One step multifunctional micropatterning of surfaces using asymmetric glow discharge plasma polymerization

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

Multifunctional micropatterned surface chemistries were deposited in one step without solvents, via plasma enhanced chemical vapour deposition. We illustrate the versatility of this technique via the controlled adhesion of proteins, geometric confinement of cells and the spatial confinement of peptide self-assembly.

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Received 9th September 2011, Accepted 12th December 2011
DOI: 10.1039/c2cc15578h

Micropatterning of surfaces with varying chemical, physical and topographical properties usually requires a number of fabrication steps. Herein, we describe a micropatterning technique based on plasma enhanced chemical vapour deposition (PECVD) that deposits both protein resistant and protein repelling surface chemistries in a single step. The resulting multifunctional, selective surface chemistries are capable of spatially controlled protein adhesion, geometric confinement of cells and the site specific confinement of enzyme mediated peptide self-assembly.

The generation of regular arrays of multiple polymer surface chemistries or features on a surface is routinely used in a small number of emerging patterning techniques, these methods require further chemical derivatization for optimal functionality. The novel technique reported here is based on PECVD and is the first example of a substrate independent, solvent free, one-step process that allows the generation of stable multifunctional micropatterns. During the deposition process, both low protein fouling and high fouling chemically patterned features are deposited simultaneously. PECVD enables the modification of various substrate materials and is scalable for use in sterile environments. Existing plasma based patterning methods such as etching, plasma lithography, and microplasmas currently require numerous production steps for use in biological applications where surface passivation and activation is necessary.

The key innovation of PIPET is the use of a patterned upper electrode (Fig. 1a and S1†) to produce an asymmetric glow discharge above a substrate. It is important to note that this is not a masking technique. The bulk plasma glow discharge and sheath below the patterned electrode vary spatially, resulting in variation to the fragmentation of the monomer being polymerized which affects the chemical structure of the resulting plasma polymer film.

The versatility of this technique is illustrated via the controlled adhesion of proteins, geometric confinement of cells and the spatial confinement of peptide self-assembly. The performance of biomaterial devices is dependent on their ability to resist or control protein and cell adhesion. The mono-mer diethylene glycol dimethyl ether (DG) was utilized in this work as it contains ether units which form the backbone of poly(ethylene glycol) (PEG) molecules. The ether functionality of PEG polymers is critical to their low-fouling nature. The use of PIPET with DG, provides patterned surfaces with regions of both high ether (PEG-like) and low ether (non PEG-like) chemistries (Fig. 1b). ‘Non-PEG-like’ chemistries occur directly under the holes of the patterned electrode which is attributable to greater monomer fragmentation in this region. The ‘PEG-like’ character of the surface increases radially from the centre of the patterned features.

The size, thickness and shape of the patterned features can be varied by manipulation of the upper electrode geometry and PECVD conditions (Fig. 1c). The increased thickness of the films within the centre of the patterned shapes was confirmed via analysis with optical profilometry (Fig. 1d). The elevated features are approximately 200 nm above the surrounding PEG-like regions which are 60 nm in thickness, when using electrode patterned features 1 mm in size. Grazing incidence Fourier Transform Infra-Red (gFTIR) microspectroscopy and time-of-flight secondary ion mass spectroscopy (ToF-SIMS) was used to analyze patterned circles. Chemical maps (Fig. 2a–c) show a higher carbonyl and lower ether content within the circle compared with the surrounding PEG-like film. This suggests that the plasma discharge and monomer fragmentation is more energetic in this region. The presence of carbonyl functionalities originates from bond scission of the monomer and post oxidation reactions.

Modeling of the plasma sheath physics using argon cross sections further validates these findings (see ESI†). Plasma sheaths are regions where charge separation can occur and
strong electron and ion density gradients are present. The model estimated the main plasma parameters for comparison with the inter-electrode distance and the patterned hole size (ESI, Table 1). The Debye length was calculated to be of the order of 100 μm which is comparable to the hole size in the upper electrode. This represents a possible limit to the pattern fidelity achievable via this technique of around 100 μm.

To demonstrate the utility of PIPET in biomedical research we proposed that it should be possible to produce site-specific ‘islands’ for the containment of protein immobilization, cell proliferation and enzyme assisted self-assembly in a micro-array format. Imaging ToF-SIMS was used to map the surface chemistries before and after incubation with bovine serum albumin (BSA) (Fig. 2d). Collective images of positive ethylene glycol related ion fragments commonly reported in PEG-like materials19 are shown in Fig. 2d and hydrocarbon ion fragments in Fig. 2e for a surface prior to incubation with BSA. Strong image contrast and good pattern fidelity is observed for all of the ions, with higher concentrations of hydrocarbon fragments being localized in the centre of the circle. More ethylene glycol related or PEG-like ion fragments are observed in the surrounding film. BSA adsorption was strongly retained within the centre of the circle, as indicated by significantly more nitrogen containing ions (Fig. 2f) in these regions. X-ray Photoelectron Spectroscopy (XPS) confirmed the spatial variation of surface chemistries (Fig. S2).

In our second example we demonstrate the preferential confinement of an adherent HeLa cell line. Optical images of the patterns used to confine cell growth are shown in Fig. 3a and b for a planar array of HeLa cells, in which individual cells attach and spread in the centre of the patterns. The ‘PEG-like’ regions support minimal cell attachment. The long term stability and robustness under physiological conditions of such patterns is a key performance characteristic. To exemplify the robustness of the PIPET films, cells were incubated on circular patterns for 1 and 7 days (Fig. 3c–d). After 7 days culture the cells remain constrained within the patterned boundary. The surrounding PEG-like regions of the film retained its biological inertness; although a small number of cells were observed initially, these were not viable after several days.
We have described a new approach for the generation of substrate independent, multifunctional, patterned surface chemistries that are cell and protein resistant/adherent in a single step. The patterning technique is extremely versatile, tunable, solvent free and scalable. We have demonstrated the broad utility of this strategy through the controlled adhesion of proteins, confinement of HeLa cells and enzyme-assisted peptide self-assembly. The method enables the production of various geometrical shapes and micropattern properties to be deposited with good spatial and chemical fidelity. This multi-functional micropatterning technique has broad applicability in the fields of cell biology, tissue engineering and biomedical science.

Notes and references