Supplementary Information:

One Step Multifunctional Micropatterning of Surfaces Using Asymmetric Glow Discharge Plasma Polymerization

Donna J. Menzies, a,b Thomas Gengenbach, a John S. Forsythe, b Nick Birbilis, b Graham Johnson, a Christine Charles, c Gail McFarland, a Richard J. Williams, a Celesta Fong, a Patrick Leech, a Keith McLean a and Benjamin W. Muir a*

a CSIRO, Materials Science and Engineering, VIC 3169, Australia
b Monash University, Dept of Materials Engineering, VIC 3800, Australia
c Australian National University, Research School of Physics and Engineering, Space Plasma, Power and Propulsion Laboratory, ACT 0200, Australia

Email: ben.muir@csiro.au
EXPERIMENTAL METHODS:

**Plasma polymer deposition:** Plasma polymerizations were performed in a custom-built reactor (Figure S1) on pre-cleaned ultra-flat single crystal, silicon wafers (<100>, 1 cm² x 0.5 mm thick,) and pre-cleaned glass slides (Biolab). Diethylene glycol dimethyl ether (BDH, 99% purity) was deposited at 125 KHz, a load power of 5 W, monomer pressure of 20 Pa for 120 seconds. Cell attachment and self-assembling peptide studies on the DGpp patterned surfaces were performed on glass substrates that were pre-coated with a heptylamine pp film (Aldrich,) (200 kHz, power of 30 W, monomer pressure of 40 Pa for 30 seconds.

**Preparation of patterned electrodes:** The electrodes comprised arrays of circles (0.9-1.6 mm diameter) or squares, and triangles with maximum cross dimension of 1.25-1.5 mm and an interspacing of approximately 2 mm, produced via lithographic etching on thin copper.

**Optical images of patterns:** Optical images of the DGpp patterned features were collected using an “Infinity X” camera (Luminera) attached to a binocular Microscope (Kyowa) at 0.7x magnification.

**Optical profilometry:** Images of the patterned films were obtained using a Wyko NT1100 Optical Profilometer (Veeco) at 5x magnification using a field of view of 1.

**giFTIR microspectroscopy:** Chemical maps across the DGpp patterned spot were acquired using a synchrotron source FTIR microscope (Australian Synchrotron). Using a Bruker Vertex 80v FTIR spectrometer coupled to a Bruker Hyperion 2000 IR microscope, spectra were collected in grazing incidence mode using a Grazing Angle Objective at 15x magnification. Spectra (256 scans) were acquired using a 20 x 20 μm aperture and step size of 50 x 50 μm (spectral resolution of 6.0 cm⁻¹). Bruker Opus software version 6.5 equipped with video mapping was used to generate 2D absorbance maps. Data
was converted to 3D chemical maps using the OPUS 6.5 software, corrected with an eight point baseline correction and normalized to the alkane stretch (~2850 to 3000 cm⁻¹).

**ToF SIMS measurements:** An ION-TOF IV, with a reflectron time-of-flight analyser was used. Positive ion spectra were collected using a 25 keV Bi⁺ primary-ion beam in high-current-bunched mode over a region of interest of 500 µm² at a resolution of 256 pixels².

**Protein adsorption:** Patterned films were incubated in BSA (Sigma) (in PBS (1 mg/mL at pH 7.4) for one hour at room temperature and thoroughly rinsed.

**Cell culture:** Patterned slides were sterilised overnight in PBS containing 200 units/ml of penicillin and 200 µg/ml streptomycin (GIBCO). HeLa cells were seeded at a density of 1x10⁶ cells/chamber in fresh Dulbecco’s Modification of Eagle’s Medium/Ham’s F12 (MP Biomedicals) supplemented with 10% (v/v) FBS (ICP Biologicals™), 100 units/ml penicillin and 100 µg/ml streptomycin. The cells were incubated for up to 7 days at 37⁰ C in humidified air containing 5% CO₂. For the final hour of incubation (either day 1 or day 7) the medium was replaced with fresh complete medium containing CellTracker™ Green (Molecular Probes,) at a dilution of 1:1,000. The culture medium was then removed and each slide rinsed with sterile PBS. Cells were fixed for 30 minutes with 4 % formol saline at room temperature, then washed with PBS and deionised water. Fluorescent images of representative cells were obtained using a Nikon Eclipse 90i microscope equipped with epifluorescence illumination. The CellTracker™ green stained cells were imaged at λ = 488 nm at 2x and 10x magnification.

**Site specific growth of self assembling peptides:** Patterned slides were incubated in Thermolysin from *Proteoloyticus rokko* (Sigma Aldrich, 2 mg/ml, 4 hours) before unbound enzyme was removed by rinsing with water. Fmoc-L and 40 mM Leu-Leu (Sigma Aldrich,) were made up at 20 mM by the addition of pH 8.5, 10 mM Tris buffer. The pH was then adjusted to 7.2 and Congo red stock solution was added to a
final concentration of 10 mM. The reactant solution was then added to the surface of the slide, and incubated for 4 hours. Hydrogel spots were dried under nitrogen, and imaged using cross polarised microscopy.

FIGURES:

Figure S1. Reactor configuration for the deposition of micropatterned DGpp surfaces. A. Photograph of the reactor as used for the procedure. B. Schematic of the major components. C. Detailed schematic of the electrode region. The live, patterned electrode is red, whereas the earthed electrode is green. The patterned electrode is brought to 1 mm of the substrate (black) upon which the patterns are to be deposited. D. A photographic image of the patterned electrode showing the specific electrode configurations (scale bar 5mm) The patterned holes allow for a variation in density of the plasma sheath and result in chemically patterned surfaces with a one step deposition.
Figure S2. High resolution XPS C 1s scans were acquired across the DGpp patterned surfaces. A and B. The C 1s curves taken both on and off the spot respectively show the spots to contain lower ether related and higher hydrocarbon groups. C. The resulting C 1s components were plotted as a percentage of total C species, showing the patterned features to contain lower COR (□) surface groups, and higher CC;CH (●) and C=O (♦) groups, with relatively uniform level of COOR (▲) detected across the surface as determined within the analysis depth of XPS.

Explanatory Note: High resolution C 1s XPS spectra were acquired across the DGpp patterned surfaces and representative spectra taken both on and off the spot are presented in Figure S2 A and B. Four main chemical components were evident and fitted to the acquired C 1s curves, including C-C;C-H (hydrocarbons), C-OR (ethers and alcohols), C=O (aldehydes and ketones) and COOR (carboxylic acids and esters). Comparison of the two C 1s curves shows variation of the COR to CC;CH intensity ratio, indicating a higher retention of monomer-like chemistry on the surrounding film compared to a lower COR concentration within the spot. Furthermore, the percentage of each C 1s component relative to total carbon is plotted as a function of distance across the patterned surface (Figure S2 C) showing that the COR content drops by approximately 10% on the patterned features compared to the surrounding, low fouling, base film. A slightly higher level of CC;CH, C=O and COOR bonded species were evident within the patterned spot compared to the surrounding film.
Figure S3. Schematic of enzyme immobilisation for peptide self-assembly. Patterned substrates were prepared as previously on glass microscope slides. A. The slide was incubated in a solution of thermolysin (from *Proteolyticus rokko*) at a concentration of 82 units/ml for a period of 24 hours. B. The slides were then washed multiple times to remove all unattached enzyme, leaving the functional enzyme only within the confines of the pattern. C. The patterns were then incubated in a solution of precursor (20mM fmoc-leucine and 40mM di-leucine) for 24 hours. During this incubation period, the enzyme performs a reversed hydrolysis reaction to form fmoc-tripeptide, which undergoes self-assembly. D. Upon removal of the precursor solution, a self-assembled hydrogel remains, which is confined to the site of enzyme activity.

Explanatory Note: Modelling of the plasma discharge

The asymmetric discharge can be electrically modelled by two sheaths at the live (upper) and earth (lower) electrodes and via the plasma bulk. The geometric electrode area ratio is $A_{\text{earth}}/A_{\text{live}} \approx 2.8$ ($A_{\text{earth}} = 78 \text{ cm}^2$ and $A_{\text{live}} = 28 \text{ cm}^2$) and most of the applied voltage will be dropped in the live electrode sheath since its capacitance will be smaller than that of the earth sheath. For the present operating pressure of 26 Pa, we assume an electron temperature $kT_e$ of $\sim 3 \text{ eV}$ (k is the Boltzmann constant) which yields a plasma potential of about $V_p \sim (kT_e/e) \ln(M_i/2P_m)^{1/2} \sim 15 \text{ V}$ ($e$ is the electronic charge, $M_i$ is the argon ion mass and $m$ is the electron mass). The presence of a ‘Pi’ resonant matching network with a blocking capacitor allows for the development of a self-bias potential $V_{sb\text{-live}}$ at the live electrode. The applied voltage at the live electrode can be written as:

$$V_{\text{live}} = Q(1.4U_{rms}) = 1.4Q(P_{rms}Z)^{1/2}$$

(1)

where $Q$ is the quality factor of the resonant circuit ($Q \sim 3$), $U_{rms}$ is the voltage at the entrance of the matching network, $Z$ is the output impedance of the generator ($Z = 50 \text{ Ohm}$), and $P_{rms}$ is the input power ($P_{rms} = 5 \text{ W}$). $V_{\text{live}}$ is about 65 V which gives a self-bias of $V_{sb\text{-live}} \sim (V_p - V_{\text{live}}) \sim -50 \text{ V}$. A simple power loss
estimate $P_{\text{loss}}$ can be carried out assuming that the plasma escapes out through the two sheaths at the Bohm speed:

$$P_{\text{ras}} = P_{\text{loss}} = e n_{\text{sheath}} v_B \left[ A_{\text{live}}(E_i + E_{\text{exc}} + V_p + |V_{\text{sheath}}|) + A_{\text{earth}}(E_i + E_{\text{exc}} + V_p + 2(kT_e/e)) \right]$$

(2)

where $n_{\text{sheath}}$ is the plasma density next to the electrodes (sheath edge density), $v_B = (kT_e/M_i)^{1/2} \approx 2.7 \times 10^3$ ms$^{-1}$ is the Bohm speed, $E_{\text{ion}} \approx 15$ V is the ionisation energy, $E_{\text{exc}} \approx 13$ V is the excitation energy, and it is assumed that each escaping electron carries $2(kT_e/e)$ of kinetic energy to the electrodes. For a 5 W input power, the calculated sheath density is $1.7 \times 10^{10}$ cm$^{-3}$.

The Debye length near the sheaths edge is:

$$\lambda_{\text{De}} = \frac{\varepsilon_0 kT_e}{e^2 n_{\text{sheath}}} \approx 10^{-4} \, \text{m} \approx 100 \, \mu\text{m}$$

(3)

where $\varepsilon_0$ is the permittivity of free space ($\varepsilon_0 = 8.85 \times 10^{-12}$ Fm$^{-1}$).

To determine the live ($S_{\text{live}}$) and earth ($S_{\text{earth}}$) electrode sheath thickness we use the Child law for a collisionless DC sheath in an argon plasma capacitively coupled between two planar electrodes:

$$S = \frac{K_i \varepsilon_0}{e v_B^2} \left( \frac{2e}{M_i} \right)^{1/2} \frac{V_i^{1/4}}{n_{\text{sheath}}^{1/2}}$$

(4)

where $K_i = 4/9 = 0.44$ and $V$ is the sheath potential ($V_{\text{live}}$ and $V_p$, for the live and earth electrode, respectively). The calculated values for $S_{\text{live}}$ and $S_{\text{earth}}$ with an input power of 5 W are 0.78 mm and 0.25 mm, respectively. At 26 Pa, the ion-neutral collision mean free path $l_i$ is about 0.15 mm and the average number of collisions in the live and earth electrode sheaths is about 5 and 1.5, respectively. The sample is
placed on the near collision-less earth electrode and the power deposited to the surface is about 0.04 Wcm\(^{-2}\). Because the hole size of the electrode is of the order of the Debye length and the live and earthed sheath thickness are about 3 and 8 Debye lengths, respectively, it is possible to have gradients in both the electron and ion density across the holes which could spatially affect the monomer fragmentation and ionisation, and contribute to the non uniform plasma polymer deposition. These results are in agreement with a similar study reported by Zelzer et al.\(^6\)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table 1.</strong> Plasma parameters estimated from the argon plasma model for an input power of 5 W and an operating pressure of 26 Pa.</td>
<td></td>
</tr>
<tr>
<td>Electron temperature, ( T_e ) (eV)</td>
<td>3</td>
</tr>
<tr>
<td>Bohm velocity, ( v_B ) (m.s(^{-1}))</td>
<td>2700</td>
</tr>
<tr>
<td>Live Sheath potential, ( V_{live} ) (V)</td>
<td>65</td>
</tr>
<tr>
<td>Earth sheath potential, ( V_p ) (V)</td>
<td>15</td>
</tr>
<tr>
<td>Sheath edge density, ( n_{sheath} ) (m(^{-3}))</td>
<td>1.7x10(^{16})</td>
</tr>
<tr>
<td>Debye length, ( l_{De} ) (m)</td>
<td>1x10(^{-4})</td>
</tr>
<tr>
<td>Live sheath thickness, ( S_{live} ) (m)</td>
<td>7.8x10(^{-4})</td>
</tr>
<tr>
<td>Earth sheath thickness, ( S_{earth} ) (m)</td>
<td>2.5x10(^{-4})</td>
</tr>
<tr>
<td>Ion-neutral mean free path, ( l_i ) (m)</td>
<td>1.5x10(^{-4})</td>
</tr>
</tbody>
</table>

**ACKNOWLEDGMENTS:**

This research was undertaken on the FTIR microscope beamline at the Australian Synchrotron, Victoria, Australia. We thank Lawry McCarthy of CSIRO for assistance with surface pattern imaging, and Robert Jones of LaTrobe University for assistance with ToF-SIMS measurements. Miss Donna Menzies is a recipient of the CSIRO OCE PhD Scheme.
REFERENCES: