

## Supplementary Information

### **Microplasma arrays: A new approach for maskless and localized patterning of materials surfaces**

*Endre J. Szili, Sameer A. Al-Bataineh, Paul Ruschitzka, Gilles Desmet, Craig Priest, Hans J. Griesser, Nicolas H. Voelcker, Frances J. Harding, David A. Steele and Robert D. Short\**

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## EXPERIMENTAL DETAILS

**Substrate preparation.** A 5% (w/v) of polystyrene (Goodfellow Cambridge Ltd.) was prepared in toluene. The solution was spin-coated onto polished silicon wafer pieces. The spin-coated samples were soft-baked at 50°C for 5 min to facilitate the removal of residual toluene. Commercial microscope glass slides were functionalized with 3-aminopropyl triethoxysilane (APTES, Sigma) to enhance protein adsorption onto the glass surface. The slides were incubated with an undiluted solution of APTES at 25°C for 45 min, rinsed in isopropanol, dried under nitrogen and then soft-baked at 120°C for 5 min.

**Surface passivation.** Bovine serum albumin (BSA) passivated polystyrene and APTES functionalized glass slides were made by incubating a 1% (w/v) solution of BSA (Sigma) in phosphate buffered saline (PBS, pH 7.4, Sigma) over the surfaces at 25°C for 4 h. The surfaces were washed in Milli-Q water and dried under nitrogen. Similarly, a 1% (w/v) solution of fluorescein conjugated BSA (Invitrogen) was incubated over the sample at 25°C for 4 h for fluorescence measurements.

**Microplasma array treatment.** A detailed description of the microplasma array fabrication and operation is provided elsewhere.<sup>1</sup> Briefly, the device consists of a dielectric barrier (SU8-50 photoresist, MicroChem Corp., USA) sandwiched between two gold electrodes. A 7 x 7 array of 250 μm diameter cavities with a depth of 55 nm and a separation distance (edge-to-edge) of 500 μm was patterned into the top gold layer using standard photolithography.

Plasma generation was carried out using a custom-built electrical system. A power supply consisted of an oscillator (Agilent Technologies, DS06034A), an audio amplifier (AMPRO, XA1400) and a step-up transformer (Southern Electronic Services) powered the microplasma array using sinusoidal AC excitation.

The microplasma array was operated at 1 kV<sub>peak-peak</sub> and 10 kHz in an atmospheric pressure (760 Torr) of helium. The microplasma array was mounted upside down on the top flange inside a custom-built microplasma reactor. Substrates were placed face-up on an insulated sample stage for surface treatment with the microplasma array. The chamber was initially pumped down to a base pressure < 5 x 10<sup>-2</sup> Torr to remove background air. For treatment, the chamber was filled with high purity helium (99.99%, BOC). A computerized stage was used to precisely control the distance between substrate and microplasma array.

The separation distance between the microplasma array and sample was kept constant at 150  $\mu\text{m}$ . The optimized treatment time for polystyrene and glass substrates was kept constant at 10 and 5 s, respectively.

**Protein patterning.** After microplasma array treatment of the BSA passivated substrates, the surfaces were incubated with 150  $\mu\text{l}$  of 20  $\mu\text{g/ml}$  Alexa Fluor<sup>®</sup> 568 conjugated streptavidin protein (Invitrogen, prepared in PBS) at 25°C for 12 h. The surfaces were washed with a solution of PBS containing 0.05% (v/v) Tween-20 (PBS-T, Sigma), rinsed in Milli-Q water and dried under nitrogen. The protein was visualized using fluorescence microscopy.

**Enzyme patterning.** Horseradish peroxidase (HRP, Sigma) was used to analyze the application of the microplasma areas in enzyme immunoassays. The samples were first incubated with 150  $\mu\text{l}$  of 2.5 mg/ml HRP at 25°C for 2 h, washed in PBS-T and then in PBS. HRP was regioselectively adsorbed to a microplasma-patterned BSA surface. A precipitating formulation of 3,3',5,5'-tetramethylbenzidine (TMB, Sigma) was then incubated over the samples at 25°C for 10 min. The localized HRP enzyme catalyzed the oxidation of soluble and colourless TMB into a dark blue insoluble product that precipitated over the enzyme-containing regions. The resulting precipitation of the oxidized TMB product was imaged by optical microscopy.

**Protein array sensing.** To demonstrate an application for protein sensing, microplasma array treated BSA-passivated polystyrene were prepared for the detection of green fluorescent protein (GFP). The samples were incubated at 25°C for 12 h with 10  $\mu\text{g/ml}$  of anti-GFP in 150  $\mu\text{l}$  of PBS or 150  $\mu\text{l}$  of PBS containing no antibody as a negative control. The surfaces were washed in PBS-T and then in PBS. After, the samples were blocked in 150  $\mu\text{l}$  of 1% (w/v) BSA-PBS solution at 25°C for 2 h and washed as above. All samples were then incubated with a protein mixture consisting of 5  $\mu\text{g/ml}$  GFP (Rockland) in 150  $\mu\text{l}$  of PBS-T supplemented with 1% (w/v) BSA, at 25°C for 2 h. This was followed by the same washing step as above. Samples were imaged via fluorescence microscopy.

**Surface characterization.** Time-of-flight secondary ion mass spectrometry (ToF-SIMS) measurements were performed using a Physical Electronics Inc. PHI TRIFT V nanoToF instrument equipped with a pulsed liquid metal <sup>79</sup>Au<sup>+</sup> primary ion gun (LMIG), operating at

30 kV. Surface analyses were performed using ‘bunched’ Au<sub>1</sub> beam settings to optimize mass resolution. The instrument software’s mosaic function was employed to collect image data over larger areas (mm scale). Spectra were collected in positive SIMS mode, typically using 100 x 100 micron raster areas. Experiments were performed under a vacuum of  $\leq 3.8 \times 10^{-8}$  Torr and in the static mode to minimize possible effects arising from sample damage.

**Analysis of Means.** A group of six positive ion ToF-SIMS spectra from regions of interest (ROI) were collected from plasma-treated regions and from the background area, respectively. The spectra were processed by analysis of means with a group of positive ion fragments related to PS and BSA, respectively (Table 1). The intensity of each fragment was normalized to the total counts of the selected fragments in each spectrum and the average was taken. The confidence intervals were calculated for  $p = 95\%$ .

**Fluorescence microscopy.** Fluorescence imaging was carried out using a Nikon Inverted Microscope TE-2000 through a 4x objective. Images of the streptavidin conjugate and RFP were captured through a Nikon filter with 510-560 nm excitation and 590 nm emission; and through a Nikon filter with 455-485 nm excitation and 500-545 nm emission for the BSA conjugate or GFP. Images were recorded with a Nikon DXM1200C digital camera and processed using NIS-Elements Basic Research v2.2 software.

**White-light optical microscopy.** Optical micrographs were acquired using a Nikon Eclipse LV150 optical microscope through a 5x objective and recorded with a digital Camera (DS-Fi1, Nikon, Japan).

**Cell-based assays.** Human SK-N-SH neuroblastoma (ATCC CRL-1573) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma) supplemented with 2 mM L-glutamine, 100 IU/ml penicillin, 100  $\mu\text{g}/\text{ml}$  streptomycin sulfate (Invitrogen) and 10 % v/v fetal bovine serum (Sigma) and maintained at 37°C in 5% CO<sub>2</sub>. Prior to use with microplasma-patterned surfaces, cells were stained with Cell Tracker Orange CMRA (Invitrogen) as per the manufacturer’s protocol.

To assess cell attachment and growth, samples were first incubated in culture media for 10 minutes prior to cell seeding. Cells were cultured in contact with microplasma-patterned arrays at a density of  $1 \times 10^5$  cells/cm<sup>2</sup> and at 37°C in 5% CO<sub>2</sub> in 12-well tissue culture dishes (Iwaki). After 4, 24 and 48 h incubation, samples were washed gently with

Dulbecco's phosphate buffered saline with calcium and magnesium (D-PBS+ Ca<sup>2+</sup>/Mg<sup>2+</sup>, 0.9 mM CaCl<sub>2</sub>, 2.67 mM KCl, 1.47 mM KH<sub>2</sub>PO<sub>4</sub>, 0.50 mM MgCl<sub>2</sub>·6H<sub>2</sub>O, 138 mM NaCl, 8.10 mM Na<sub>2</sub>HPO<sub>4</sub>). Cells were fixed with 3.7% formaldehyde solution for 10 minutes, then stained with 2 µg/ml Hoechst 33342 (Sigma) in culture media for 10 minutes. Samples were finally washed with D-PBS+ Ca<sup>2+</sup>/Mg<sup>2+</sup> and mounted in Fluoro-Gel/Tris buffer (ProSciTech) for analysis. Mounted samples were observed on an Eclipse50i fluorescence microscope (Nikon) with a DS-U2 digital camera (Nikon). CellTracker Orange CMRA was observed through excitation filter 540-557 nm and emission filter 605-625 nm and Hoechst 33342 through excitation filter 340-380 nm and emission filter 435-485 nm. All images were processed and analyzed by NIS-Elements BR 3.0 software.

**Fabrication of glass microfluidic chips.** Glass microfluidic chips were prepared using a combination of UV-photolithography and deep-reactive ion etching (DRIE). Pyrex<sup>TM</sup> plates were spin-coated (2000 rpm) with SU8-10 photoresist and baked on hotplates for 2 min and 5 min at 65°C and 95°C, respectively. The sample was then exposed (180 mJ/cm<sup>2</sup>, 360 nm) through a chrome-glass photomask patterned with the microchannel design, and post-exposure baked for 1 min and 3 min at 65°C and 95°C, respectively. The pattern was developed in the photoresist in SU8 developer solution for 3 min, was rinsed in isopropanol, and hard-baked for 1 min and 5 min at 95°C and 150°C, respectively. DRIE (ULVAC NLD570) was carried out using fluorocarbon plasma (C<sub>4</sub>F<sub>8</sub>) at an etch rate (in Pyrex<sup>TM</sup> glass) of ~ 0.3 µm/min. The final etch depth was 18 µm. Integration of the electrodes into the glass microchip was carried out using molten gallium metal according to the methodology given elsewhere.<sup>2</sup>

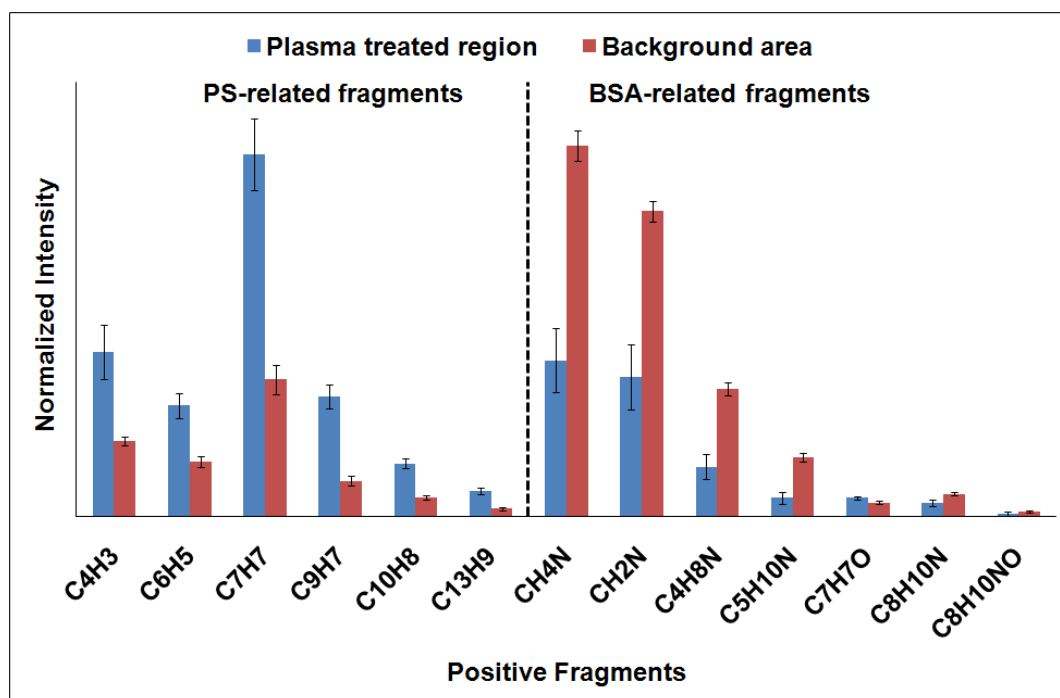
**Protein patterning in microfluidic chips.** The microchannel wall was first functionalized with 3-aminopropyl triethoxysilane (APTES) by incubation with 100 mM 3-aminopropyl triethoxysilane (APTES), prepared in toluene at 25°C for 1 h. The microchannel was rinsed in toluene and then dried under nitrogen. The microchannel was then passivated by incubating with 1% (w/v) BSA prepared in PBS at 25°C for 4 h. The microchannel was then flushed with Milli-Q water and dried under nitrogen. Microplasma array treatment was performed at 5 kV<sub>peak-peak</sub> and 10 kHz in a helium flow of 5 ml/min. A solution of 20 µg/ml Alexa Fluor<sup>®</sup> 568 conjugated streptavidin protein (in PBS) was incubated in the microchannel at 25°C for 12 h. The microchannel was flushed with PBS-T, then Milli-Q

water and finally dried under nitrogen. The protein was visualized using fluorescence microscopy as described above in the Experimental Section.

**Table 1.** Positive fragments used in the evaluation of BSA treatment with microplasma by analysis of means.

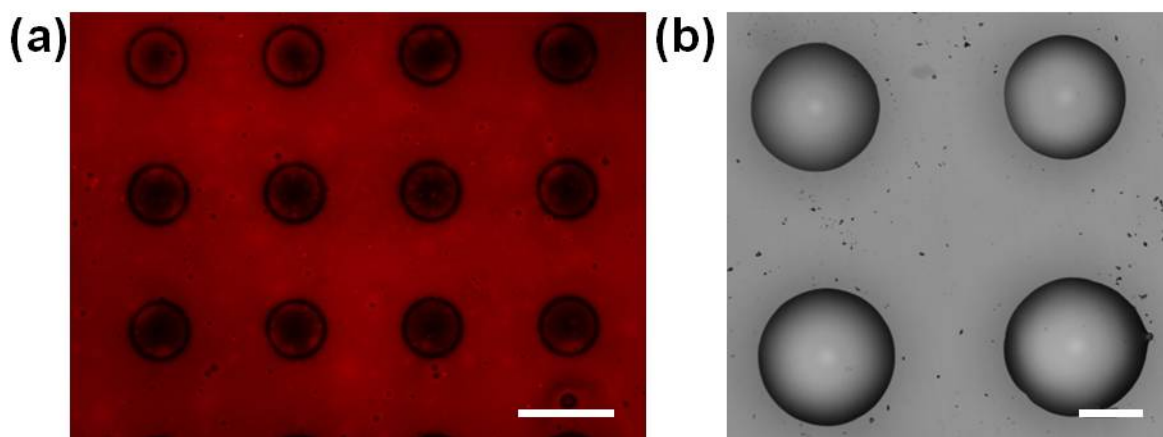
PS-derived fragments	<i>m/z</i>	BSA-related fragments	<i>m/z</i>
C <sub>4</sub> H <sub>3</sub> <sup>+</sup>	51	CH <sub>2</sub> N <sup>+</sup>	28
C <sub>6</sub> H <sub>5</sub> <sup>+</sup>	77	CH <sub>4</sub> N <sup>+</sup>	30
*C <sub>7</sub> H <sub>7</sub> <sup>+</sup>	91	C <sub>4</sub> H <sub>8</sub> N <sup>+</sup>	70
C <sub>9</sub> H <sub>7</sub> <sup>+</sup>	115	C <sub>5</sub> H <sub>10</sub> N <sup>+</sup>	84
C <sub>10</sub> H <sub>8</sub> <sup>+</sup>	128	C <sub>7</sub> H <sub>7</sub> O <sup>+</sup>	107
C <sub>13</sub> H <sub>9</sub> <sup>+</sup>	165	C <sub>8</sub> H <sub>10</sub> N <sup>+</sup>	120
		C <sub>8</sub> H <sub>10</sub> NO <sup>+</sup>	136
		C <sub>10</sub> H <sub>11</sub> N <sub>2</sub> <sup>+</sup>	159
		C <sub>11</sub> H <sub>8</sub> NO <sup>+</sup>	170

\* This fragment ion is also expected from phenylalanine in BSA<sup>[1]</sup>

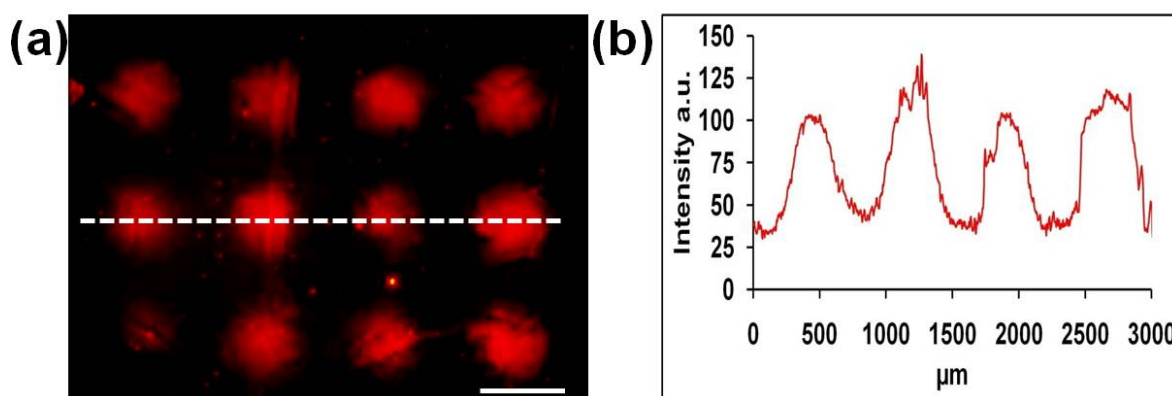


**Figure S1.** Normalized intensities of positive fragments of microplasma-treated BSA-passivated polystyrene substrate. ToF-SIMS region of interest (ROI) spectra were acquired within microplasma-treated regions (blue) and in the background area (red) within the array. While clearly the signals derived from BSA are greatly reduced in intensity after microplasma array treatment, the observation that the spectra of the treated areas do not exclusively contain only polystyrene-derived fragment ions suggests that while ablation dominates, there is a residual small amount of plasma-crosslinked BSA on the surface.





**Figure S2.** (a) Fluorescence micrograph of fluorescently-labelled streptavidin on microplasma array treated polystyrene. (b) Brightfield microscopy image of condensed water droplets (upon exposure to a stream of water vapour) on microplasma array treated polystyrene. Scale bars = 500  $\mu\text{m}$  for (a) and 200  $\mu\text{m}$  for (b).



**Figure S3.** Microplasma array patterning of protein on microscope glass slides. Patterned fluorescently-labelled streptavidin is shown in (a) and the corresponding fluorescence intensity line scan across a section of the array, as indicated by the broken white line, is shown in (b). The glass was prepared by first incubation with 100 mM 3-aminopropyl triethoxysilane (APTES), prepared in toluene at 25°C for 1 h, rinsed in toluene, dried under nitrogen and then soft-baked at 120°C for 5 min. The samples were then passivated with BSA, microplasma array treated, patterned with fluorescently-labelled streptavidin and imaged by fluorescence microscopy as described above in the Experimental Section. Scale bar = 500  $\mu\text{m}$ .

## REFERENCES

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