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Fibronectin Terminated Multilayer Films: Protein Adsorption and Cell Attachment Studies

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Abstract

Electrostatically driven layer-by-layer (LbL) assembly is a simple and robust method for producing structurally tailored thin film biomaterials, of thickness ca. 10 nanometers, containing biofunctional ligands. We investigate the LbL formation of multilayer films composed of polymers of biological origin (poly(L-lysine) (PLL) and dextran sulfate (DS)), the adsorption of fibronectin (Fn) - a matrix protein known to promote cell adhesion - onto these films, and the subsequent spreading behavior of human umbilical vein endothelial cells (HUVEC). We employ optical waveguide lightmode spectroscopy (OWLS) and quartz crystal microgravimetry with dissipation (QCMD) to characterize multilayer assembly in situ, and find adsorbed Fn mass on PLL terminated films to exceed that on DS terminated films by 40%, correlating with the positive charge and lower degree of hydration of PLL terminated films. The extent and initial rate of Fn adsorption to both PLL and DS terminated films exceed those onto the bare substrate, indicating the important role of electrostatic complexation between negatively charged protein and positively charged PLL at or near the film surface. We use phase contrast optical microscopy to investigate the time dependent morphological changes of HUVEC as a function of layer number, charge of terminal layer, and the presence of Fn. We observe HUVEC to attach, spread, and lose circularity on all surfaces. (Positively charged) PLL terminated films exhibit a greater extent of cell spreading than do (negatively charged) DS terminated films, and spreading is enhanced while circularity loss is suppressed by the presence of adsorbed Fn. The number of layers plays a significant role only for DS terminated films with Fn, where spreading on a bilayer greatly exceeds that on a multilayer, and PLL terminated films without Fn, where initial spreading is significantly higher on a monolayer. We observe initial cell spreading to be followed by retraction (i.e. decreased cell area and circularity with time) for films without Fn, and for DS terminated films with Fn. Overall, the Fn coated PLL monolayer and the Fn coated PLL terminated multilayer are the best performing films in promoting cell spreading. We conclude the presence of Fn to be an important factor (more so than film charge or layer number) in controlling the interaction between multilayer films and living cells, and thus to represent a promising strategy toward in vivo applications such as tissue engineering.

Keywords

fibronectin; poly(L-lysine); dextran sulfate; protein adsorption; endothelial cell; layer-by-layer

1. Introduction

Living cells receive important signals through biological recognition events at their surface. Biomaterials capable of mimicking these signals are potentially valuable as tissue engineering substrates, biosensing surfaces, and drug delivery vehicles. Materials must be capable of signal

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transmission and also meet stringent structural, mechanical, and degradation requirements - a scenario best realized through a coating approach where bulk material and surface properties are effectively decoupled. Multilayer nanofilms, formed by the layer-by-layer (LbL) method [1-7], are promising in this regard. LbL assembly occurs through the alternate adsorption of positively and negatively charged polyelectrolytes, and the resultant multilayer films can be rendered bioactive through the adsorption of protein or other biological molecules capable of transmitting signals to contacting cells [8-13]. Since only physical interactions are involved, LbL assembly represents a simple, controllable, and broadly applicable route to bioactive thin film materials.

Fibronectin (Fn) is an extracellular matrix protein known to promote cell attachment and spreading [14]. The mechanism is thought to involve attachment of $\alpha_5\beta_1$ transmembrane integrin receptors to Fn's cell binding site, located on the 10th type III repeat module and containing the specific amino acid sequence RGD, as well as to synergy sites located on the 8th and 9th type III repeats. Thus, materials coated with Fn are promising for a variety of cell contacting applications. Indeed, a number of studies attest to the enhanced cell attachment and spreading of surfaces coated with Fn, compared to identical materials in the absence of Fn [15-36].

Despite the promise of multilayer films in cell contacting applications, and the proven utility of Fn as a cell adhesion motif, only a few investigations of Fn coated multilayer films have appeared [37-41]. Ai et al. showed cerebellar neurons to attach, grow, and differentiate on silicone coated with multilayer films consisting of three bilayers of poly(styrene sulfonate) (PSS)/poly(ethylene imine) (PEI) and four bilayers of Fn/poly(D-lysine) (PDL) [37]. Interestingly, similar cell behavior occurred on silicone coated with a monolayer of PDL without any Fn. Ngankam et al. analyzed Fn adsorption to poly(allylamine hydrochloride) (PAH)/PSS films and found the extent of adsorption to be significant: films of mass 1.1 and 0.5 $\mu\text{g}/\text{cm}^2$ were observed on PAH and PSS terminated films, respectively, with considerable surface aggregation present in the latter system [38]. Kreke et al. investigated osteoblast adhesion strength, via a flow-induced detachment assay, onto Fn coated PAH/heparin sulfate films formed at various values of solution pH [39]. Although Fn adsorption was modest (always less than 0.01 $\mu\text{g}/\text{cm}^2$), they observed the force required for cell detachment to scale with quantity of adsorbed Fn. Li et al. found a Fn coating to enhance smooth muscle cell attachment and growth on a PAH/PSS film, and the degree of enhancement to increase with the number of layers [40]. In contrast, Olenych et al. found only a minor influence of adsorbed Fn on the spreading and motility of smooth muscle cells cultured on multilayer films ranging from cell adherent to cell resistant [41].

We investigate here the formation and cell attachment properties of Fn terminated multilayer films composed of the linear polyelectrolytes poly(L-lysine) (PLL) and dextran sulfate (DS). Films composed of biological polymers, such as polyamino acids and polysaccharides, are ideal for *in vivo* applications (e.g. tissue engineering) owing to their biological origin and biodegradability. We employ optical waveguide lightmode spectroscopy (OWLS) and quartz crystal microgravimetry with dissipation (QCMD) to measure the kinetics of LbL assembly and Fn adsorption, and phase contrast optical microscopy to analyze the attachment and spreading of human umbilical endothelial cells (HUVEC) in contact with multilayer films. Our goal is to understand the time-dependent morphological response of a model cell system - in contact with a multilayer film composed of polyelectrolytes of biological origin - in terms of number of polyelectrolyte layers, the charge of the terminal layer, and the presence of Fn.

2. Materials and Methods

2.1. Optical Waveguide Lightmode Spectroscopy (OWLS)

OWLS is a highly sensitive method (precision $\sim 1 \text{ ng/cm}^2$) for measuring macromolecular adsorption kinetics at the solid-liquid interface [42-45]. Detection is based on excitation of guided modes via a polarized laser light beam directed upon a grating coupler at the surface of an optical waveguide. The mass and thickness of an adsorbed layer can be related to changes in the guided modes through an optical model, such as one assuming an optically uniform adsorbed layer [46]. Solvent trapped within an adsorbed layer does not contribute to the layer mass calculated this way; in this sense, OWLS provides a measure of the “dry mass”. Our OWLS instrument (OWLS 110, MicroVacuum, Hungary) is composed of a parallel plate flow cell whose bottom surface is a OW 2400 Sensor Chip (MicroVacuum), consisting of a planar $\text{Si}_{1-x}\text{Ti}_x\text{O}_2$ waveguide ($x=0.25 \pm 0.05$) coated onto a glass substrate.

2.2. Quartz Crystal Microgravimetry with Dissipation (QCMD)

A QCM consists of a thin quartz disc sandwiched between a pair of electrodes. The resonant frequency of the crystal, when excited by an AC voltage, depends on the total oscillating mass, including coupled water. (QCM thus provides a measure of the “wet mass”.) A “soft” (viscoelastic) adsorbed layer will dampen the crystal’s oscillation [47-51]. The dissipation may be measured at multiple frequencies and by applying a viscoelastic model, the mass, thickness, elastic shear modulus, and shear viscosity of the adhering film can be determined. Our QCMD instrument (D300, Q-Sense, Sweden) is composed of a parallel plate flow cell whose bottom surface is a QSX 303 Sensor Chip (Q-Sense), consisting of a planar SiO_2 coating on a quartz crystal.

2.3. Cell Culture

Cryopreserved human umbilical endothelial cells (HUVEC) and media are purchased from Cambrex Bio Science (Walkersville). The complete medium is composed of Endothelial Cell Basal Medium supplemented with 1mL/L of human recombinant Epidermal Growth Factor at a concentration of 10 $\mu\text{g/L}$, 1mL/L Hydrocortisone at a concentration of 1.0 g/L , 1 mL/L of Gentamicin at a concentration of 50 g/L , 1mL/L of Amphotericin-B at a concentration of 50 g/L , 4 mL/L of Bovine Brain Extract at a concentration of 3 g/L , and 20 mL/L of Fetal Bovine Serum. The serum-free medium is composed solely of the Endothelial Cell Basal Medium. Prior to use, the HUVEC are propagated (1:20) and cryo-preserved a second time. HUVEC are then cultured at 37°C in 5% CO_2 in a small tissue culture dish of 10 cm^2 in complete medium until they reach 70%-90% confluence. They are then detached using PBS with 0.5% EDTA. To preserve the integrin receptors, no trypsin is used. The HUVEC are then centrifuged and diluted in serum-free medium before being introduced to LbL films.

2.4. Multilayer Film Formation/Protein Adsorption

Poly(L-lysine) (PLL) hydrobromide of MW 70,000-150,000, dextran sulfate (DS) of MW ca. 500,000, and fibronectin (Fn) are obtained from Sigma Chemical. Fig 1. gives the chemical formulas of these biological polymers. PLL, DS, and Fn are diluted in a buffer solution at the respective concentrations 0.4 g/L , 0.5 g/L and 0.04 g/L . The buffer is 10 mM N-[2-hydroxyethyl]piperazine-N'-ethanesulfonic acid (HEPES) and 100 mM NaCl adjusted to pH 7.4 by the addition of NaOH. To avoid bubble formation, the buffer is degassed in an ultrasonic bath for 25 min prior to use.

The same experimental protocol is employed for both OWLS and QCMD experiments. A sensor chip is washed with 2% Hellmanex (Hellma, Mulheim, Germany), a commercial glassware detergent, and then allowed to soak overnight in buffer. The sensor chip is then

rinsed with deionized water and introduced to the flow cell. A buffer solution is continuously introduced to the flow cell at a flow rate of 60 $\mu\text{L}/\text{min}$ until a stable baseline is achieved. A solution of PLL is then introduced for 12 min and followed by a buffer rinse of 20 min. A solution of DS is then introduced for 10 min and rinsed for 20 min. Each subsequent layer is introduced by a 10 min adsorption step and a 20 min rinsing step. The layer of Fn is adsorbed for 40 min and rinsed until desorption becomes negligible. In this study, we test a PLL monolayer, a PLL-DS bilayer, a PLL terminated multilayer, and a DS terminated multilayer, all with and without a final layer of Fn. As controls, we also examine the bare OWLS sensor chip surface with and without adsorbed Fn.

2.5 Cell Spreading

After film formation, the sensor chip is removed from the OWLS apparatus and, while maintaining hydration, placed in a Petri dish above an Inverted Optical Microscope (Olympus IX 71). A solution containing 1×10^4 HUVECs per ml are placed in the dish, the temperature is maintained at 37°C with a dish heater and the pH maintained at 7.4 by addition of 0.3% HEPES of concentration 1 M. The medium is covered with mineral oil to prevent evaporation. Images of the cells are taken 15, 45, 90 and 180 min after seeding using a Qimaging QICAM Digital Camera. The area and circularity of individual cells are obtained using Image J NIH software (circularity = $4\pi \cdot \text{area} / \text{perimeter}^2$). Cell position and cell motility are not measured.

3. Results

3.1 Multilayer Film Formation

Fig. 2A shows OWLS and QCMD measurements of film mass versus time for the LbL growth of PLL-DS films, as described in Section 2. OWLS provides a measure of the polymer contribution to the film mass (i.e. the “dry mass”); the increase in dry mass is essentially irreversible during both PLL and DS adsorption steps, and plateau levels scale roughly exponentially with layer number. (Schaaf, Voegel, and co-workers have recently linked exponential film scaling to intra-film mobility of at least one polyelectrolyte component [52, 53].) The (PLL-DS)₃ film has a thickness of about 10 nm (see Table 1). QCMD provides a measure of the polymer plus solvent contribution to the film mass (i.e. the “wet mass”); the wet mass significantly exceeds the dry mass following the second layer. The difference between the wet and dry masses is a reasonable measure of film hydration: films containing at least one DS layer are ca. 60% hydrated (see Fig. 2B). DS terminated films are modestly, but systematically and statistically significantly, more hydrated than PLL terminated films. The standard deviation of the mean change in film water content decreases rapidly with layer number. After three bilayers, the deviation is less than 1%; this result serves to demonstrate the negligible effect of the underlying substrate on thicker films.

QCMD also allows for determination of film visco-elastic properties. We find (PLL-DS)₂-PLL to have shear modulus 0.95 GPa and viscosity 8.8 mPa*s, and (PLL-DS)₃ to have shear modulus 0.49 GPa and viscosity 6.2 mPa*s. Based on these data (two-fold greater shear modulus, 40% greater viscosity), the PLL terminated multilayer is much more rigid than the DS terminated multilayer.

3.2 Fibronectin Adsorption

The adsorption of Fn to the bare silica titania (ST) sensor chip, a single PLL layer, a PLL-DS bilayer, a PLL terminated multilayer, and a DS terminated multilayer is compared in Fig. 3A. We observe the plateau mass of Fn adsorbed to PLL terminated films to be roughly 40% greater than that on DS terminated films. Fn adsorption to PLL-DS and (PLL-DS)₃ is nearly identical, while that onto (PLL-DS)₂-PLL exceeds slightly that onto PLL. (Comparing adsorption onto multilayer with that onto bilayer or monolayer films allows one to assess the degree of protein

penetration into the film, as demonstrated by Salloum et al. [54]. Our results therefore suggest little or no film penetration.) Interestingly, Fn adsorption onto DS terminated films exceeds that onto bare silica titania, despite the expected strong negative charge of the outer DS layer. We attribute this behavior to interaction with PLL chains near the film surface. The fractional degree of reversibility is significantly greater on DS than on PLL terminated films; the mass of irreversibly attached Fn is roughly double on PLL compared to DS terminated films. It is interesting to note the absence of a true plateau in many of the Fn adsorption curves. While polyelectrolyte adsorption is typically limited by charge build-up and thus tends to saturate abruptly, protein adsorption is controlled asymptotically by steric effects, so the approach to saturation is slower [55].

An example plot of the adsorption rate versus adsorbed mass is shown in Fig. 3B. As described previously, the extrapolated intercept of the linear, surface-limited regime provides a measure of the apparent adsorption rate constant, k_a' [55-57]. Table 1 lists values of k_a' determined in this way, and shows initial Fn adsorption to be nearly equally rapid on a PLL monolayer, a PLL terminated multilayer, and a DS terminated multilayer; to be somewhat lower on a DS terminated bilayer; and to be lower still onto the bare sensor chip. It is especially interesting to note the nearly identical initial adsorption rate onto PLL and DS terminated multilayers, despite the negative charge and hydrated nature of the latter. Based on this result, we conclude initial adsorption to be driven primarily by the rapid complexation of the negatively charged Fn with PLL at or very near the top of the films. (The high uncertainties in some of the measured rate constants may reflect their sensitivity to small fluctuations in the amount of intra-film PLL.)

3.3 Cell Spreading

The kinetics of HUVEC spreading on several substrates are shown in Fig. 4A, with particular emphasis on the influence of the terminal layer and the presence of Fn. To simplify the presentation, cell area is reported relative to the average area of HUVEC on the bare silica titania (control) surface following three hours. (The uncertainty represents the standard error of the mean.) The general trend in all of these systems is for cells to spread for the first 50-100 minutes, and then to cease spreading or even to retract somewhat. The highest degree of spreading is always observed with Fn-containing systems. The Fn layer without any polyelectrolyte exhibits the greatest spreading, reaching 140% of the control level and then remaining roughly constant. Multilayer films ending in PLL-Fn and DS-Fn exhibit strong initial spreading to about 120% of the control value at 100 minutes, after which the former plateaus and the latter exhibits significant retraction. Only modest spreading occurs on Fn-free multilayers: initial spreading fails to reach the control value and significant retraction follows. Interestingly, spreading on the PLL terminated multilayer is higher initially (during the first 50 minutes) in the absence of Fn. HUVEC generally spread to a greater extent on PLL versus DS terminated films. At 3 hours, only Fn alone and Fn on a PLL terminated multilayer induce significantly greater cell spreading than does the control surface.

Layer number plays a significant role only for DS terminated films with Fn, where spreading on a bilayer (Fig. 4B) greatly exceeds that on a multilayer (Fig. 4A), and PLL terminated films without Fn, where significant initial spreading occurs only for a monolayer (Figs. 4A and 4B). During the first 25 minutes, HUVEC spreading on a PLL monolayer is decreased in the presence of Fn.

The kinetics of shape change, as measured by circularity, for HUVEC on these surfaces is shown in Fig. 5. Since the cells are nearly circular (i.e. circularity=1) at $t=0$ for all experiments, the general trend is for cells to lose circularity fairly rapidly over 50 minutes and slowly thereafter. Exceptions are DS terminated films in the absence of Fn, where significant circularity loss continues up to at least 3 hours. As seen in Fig. 6, cells on the DS terminated

multilayer film appear to be more “star-shaped” than those on any other surface. All of the surfaces examined show greater circularity loss than the control (ST), and only the DS terminated multilayer exhibits circularity loss that is considerably larger than the other (non-control) systems. While Fn alone increases the circularity loss compared to the bare substrate, Fn with each polyelectrolyte film results in a reduced extent of circularity loss.

4. Discussion

We investigate the LbL film formation of a polyamino acid/polysaccharide multilayer film, the adsorption of the matrix protein Fn to films composed of various numbers of layers, and the time dependent morphological changes of a model cell system (HUVEC) as functions of layer number, charge of terminal layer, and the presence of Fn. The PLL/DS multilayer film grows exponentially with layer number, probably due to the intra-film mobility of PLL, and contains a significant quantity of water, most likely due to the hydrogen bonding capabilities of DS. Fn adsorption is greater and more irreversible on PLL than on DS terminated films, owing to the positive charge and lower degree of hydration of the PLL terminated films. Interestingly, the initial adsorption rate is nearly identical on PLL and DS terminated films, suggesting initial attachment to involve complexation with a common factor, probably PLL at or near the film surface. Fn adsorption differs only slightly between a PLL monolayer and a PLL terminated multilayer, and between a PLL-DS bilayer and a DS terminated multilayer, suggesting Fn to remain principally at the film surface. All films exhibit increased HUVEC spreading and decreased HUVEC circularity in the presence of Fn, and the Fn coated PLL monolayer and the Fn coated PLL terminated multilayer provide the greatest extent of cell spreading.

HUVEC spreading behavior can be understood in terms of multilayer film biofunctionality, hydration, charge, and rigidity. Clearly, cell interactions with Fn are playing a large role: all Fn containing systems exhibit HUVEC spreading up to at least 120% of the control value at some time during the experiment, while none of the systems without Fn reach this value. We attribute this effect to the well-known integrin binding mechanism [14]. However, the magnitude of the Fn influence differs depending on the composition of the film: we observe the enhancement to be greatest for DS terminated films. Film hydration appears to play a key role as well: HUVEC spreading on a weakly hydrated PLL monolayer significantly exceeds that on a more hydrated PLL terminated multilayer. In fact, cell spreading on a PLL monolayer is initially as strong as on any Fn containing film. The influence of terminal layer charge is also significant. Generally, a lesser degree of spreading occurs on (negatively charged) DS terminated films than on (positively charged) PLL terminated films. The initial inhibitory effect of Fn on PLL terminated films is likely a charge effect: Fn adsorption actually makes the film more negative. Along these lines, it is possible that the enhanced short time spreading on DS terminated films, in the presence of Fn, is partially due to its less negative charge (compared to DS). It should be stressed that the positive multilayer film considered here is also much more rigid than the negative one, so increased rigidity may at least partially explain the enhanced cell spreading on the positive film.

A few recent studies have addressed the issue of Fn-containing LbL films [37-41]. In light of these earlier efforts, our study is distinguished by its focus on multilayer film polymers of biological origin, and by its explicit consideration of the influence of layer number, terminal layer charge, and film hydration on the adsorption of Fn and the kinetics of cell spreading. A number of other studies address the more general question of LbL films as cell-contacting biomaterials [10,12,37,39,40, 58-70]. A typical goal is to control the attachment and spreading of cells contacting the films. In addition to matrix proteins like Fn, short peptides may also serve as integrin binding biochemical motifs. Using this latter approach, Berg et al. investigated the chemical grafting of the three amino acid sequence arginine-glycine-aspartic acid (RGD)

to the terminal layer of a multilayer film, and found an increased murine fibroblast adhesion with increased RGD density. Another strategy to control cell behavior involves the mechanical rigidity of the film. Richert et al. have shown the attachment and spreading of chondrosarcoma [62] and smooth muscle [63] cells to increase significantly when films were rigidified through a post-formation chemical cross-linking step. Mendelsohn et al. found the adhesion of murine fibroblasts to be increased on films with greater ionic cross-linking, and thus with a lower degree of hydration, as controlled through the formation pH [59]. Boura et al. found endothelial cell attachment to decrease with multilayer film hydration, and to decrease on a monolayer as compared to a multilayer film [61]. They also found the degree of intracellular FAK phosphorylation to be unchanged on certain multilayer films, suggesting these films to promote an undisturbed transduction of the adhesion signal (an impressive sign of film biocompatibility) [70]. Our observation of enhanced HUVEC spreading on PLL monolayer compared to PLL multilayer films is consistent with these earlier observations. An interesting question is how mechanical properties and biochemical signaling work together: Picart et al. investigated the influence of chemical cross-linking together with RGD functionalization and found both to be important factors in the adhesion of primary osteoblasts [12]. Indeed, the significant enhancement of spreading due to Fn on our weakly adherent DS terminated films demonstrates how these factors together govern cell behavior. Finally, another strategy to control cell behavior is through film hydrophobicity: Salloum et al. found the adhesion and spreading of smooth muscle cells to increase with multilayer hydrophobicity as controlled through polymer chemistry, with the weakest adhesion and highest cellular mobility on a novel zwitterionic film [69].

Our observation of enhanced cell spreading on polycation-terminated films does not appear to be universal. While Richert et al. found increased cell adhesion on polycation-terminated films [62,63], Kidambi et al. found the strongest hepatocyte attachment and spreading on negatively charged, sulfonated polymer terminated multilayers [64]. Other studies report no terminal layer effect [59,67]. For example, Hwang et al. show monocyte adhesion and cytokine activation to be identical on polycation- and polyanion-terminated multilayer films [67].

The mechanism of Fn-modulated cell spreading is thought to involve attachment of its cell binding site, located in the 10th type III module, and its synergy sites, located in the 8th and 9th type III module, to $\alpha_5\beta_1$ integrin receptors within the cell membrane [14]. Fn's cell binding site consists of the amino acid sequence arginine-glycine-aspartic acid (RGD), and many efforts have been made to biofunctionalize surfaces through the chemical grafting of RGD sequences [71-80]. Materials presenting the entire Fn molecule, such as the Fn terminated multilayer assemblies described herein, may however be preferable to these chemically modified surfaces for reasons discussed previously [81-85]. First, many different integrins bind to RGD, but due to the presence of the synergy site, Fn bind preferentially to the $\alpha_5\beta_1$ integrin (leading to the formation of fibrillar adhesion sites and ultimately to the generation of traction forces). Second, cells may physically modulate the biochemical influence of Fn. For example, mechanical stretching can reveal hidden, cryptic sites within the protein, contributing to fibril formation, or alter the conformation of the RGD loop or its distance from the synergy site, affecting integrin binding. Although ours is not a detailed study of Fn's biochemical signaling mechanism, the significant influence observed here for Fn, immobilized within a multilayer architecture, on cell morphology suggests its effectiveness in transmitting signals to contacting cells.

5. Conclusion

We investigate the LbL formation of a multilayer film composed of polyelectrolytes of biological origin; the adsorption of the matrix protein Fn to these films; and the morphological response of a model cell system in terms of number of polyelectrolyte layers, the charge of the

terminal polyelectrolyte layer, and the presence of Fn. We find Fn to adsorb more strongly to (positively charged and less hydrated) polycation terminated films, and for cells to generally spread - to a greater extent and more symmetrically - on films coated with a layer of Fn. Cell retraction may follow initial spreading, and is most pronounced in the absence of Fn or for DS terminated films. Fn coated multilayer films composed of biological polymers thus represent a promising strategy for modulating cell interactions with materials, a capability that may be useful for *in vivo* applications such as tissue engineering.

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(a)

(b)

Figure 1.

Chemical formulas of the biological polyelectrolytes employed here to construct multilayer films: (A) anionic dextran sulfate (DS) and (B) cationic poly(L-lysine) (PLL).

Figure 2.

A) Film mass versus time during LbL formation as measured by optical waveguide lightmode spectroscopy (OWLS) and quartz crystal microgravimetry with dissipation (QCMD). The former (latter) being a measure of "dry mass" ("wet mass"), i.e. with solvent excluded (included). B) Change in the percentage of film mass attributable to water, upon placement of a new layer, versus layer number. Water mass is estimated by the difference between the "wet mass" and the "dry mass".

Figure 3.

(A) Mass versus time for fibronectin (Fn) adsorbed to the bare silica-titania sensor chip (ST), to a monolayer of poly(L-lysine) (ST-PLL), to a poly(L-lysine)/dextran sulfate bilayer (ST-PLL-DS), to a poly(L-lysine) terminated multilayer (ST-(PLL-DS)₂-PLL), and to a dextran sulfate terminated multilayer (ST-(PLL-DS)₃). The nearly identical adsorption to a PLL terminated multilayer and monolayer, and to a DS terminated multilayer and bilayer, suggest little or no penetration of Fn into multilayer structures. (Arrow 1 points to the desorption curve of Fn on ST-PLL-DS, and arrow 2 points to that of Fn on ST-(PLL-DS)₃.) (B) An example curve of adsorption rate versus adsorbed mass, as measured by OWLS. The line represents a best fit to data in the initial surface limited regime, the intercept being proportional to the adsorption rate constant k_a .

Figure 4.

The average normalized area of HUVEC versus time in contact with silica titania (ST), a Fn layer on silica-titania (ST-Fn), a poly(L-lysine) monolayer on silica-titania (ST-PLL), a Fn layer on a poly(L-lysine) monolayer (ST-PLL-Fn), a poly(L-lysine)/dextran sulfate bilayer (ST-PLL-DS), a Fn layer on a poly(L-lysine)/dextran sulfate bilayer (ST-PLL-DS-Fn), a poly(L-lysine) terminated multilayer (ST-(PLL-DS)₂-PLL), a Fn layer on a poly(L-lysine) terminated multilayer (ST-(PLL-DS)₂-PLL-Fn), a dextran sulfate terminated multilayer (ST-(PLL-DX)₃), and a Fn layer on a dextran sulfate terminated multilayer (ST-(PLL-DX)₃-Fn), as measured by phase contrast optical microscopy. Normalization involves dividing the average area by that on the ST (control) surface after 3h.

Figure 5.

The average circularity of HUVEC versus time in contact with various multilayer systems (see legend and caption of Fig. 4), as measured by phase contrast optical microscopy.

Surface	Schematic	Picture	Area (%)	Circularity
ST			100	0.77 ± 0.03
ST-Fn			135 ± 1	0.55 ± 0.01
ST-PLL			101 ± 3	0.53 ± 0.07
ST-PLL-Fn			122 ± 2	0.60 ± 0.02
ST-PLL-DS			83 ± 2	0.46 ± 0.03
ST-PLL-DS-Fn			108 ± 16	0.59 ± 0.06
ST-(PLL-DS) ₂ -PLL			82 ± 8	0.52 ± 0.03
ST-(PLL-DS) ₂ -PLL-Fn			120 ± 7	0.59 ± 0.02
ST-(PLL-DS) ₃			79 ± 2	0.33 ± 0.03
ST-(PLL-DS) ₃ -Fn			93 ± 9	0.63 ± 0.03

Figure 6.

Phase contrast optical microscopy images of human umbilical endothelial cells (HUVEC) seeded onto various multilayer systems for 3 hours (see legend of Fig. 4). Imaged cells are of roughly the average area and shape factor. Schematics of the protein/polyelectrolyte films are also shown. Images are $1.25 \mu\text{m}$ by $1.25 \mu\text{m}$.

Thickness of poly(L-lysine)/dextran sulfate multilayer film, thickness and mass of fibronectin (Fn) layer, percent Fn mass loss during buffer rinse, and initial Fn attachment rate constant. ST = silica titania (the bare sensor ship), PLL = poly-L-lysine, DS = dextran sulfate, and Fn = Fibronectin.

Table 1

Multilayer Film	Polyelectrolyte Film Thickness (nm)	Fn Layer Thickness (nm)	Fn Layer Mass (ng/cm ²)	% Fn Mass Loss During Buffer Rinse	Fn Adsorption Rate Constant k_a , (10 ⁻⁵ cm/s)
ST-Fn	--	12 ± 2	234 ± 15	9.8 ± 0.3	6.2 ± 0.8
ST-PLL-Fn	0.5 ± 0.1	11 ± 1	492 ± 6	3.9 ± 0.7	18 ± 4
ST-PLL-DS-Fn	1.1 ± 0.1	18 ± 3	262 ± 43	20.7 ± 3.6	11.0 ± 0.4
ST-(PLL-DS) ₂ -PLL-Fn	6.9 ± 0.7	16 ± 1	502 ± 8	9.4 ± 1.9	20 ± 6
ST-(PLL-DS) ₃ -Fn	11.5 ± 2.5	22 ± 8	232 ± 51	30.2 ± 7.8	19 ± 8