Plasma nanotextured microfluidics: 
New methods for passive flow control 
and separation of biomolecules 
with affinity chromatography

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Introduction: Plasma processing at a glance

- Plasma is a quasi neutral gas containing electrons, ions, neutrals and free radicals.
- It is produced in a vacuum plasma reactor
- “Cold” Plasma has room temperature gas and hot electrons causing chemical reactions

\[
\begin{align*}
\text{O}_2 + e & \rightarrow O + O + e \quad \text{(decomposition)} \\
\text{O}_2 + e & \rightarrow \text{O}_2^+ + 2e \quad \text{(ionization)} \\
\text{O}_2 + e & \rightarrow \text{O}_2^* \rightarrow \text{O}_2 + h\nu \quad \text{(light emission)} \\
\text{O} + \text{Organic Substrate} & \rightarrow \text{Volatile products}
\end{align*}
\]
Motivation

• Microfluidics-Lab-Chip have large Surface to Volume ratios S/V

• Large S/V results in fast-efficient diffusion / reaction / separation.

• Thus, one needs to precisely control surface properties:
  • Wetting properties, biomolecule binding, cell binding

• One also needs tools to achieve flow control with minimal mechanical moving parts.

We have developed a technology toolbox using plasma processing to meet the above challenges
We overview our results in this presentation
1. Control of Nanotopography: The concept of plasma nanotexturing

**PMMA after Oxygen plasma**

- 5 min
- 20 min
- 60 min

**PEEK after O2 plasma**

- 60 min


1. Control of Nanotopography: The concept of plasma nanotexturing for poly-dimethylsiloxane (PDMS) in F containing plasmas (SF6)

- 1 min SF6 treatment: Rms: 25nm. SAD%: 40%
- 2 min SF6 treatment: Rms: 91nm. SAD%: 298%
- 4 min SF6 treatment
- 6 min SF6 treatment. Height of nanocolumns: 2µm
- 8 min SF6 treatment
- 15 min SF6 treatment

The evolution of the average height and diameter of nanocolumns with SF6 plasma duration

“Islands” of nanocolumns are formed with increasing SF6 treatment time
1. Control of the amount of nanotexture in a microchannel

PMMA Rough channel after 20 min O₂ plasma etching, depth: 20 μm

Smooth channel after tuning the electrode temperature, depth: 20 μm

Smooth channel after the post-etching procedure, depth: 20 μm
2. Control of Wetting Properties: From Superhydrophilic to Superoleophobic

Oxygen Plasma Etching of PMMA produces superhydrophilic surfaces.

Plasma Etching followed by fluorocarbon deposition produces superhydrophobicity.

A Tserepi *et al.*, *Nanotechnology* 17(15), 3977-3983 (2006)
Patent.: GR 20050100473, PCT/GR06/000011

MiNaSens Workshop, Aghia Paraskevi, March 7-8, 2013
3. Control of liquid flow: Stable Capillary pumping

Nanotexturing with O₂ Plasma induces Super-hydrophilicity, which is stable for a long time.

Increased roughness by O₂ plasma strongly delays the hydophobic recovery.
3. Control of liquid flow: Passive superhydrophobic valves

- Different plasma conditions lead to different final contact angle,
- hence different surface wetting fraction.
- Solve the Cassie-Baxter equation for the surface fraction $\Phi$
- substituting to find the threshold pressure

$$P_{th} = \gamma_v \left[ \frac{2}{w_0} \cos \theta_v + \frac{1}{h} \left( \cos \theta_v - 1 \right) + \frac{\phi_s}{h} \left( \cos \theta_v + 1 \right) \right]$$

<table>
<thead>
<tr>
<th>Etch time\ channel depth</th>
<th>10 µm</th>
<th>20 µm</th>
<th>40 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 sec</td>
<td>0.118 bar</td>
<td>0.062 bar</td>
<td>0.034 bar</td>
</tr>
<tr>
<td>60 sec</td>
<td>0.134 bar</td>
<td>0.070 bar</td>
<td>0.038 bar</td>
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<tr>
<td>120 sec</td>
<td>0.136 bar</td>
<td>0.076 bar</td>
<td>0.042 bar</td>
</tr>
<tr>
<td>240 sec</td>
<td>0.149 bar</td>
<td>0.08 bar</td>
<td>0.043 bar</td>
</tr>
</tbody>
</table>
3. Control of liquid flow: Passive superhydrophobic valves

- We measured valve threshold pressure in 20μm deep, 175μm wide microchannel with SH stripe.
- Four Plasma Nanotexturing times, give four different pressures.
- Agreement with theoretical prediction (unpublished results).

3. Control of liquid flow: Programmable liquid dispensing
(a liquid switchboard)

✓ One inlet is connected with 3 outlets via 3 “smart” superhydrophobic valves having different threshold pressures.
✓ All channels are superhydrophilic due to oxygen plasma treatment
✓ The valve is superhydrophobic due to fluorocarbon deposition
✓ Operation: Fill in first channel 1, then 2, then 3
(unpublished results)

Approach analogous but simpler compared to:
4. Contol of biomolecule capturing: 
Enhanced and binding of proteins

- plasma-nanotextured polymeric surfaces adsorb 3x-5x more protein compared to untreated surfaces, resulting in highly sensitive, very intense microarrays

M. Vlachopoulou et al, Colloids and Surfaces 2011.
Application: Sensitive DNA Microarrays Through Protein Conjugates

Four deleterious BRCA1 gene mutations selected: Cover >60% of mutations in Greek population: Deletions, insertions or single nucleotide polymorphisms

Hybridization and Detection through oligonucleotides array: probes corresponding to both wild- and mutant-type sequences
4. Control of biomolecule capturing: Selective protein binding in microfluidics with controlled wetting

Proteins do not bind on superhydrophobic areas but selectively only to hydrophilic


MiNaSens Workshop, Aghia Paraskevi, March 7-8, 2013
5. Control of Cell adhesion

Example of human fibroblasts on different nanotopography

Untreated Surface

0 V plasma bias, 1 min, 5nm Roughness

100 V plasma bias, 1 min, 50nm Roughness

(unpublished results)
Affinity separation of phosphopeptides in plasma nanotextured TiO2 microfluidics

Stationary phase

Binding

Elution

O

peptide

P

O

TiO2

TiO2

XRD

Phosphopeptide Chromatogram

TiO2 Anatase
ZrO2 Monoclinic

Sample in (CF3COOH)

NH4OH at detector

Mono

Tetra

Absorbance units (mAU)

Time (min)

20 30 40 50 60 70 80

100 µm

1 µm
Affinity chromatography separations on chip:
Complex β-casein tryptic digest mixture

Maldi-MS spectrum before its injection in the column

K. Tsougeni et al, Lab Chip 11(18), 3113-20, 2011

Maldi-MS spectrum after its injection in the column
Summary & Conclusions

- Plasma processing may be used to 1) pattern, 2) texture, 3) selectively modify polymer surfaces at the micro and nanoscale.
- We have shown that we can control:
  a) The topography
  b) The wetting properties (contact angle from 0-175 degrees for water and oils)
  c) The flow (capillary pumping and hydrophobic valving)
  d) The biomolecule binding (selectivity, enhancement, binding)
  e) The cell adhesion

- Such microfluidics/microcolumns are ideal for affinity separations
- Applications in phosphopeptide enrichment were demonstrated
- A toolbox is available for further applications
Thank you

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