Fibronectin/polyelectrolyte multilayered assemblies: film formation and cell attachment studies

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Introduction

Biomimetic materials capable of specific interactions with a biological environment represent the current biomaterial paradigm within tissue engineering and drug delivery [1]. Electrostatically driven layer-by-layer (LbL) self-assembly [2,3] is a simple and robust method for realizing structurally tailored biomaterial coatings, of thickness ca. 10 nanometers, containing biofunctional ligands. We investigate the placement of fibronectin – a matrix protein useful in tissue engineering applications onto multilayered films formed by the alternate deposition of poly-l-lysine (PLL) and dextran sulfonate (DXS).

Experiment.

Cationic poly-l-lysine hydrobromide (mw= 70,000-150,000), anionic dextran sulfate (mw= 500,000) and Fibronectin were purchased from sigma. The layer-by-layer self-assemblies were built from polyelectrolytes solutions at 0.5 mg/ml (DXS), 0.4 mg/ml (PLL) and 40 μg/ml Fibronectin(FN) dissolved in hepes buffer. Human Umbilical Vein Endothelial Cells were purchased cryopreserved from Biowhittaker and were culture in medium with serum. Following multiplication and second cryopreservation, they were then used for an experiment at their first passage. In order to preserve the transmembrane cell adhesion protein integrin $\alpha_5\beta_1$ that interact with extracellular matrix protein Fibronectin and allow the activation of an attachment and spreading process, no trypsinization was used; instead PBS solution with 0.5% EDTA was employed. Optical waveguide lightmode spectroscopy (OWLS) was used to characterize film formation in situ. OWLS is an evanescent wave technique based on integrated optics [4] used to measure the adsorption of macromolecules at the waveguide/solution interface. More precisely, this technique enables us to determine the adsorbed density of every layer of polymer and protein as well as the thickness of every one of them. In this study $(PLL\text{-}DXS)_3$-FN and $(PLL\text{-}DXS)_2$-PLL-FN were constructed on SitiO2 (STO) waveguide coated sensor chipsand their impact on Human Umbilical Vein Endothelial Cells using optical phase contrast microscopy. Cells were in a medium which is serum free for the experiment and pictures of the cells were taken 30 min and 45 min after being seeded on the biofilms we are investigating. Measures of area and circularity were performed using NIH Image J software.

Results

As we show in Figs. 1 and 2, each layer of PLL or DXS is not identical during the build up; instead, the layers becomes denser and thicker indicating a exponential growth. This is in agreement with the fact that the polymers are weakly charged. The common interpretation for this phenomenon is the fact that instead of having a layer of polymer on the top of another one with a strict delimitation between them (as occurs for linear growth), here the layers evolve by a complexation of the two kinds of polymers [5]. We also observe that the Fibronectin density and

thickness on a (positively charged) PLL terminated film to exceed, by factors of 6.0 and 2.7 respectively, those on a (negatively charged) DXS terminated film; these results are consistent with predictions based upon electrostatics.

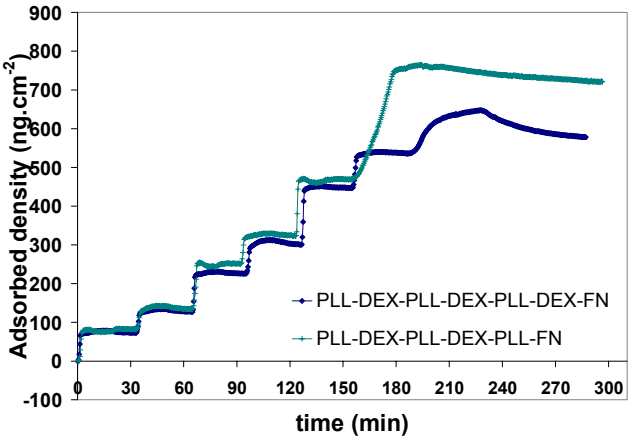

Figure 1

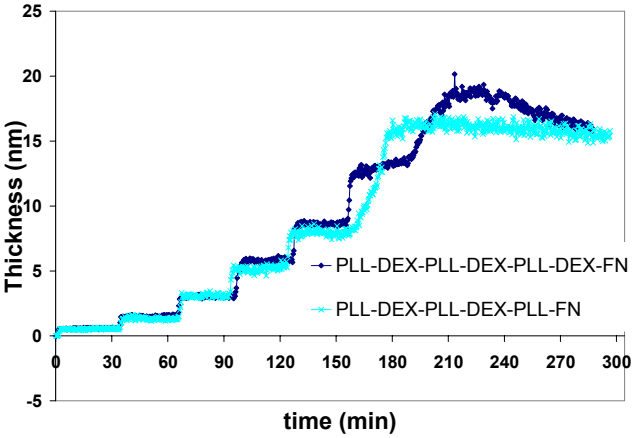

Figure 2

In table 1, we summarize the average area and circularity of the cells. The greater area is on the self-assembly $(PLL\text{-}DXS)_3$-FN. The average area doesn't change between 30 min and 45 min but the shape changes considerably; they are less round and more "edgy". The area on PLL and STO are comparable, the shape is per opposition very different, while on the WG they stay round, they become very edgy on PLL. On the $(PLL\text{-}DXS)_3$-FN, the area decreases considerably showing a strong retraction of the cells, while retracting, the cells becomes more elongated. On $(PLL\text{-}DXS)_3$, the cells don't spread, this is not surprising knowing the repellant properties of DXS. What's interesting is that FN on the top of this structure seems to be able to delay the effect of the DXS.

Table 1

	$(PLL\text{-}DXS)_3$-FN	$(PLL\text{-}DXS)_3$	$(PLL\text{-}DXS)_2$-PLL-FN	$(PLL\text{-}DXS)_2$-PLL-FN	STO
Area (μm) 30 min	2350 ± 185	--	2916 ± 200	2444 ± 146	2425 ± 182
Area (μm) 45 min	1746 ± 120	--	2853 ± 198	2250 ± 138	2217 ± 141
Circularity 30 min	0.51 ± 0.03	--	0.55 ± 0.02	0.60 ± 0.02	0.77 ± 0.02
Circularity 45 min	0.37 ± 0.03	--	0.50 ± 0.02	0.44 ± 0.02	0.70 ± 0.02

Conclusion

We investigate here HUVEC spreading on Fibronectin coated, polyelectrolyte multilayer films, while spreading is enhanced on PLL terminated films, cells lose their circularity much more rapidly on DXS terminated films, despite the presence of fewer Fibronectin molecules.

References

[1] J.A. Hubbel, Current opinion in biotechnology, 1999, 10, 123-129.
[2] Decher, G., science, 1997. 277, 1232-1237
[3] Decher G and Schlenoff JB. Eds. Multilayers thin films. 2003, Wiley-VCH: Weinheim.
[4] Tiefentaler,K.; Lukosz, W. J. Opt. Soc. Am. B. 1989, 6, 209-220.
[5] Picart et al, Proceedings of the national academy of sciences of the United States of America, 2002, 99, 12531- 12535