JRC
EUROPEAN COMMISSION

ihcp
Institute for Health and Consumer Protection

Application of plasma technologies to biological interface design

M. Perez Roldan, A. Ruiz, , G. Ceccone, P. Colpo, Fr. Rossi

Institute for Health and Consumer Protection Ispra
http://www.jrc.cec.eu.int

JRC
EUROPEAN COMMISSION

IHCP
Institute for Health and Consumer Protection

Outline

- Background
- Plasma polymerization as surface functionalisation technique
- Surface Micro-Nano Patterning
- PeO like film as Cell culture platform
- Conclusions

JRC work-programme

Support to the European Policy on:

- Exposure monitoring
  - Air, water, food quality monitoring
  - Indoor exposure measurements

(Bio)sensors

- Chemical policy (REACH)
  - Toxicity evaluation of 30000 chemicals compounds
  - Reduction of animal testing
  - Validation of alternative methods

In vitro tests
Cell on chip

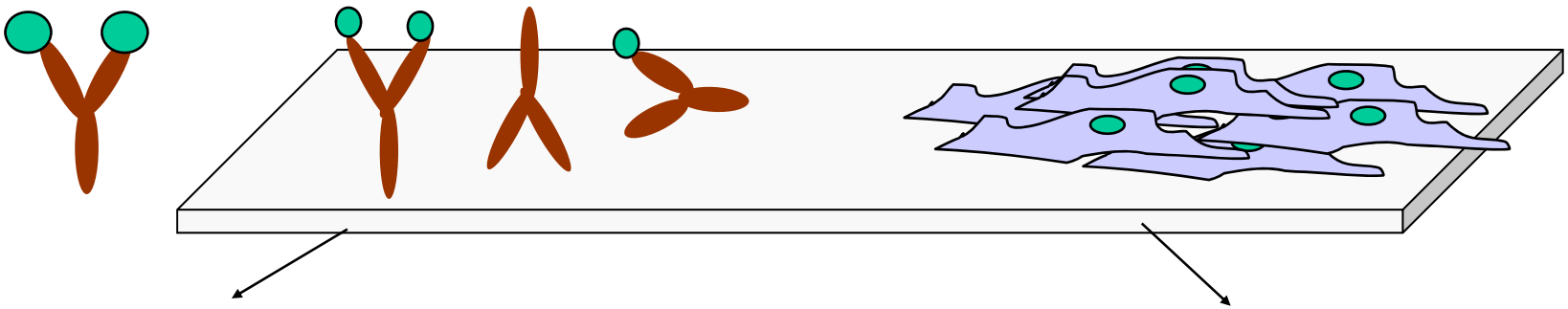
- Nanotoxicology
  - In vitro tests
  - Nanoparticles - proteins interactions
  - Nanoparticles – cell interactions

Protein surface interaction

Cells and proteins on solid surfaces

Proteins

Cells



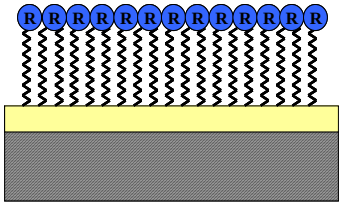
Biosensing for environment monitoring, medical application…

Implants, tissues engineering Biology study, cell therapy
Toxicology assays

Reliability and relevance of assays depend on bioactivity of biomolecules/cells i.e. Bio interfaces

Two Strategies for Surface Functionalisation

1. Self Assembled Monolayers (SAM)

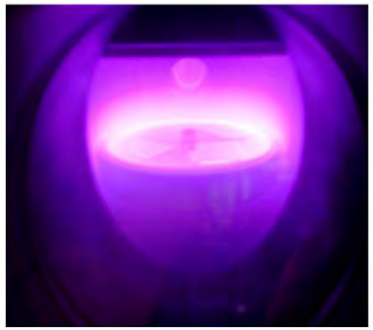


Alkane thiols or alkyl silanes self-assemble on activated Au (111) or -OH surfaces. They are terminated with different functionalities (COOH, NH_2, CF_x, PEG)

- chemical purity
- easy and "cheap" (for research)

- non-homogeneous coverage
- Oxydation
- need of "special" substrate

2. Polymer Plasma Deposition (PE-CVD)



Capacitive coupled plasma reactor. By using different gas precursors in the discharge it is possible to deposit polymers with different functionalities (COOH, NH_2, CF_x, PEG).

- control of the film properties by plasma parameters
- any substrate can be functionalized
- homogeneous coverage
- compatible with industrial production

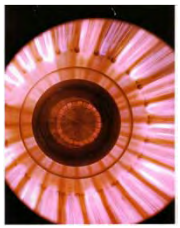
- no chemical purity
- need of special equipment

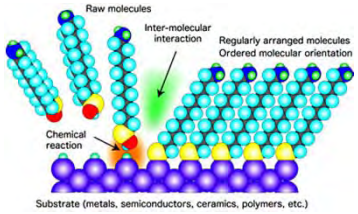
Functional surface production

Production of films with controlled properties

Plasma deposition

Self Assembled Monolayers (silanes, thiols)





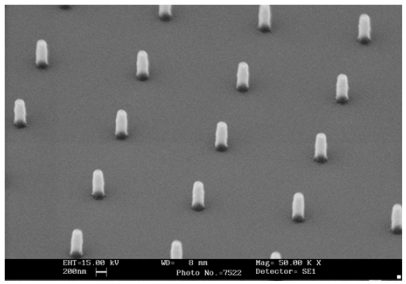
Characterisation/ Functional properties

Composition, Physico chemical properties (surface energy wettability charge)

Surface engineering to
Control surface
Physical/chemical properties
at micro and nanoscale

Micro and nanopatterning

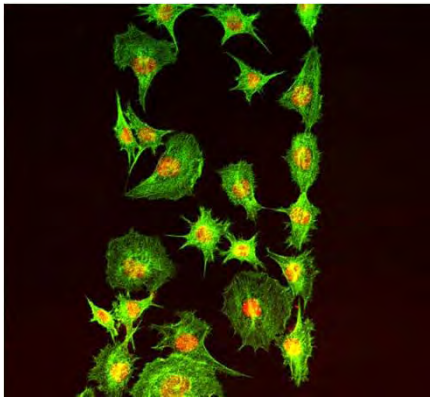
Photolithography,
E-Beam
colloidal lithography



Applications :

biosensors

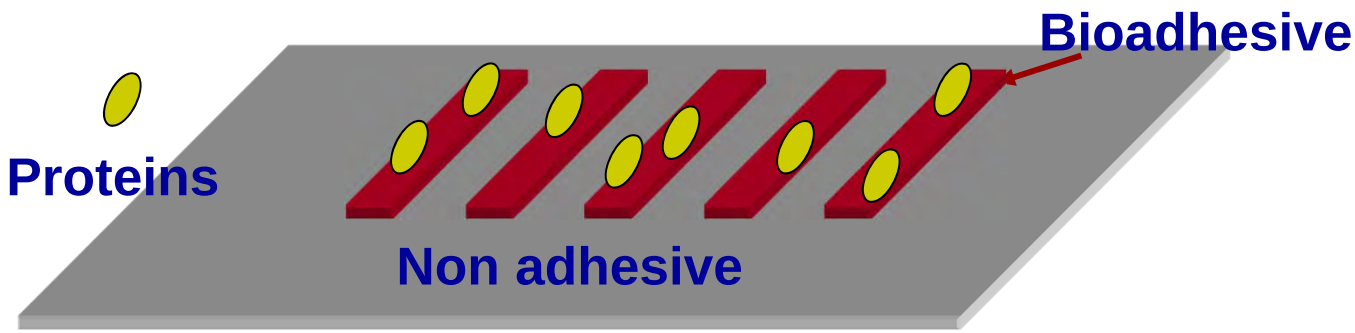
Cell biology



OBJECTIVES

Create chemical contrast 'Bio adhesive – non adhesive' at micro - nanoscale

Combination of Functionalisation and patterning techniques



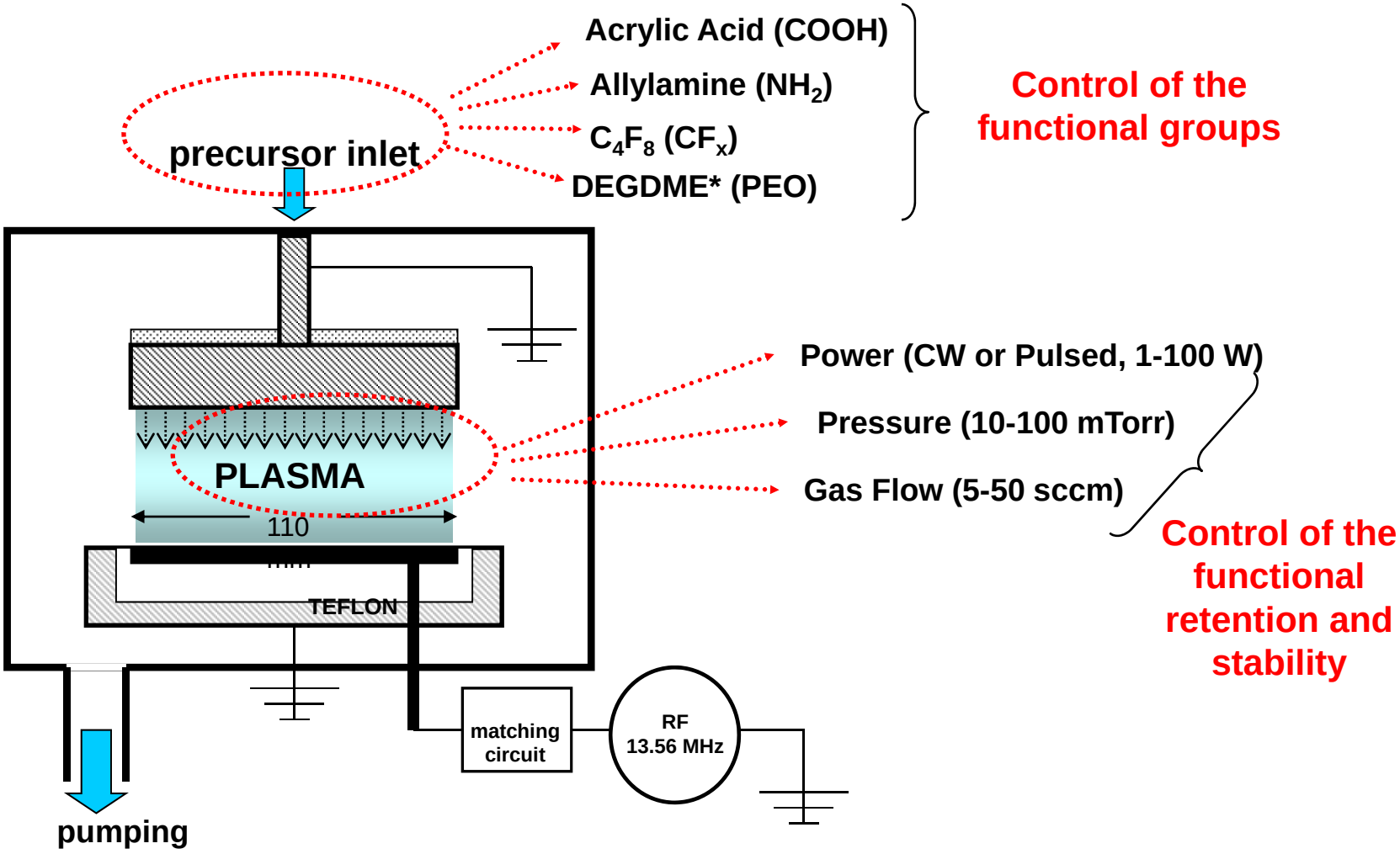
Improve biosensor performance
Miniaturization

Control cell micro-environment

E Beam lithography +

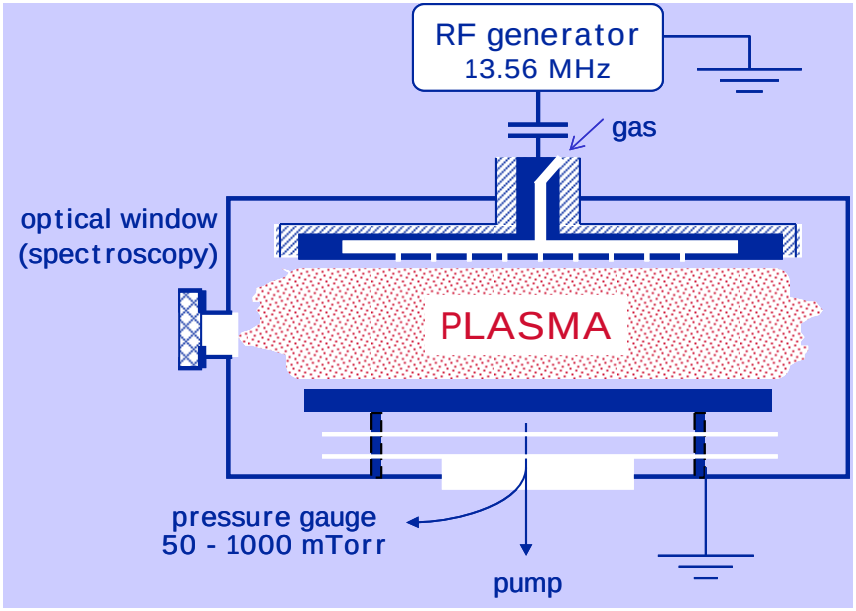
Plasma polymer Poly-ethylene- Oxide as universal platform

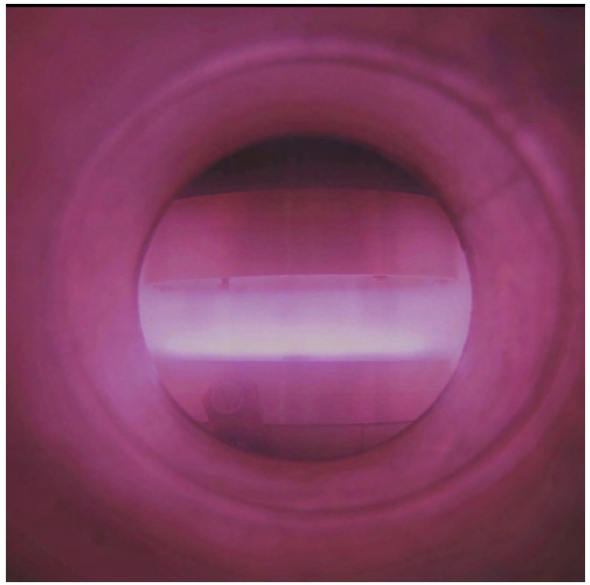
Polymer Plasma Deposition (PE-CVD)



*Di-EthyleneGlycoleDiMethylEther

Plasma deposited PEO-like coatings





0.4 sccm DEGDME
5 sccmAr
400 mTorr
5-15 W

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

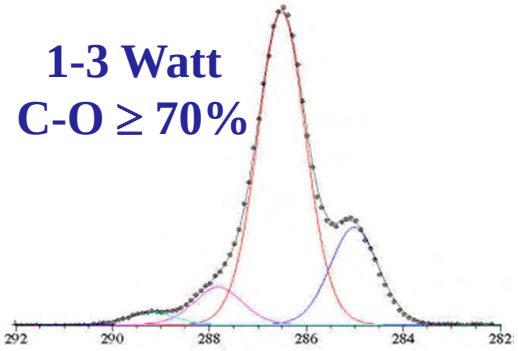
Diethylene glycol dimethyl ether
DEGDME

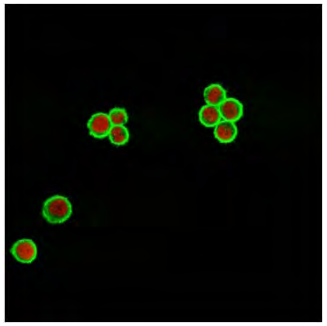
Anti-fouling properties of plasma PEO

Optimization of PEO-like deposition

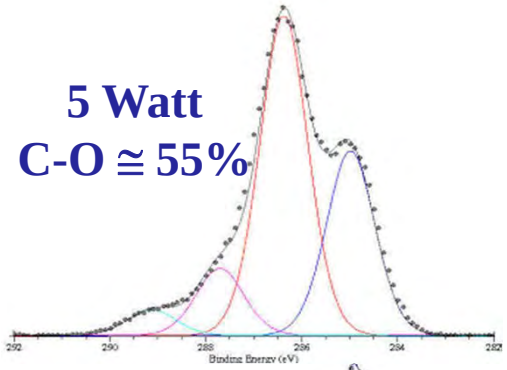
Deposition parameters: Di-Ethylene Glycole DiMethylEther precursor, 20mTorr, 2W, 30 min

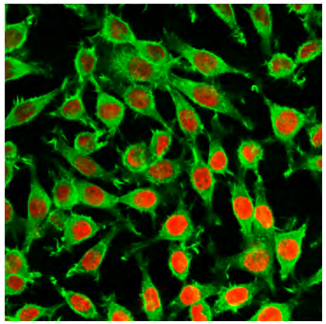
1-3 Watt
C-O ≥ 70%



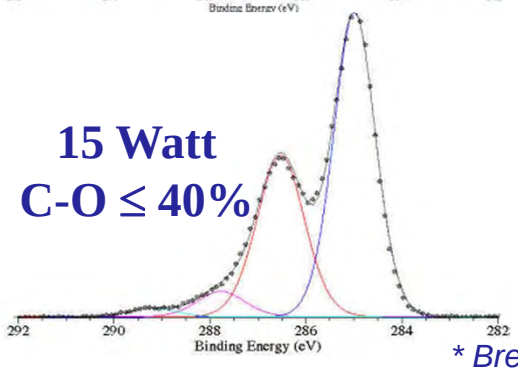


5 Watt
C-O ≅ 55%

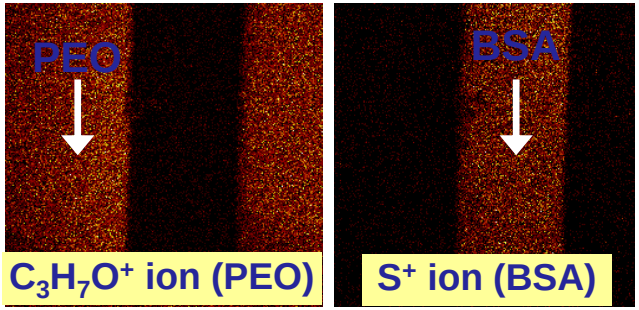




15 Watt
C-O ≤ 40%



- High PEO-like character (C-O>70%) for P=2W
- High coating stability (water, ethanol, PBS...)
- 95% protein adhesion reduction for P<5watts



Chemical characterization of PEO patterns after incubation in BSA solution by ToF-SIMS

** Bretagnol, et al. Acta Biomat. 2006, 2:165.*

Plasma polymer films properties

Evaluation of the absorbed protein mass (QCM)

Surface	*Functionality*	*Contact angle (degrees)*	*BSA Absorbed mass (@ pH=7.5) (45 μg/ml) ($ng*cm^{-2}$)*
PAA	COOH	38 ± 2	330
PAL	NH_2	45 ± 3	480
Teflon	CF_x	110 ± 2	290
PEO	C – O	50 ± 3	<10

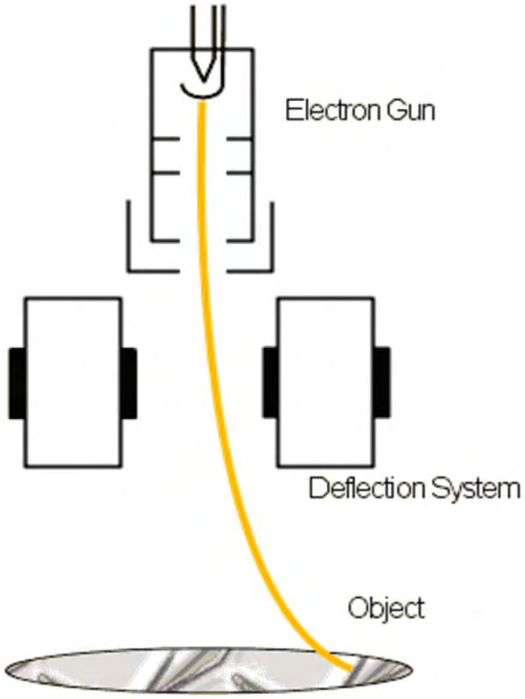
Bio-Adhesive

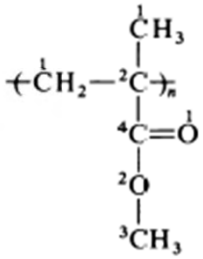
Anti-adhesive

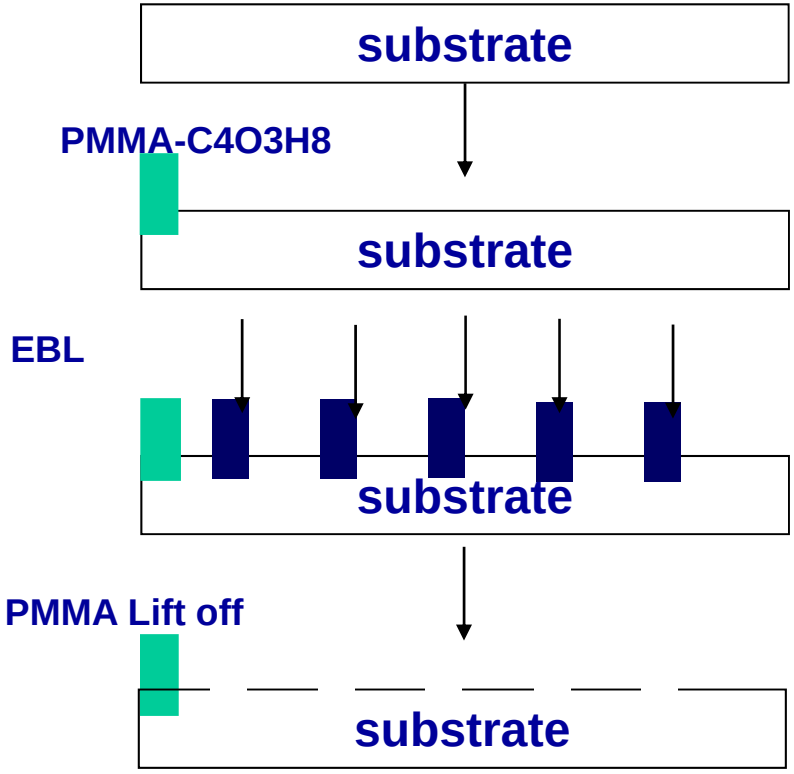
Electron Beam Lithography of plasma polymers

General principle:

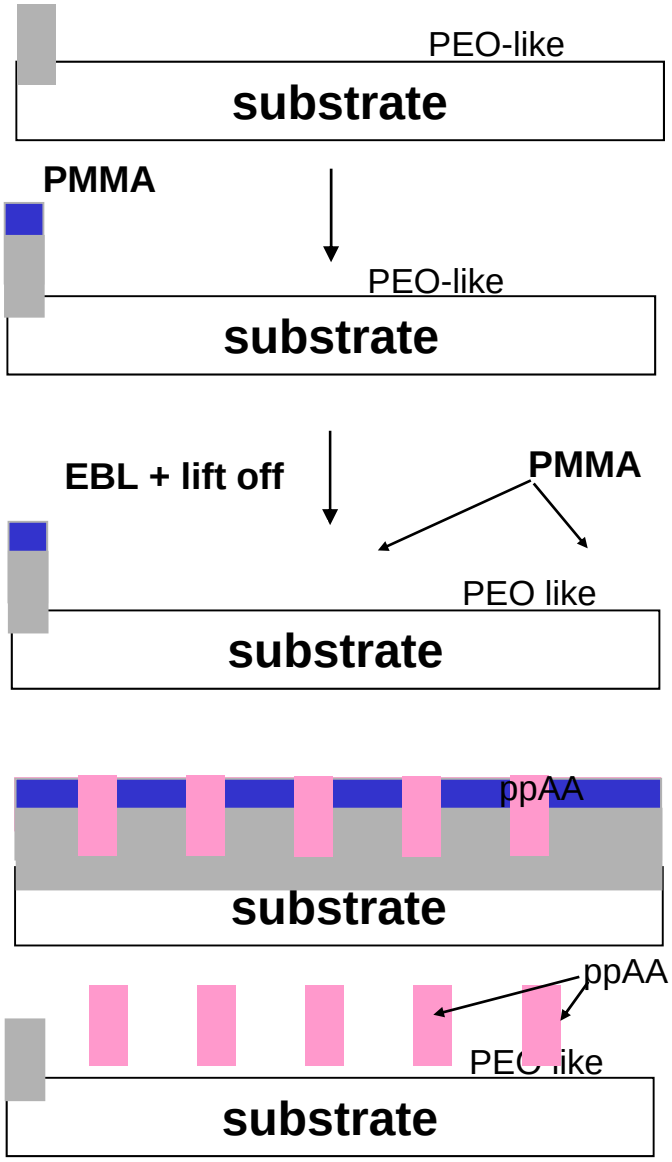
- Higher definition (≈10nm). Use PMMA as electron sensible resists
- Electrons break modify polymer chain -> can be dissolve by adapted solvent.
- metallic electrodes, nanostructure of PMMA or Si.

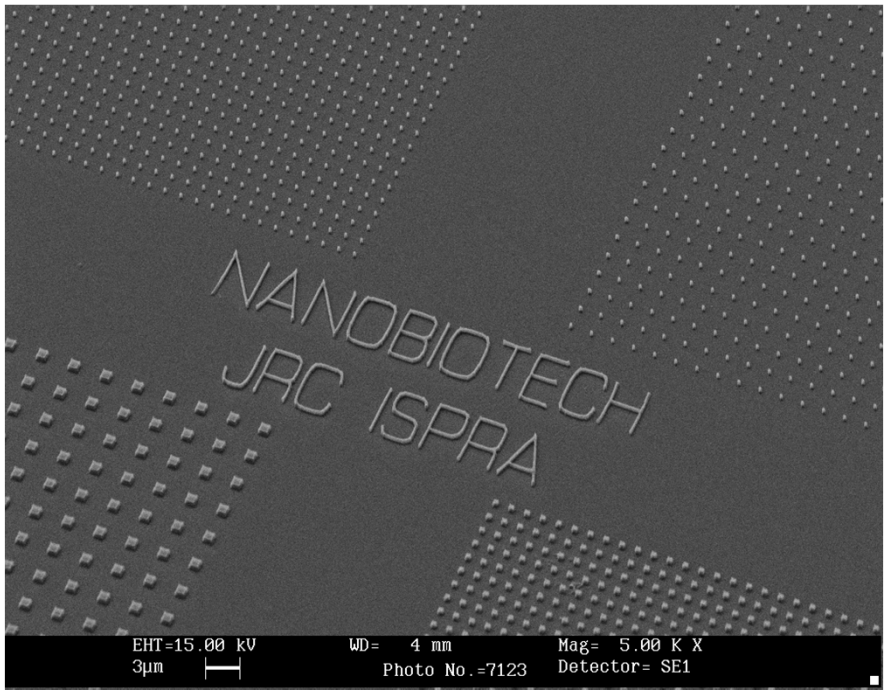






EBL & plasma polymerization: PEO-like/ppAA nanostructures





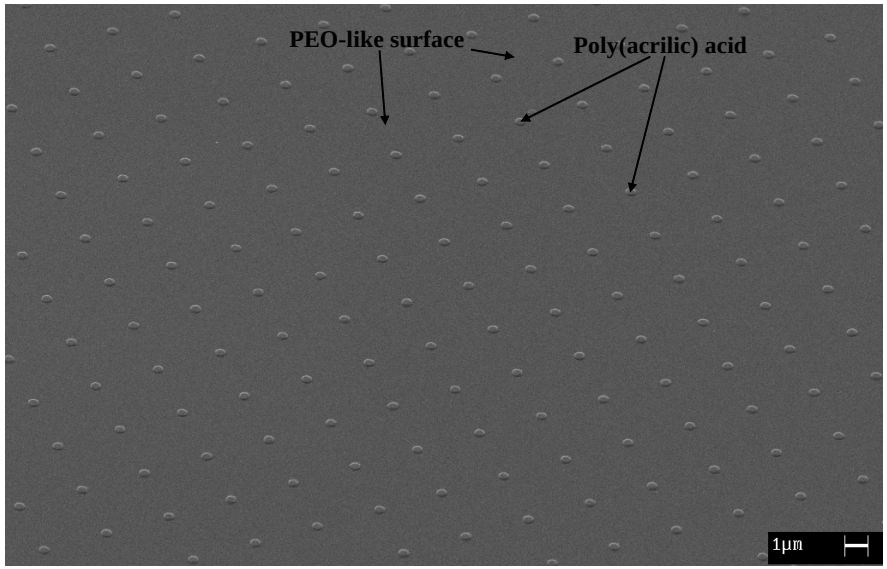
Micro and nano structures of pPAA on PEO-like matrix.

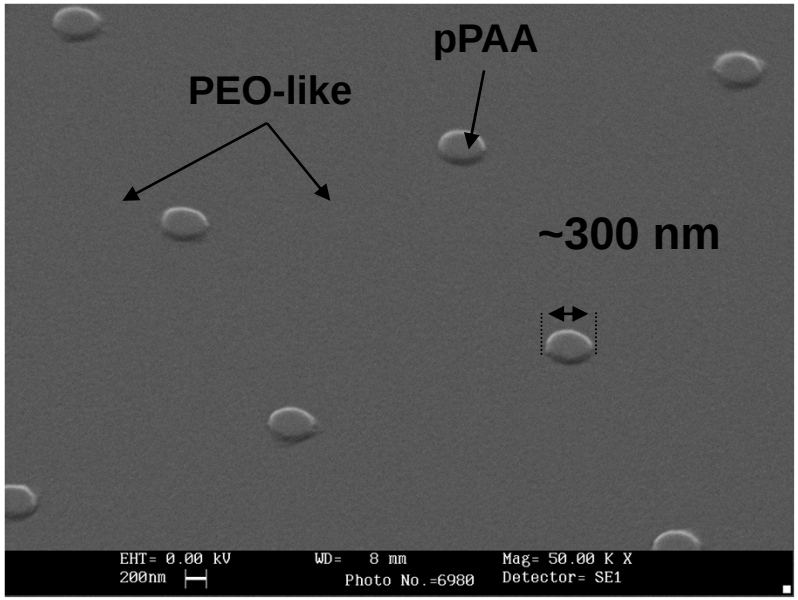
- No Baking of PMMA
- Removal of PMMA at the end of the process with MIBK/IP

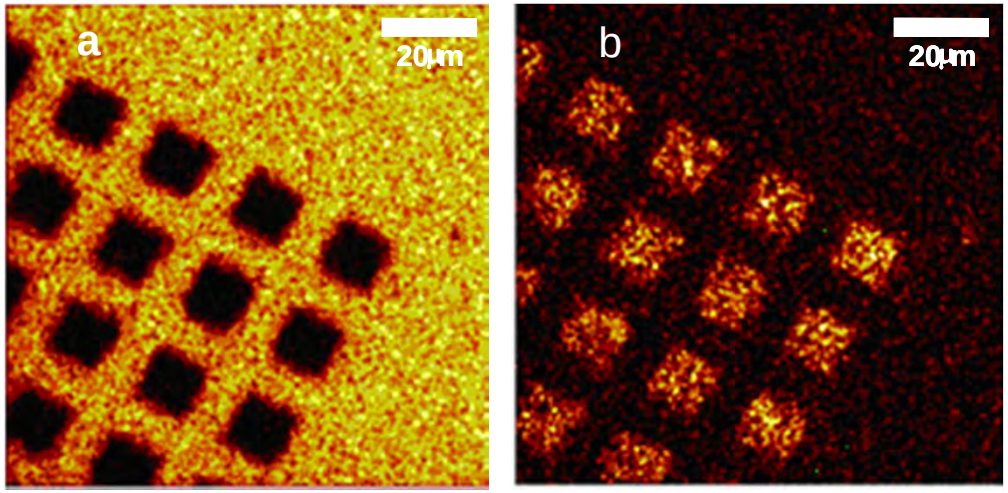
-methyl isobutyl ketone (MIBK) and isopropyl alcohol (IPA), 1/3

F. Bretagnol, et al. Nanotechnol.18 (2007), 135303

EBL & plasma polymerization.



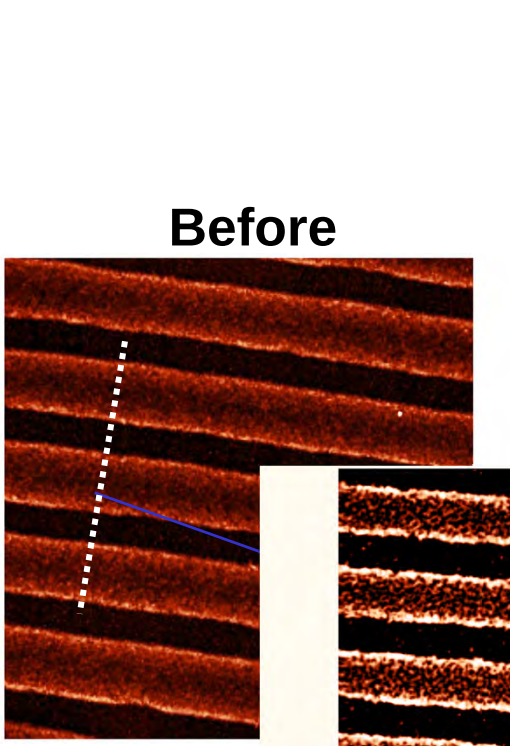


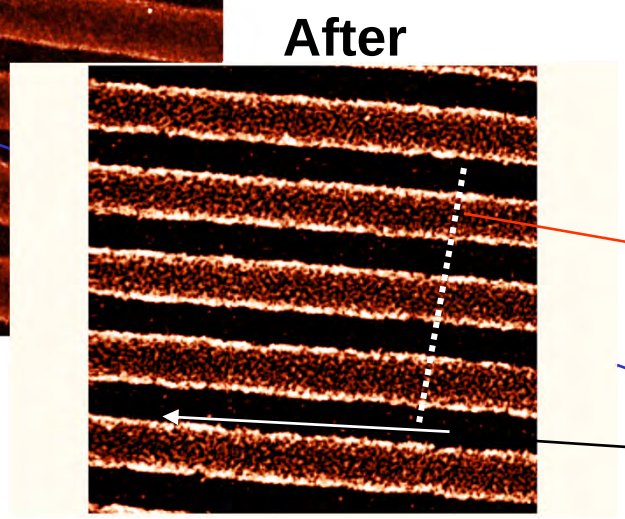


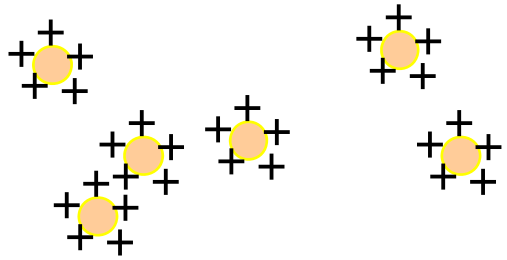
ToF-SIMS analysis after immersion in a BSA solution (a) C3H7O+ ions (59 amu) (b) sum of BSA amino acid fragments (C3H6N+, C3H7N+ , C3H8N+ C4H8N+).

Evidence of the chemical contrast

Positively charged particles are mainly adsorbed on the pPAA







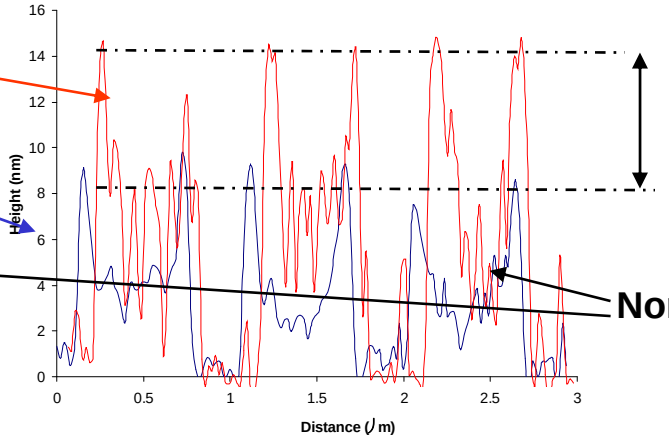
Au-NPs (~8-10 nm)

PH=3-4

10 mn and water washing

COOH (-)

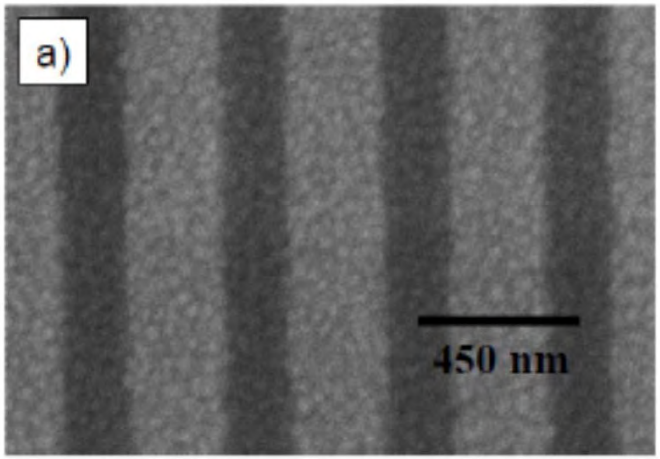
PEO-like (slightly +)



Dh ~ 6-8 nm

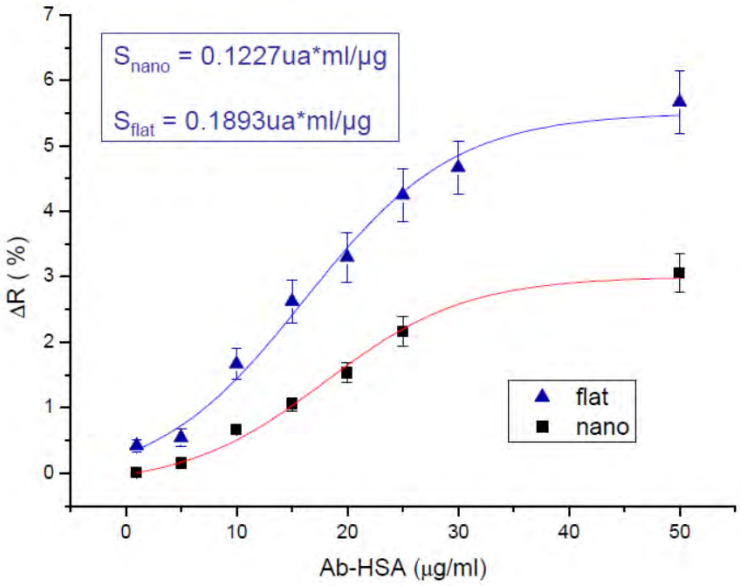
Non specific adsoprtion

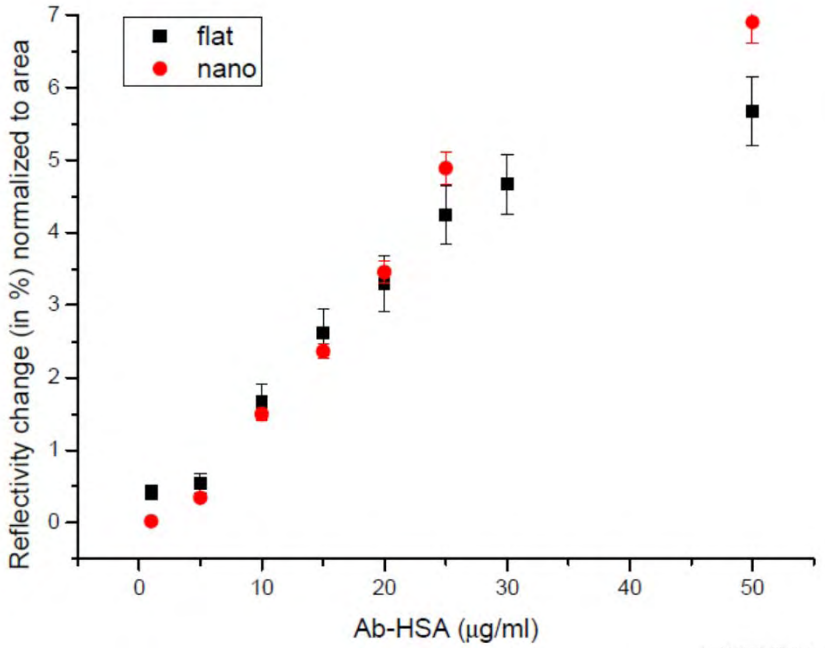
Protein nanostructure interactions



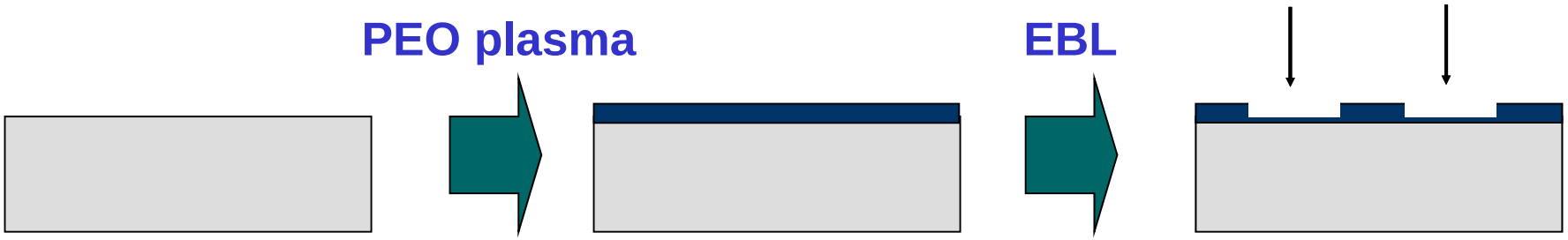
SURFACE PLASMON RESONANCE

HSA (20 µg/ml) Blocking with BSA
Ab-HSA at different concentrations (1-50 µg/ml)





Biointerfaces: PEO +EBL

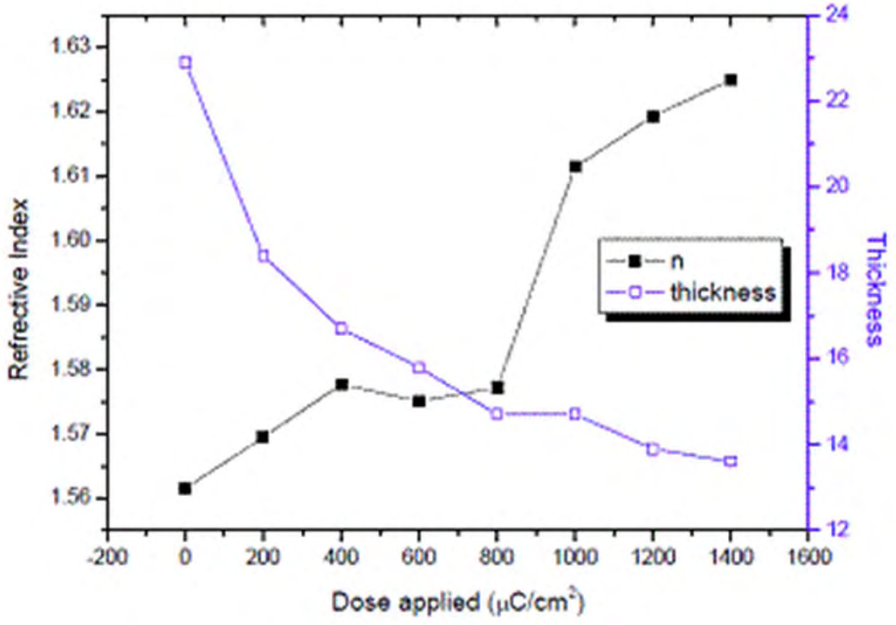


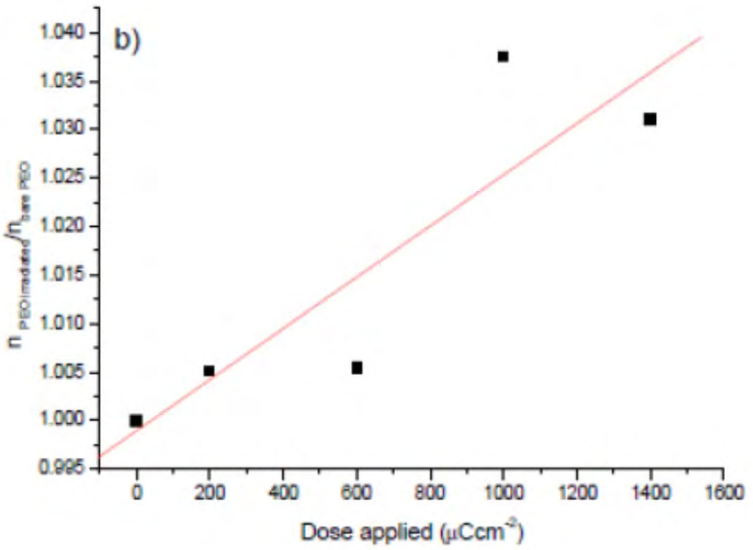
Fabrication step optimisation PEO on SPR prism
EBL with different doses and energies

Energy	Dose
10kV	500-7000 μC/cm²
5kV	250-3500 μC/cm²
2kV	100-1400 μC/cm²

Film characterisation by Ellipsometry

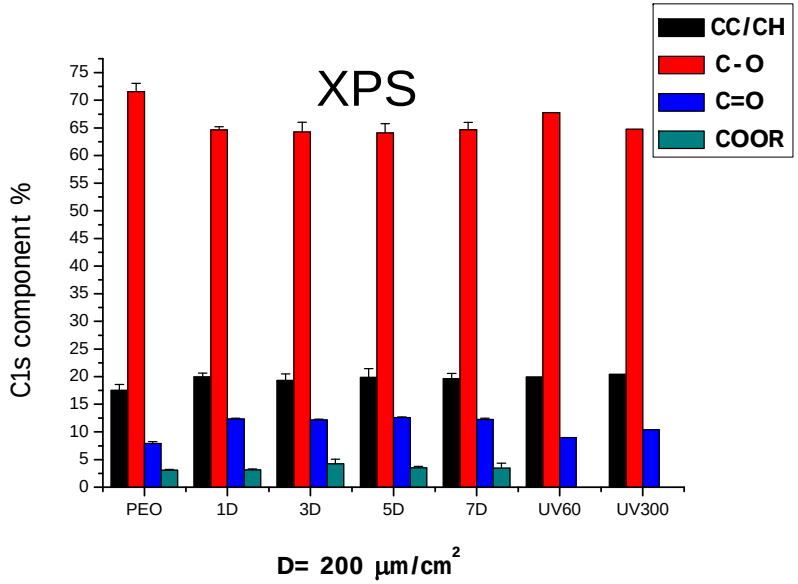
Energy: 2KeV





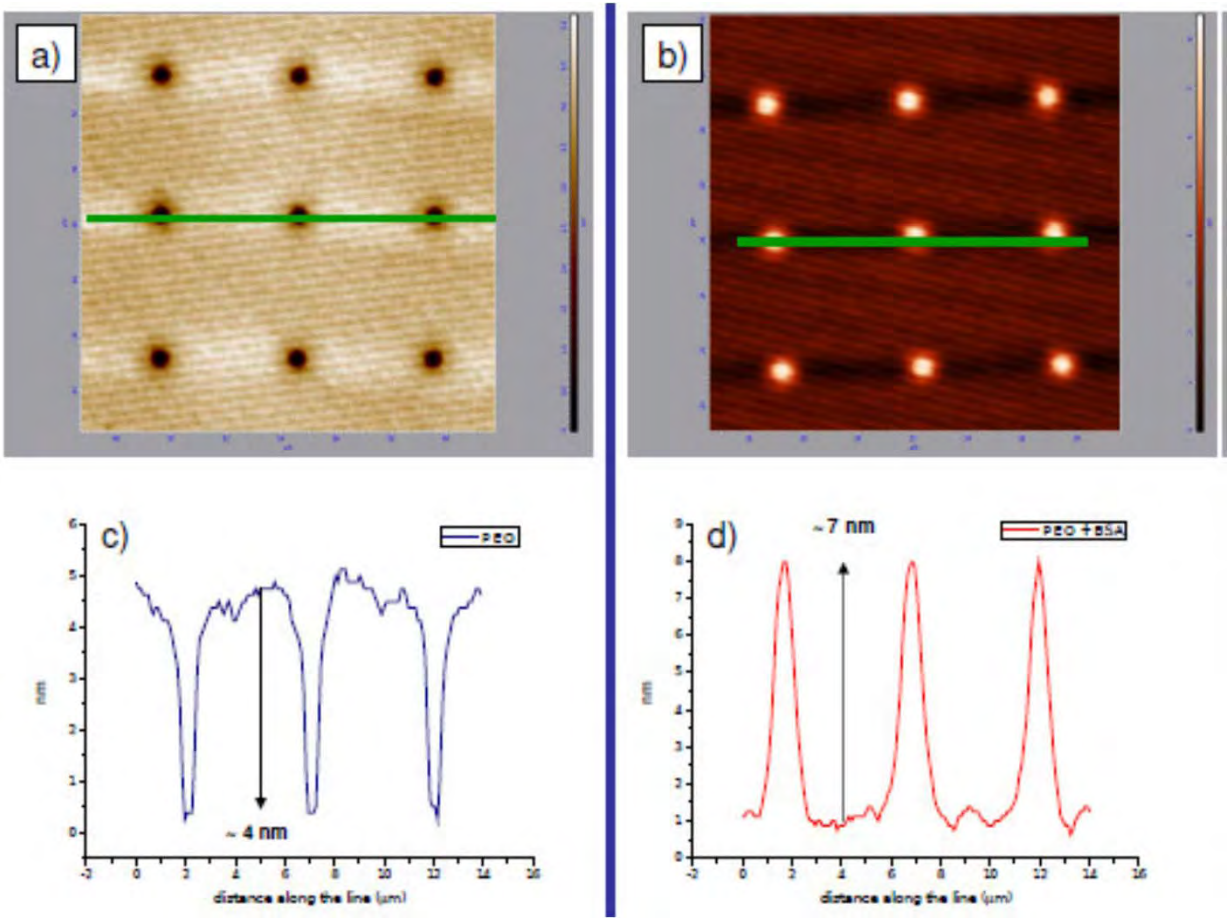
Densification of the film after irradiation.

Micro spot = 200 x 200 μm



AFM characterisation

500 nm spots, pitch = 5 μm

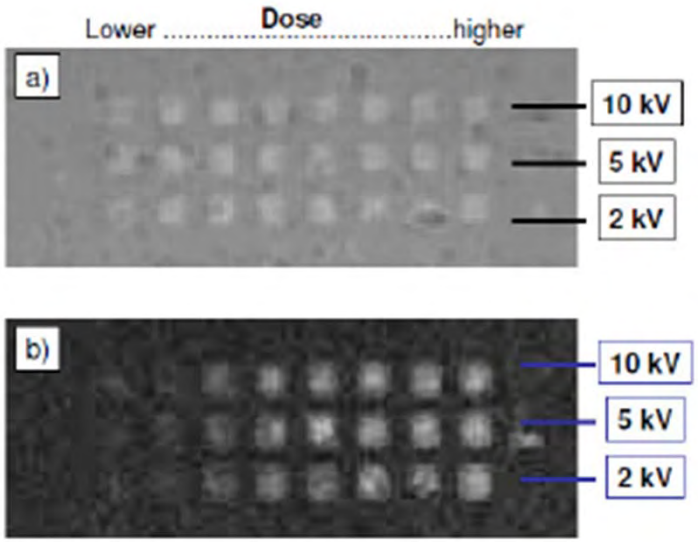


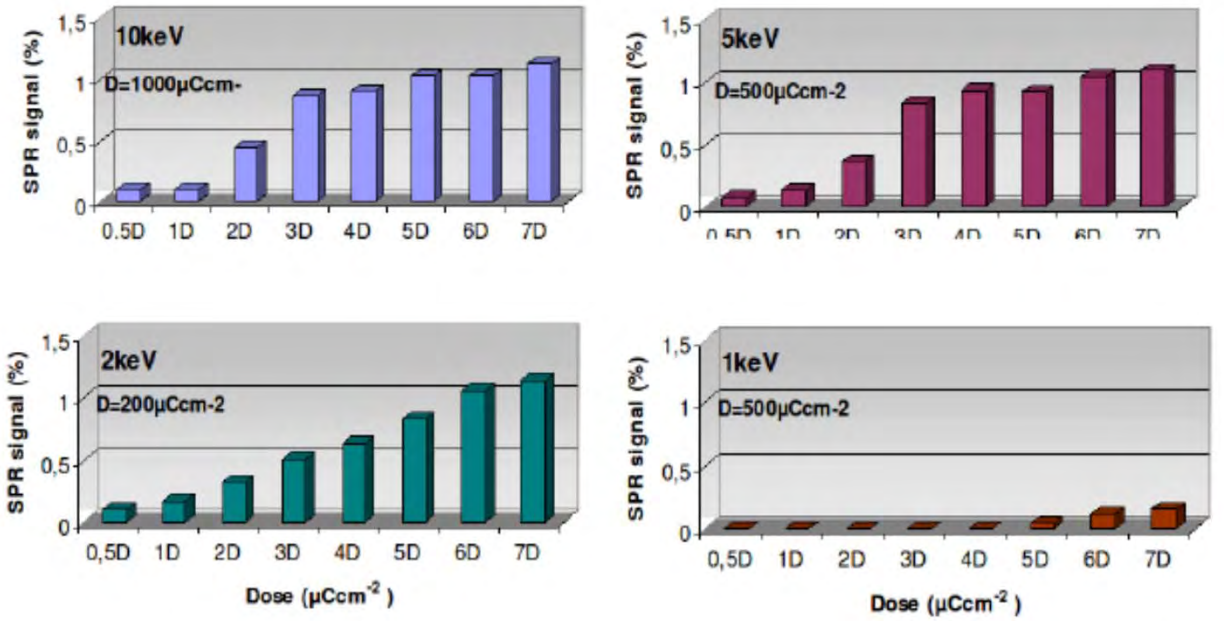
Without Proteins

+ BSA 20 μg/ml

Protein interactions

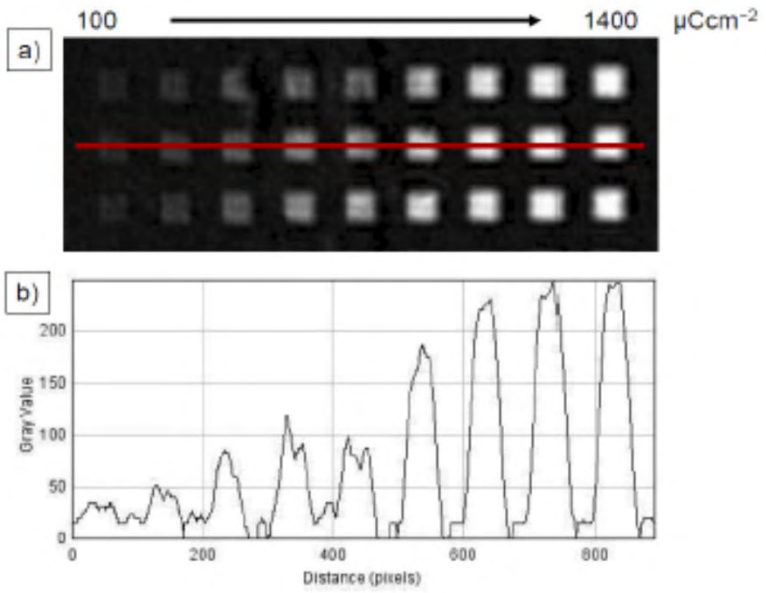
SPR images

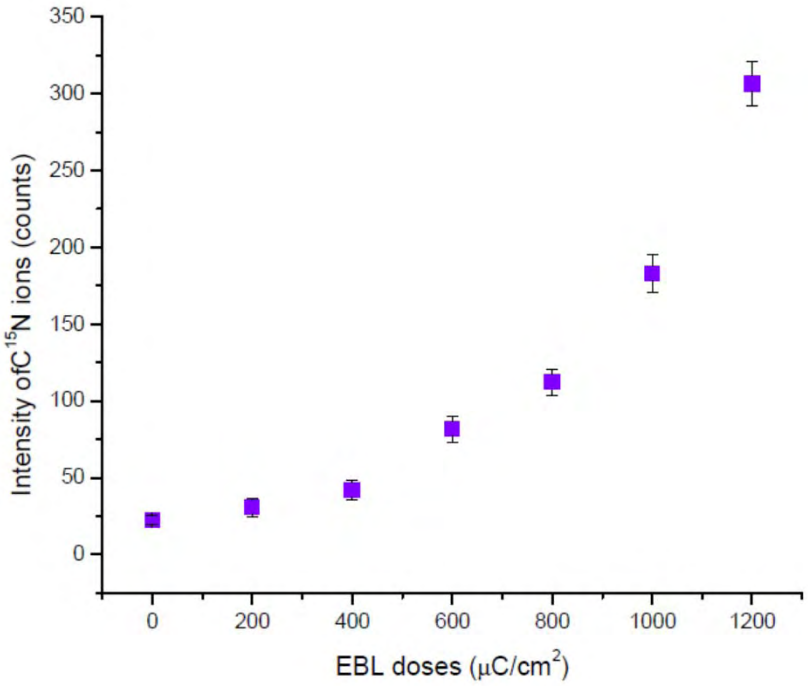




SPR image before and after IgG injection (20 µg/ml)

Microspots = 100 x 100 μm^2

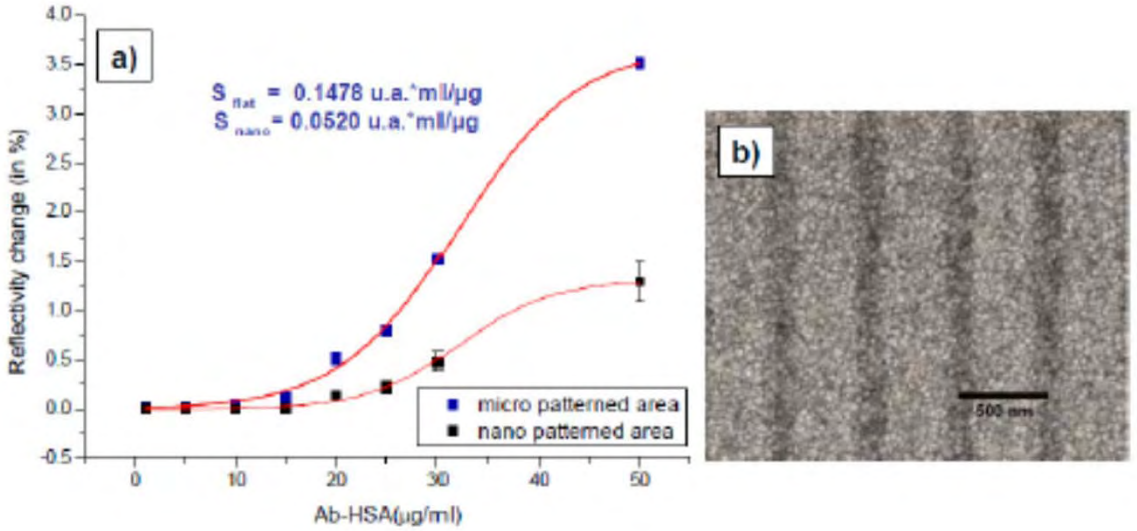




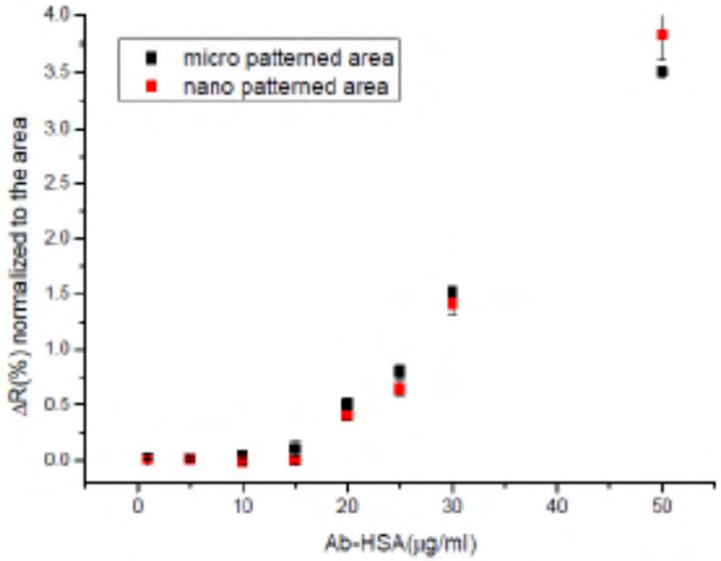
Microspots = 100 x 100 μm^2

Tof-Sims analysis with Ubiquitin–N^{15}

20 μg/ml



Nanostructures: line 170 nm width
picth = 500 nm

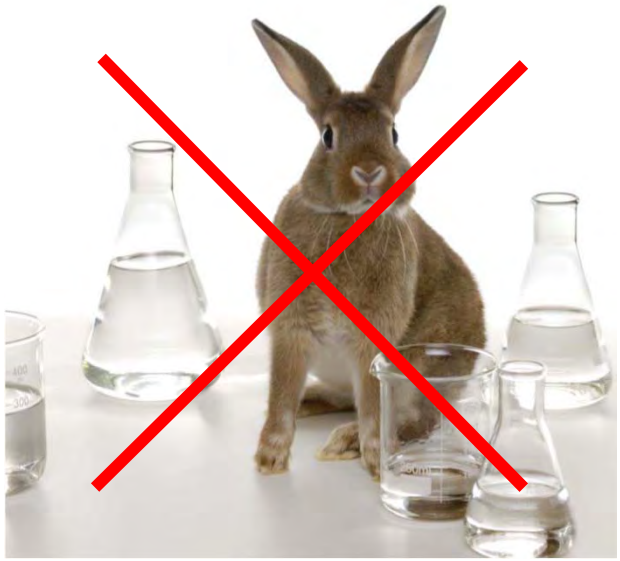


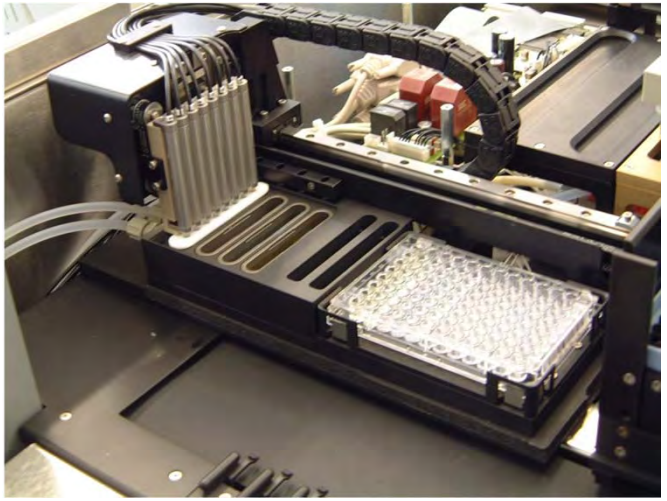
Background

Support to the implementation of EU policies

- **R**egistration, **E**valuation and **A**uthorisation of **CH**emicals (REACH)(EC/1907/2006)
- 7th Amendment to the Cosmetics Directive (2003/15/EC)
- Pharmacological Testing and Toxicity Testing in drug development (ICH M3(R1))

⇒ Request for validated *in vitro* testing methods as alternatives to animal testing





Alternative methods

- Based on cell lines $\xrightarrow{?}$ Effects of chemicals on **human Health**
- Primary cells : Reproducibility, availability
- stem cells : difficult to work with:
  - Maintenance as non differentiated
  - Control of differentiation
  - Different types of cells can be produced simultaneously

➔ *Interpretation of in vitro tests results??*

OBJECTIVE OF THE WORK

- Control of stem cells developmental processes by surface chemistry: use of biomolecules microarrays.
- Application to developmental neurotoxicity

Our approach

Micro-environment engineering

Surface Chemistry
Surface Micro/Nanostructuring
Spot size, spacing, nature

Bio functionalisation

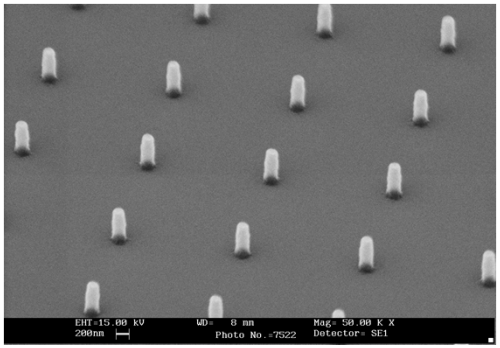
Specific/non-specific binding
ECM proteins: Fibronectin, Laminin, RGD peptides

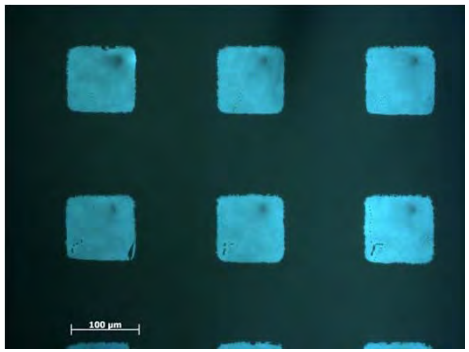
Cell biology

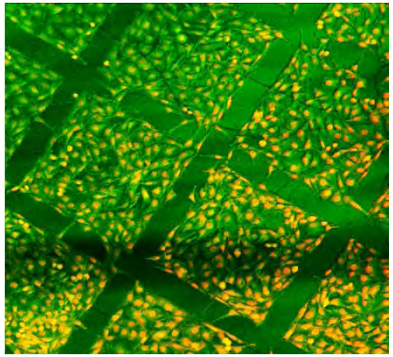
HUCB stem cell response:
Survival
Migration
Differentiation

Surface engineering

Biology

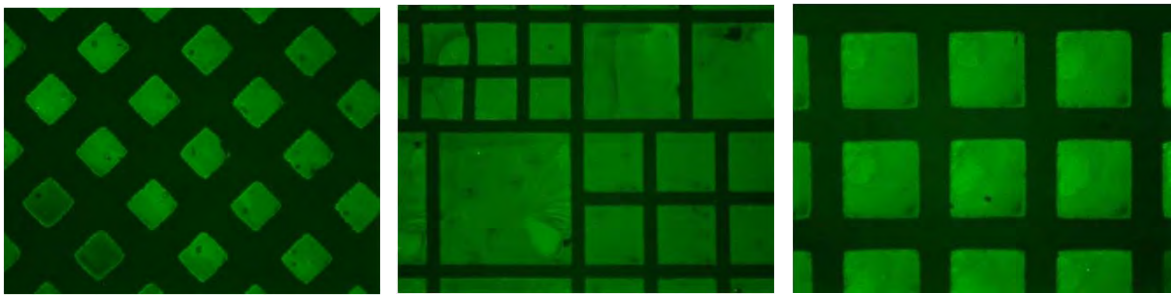


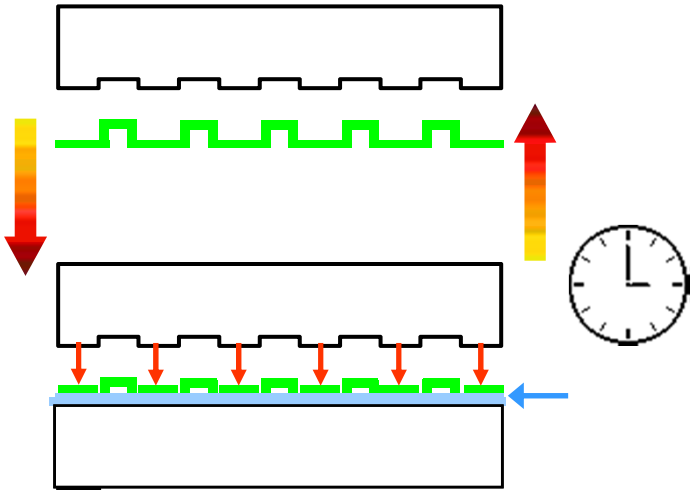




Dual properties of PEO like film:

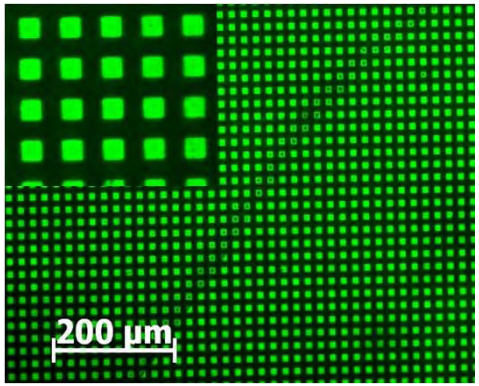
- Anti adhesive properties in wet condition
- Adhesive in dry condition



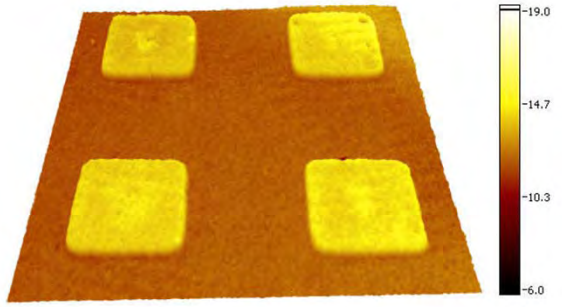


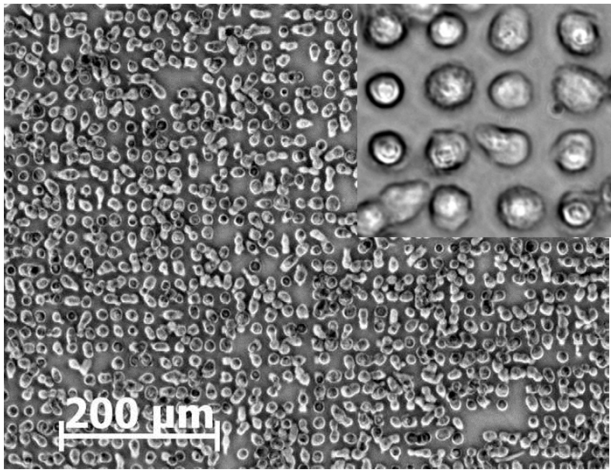
Fluorescense microscopy after print and rinsing

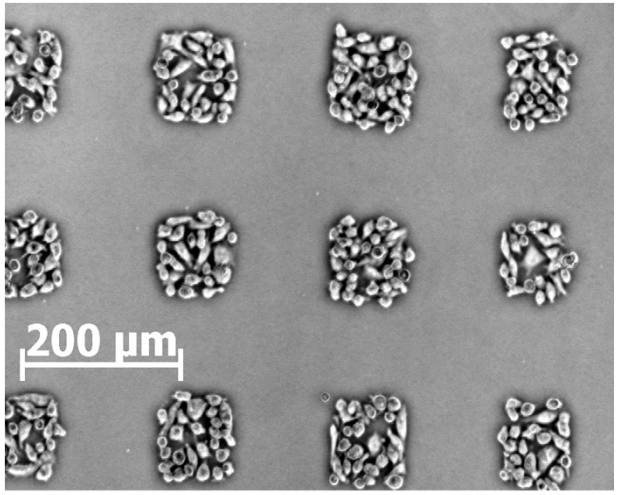
PLL, 10 μm patterns

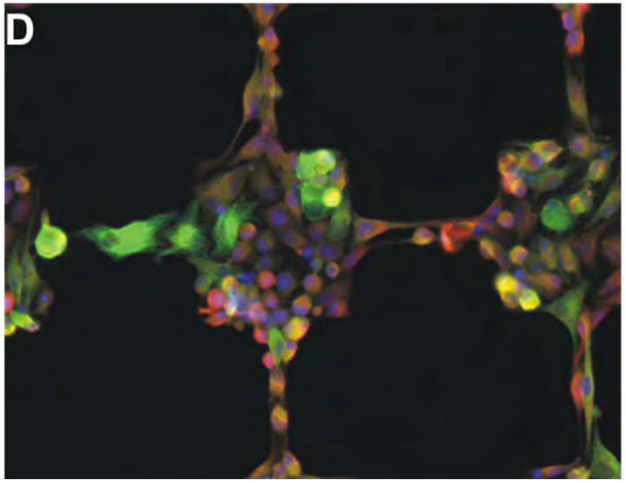


Fn, 120μm patterns

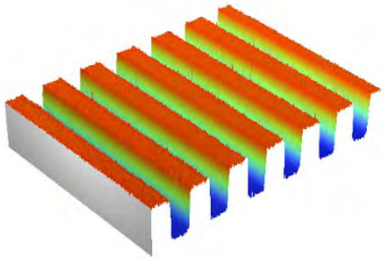




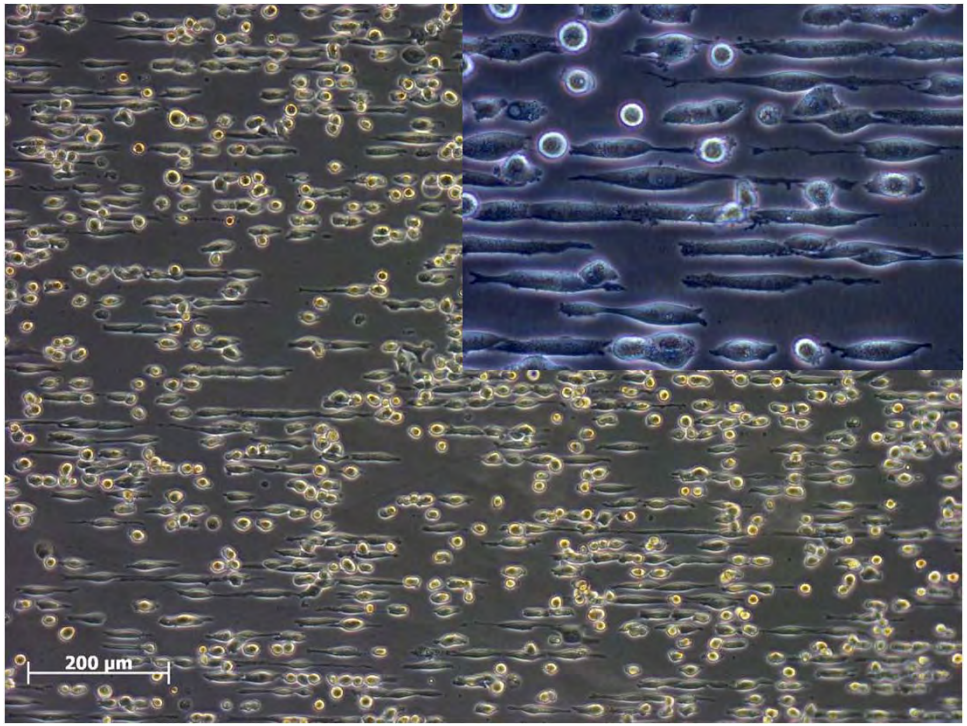


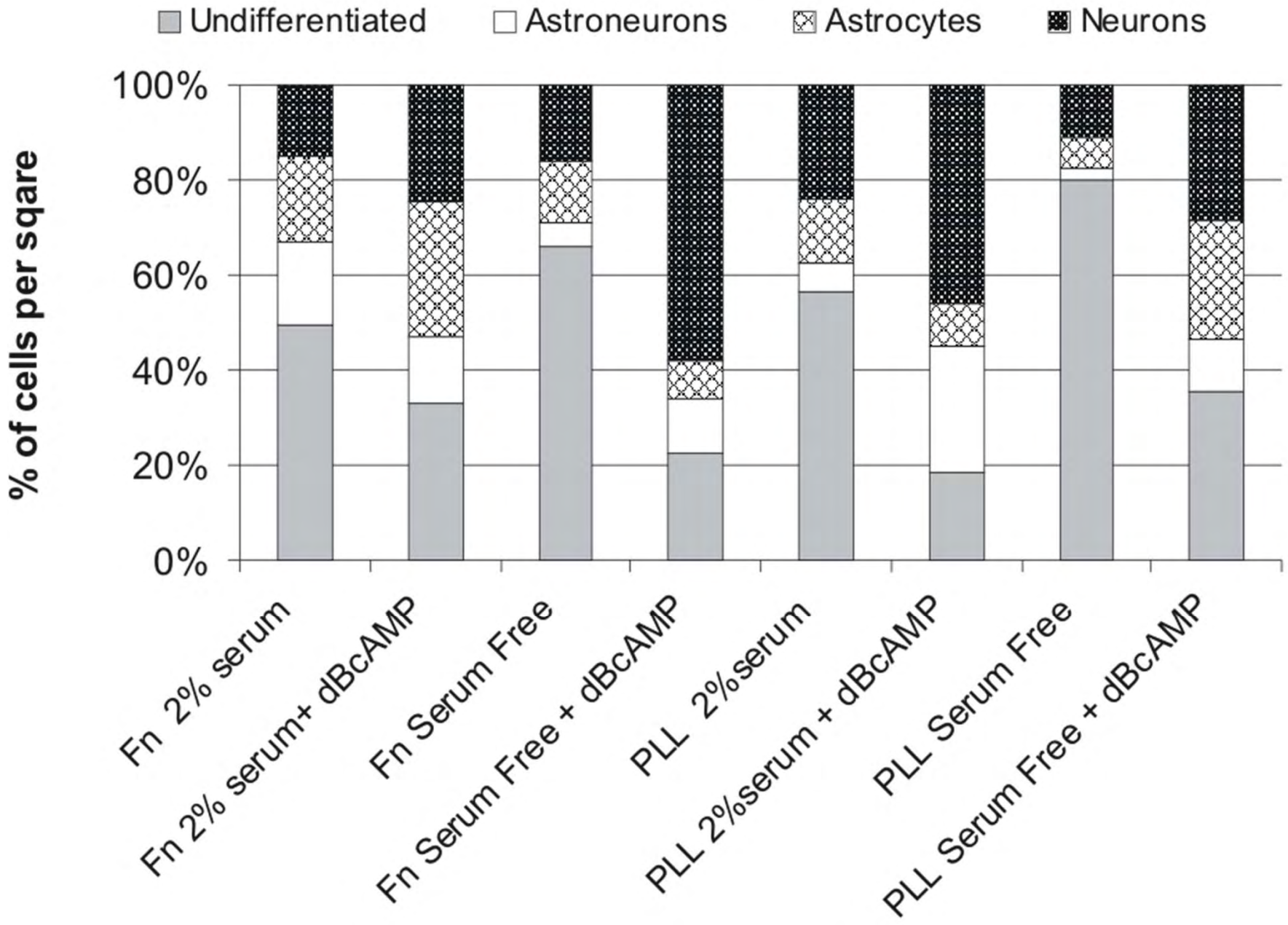


β-TubIII → neurons , GFAP → astrocytes / stem cell, Hoechst → nuclei.




50 µm
200 µm


200 µm


Undifferentiated
Astroneurons
Astrocytes
Neurons
% of cells per sqare
100%
80%
60%
40%
20%
0%
Fn 2% serum
Fn 2% serum+ dBcAMP
Fn Serum Free
Fn Serum Free + dBcAMP
PLL 2%serum
PLL 2%serum + dBcAMP
PLL Serum Free
PLL Serum Free + dBcAMP

Conclusion

✓ Plasma polymerization allows the fabrication of complex layers with a whole range of controlled biological properties.

✓ Plasma polymerization is compatible with different patterning methods such as e-Beam allowing the creation of ordered micro and nano-patterned surfaces with complementary chemical properties.

✓ Direct printing of proteins patterns on PEO is possible :

- Control of cell physico/bio/chemical environment
- Control of cell cluster sizes, distances, interactions
- Control of stem cells maintenance and commitment

JRC
EUROPEAN COMMISSION

Institute for Health and Consumer Protection

Special thanks to Jakub Nowak, Dora Mehn, Marzena Zichowicz, Ana Ruiz & Lena Buzanska

JRC Action. NanoBioTech – NanoBiotechnologies for Health Applications (JRC-15024)