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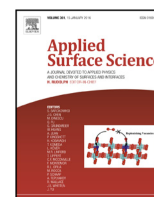

Investigation of the growth mechanisms of diglyme plasma polymers on amyloid fibril networks

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ABSTRACT

Within the area of biomaterials research, the ability to tailor a materials surface chemistry while presenting a biomimetic topography is a useful tool for studying cell–surface and cell–cell interactions. For the study reported here we investigated the deposition of diglyme plasma polymer films (DGpp) onto amyloid fibril networks (AFNs), which have morphologies that mimic the extracellular matrix. We extend our previous work to observe that the nanoscale contours of the AFNs are well preserved even under thick layers of DGpp. The width of the surface features is positively correlated to the DGpp thickness. DGpp film growth conformed to the underlying fibril features, with a gradual smoothing out of the resultant surface topography. Further, to understand how the films grow on top of AFNs, X-ray photoelectron spectroscopy depth profiling was employed to determine the elemental composition within the coating, perpendicular to the plane of the substrate. It was found that AFNs partially fragment during the initial stage of plasma polymerisation, and these fragments then mix with the growing DGpp to form an intermixed interface region above the AFN. The findings in this study are likely applicable to situations where plasma polymerisation is used to apply an overcoat to adsorbed organic and/or biological molecules.

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1. Introduction

The host response to a particular biomaterial is largely governed by the surface chemistry and structure of that material [1]. Modification of the outermost layers of a biomaterial is commonly employed as a means to provide a desired biological response while retaining device functionality [2–5]. Subtle variations in surface morphology can lead to changes in cell attachment and proliferation [6–8], and in the case of stem cells, differentiation [9–11]. A number of methods are available for creating nano- to micro-scale features on a substrate surface [12–16]. A drawback of these techniques is that the surface structures produced do not mimic the typical nanoscale fibrous morphology of the extracellular matrix (ECM) where cells normally reside. Therefore, approaches to obtain fibrous biomimetic surface architectures have attracted attention.

Biomimetic nanostructured fibrillar networks can be fabricated in two or three dimensions through the self-assembly of peptides or proteins into nanoscale fibrils [17–20]. Non-toxic amyloid fibrils represent one promising subset of such self-assembled materials due to their ease of formation and the ability to accurately control the fibrous morphology. Amyloid fibril networks (AFNs) deposited on solid supports have been previously shown to promote cell adhesion and spreading in a variety of cell types [21,22]. To mask the effect of native functional groups present on the surface of AFNs and study the topographical effects in isolation, we have previously reported a technique utilising plasma polymerisation to coat the AFN construct [23].

Plasma polymerisation is a solvent free process that can produce uniform, defect free coatings in one step. It is frequently used to produce functional coatings for biological applications [24–27]. This convenient technology permits the use of an exceptionally wide range of precursors, including gases, volatile compounds, and solids, which can release vapour upon sublimation. Recently, peptides, amino acids [28–30] and other biological molecules [31] have been exploited as precursors for plasma polymerisation. In other studies, antibiotics [32,33] were first deposited onto a substrate before subsequent coating with a plasma polymer film, which served as a barrier for the controlled diffusion of the underlying antibacterial molecules.

In our previous study, plasma polymer films were prepared from a diethylene glycol dimethyl ether (diglyme) precursor on top of AFNs first adsorbed on mica [23]; diglyme was chosen as it can

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provide low-fouling or protein adhesive surface chemistry via tailoring of the deposition conditions. The diglyme plasma polymer (DGpp) coatings displayed the contours of the AFN, preserving the fibrous topography with only a slight broadening effect of the fibril widths. Here, we employed a series of diglyme plasma polymer deposition times to explore this phenomenon in DGpp films of different thickness. Remarkably, the fibrous structure was found to be visible via atomic force microscopy (AFM) on the surface of DGpp films up to 1 μm thickness, albeit with a 3 fold increase in the fibril width. We also investigated the growth mechanisms of the AFN-DGpp constructs. During plasma deposition onto the substrate, AFN, and the growing coating will be exposed to energetic ions from the plasma. We hypothesised that the AFNs will be fragmented to a certain extent in this environment, but then be eventually buried and protected by the growing DGpp films. The distribution of AFN fragments throughout the constructs was probed by X-ray photoelectron (XPS) depth profiling, with a model of the growth process presented.

2. Experimental details

2.1. Substrate preparation

Ultra-flat single crystal silicon wafers ($\langle 1\,0\,0 \rangle$, 1 cm^2 × 0.5 mm thick, M.M.R.C. P/L) and freshly cleaved ruby mica (1 cm^2) (GMS Composites, Melbourne) were used as substrates for the deposition of diethylene glycol dimethyl ether (BDH, 99% purity) plasma polymer films, or amyloid fibril network followed by plasma polymer deposition, respectively. Si wafer substrates were cleaned immediately prior to plasma polymer deposition by ultrasonication in a surfactant solution of 2% RBS-35 (Pierce, USA) with 2% ethanol for 30 min, followed by rinsing with copious amount of Milli-Q water, and finally dried in a high-pressure stream of nitrogen.

2.2. Amyloid fibril network preparation

Hen egg white lysozyme (HEWL) (Sigma) was self-assembled into amyloid fibrils in controlled reaction conditions. HEWL was dialysed according to the protocol used in Jung et al. [34] HEWL solutions (2 wt %) were prepared by dissolving the purified protein in Milli-Q water and adjusting the pH to 2 using HCl. Solutions were transferred to sealed glass vials and placed in an oil bath at 90 °C for 24 h while undergoing constant stirring at 300 rpm using a 20 mm × 5 mm Teflon magnetic stirrer bar, as described in Lara et al. [17]. After the reaction, solutions were quenched by immersion in a water–ice bath. Amyloid fibril networks (AFNs) were prepared by incubating 100 μL of the fibril solution for 10 min on the freshly cleaved mica substrates, followed by rinsing in Milli-Q water (1 mL), and drying under a gentle stream of nitrogen.

2.3. Plasma polymerisation

Deposition of plasma polymer films was carried out in a custom-built reactor via radio frequency glow discharge (RFGD) plasma polymerisation. The plasma reactor consists of a cylindrical glass chamber (height of 35 cm and width of 17 cm) and was fitted with two capacitively coupled electrodes, spaced 10 cm apart. The top electrode (diameter = 9.5 cm) was connected to a RF power supply (125 kHz), while the bottom electrode (diameter = 14 cm) was grounded. The monomer (diglyme) was contained in a round-bottom flask that was connected to the reactor chamber via a stainless steel line, and the flow of the monomer vapour was controlled via a manual valve. Substrates were placed on the bottom electrode. Prior to deposition, the reactor was evacuated to a base pressure of less than 0.1 Pa via a rotary pump. The diglyme monomer was degassed three times prior to plasma polymerisation. DGpp films were deposited at a load power of 50 W with an initial monomer pressure of 20 Pa and treatment times ranged from 30 s to 33 min. For treatments of more than 3 min, the deposition was performed in 3 min increments, with 15 min intervals to avoid overheating of the electrodes. After deposition, the reactor was pumped down to base pressure before venting.

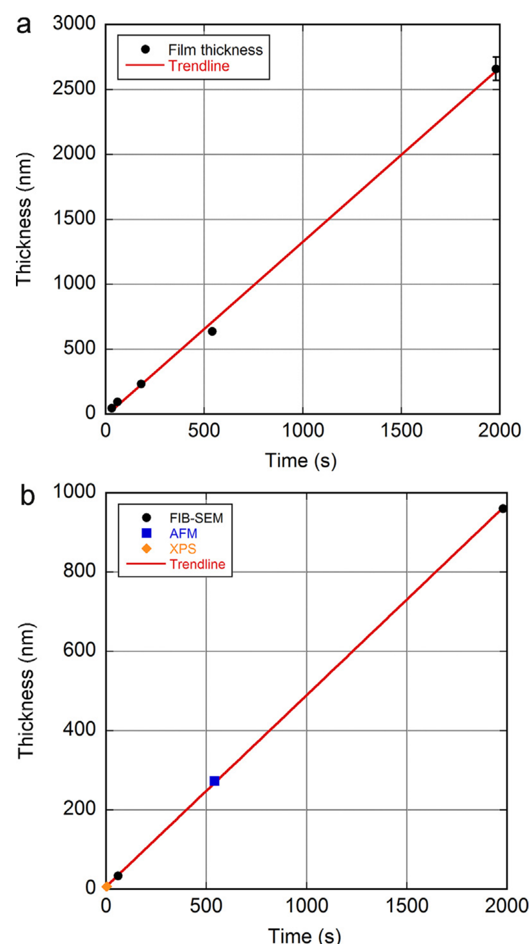

Fig. 1. Film thickness vs. deposition time for (a) DGpp on Si wafer measured using profilometry and (b) DGpp + AFN on mica measured using a combination of three alternative techniques; (i) FIB-SEM, (ii) AFM measurements of a masked area, and (iii) XPS overlayer thickness calculations for very thin samples.

2.4. Profilometry

Plasma polymer film thicknesses on Si wafer were measured using a Veeco Dektak 6M Stylus profilometer. Masked areas were prepared using 10 (w/v %) solution of poly(D,L-lactide) (Boehringer Ingelheim) in acetone [35]. One drop of the solution was placed on a substrate and dried in air 10 min prior to film deposition. Following plasma polymerisation, the mask was lifted off from the substrate using tweezers without damaging the surrounding film. The profilometry stylus (width 12.5 μm), with force set to 10 mg, was drawn a distance of 400 μm across the edge of the masked area and the film over 10 s.

2.5. Focused ion beam scanning electron microscopy

The thickness of the 50 W DGpp films deposited on AFN coated mica was determined using a focused ion beam scanning electron microscope (FIB-SEM) (FEI Helios NanoLab 600 DualBeam FIB-SEM, Eindhoven, Netherlands). Prior to imaging the samples were coated

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