Atmospheric Pressure Plasma Based Fabrication of Paper Biosensors

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Research Area

In-situ Resource Utilization for Mars Mission

- Biosensors
- Air treatment
- Printable Electronics
- Water and Surface treatment

Funding
- NASA Advanced Exploratory Systems (AES)
- NASA ARC Center/Director’s Innovation Fund
- NASA Innovative Advanced Concepts (NIAC)
Technologies for Space Mission

- NASA’s manned mission to Mars in 2030’s
- NASA’s Orion Multi-purpose Crew Vehicle - human missions beyond LEO
- Public and Private Outer Space Explorations
- Currently 1 year long mission in ISS by NASA astronauts and Russian cosmonauts
- Mars 500 mission by Russia, China and ESA-finished in 2011. 520 days of isolation to study the effect of isolation on human physiology and psychology.
- NOT enough data on changes in human physiological conditions in space
- Insitu resource utilization - Mars mission
A Biomarker is a parameter that can be used to measure the presence of or severity or progress of some disease or the effects of treatment. The parameter can be chemical, physical or biological.

**Key scientific and technological goals**

- Reliable and reproducible fabrication of paper sensors
- Efficient surface functionalisation for flow control, biomolecule attachment and reduced non-specific binding
- Incorporation of nanostructures for signal transduction/amplification (Gold NPs, CNT)
- DNA hybridization
Surface Functionalization

- To retain biological activity of the ligand
- Physisorption
  - Protein conformational change
  - Loss of antigen binding activity
- To minimise non specific binding - High signal to noise
- Film wettability, surface roughness
- To achieve high immobilization yield
- To ensure reproducibility and reliability of measurement
  - NH2- Amines; COOH – Carboxy;
  - SH – Mercapto; (OH)$_n$ poly ethylene glycol (PEG)
Why Paper Sensors:
- Healthcare technologies for long duration mission require: low weight & long shelf life or easy fabrication,
- Cellulose: Light weight, capillary flow - independent of gravity, in-space fabrication
- Bacterial cellulose can be prepared using synthetic biology in Mars

Wax printed paper before heating

Wax printed paper after heating

\[ L = \sqrt{\frac{\gamma D t}{4 \eta}} \]  
Washburn equation

(surface tension, distance)
Fabrication of Biosensor Chips

- Can form fluidic channels, patterning, organic functionalities, nanostructures in paper
- Gandhiraman et al., US Patent application 14/515, 072
Fabrication of Biosensor Chips

Organic functionalization & nanomaterial deposition

Wax coated paper
Plasma functionalized paper
SEM
DNA Hybridization

Amine functionalization for covalent binding
Polyethylene glycol (PEG) functionalization for reduced non-specific binding

Gandhiraman et al., ACS Applied Materials & Interfaces 2014, 6, 22751
Nanomaterials Deposition

Cellulose

Silver nanowire

Silver nanowire coated cellulose
Gold nano particle (NP)

Aminated DNA containing Cy3 Fluorophore

NH₂ ssDNA on Gold – Strong binding
NH₂ ssDNA on aminated (Aptes) surface – repulsion
NH₂ ssDNA on PEG surface - repulsion

Amine Surface

PEG Surface

Amine + Gold NP

PEG + Gold NP
Multiple bands between 2800 and 3000 cm\(^{-1}\) C–H vibrations in the ethyl components

Intense bands between 800 and 1200 cm\(^{-1}\) arise from Si–O molecular vibrations of the ortho silicate component of the precursor.

Asymmetric and symmetric N–H stretching bands between 3380 - 3350 cm\(^{-1}\) and between 3310 - 3280 cm\(^{-1}\)

N–H deformation between 1650 cm\(^{-1}\) and 1580 cm\(^{-1}\)

N–H bending vibration of free amine 1630 cm\(^{-1}\)

N–H bending vibration of amine group, which is hydrogen-bonded either to hydroxyl groups in silanol in the bulk of the film or to cellulose at the interface 1585 cm\(^{-1}\)
X-ray photons with energy level close to the core level excitation are incident on the sample, transition of the core level electrons to unoccupied valence orbitals takes place upon absorption of photon energy.

C 1s XPS

Linear relationship between $\sigma^*$ transition energies and bond lengths to atoms adjacent to the core excited atom, bond-length with a ruler.
### Table 1. C K-Edge NEXAFS Peak Positions and Attribution

<table>
<thead>
<tr>
<th>Energy (eV)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>284.8 (shoulder)</td>
<td>probable beam damage</td>
</tr>
<tr>
<td>285.9 (intense)</td>
<td>$\pi^<em>$ C==C; $\sigma^</em>$ at 308</td>
</tr>
<tr>
<td>286 (intense)</td>
<td>$\pi^*$ C==N</td>
</tr>
<tr>
<td>287.8 (shoulder)</td>
<td>carbonyl C==O $\pi^*$</td>
</tr>
<tr>
<td>288.1 (shoulder)</td>
<td>probably corresponds to excitations into orbitals of dominantly C–H* resonance; $\sigma^*$ CNH</td>
</tr>
<tr>
<td>289.1 (intense)</td>
<td>clearly not present in cellulose</td>
</tr>
<tr>
<td>290.1 (intense)</td>
<td>1s to $\sigma^*$ C–H/3p</td>
</tr>
<tr>
<td>292.5 (intense)</td>
<td>C–H resonance</td>
</tr>
<tr>
<td>297 (broad)</td>
<td>$\sigma^*$ O–C–O</td>
</tr>
<tr>
<td>299 (broad)</td>
<td>1s to $\sigma^*$ C–N of carbon in –CONH</td>
</tr>
<tr>
<td>304 (broad)</td>
<td>$\sigma^*$ C==C</td>
</tr>
<tr>
<td>308 (broad)</td>
<td>C==O $\sigma^*$</td>
</tr>
</tbody>
</table>

### Table 2. N K-Edge NEXAFS Peak Positions and Attribution

<table>
<thead>
<tr>
<th>Peak Position (eV)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>399.3 (intense)</td>
<td>$\pi^*$ N==C</td>
</tr>
<tr>
<td>400.6 (minor)</td>
<td>$\pi^<em>$ C==N/$\sigma^</em>$ N–H</td>
</tr>
<tr>
<td>401.9 (intense)</td>
<td>$\pi^*$ CONH</td>
</tr>
<tr>
<td>404.8 (shoulder)</td>
<td>nitro compound</td>
</tr>
<tr>
<td>405.9 (intense)</td>
<td>$\sigma^*$ N–C</td>
</tr>
<tr>
<td>408.9 (shoulder)</td>
<td>$\sigma^*$ NH2</td>
</tr>
<tr>
<td>411 (broad)</td>
<td>$\sigma^*$ NH</td>
</tr>
<tr>
<td>403.5</td>
<td>$\sigma^*$ N==C</td>
</tr>
<tr>
<td>406</td>
<td>$\sigma^*$ CONH</td>
</tr>
</tbody>
</table>
Plasma Surface Functionalisation
Carboxy Functionalisation on Plastic

Sequential deposition of Tetra ethyl ortho silicate and Acrylic acid

Teos: Adhesion and network building layer
Acrylic acid: carboxy functionality

Percentage Carboxy:
6.5% in Acrylic acid
16.4% in Teos AA coating
Plasma Functionalisation on Gold

Carboxy functionalities are deposited on gold surface by plasma enhanced chemical vapor deposition (PECVD)

1) Siloxane as an adhesion layer on gold using Tetra ethyl orthosilicate (Teos)
Sequential deposition of acrylic acid for carboxy functionalisation

2) Mercapto silane deposition- Mercapto group adhesion layer on gold, siloxane for network building for further functionalisation
Sequential deposition of acrylic acid for carboxy functionalisation

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<table>
<thead>
<tr>
<th>PECVD</th>
<th>Biacore – CM5</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 mins</td>
<td>12 hrs to 24 hrs</td>
</tr>
<tr>
<td>No refrigeration required</td>
<td>storage in fridge</td>
</tr>
<tr>
<td>Reduced cost due to bulk production</td>
<td>Sq.cm chip costs 120 euro.</td>
</tr>
</tbody>
</table>
Infrared Red Spectroscopy and SIMS
### XPS - Quantitative analysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>C-C</th>
<th>C-S</th>
<th>C-O</th>
<th>O-C=O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Binding energy (eV)</td>
<td>% Area of Peak</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>285.0</td>
<td>286.5</td>
<td>289.1</td>
<td>60.8%</td>
</tr>
<tr>
<td>MA</td>
<td>285.4</td>
<td>286.5</td>
<td>289.0</td>
<td>39.0%</td>
</tr>
</tbody>
</table>

Percentage carboxy group has increased from 2.1% in mercapto based coating to 19.1% in Teos/AA coating.
Specific binding: attachment of Cy-5 labelled amino terminated DNA
Non specific binding: attachment of Cy-5 labelled DNA without NH2
Samples Treated with Whole Blood

SiOCH

COOH
Whole Blood - Labelled Detection of NSB

SiOCH coating

Fibrinogen capture layer

Platelets bound from whole blood

Carboxy coating

Fibrinogen capture layer

Platelets bound from whole blood
# Commercial Chips

http://www.gelifesciences.com

## PURCHASE ORDER

**CM5 chips - DCU**  
GE Healthcare UK Ltd  
Amersham Place  
Little Chalfont  
Buckinghamshire  
HP 7 9NA  
UK  
United Kingdom

**Order Number**  
300136795

**Order Date**  
12/04/2011

**Supplier Number**  
10091

**Requisitioned By**  
Ram Gandhiraman

**Authorised By**  
Mary Fallon

**Tax Clearance:** Q072611  
**Certificate Expiry Date:** 13/10/2011

**Deliver To:**  
BDI  
Dublin City University  
Collins Avenue  
Dublin 9  
Ireland

**Invoice To:**  
Dublin City University  
Finance office  
Dublin City University  
Glasnevin  
Dublin 9

### Dr. Ram Prasad Gandhiraman

<table>
<thead>
<tr>
<th>PRODUCT CODE</th>
<th>DESCRIPTION</th>
<th>QTY</th>
<th>UNIT</th>
<th>UNIT PRICE</th>
<th>VAT %</th>
<th>NET AMOUNT (EX VAT)</th>
<th>GROSS (INC VAT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40000</td>
<td>Br-1000-12 CMS sensor chips</td>
<td>1.0</td>
<td>EA</td>
<td>410.00</td>
<td>0.0</td>
<td>410.00</td>
<td>410.00</td>
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<tr>
<td>40000</td>
<td>BR-1000-50</td>
<td>1.0</td>
<td>EA</td>
<td>263.00</td>
<td>0.0</td>
<td>263.00</td>
<td>263.00</td>
</tr>
<tr>
<td>40000</td>
<td>Shipping charge</td>
<td>1.0</td>
<td>EA</td>
<td>30.00</td>
<td>0.0</td>
<td>30.00</td>
<td>30.00</td>
</tr>
</tbody>
</table>
Sensor Chip CM5, pack of 3

The most versatile chip available – the first choice for immobilization via -NH2, -SH, -CHO, -OH or -COOH groups.

- Use for immobilization via -NH2, -SH, -CHO, -OH, or -COOH groups.
- Suitable for ligand fishing.
- Suitable for high capacity capture.
- Supports a wide range of immobilization levels.
- Attach proteins, nucleic acids, carbohydrates or small molecules.

Matrix: carboxymethylated dextran covalently attached to a gold surface. Molecules are covalently coupled to the sensor surface via amine, thiol, aldehyde or carboxyl groups. Interactions involving small organic molecules, such as drug candidates, through to large molecular assemblies or whole viruses can be studied. A high binding capacity gives a high response, advantageous for capture assays and for interactions involving small molecules. High surface stability provides accuracy and precision and allows repeated analysis on the same surface.

Product Name: Sensor Chip CM5, pack of 3

Price: On request
Product code: BR100012

Sensor Chip CM5, pack of 3

Complete Packsize 3 pieces
Application For most interaction analyses in Biacore systems.
Includes 3 x sensor chips
Surface Carboxymethylated dextran covalently attached to a gold surface.
For Use With Biacore X100, Biacore 3000, Biacore 2000, Biacore 1000, Biacore Upgrade, Biacore C, and Biacore J
Storage Conditions 11 REFRIGERATED
Min. Order Quantity 1
## Comparision with Biacore/GE Lifesciences

<table>
<thead>
<tr>
<th>Samples</th>
<th>PECVD</th>
<th>Response Mass Units (anti-IgG) (ng/mm²)</th>
<th>Response Mass Units (IgG) density (ng/mm²)</th>
<th>Fibrinogen Mass density of Non specifically bound Fibrinogen (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM5 dextran (GE Lifesciences)</td>
<td>7149.0</td>
<td>7.2</td>
<td>810</td>
<td>1 mg/mL</td>
</tr>
<tr>
<td>PECVD</td>
<td>3874.0</td>
<td>3.9</td>
<td>588</td>
<td>1 mg/mL</td>
</tr>
</tbody>
</table>

- **Deposition time**: PECVD: <10 mins, Biacore - CM5: 12 hrs to 24 hrs
- **Cost**: PECVD: High initial investment, low running cost, Several hundred chips can be deposited in an hour. Biacore - CM5: No initial investment, high manual intervention, Each chip costs 135 euro.
- **Sensitivity**: PECVD: Performs similar to Biacore chips. Biacore - CM5: Highly sensitive
- **Stability**: PECVD: Highly stable over long period of time - No refrigeration necessary. Biacore - CM5: Long chain films poor stability, storage in fridge
- **Organic wastes**: PECVD: None. Biacore - CM5: Industrial liquid wastes

Gandhiraman et.al., ACS Applied Materials and Interfaces 2011, 3 (12), pp 4640–4648
Surface Plasmon Enhanced Ellipsometry

Poksinski, M. [2003], Total Internal Reflection Ellipsometry, 1st ed, Institute of Technology, Linköping University. Licentiate Thesis No. 1016
Ellipsometry

- Linearly polarized light upon reflection becomes elliptically polarized.
- Fresnel reflection co-efficients of p and s components are different
- phase change of the reflected field relative to the incident field occurs, depending on the refractive indices of the materials involved

Relative attenuation of s and p polarized light determines tilt of ellipse (psi) $\psi$
Relative phase shift of s and p polarized light is related to ellicity of ellipse (delta) $\Delta$
- $r_s, r_p$: normalized amplitude of s and p component after reflection;
- $\tan(\Psi)$: amplitude ratio upon reflection and $\Delta$: phase shift

\[ r_{12}^p = \frac{\hat{N}_1 \cos \phi_1 - \hat{N}_1 \cos \phi_2}{\hat{N}_2 \cos \phi_1 + \hat{N}_1 \cos \phi_2} \]
\[ r_{12}^s = \frac{\hat{N}_1 \cos \phi_1 - \hat{N}_1 \cos \phi_2}{\hat{N}_1 \cos \phi_1 + \hat{N}_2 \cos \phi_2} \]
\[ R^p = \frac{r_{12}^p + r_{23}^p \exp(-j2\beta)}{1 + r_{12}^p r_{23}^p \exp(-j2\beta)} \]
\[ R^s = \frac{r_{12}^s + r_{23}^s \exp(-j2\beta)}{1 + r_{12}^s r_{23}^s \exp(-j2\beta)} \]
\[ \rho = \frac{R^p}{R^s} \quad \rho = \tan(\Psi) e^{j\Delta} \]
Surface Plasmon Resonance

Surface plasmons- collective electron density waves propagating at the interface between metal and dielectric

Metal- dielectric interface
At certain launch angle resonance occurs, when the wave vector of the component of the incident light parallel to the metal surface is equal to the wave-vector of the surface plasmons. A travelling wave parallel to the surface alone exists while the amplitude of the electric field exponentially decays along the surface perpendicular direction. Energy of the incident photon is then absorbed by surface charge density.

The wave vector of the surface plasmon oscillations $K_x$ is defined as,

$$K_x = \frac{2\pi}{\lambda} \left( \frac{n^2_m \times n^2_d}{n^2_m + n^2_d} \right)$$

$n_m =$ refractive index of the metal ; $n_e = $ refractive index of the dielectric material.

The refractive index of the silver films is higher than that of gold, they exhibit longer and enhanced evanescent field and hence more sensitive than gold.
Surface Plasmon Resonance

An electromagnetic plane wave that propagates in a medium with refractive index $n$ can mathematically be described by an electric field $E$:

$$E = E_0 \exp(j\omega t - jk \cdot r) = E_0 \exp(j\omega t -jk_x x -jk_y y -jk_z z)$$

wavevector $k$: its direction is parallel to that of the wave propagation; its magnitude is

$$k = \sqrt{k_x^2 + k_y^2 + k_z^2} = n \frac{2\pi}{\lambda} = n \frac{\omega}{c}$$

$$n_1 \sin \alpha = n_2 \sin \beta$$

$$k_{x1} = k_{x2} \equiv k_x$$

$$k_{y2} = n_1^2 \left(\frac{2\pi}{\lambda}\right)^2 \left(\frac{n_2^2}{n_1^2} - \sin^2 \alpha\right)$$

For $\sin \alpha > n_2/n_1$ right part is negative and $K_y$ is imaginary

$$E_2 = E_0 e^{-\kappa y_2 y} \exp(j\omega t -jk_x x)$$

In medium 2 there is only a travelling wave parallel to the interface, with the amplitude of the electric field exponentially decaying along the y direction.
Maximising SPR Sensing

Effect of thickness of the metal layer
To obtain maximum SPR sensitivity, it is advantageous to have the reflected light intensity as minimum as possible at the SPR wavelength.
If the metallic film is too thick that the evanescent wave decays before reaching the dielectric area. The evanescent wave decays within a sub-wavelength scale

\[ A(z) = e^{-\left(\frac{2\pi n_m}{\lambda}\left[\sin^2 \theta - \left(n_d/n_m\right)^2\right]\frac{1}{2} + \alpha\right)z} \]

\( n_m = \) refractive index of the metal,
\( n_d = \) refractive index of the dielectric,
\( \theta = \) angle of illumination at the metal surface and
\( z = \) any location at which the evanescent field is measured.

Penetration depth \( d_p \) of the evanescent field is defined as given below.

\[ d_p = \frac{\lambda}{2\pi \sqrt{n_m \sin^2 \theta - n_d^2}} \]

The decay of evanescent field continues with distance.

\( \omega_p = \) plasmon frequency
\( n_e = \) Free electron density
\( m_e = \) mass
\( e = \) electron charge

Patents

Patents:

• Ram P. Gandhiraman, Vivek Jayan, M. Meyyappan, Jessica Koehne. Atmospheric pressure plasma based fabrication of printable electronics and functional Coatings. US Patent application 14/515,072


Invention Disclosure:

• Niall O Connor, Ram P. Gandhiraman, David Williams, Stephen Daniels. “Aerosol assisted atmospheric plasma based surface modification and patterning for biosensors”

• Ram P. Gandhiraman, Gowri Manickam, Michael Berndt, David Williams, Stephen Daniels. “Plasma polymerized films with encapsulated metal nanoparticles as a substrate for SPR detection”.
Acknowledgements

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• Jessica Koehne - NASA ARC
• Meyya Meyyappan - NASA ARC