ELECTRONIC SUPPORTING INFORMATION

Micropatterned Substrates Made by Polymer Bilayer Dewetting and Collagen Nanoscale Assembly Support Endothelial Cell Adhesion

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Atomic Force Microscopy (AFM) Images

Topographical AFM images are provided of PS and PNVP films after air plasma treatment (denoted PSox and PNVPox within the manuscript)) which were omitted from Figure 1 for clarity. There is essentially no change in surface topography or roughness after plasma treatment (PSox RMS roughness = 0.32 nm; PNVPox roughness = 0.22 nm).



Figure S1. Topographical AFM images of PSox (left) and PNVPox (right).

Root-Mean-Square (RMS) Roughness Measurements

RMS roughness measurements results for quick dried deposition of collagen on various polymer films at different collagen concentrations are shown in Figure S2. Pamula *et al.*¹ have previously shown that the surface roughness increases with increasing mass of adsorbed collagen, particularly under sub-monolayer coverage on the polymer film. We observe that the average RMS roughness of collagen on PS increases slightly, from 0.75 ± 0.11 nm to 0.87 ± 0.22 nm, when the collagen concentration is increased from 2 to $10 \,\mu g \, \text{mL}^{-1}$. Collagen deposited on PSox produces slightly less rough surfaces, however the roughness is much greater than the original PSox film (RMS roughness of native film = 0.33 ± 0.10 nm). Both PS and PSox are much rougher than PNVP and PNVPox after collagen deposition. Surface roughness of the polymers (prior to adsorption of collagen) is unaffected by air plasma treatment.



Figure S2. RMS roughness values for different polymer surfaces after collagen deposition and rapid drying at two different concentrations. The results shown correspond to the average RMS roughness values for at least two samples, analyzed at three different $5 \times 5 \ \mu m^2$ regions on each sample.

Spectroscopic Ellipsometry Measurements

Spectroscopic ellipsometry was used to measure the thickness of mat-like collagen layers deposited on the polymeric substrates used in this work. Under all surface preparation conditions and collagen concentrations used, thicker collagen films were adsorbed on PS and PSox surfaces compared to the corresponding PNVP and PNVPox surfaces (Figure S3). In the case of the PNVP, when the collagen concentration was 2 μ g mL⁻¹, no collagen layer could be measured on the substrate, indicating the low amount of adsorbed protein. Even when using the high collagen concentration of 10 μ g mL⁻¹, the estimated collagen layer thickness on both PNVP and PNVPox is smaller than the collagen layer thickness measured on any PS substrate under any adsorption conditions. The adsorption onto PS and PSox is comparable in terms of collagen layer thickness, with layer thicknesses varying from 0.7 – 1 nm through to ~ 5.3 nm at high collagen concentrations.



Figure S3. Collagen layer thickness after deposition and rapid drying as a function of surface type and collagen concentration, as measured by spectroscopic ellipsometry.

Contact Angle Data Measurements

The surface coverage of mat-like collagen surfaces on polymer films was evaluated by static contact angle measurements. Contact angle data is presented in Table S1. The employed polymer films are all very hydrophilic, with the exception of PS with a high contact angle (94.2° \pm 0.6°). Both plasmatreated PS (PSox) and PNVP (PNVPox) are hydrophilic due to surface oxidation. Upon collagen adsorption, the contact angle on all surfaces converges (albeit at different rates) to the previously reported values of contact angle of Type 1 collagen of $31.9 \pm 0.7^{\circ}$, regardless of the initial wettability of surface used². The results at the high collagen concentration (10 µg mL⁻¹) suggest that (at least) monolayer coverage of collagen has been achieved on all films except for PNVPox. The variation of contact angles at the lower collagen concentration (2 µg mL⁻¹) suggests sub-monolayer coverage and greater difference in adsorption between the substrates. If we assume the validity of the Cassie-Baxter³ expression for these composite materials (a crude approximation given the collagen layer is neither smooth nor impenetrable to water), the fractional surface coverage of collagen on PS (0.68 ± 0.04) is approximately twice as large as PNVP (0.3 ± 0.11), while the fractional surface coverage of collagen on PS (0.2 ± 0.03) is much greater than on PNVPox (negligible).

Table S1. Static water contact angle measurements on polymer films used in this work after collagen deposition and rapid drying.

| | Static Contact Angle [°] | | |
|-----------------|--------------------------|--------------------------------------|---------------------------------------|
| | | | |
| Surface Type | Bare Surface | Collagen (2 µg mL ⁻¹) | Collagen (10 µg mL ⁻¹) |
| PS | 94.2 ± 0.6 | 56.2 ± 0.8 | 30.7 ± 1.3 |
| PSox | 10.9 ± 1.2 | 17.2 ± 0.5 | 33.3 ± 3.9 |
| PNVP | 16.5 ± 3.3 | 22.3 ± 0.4 | 34.2 ± 1.1 |
| PNVPox | 13.5 ± 0.1 | 13.3 ± 1.0 | 26.7 ± 1.5 |

Quartz Crystal Microbalance (QCM-D) Measurements

Quartz crystal microbalance (QCM-D) studies were performed for the adsorption of collagen at different concentrations ($2\mu g m L^{-1}$ and $10 \mu g m L^{-1}$ in phosphate-buffered saline) onto different types of polymer films. The change in frequency (Δf) and dissipation (ΔD) was measured for several harmonics of the quartz crystal during the adsorption and washing processes.

We observe that in addition to a concentration-dependent frequency change during collagen adsorption, there is also a significant increase in the measured dissipation. This is typical for a nonrigid, deformable layer such as collagen.⁴ Additionally, Gurdak *et al.*⁴ have stated that adsorbed collagen layers on polymer films are highly hydrated, and the presence of adsorbed water at the interface can also contribute to the change in ΔD . In **Figure S4** we present the ΔD - Δf curves (5th harmonic) for PS and PSox at the two different collagen concentrations studied, observing two key features. Firstly, as mass is deposited on the polymer film (giving a decrease in frequency), there is a concomitant increase in *D*, indicating the non-rigid nature of the adsorbed layer. Secondly, adsorption onto PSox gives significantly larger ΔD values than adsorption onto untreated PS, regardless of collagen concentration. We interpret this result to be due to the affinity of water to our (now hydrophilic) polymer layer, changing the hydration state of the adsorbed material. Interestingly, there is a significantly larger change in ΔD at the high collagen concentration on PSox compared to the lower concentration, despite approximately the same change in Δf (see blue and green curves).



Figure S4. Simultaneous change in dissipation (ΔD) and frequency (Δf) of the fifth harmonic during QCM-D measurements of collagen adsorption onto polystyrene (PS) and plasma-treated polystyrene (PSox) at two different collagen concentrations. Data shown corresponds to PS ([collagen] = 2 µg mL⁻¹ (black curve) and 10 µg mL⁻¹ (red curve)) and PSox ([collagen] = 2 µg mL⁻¹ (green curve) and 10 µg mL⁻¹ (blue curve))

Due to significant changes in ΔD upon the adsorption of collagen under certain conditions, two models were used to determine the mass per unit area of adsorbed protein – the Sauerbrey model and the Voigt viscoelastic model.⁵ The Sauerbrey equation (see manuscript) calculates the change in mass on the crystal solely from the change in frequency, whereas the Voigt model takes into account the viscoelastic nature of the adsorbed material. Because of this it is generally accepted that the Voigt model will provide a more accurate measure of adsorbed mass on materials where the change in dissipation is significant.⁴

The data presented in **Figure S5** relates the adsorbed mass calculated by both the Sauerbrey and Voigt models for all flat polymer films studied in this work. We observe that the Voigt model, across all samples, estimates a larger adsorbed mass than the 'simpler' Sauerbrey equation. The variation between the two models is greatest at high collagen concentrations (see red data), which correspond to experiments where ΔD was typically large. The variation between the two models is always larger on PSox and PNVPox than their untreated counterparts, which again is a likely effect of the differing wettabilities of the substrates (changing the amount of adsorbed water within the protein layer). Generally, we consider the two models to be in reasonable agreement with one another, with the key trend of PS materials (plasma-treated or untreated) adsorbing more protein than their PNVP counterparts. For this reason, mass changes as determined by the Sauerbrey equation are presented within the manuscript for simplicity.





The information provided by using the Voigt model to fit QCM-D data to our polymer films allows a simple measure of the extent of hydration of the adsorbed layer. We present in **Figure S6** the adsorbed layer thickness as predicted by the Voigt model (at a collagen concentration of 10 μ g mL⁻¹), and the layer thickness calculated solely from the adsorbed mass of protein (i.e. that the collagen layer is 'bulk-like' with a density corresponding to the bulk material). We observe that the thickness of the adsorbed layer is typically twice as large as a 'dense' collagen layer, regardless of the substrate. This indicates that the adsorbed collagen is significantly hydrated during adsorption onto the polymer surface.



Figure S6. Calculated adsorbed layer thickness (by the Voigt model, purple column) of the collagen layer deposited on various polymer films at a concentration of 10 μ g mL⁻¹. Also shown is the estimated layer thickness (blue column) assuming a bulk-like collagen layer (the density of collagen was estimated to be 1.4⁴.)

QCM-D measurements were also performed for the adsorption of collagen onto patterned substrates produced by the dewetting of PS/PNVP bilayer films (see manuscript). Two different samples were studied – a film consisting of small uncorrelated holes of exposed PS within a PNVP background, and a highly dewetted network with a very high fractional surface area of exposed PS. The effect of plasma treatment on the amount of adsorbed collagen was studied for both substrates (see Figure S7). Two trends were observed, namely that the amount of adsorbed collagen increases with

increasing PS surface area, and that plasma-treated substrates adsorb more collagen than their untreated counterparts.



Figure S7. The mass of adsorbed collagen (as measured by QCM-D) onto micropatterned PNVP/PS substrates of varying pattern size at a concentration of 2 μ g mL⁻¹. Data is shown for untreated films (black columns) and plasma-treated films (striped columns). The adsorbed mass is determined from changes in the frequency of the fifth overtone of the QCM crystal via the Sauerbrey equation.

Surface Morphologies via the Dewetting of Polymer Bilayer Films

Recent work by our group⁶ demonstrated that upon thermal annealing above the glass transition temperature of PNVP, a thin PNVP film will dewet from a PS substrate, enabling the formation of surface micropatterns. The pattern hole size is a function of annealing temperature and time, with the additional effect that PNVP undergoes thermal cross-linking during the annealing process.⁷ Dewetting occurs via the nucleation and growth of uncorrelated holes, (**Figure S8**) and the final dewetted state achievable using PS-PNVP bilayers consists of a highly dewetted, polygonal network (**Figure S8F**). Dewetted PS-PNVP films do not decompose into a series of discrete PNVP droplets on a PS background due to the cross-linking of the PNVP layer, preventing complete dewetting from taking place.



Figure S8. Optical microscopy image sequence of the dewetting of a PS-PNVP bilayer film, annealed at 200 °C. Shown are A) initial film; B) after 30 seconds; C) after 60 seconds; D) after 90 seconds; E) after 120 seconds; F) after 180 seconds. All scale bars = 100 μ m.

Fluorescence Microscopy

Site-selective protein adsorption was also demonstrated using fluorescence microscopy via the adsorption of a fluorescently (FITC tagged) protein, bovine serum albumin (BSA), onto micropatterned surfaces. Micropatterned surfaces of varying pattern size were prepared and then exposed to a solution of FITC-BSA, with typical fluorescence microscopy images (and their white light counterparts) shown in **Figure S9**. We observe that the base of the PS holes (and the hole rims)

fluoresce an intense green, with the surrounding PNVP matrix appearing black. Adsorption of FITC-BSA occurs within the PS holes, regardless of micropattern size, visually demonstrating site-selective protein adsorption on our patterned surfaces.



Figure S9. White-light and fluorescence optical microscopy to visualize FITC-tagged BSA adhesion on patterned substrates. Shown here are: A) and B): small PS holes (diameter = $13.3 \pm 5.3 \mu$ m); C) and D) intermediate-size PS holes ($47.0 \pm 2.2 \mu$ m; E) and F) highly coalesced patterned network. The FITC-BSA concentration used was 500 µg mL⁻¹. All scale bars shown are 200 µm.

Examples of Endothelial Cell (EC) Morphologies on Flat and Micropatterned Substrates

EC morphologies after adhesion onto different types of flat and micropatterned polymer substrates after deposition of a mat-like collagen layers are shown in Figure S10. The difference in cell morphology on flat PSox and PNVPox films is shown in Figure S10A and Figure S10B. On flat PSox films (Figure S10A), a cluster of ECs can be observed displaying a flattened, cuboidal morphology. The cells are also non-reflective in appearance, which is consistent with viable cells attached to the substrate. In contrast, ECs on flat PNVPox films (Figure S10B) display a significantly elongated morphology and are also reflective in appearance. This observation is consistent with non-viable cells either not attached or in the process of detaching from the surface.

EC morphologies on micropatterned substrates are shown in **Figures S10C and S10D**. Cells displaying a flattened, non-reflective appearance within the micropatterned PS domains are shown in **Figure S10C**; there is one cell bound in each hole within the image. A rounded, reflective cell, considered unattached to the substrate, is highlighted by the arrow in **Figure S10D**.



Figure S10. Optical microscopy images of ECs adhered to different flat and patterned polymer substrates, following adsorption of a mat-like surface of collagen. A) ECs on a flat PSox film; B) ECs on a flat PNVPox film; C) and D) ECs on micropatterned substrates prepared by dewetting of PS-PNVP bilayer films. Cell adhesion within individual PS domains is shown in (C), while an example of an unattached EC is shown in (D) (highlighted by arrow). Scale bar in (A) and (B) = 50 μ m; scale bar in (C) and (D) = 25 μ m.

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