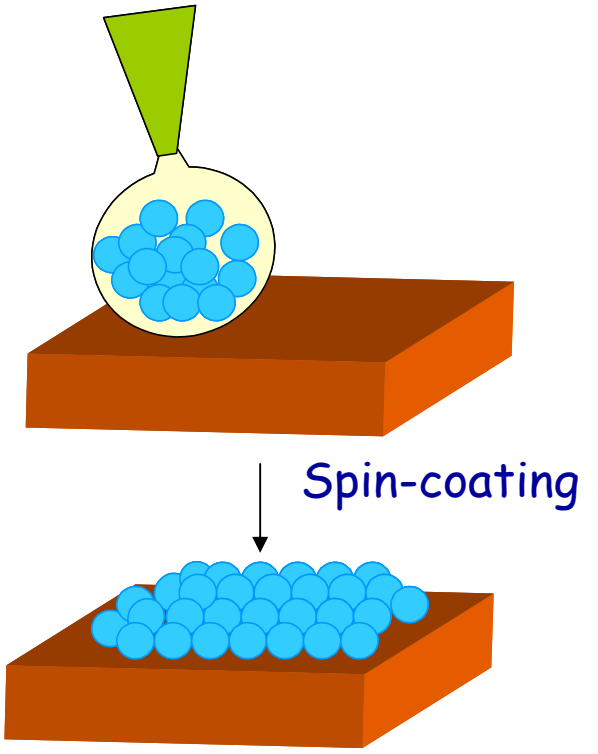
COLLOIDAL LITHOGRAPHY



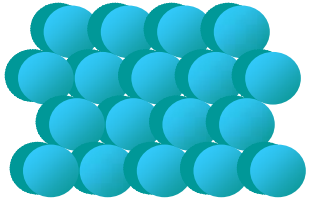
PS = polystyrene

PRODUCTION OF THE PHYSICAL MASK





PS: polystyrene

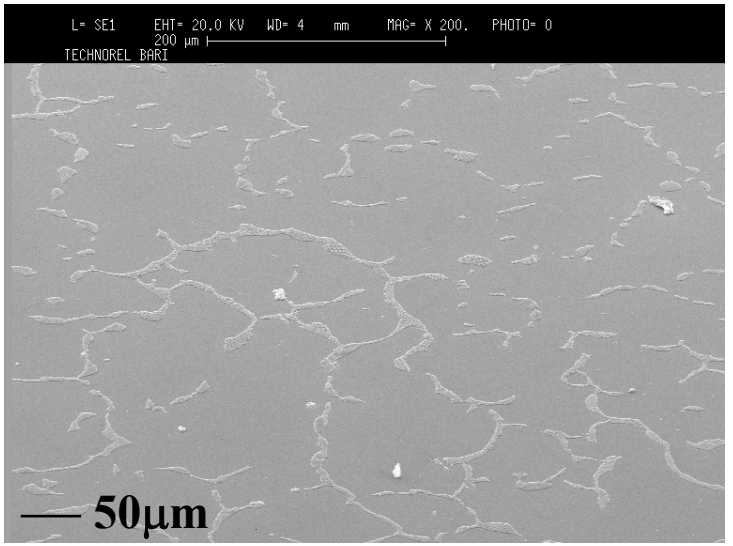


Ordered array of hexagonal lattices

PLASMA GRAFTING TO IMPROVE SUBSTRATE WETTABILITY BEFORE PATTERNING

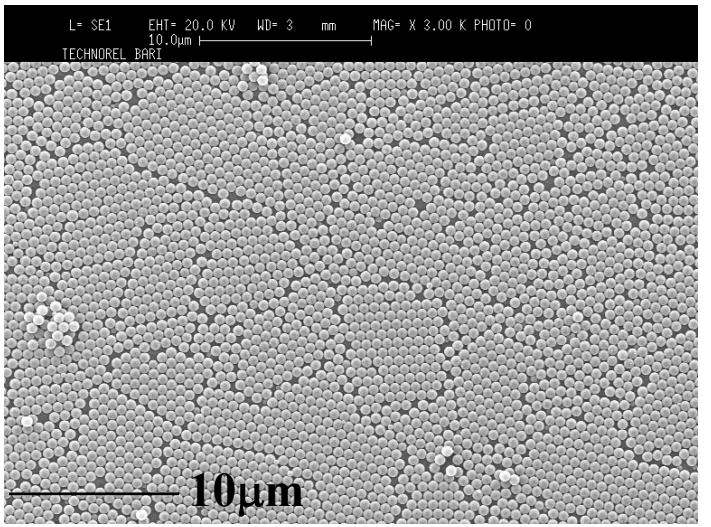
Native polystyrene (PS)

θ_{adv} = 90°±3°



NH_3- plasma treated PS

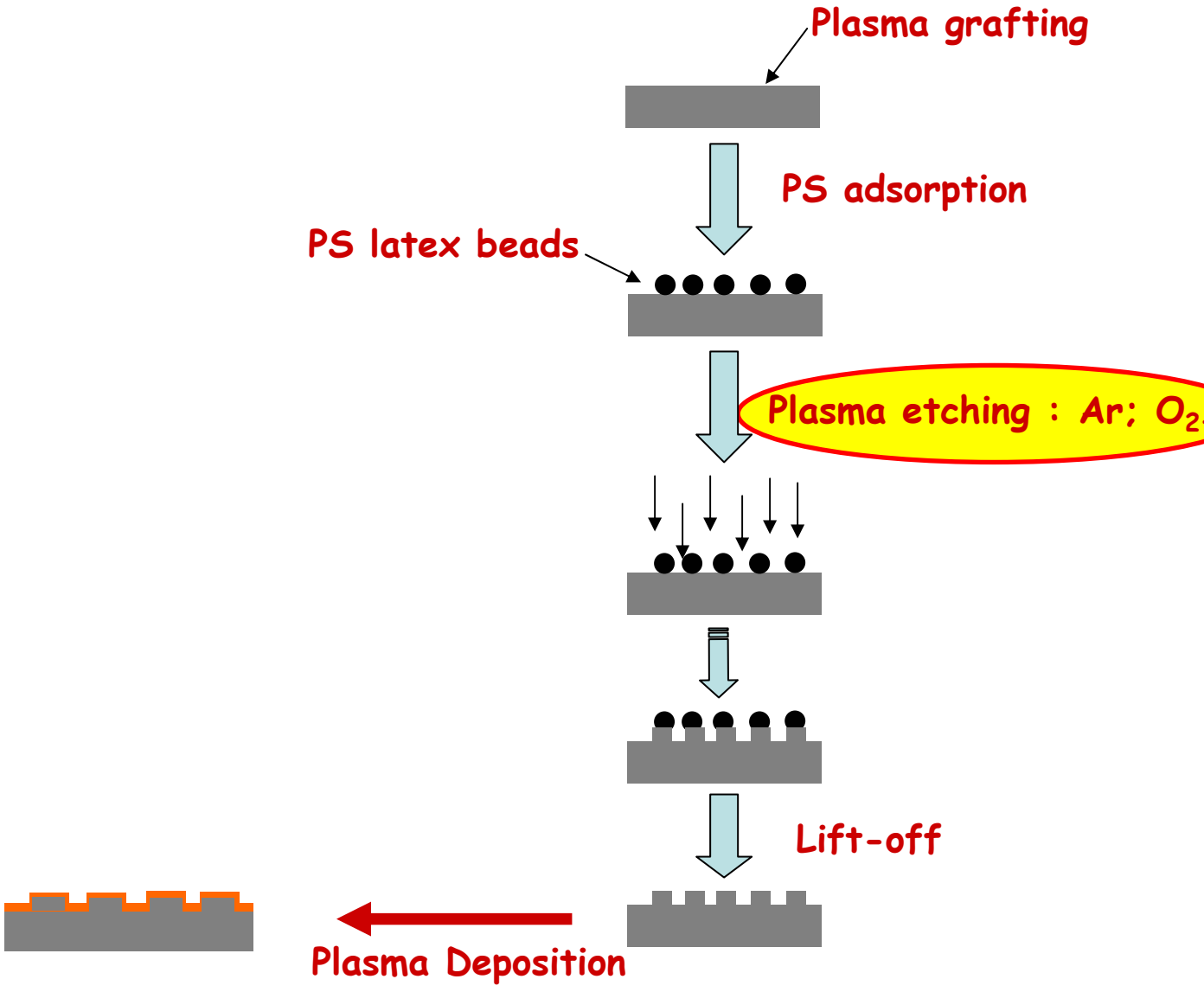
θ_{adv} = 20°±3°



(10 sccm NH_3, 200mtorr, 20 W, 1 min)

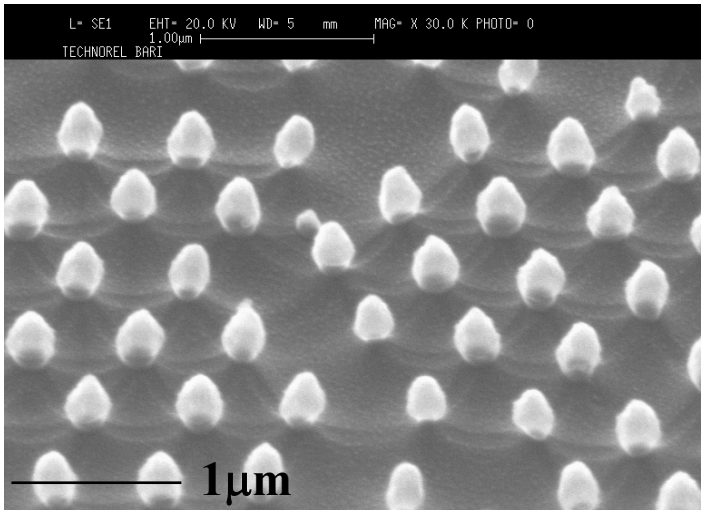
THE SUBSTRATE MUST BE WETTABLE!

COLLOIDAL LITHOGRAPHY

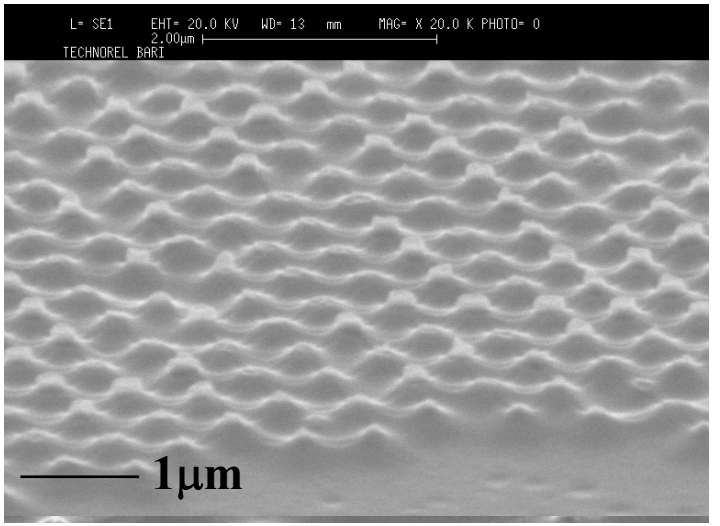


PATTERN BY MEANS OF O_2 and Ar

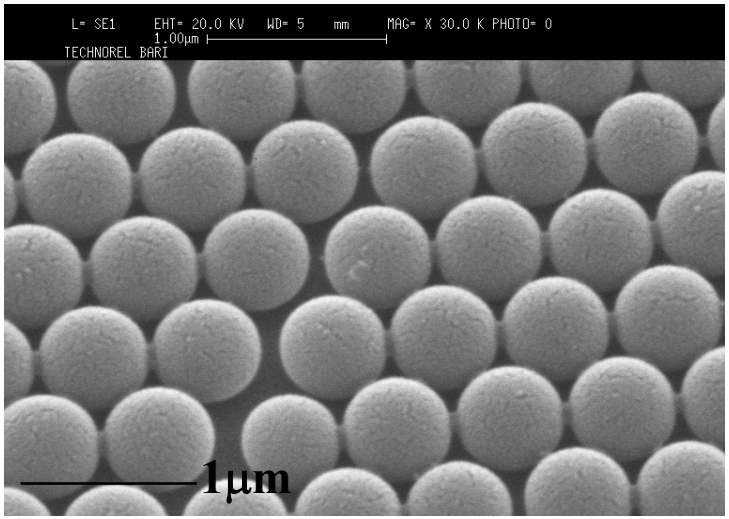
10min O_2 treatment



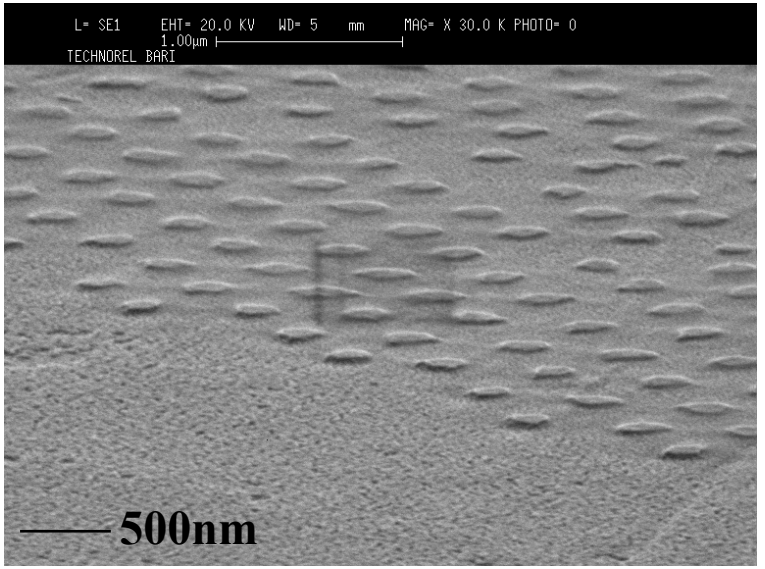
Lift-off



60min Ar treatment



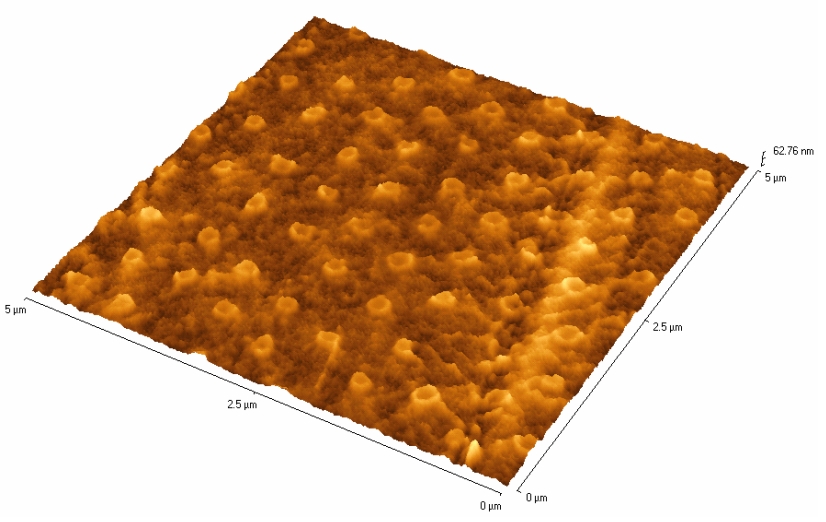
Lift-off

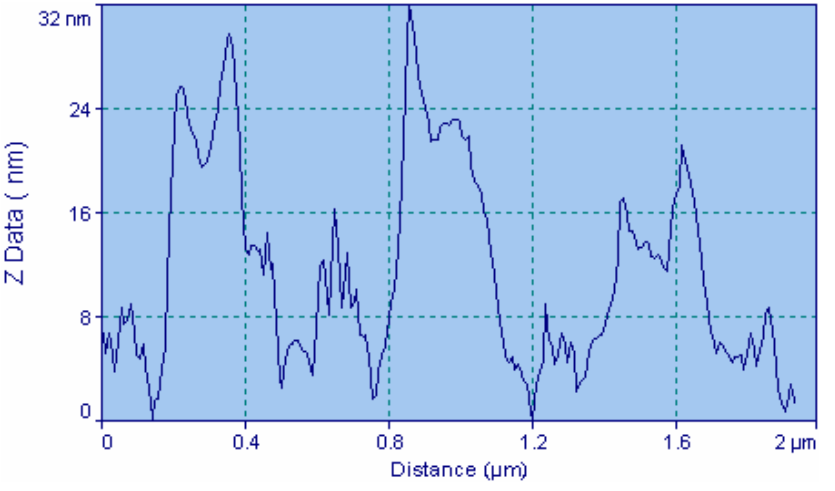


O_2(Ar) 10sccm; 50W; 200mTorr

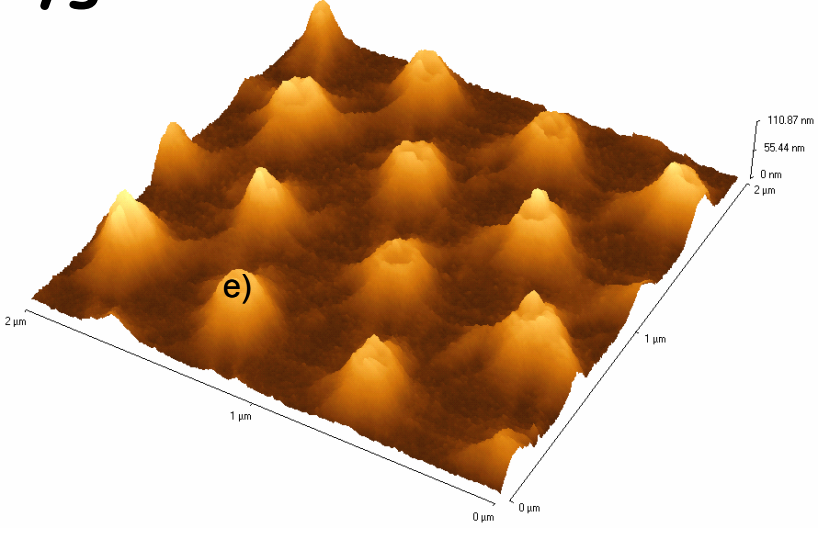
PATTERN BY MEANS OF O_2 and Ar

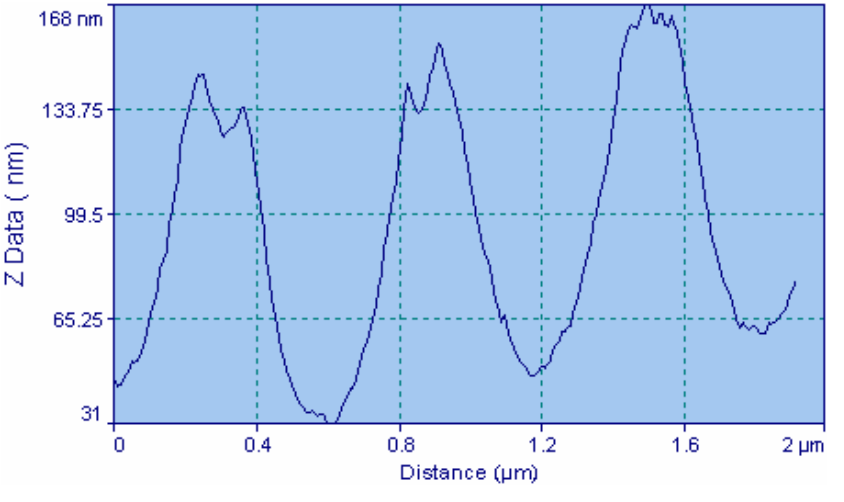
Argon





Oxygen





NOW

We are studing the fundamentals of combination of CL with plasmas :

- effect of the chemical composition of substrate surface (H_2O and O_2 pre-treatments)

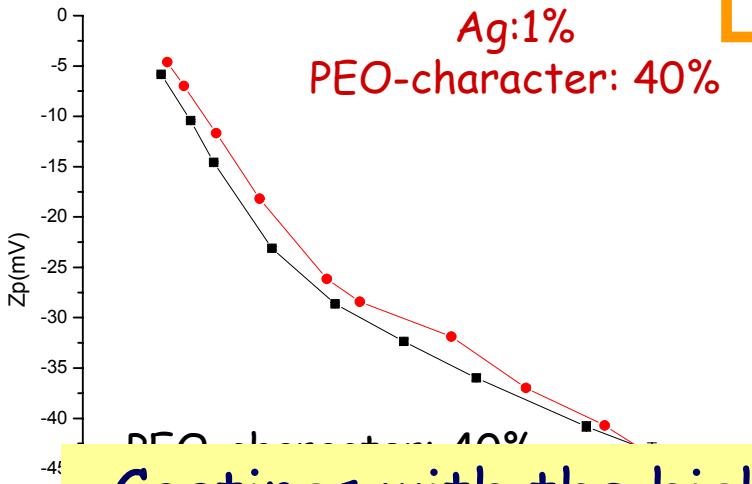
- effect of the plasma-etching during the transfer of the pattern

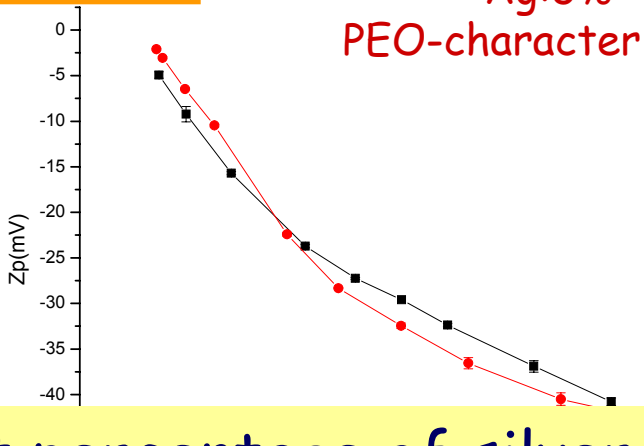
FUTURE

Compare the effect of chemistry and/or topography on cell adhesion

Producing chemical nano-domains spatially resolved (biosensors)

Z potential

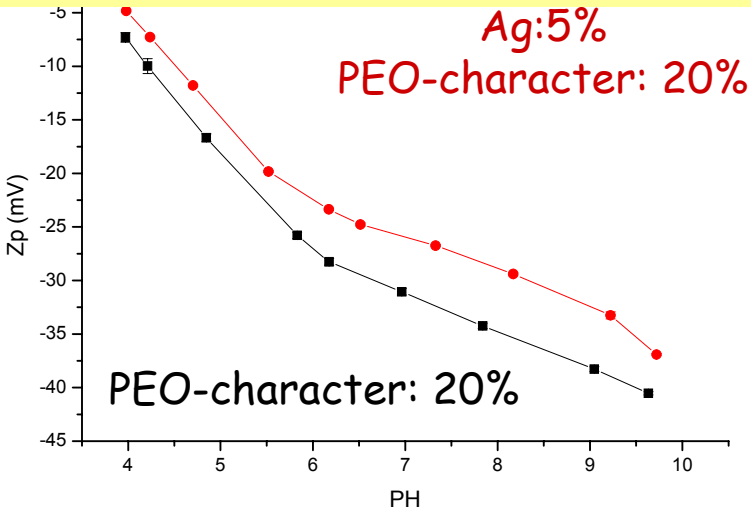




Coatings with the highest percentage of silver have

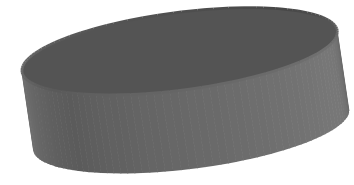
a more positive Z potential

with respect to the other ones



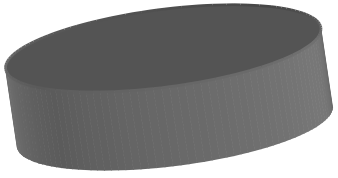
RISULTATO:

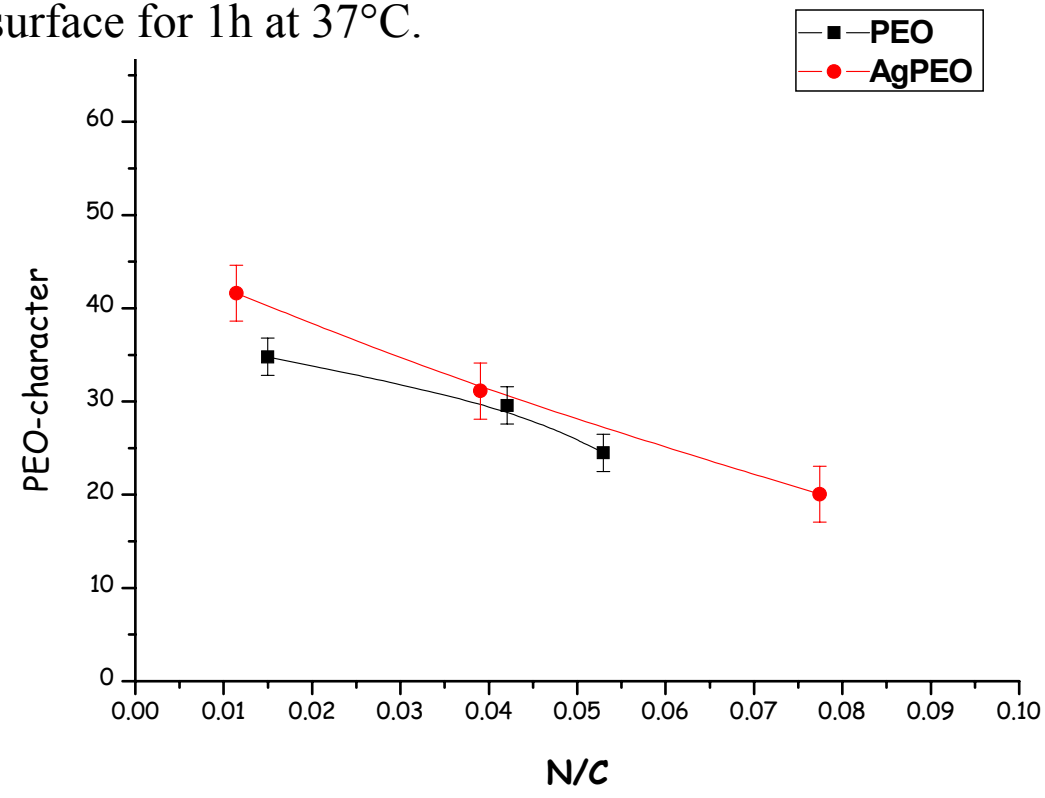
OBJECTIVE 3: Correlate protein adsorption and cell (prokaryotic and eukaryotic) behaviours with chemical-physical characteristics of the coatings;

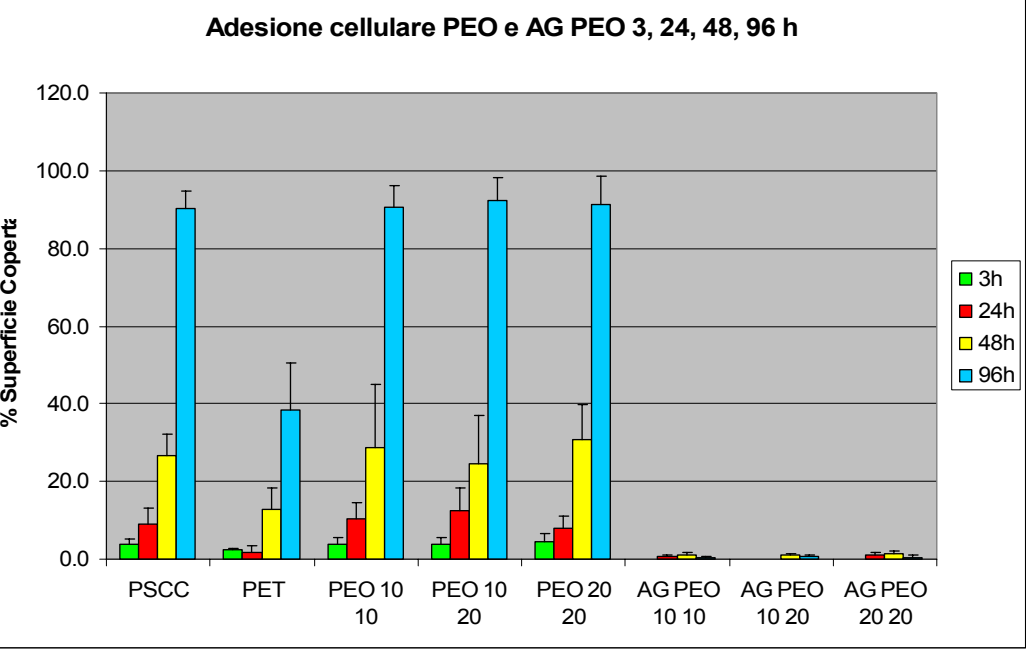


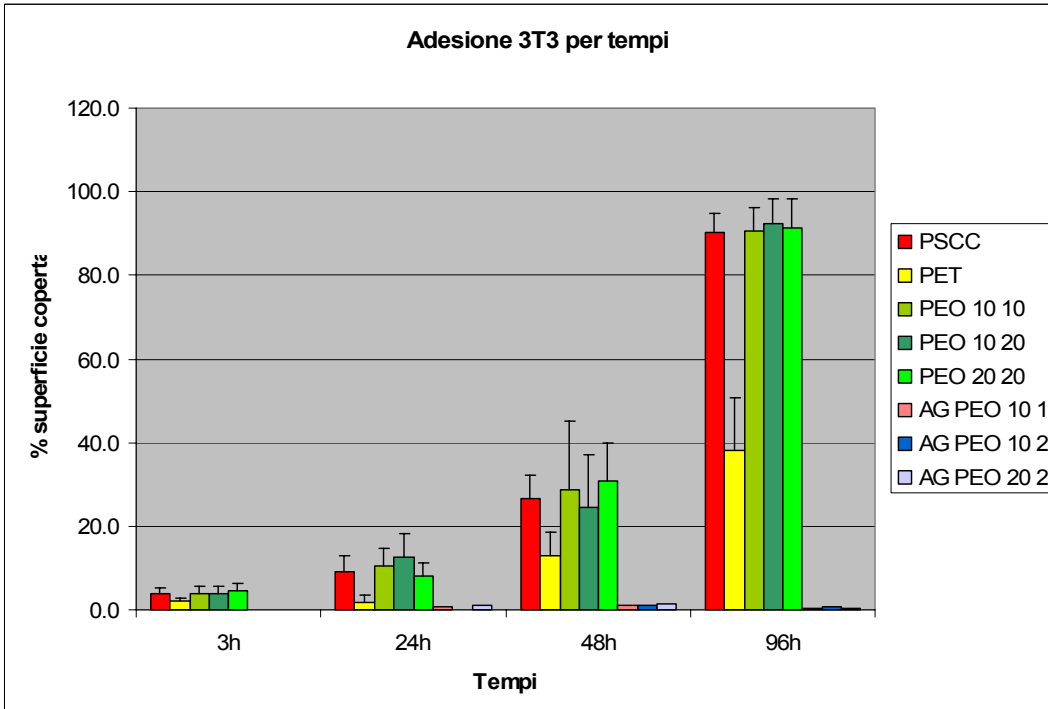
Drop of fibronectin solution (50µg/ml) that covers the whole substrate surface for 1h at 37°C.

Twice distilled water rinsing






Adesione cellulare PEO e AG PEO 3, 24, 48, 96 h
% Superficie Coperta
120.0
100.0
80.0
60.0
40.0
20.0
0.0
PSCC
PET
PEO 10 10
PEO 10 20
PEO 20 20
AG PEO 10 10
AG PEO 10 20
AG PEO 20 20
3h
24h
48h
96h


Adesione 3T3 per tempi
% superficie coperta
120.0
100.0
80.0
60.0
40.0
20.0
0.0
3h
24h
48h
96h
Tempi
PSCC
PET
PEO 10 10
PEO 10 20
PEO 20 20
AG PEO 10 1
AG PEO 10 2
AG PEO 20 2