Application of plasma technologies to biological interface design

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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

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

• Nanotoxicology
  – In vitro tests
  – Nanoparticles - proteins interactions
  – Nanoparticles – cell interactions
Cells and proteins on solid surfaces

Proteins

Biosensing for environment monitoring, medical application...

Cells

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, NH₂, CFₓ, PEG).
   - Chemical purity
   - 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, NH₂, CFₓ, PEG).
   - Control of the film properties by plasma parameters
   - Any substrate can be functionalized
   - Homogeneous coverage
   - 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)

Micro and nanopatterning
Photolithography, E-Beam colloidal lithography

Applications:
biosensors
Cell biology

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

Create chemical contrast ‘Bio adhesive – non adhesive’ at micro - nanoscale

Combination of Functionalisation and patterning techniques

- Improve biosensor performance
- Control cell micro-environment

E Beam lithography +

Plasma polymer Poly-ethylene- Oxide as universal platform

TNT 2011 - Tenerife
Polymer Plasma Deposition (PE-CVD)

Control of the functional groups
- Acrylic Acid (COOH)
- Allylamine (NH₂)
- C₄F₈ (CFₓ)
- DEGDME* (PEO)

Control of the functional retention and stability
- Power (CW or Pulsed, 1-100 W)
- Pressure (10-100 mTorr)
- Gas Flow (5-50 sccm)

*Di-EthyleneGlycoleDiMethylEther

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Plasma deposited PEO-like coatings

- **RF generator**: 13.56 MHz
- **Gas**: 0.4 sccm DEGDME, 5 sccm Ar, 400 mTorr
- **Pump**: 50 - 1000 mTorr
- **Optical window**: (spectroscopy)

**Chemical Reaction**

\[
\text{CH}_3\text{O(CH}_2\text{CH}_2\text{O})_2\text{CH}_3 + \text{Ar} \xrightarrow{\text{RF (13.56 MHz)}} \text{Glow discharge} \rightarrow \text{PEO-like films}
\]

- **Diethylene glycol dimethyl ether**: DEGDME

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**Anti-fouling properties of plasma PEO**

**Optimization of PEO-like deposition**

Deposition parameters: Di-Ethylene Glycol Dimethyl Ether precursor, 20mTorr, 2W, 30 min

- **1-3 Watt**
  - C-O ≥ 70%
  - High PEO-like character (C-O>70%) for P=2W

- **5 Watt**
  - C-O ≅ 55%
  - High coating stability (water, ethanol, PBS...)
  - 95% protein adhesion reduction for P<5 watts

- **15 Watt**
  - C-O ≤ 40%


**Chemical characterization of PEO patterns after incubation in BSA solution by ToF-SIMS**
# Plasma polymer films properties

## Evaluation of the absorbed protein mass (QCM)

<table>
<thead>
<tr>
<th>Surface</th>
<th>Functionality</th>
<th>Contact angle (degrees)</th>
<th>BSA Absorbed mass (@ pH=7.5) (45 μg/ml) (ng*cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAA</td>
<td>COOH</td>
<td>38 ± 2</td>
<td>330</td>
</tr>
<tr>
<td>PAL</td>
<td>NH₂</td>
<td>45 ± 3</td>
<td>480</td>
</tr>
<tr>
<td>Teflon</td>
<td>CFₓ</td>
<td>110 ± 2</td>
<td>290</td>
</tr>
<tr>
<td>PEO</td>
<td>C – O</td>
<td>50 ± 3</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

- **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

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

Before

After

Au-NPs (~8-10 nm)
PH=3-4
10 mn and water washing

COOH (-)

PEO-like

PEO-like (slightly +)

Dh ~ 6-8 nm

Non specific adsorption
Protein nanostructure interactions

SURFACE PLASMON RESONANCE

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

\[
S_{\text{nano}} = 0.1227 \text{ua*ml/μg} \\
S_{\text{flat}} = 0.1893 \text{ua*ml/μg}
\]
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

XPS

D= 200 μm/cm²
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 \( \mu \text{g/ml} \))

Microspots = 100 x 100 \( \mu \text{m}^2 \)
Microspots = 100 x 100 μm²

Tof-Sims analysis with Ubiquitin–N¹⁵

20 μg/ml
Nanostructures: line 170 nm width
pitch = 500 nm
Advanced cell culture platform development

Background

Support to the implementation of EU policies

- Registration, Evaluation and Authorisation of CHemicals (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 → 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

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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.

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PLL micropatterns allow maintaining the neural stem cells attached to the surface in non-differentiated state.

Attachment to Fn without serum promotes neuronal differentiation.
✓ 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
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