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Discovery of Low Mucus Adhesion Surfaces

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Abstract

Mucus secretion from the body is ubiquitous and finding materials that resist mucus adhesion is a major technological challenge of medical and consumer import. Here, using a high throughput platform (HTP) with photo-induced graft polymerization, we first rapidly synthesized, screened and tested a library of 55 different surfaces from six functional monomer classes to discover porcine intestinal low mucus adhesion surfaces using a 1 hr static mucus adsorption protocol. From this preliminary screen, two chemistries, a zwitterionic ([2-(acryloyloxy)ethyl] trimethylammonium chloride) and a multiple hydroxyl (N-[tris(hydroxymethyl)methyl]acrylamide) surface, exhibited the significantly low mucus adhesion from a Langmuir-type isotherm when exposed to increasing concentrations of mucus for 24 hr. Apolar or hydrophobic interactions were likely the dominant attractive forces during mucus binding since many polar or hydrophilic monomers reduced mucus adhesion. Hansen solubility parameters were used to illustrate the importance of monomer polarity and hydrogen-bonding in reducing mucus adsorption. For a series of PEG monomers with changing molecular weight from 144 g/mol to 1100 g/mol, we observed an excellent linear correlation ($R^2 = 0.998$) between relative amount adsorbed and the distance from a water point in a specialized HSP plot, emphasizing the role of surface-water interactions for PEG modified surfaces.

Keywords

Low mucus adhesion; high throughput; UV-induced graft polymerization; membrane surface; monomer

Introduction

Mucus is a viscoelastic secretion (glycoprotein, proteoglycans and lipids) that coats epithelia cells as a protective barrier and lubricative layer [1, 2]. It is mainly composed of lipids, proteins, mucin and DNA [3]. Mucus is present in many apertures (noses, eyes, mouth, vagina etc.) of the body. It is semipermeable for exchanging of nutrients and water and blocking bacteria and pathogens [1, 2]. Mucus excretion at various epithelial surfaces of the body has important medical and technological significance. Medically related examples

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include cervicovaginal mucus impact on fertility, ocular mucus lubrication and potential adherence to contact lenses, and respiratory mucus protection of underlying epithelium from foreign particles, and possible obstruction of airways in respiratory disorders. Control of mucus adhesion on solid substrates can play a significant role in enabling effective treatments. Cystic fibrosis (CF) is a genetic disease in which the body's exocrine glands secrete an excess amount of mucus. DNA from necrotic cells contributes to mucus "adhesiveness" or viscosity in CF patients. Removing highly viscous adhesive mucus from the throat is challenging, as mucus adheres strongly to the suction tubes used by pulmonologists (Dr. Robert Brown, Mass. General Hospital, Boston, private communication).

The technological significance of mucus is impacted by the high viscosity of mucus (10,000-fold higher than water) and its adhesion to surfaces through hydrophobic interactions. These properties limit the efficacy of nanoparticle-based drug and gene delivery, for example to the gastrointestinal tract or the lungs [3-6]. Several modified surfaces functionalized with polyethylene glycol (PEG), acid and amine were developed to reduce mucus adhesion and improve the transport of the nanoparticles [3-6]. Crater and Carrier [4] found that carboxylate, sulfate, and amine modified polystyrene nanoparticles exhibited diffusion coefficients in gastrointestinal mucus that were 20-40 fold reduced compared to those in water, and highly dependent on particle surface properties. Large industrial companies produce a series of products that would benefit from repelling/attracting mucus. These include throat suction tubes, tampons/pads, nasal inserts and other devices that need to either repel or attract mucus. Little is known on how to choose surfaces rationally with these goals in mind, so, in this study, we report on a high throughput search to find such surfaces at short and long times.

Previously, in order to mitigate membrane fouling during synthetic membrane filtration, we developed a surface modification technique, photo-induced graft polymerization (PGP) [7-11]. The advantage of using this surface functionalization approach is that in place of developing new bulk materials, which involve great expense, one can modify the surfaces of known materials without changing their bulk physical properties. The strategy of our earlier work was to modify material surfaces with graft polymers so as to reduce the affinity between protein and material surface. Together with a novel high throughput platform (HTP), we rapidly synthesized and screened a large number of polymer membrane surfaces for repelling natural organic matter and separately for protein resistance [12-14]. The surfaces grafted with PEG, amine and zwitterionic monomers exhibited much lower protein adhesion and hence fouling (plugging) than the control or as-received poly(ether sulfone) (PES) filtration membrane [12-14].

In this work, we adopt this rapid method (HTP-PGP) to synthesize, screen and test novel low mucus-adhesive surfaces from a 55-monomer library (hydroxyls, PEGs, acids, amines, basic&zwitterionics, methacrylates) from 1 hr mucus adsorption. In order to compare the mucus-resistant difference for the monomer classes, we also tested the adsorption behavior of the 5 winners from each monomer class for 24 hr mucus adsorption. As expected the order of best resistance was changed due to different adsorption behavior. We explained the results in terms of Hansen solubility parameters and showed the importance of monomer polarity and hydrogen-bonding in reducing mucus adsorption, especially for PEG monomers.

Experimental

Materials

96-well filter plate—Polypropylene 96-well filter plates (Seahorse Labware, Chicopee, MA) were used in the HTP-PGP experiment. 100 kDa molecular weight cut-off PES membrane coupons (effective area 19.35 mm²) were mounted and sealed by the manufacturer on the bottom of each of the 96 wells (400 µl volume). The membranes were washed several times with de-ionized (DI) water and soaked in DI water overnight before use in order to remove surfactant. The hydraulic resistance of the 96 membranes ranged from 8.12×10¹¹ to 9.49 ×10¹¹ m⁻¹ with a coefficient of variation equal to 4.0% [12-14].

Monomers—55 commercial vinyl monomers (Fig. 1) were purchased from Sigma-Aldrich (Saint Louis, MO) and were used without further purification. The name, structure, and molecular weight (MW) of these monomers are shown in Table S1 (Supporting Information). Except for monomer #4, which used water/ethanol 80/20 wt %/wt %, and #s 44-55, which used water/ethanol 20/80 wt %/wt %, the other monomers used only water as a solvent.

Collection and preparation of native mucus samples—Native porcine intestinal mucus was collected from pig jejunum within 2 hr of slaughter. Pig intestines (Research 87Inc, Boylston, MA) were acquired from a local abattoir and stored on ice prior to mucus collection. The jejunum was isolated and cut open, and any bulk waste material was removed. Mucus was gently scraped with a spatula and stored at -80°C until experimentation. The mucus sample was thawed for approximately 30 minutes at 37°C temperature before incubation with the modified membrane surfaces. It was then reconstituted in PBS and mixed by vortexing to prepare mucus solutions with concentrations of 0.5, 1, 2, 4, 8, 10, 15, 20, 25 and 30 mg/l. The PBS contained 10 mmol L⁻¹ phosphate buffer, 2.7 mmol L⁻¹ potassium chloride, and 137 mmol L⁻¹ sodium chloride with pH 7.4 at 25°C.

Methods

HTP UV-induced graft polymerization—As PES is intrinsically photoactive, the radicals can be generated on the PES membrane surfaces by breaking the chemical bonds of sulfone groups which initiate the graft polymerization (Fig. S1). For the HTP grafting (Fig. S2), the PES membranes at the base of each well of a 96-well filter plate (19.35 mm²) were covered with 200 µl of monomer solutions for 1 hr while shaking at 22 ± 1°C. The monomer solutions were removed and the filter plates were sealed in a glass container, and then nitrogen gas was purged for 10 min to remove oxygen. Then UV irradiation was applied in a chamber (F300S, Fusion UV Systems, Inc. Gaithersburg, MD) containing an electrode-less microwave lamp (~ 7% of the energy was at < 280 nm). A band-pass UV filter (UG-11, Newport Corporation, Franklin, MA) was placed between the 96-well filter plate and the UV lamp to reduce the energy at wavelengths below 280 nm to < 1% of the exposed wavelength [11]. After photo-grafting, the membranes were washed with DI water for 24 h to remove homopolymer and unreacted monomer residue from the membranes [10].

Protein adsorption and assay—100 µl of native mucus solution was added to each well of the 96-well filter plates, and the plates were incubated on an orbital shaker for 1 hr (non-equilibrium) or 24 hr (equilibrium) at 100 rpm mixing rate. In order to remove the free mucus from the surfaces, 100 µl of PBS solution was added into each well, and the plates were shaken using a plate reader for 15 s. The 200 µl of mucus/buffer mix was then aspirated out of the wells by vacuum. Two additional rinses with 200 µl of PBS each were

applied and then aspirated from each well. The amount of mucus adsorbed, q (mg/cm²), was calculated as:

$$q = (C_0 - C_t) \times V/A \quad (1)$$

where, C_0 (mg/ml) is the initial mucus concentration, C_t (mg/ml) is the mucus concentration after adsorption, V (ml) is the volume of the mucus solution and A (cm²) is the effective area (0.20 cm²) of each membrane in the 96 well filter plate. C_t was measured using a bicinchoninic acid (BCA) assay kit (Pierce, Rockford, IL). Simply, the mucus solution after adsorption was pipetted from the 96 well filter plate and dipped into a UV transparent 96-well received plate (Costar 3635, Corning, Lifescience, Lowell, MA). A Microplate Spectrophotometer (PowerWave XS, BioTek Instruments Inc., Winooski, VT) was used to measure the absorbance at 562 nm of the mucus solution in the receiver plate wells, from which C_t in each receiver well was calculated.

The adsorption ratio, q is defined as the amount of mucus adsorbed on the modified PES membrane surfaces ($q_{\text{mucus, mod}}$) to that on the as-received or control surface ($q_{\text{mucus, control}}$). q for each monomer was obtained four quadruplicate in average for both non-equilibrium (1 hr) and equilibrium (24 hr) measurements. Mucus-surface affinity is evaluated based on the Langmuir adsorption isotherm [15]:

$$q = \frac{q_s K [C]}{1 + K [C]} \quad (2)$$

Where q (mg/cm²) is the amount adsorbed, $[C]$ (mg/ml) is the concentration of mucus solution, and a saturated amount of adsorption q_s (mg/cm²) and the binding constant K (ml/mg) indicated the affinity of adsorbent to adsorbate.

Surface structure and degree of grafting (DG) by ATR/FTIR—Attenuated Total Reflection/Fourier Transform Infrared spectroscopy (ATR/FTIR, Magna-IR 550 Series II, Nicolet Instruments, Madison, WI) was used to characterize the surface structure of the PES membrane. Penetration of the IR beam was ~ 0.1 to 1.0 μm . DG is defined as the ratio of the absorbance peak height at ~ 1735 cm⁻¹ (carbonyl stretching) of grafted monomer, to a reference peak at ~ 1578 cm⁻¹ of the benzene carbon-carbon double bond of PES membrane.

Hansen solubility parameters—Hansen solubility parameter (HSP) are defined as the square root of the cohesive energy density of a molecule and used here to characterize the physical properties of the chemistry of modified surfaces. HSP consists of three components (i) “non-polar” or dispersion interactions (δ_d), (ii) “polar” or permanent dipole-permanent dipole interactions (δ_p), and (iii) hydrogen bonding interactions (δ_h). δ_d , δ_p , and δ_h . HSPs are estimated from the molecular physical properties of each molecular group in a monomer. This approach is considered a group contribution method [16, 17]. In an attempt to understand the role of water in protein (mucus) adhesion to surfaces, we plot, the polar and hydrogen bonding components, $(\delta_p^2 + \delta_h^2)^{1/2}$ versus the dispersion (non-polar) component, δ_d , for the grafted monomers and water. The distance, $d_{\text{M-H}_2\text{O}}$, between the data points for the monomers and that for water is a measure of hydrophilicity of the surface chemistry.

Results

First, we synthesized and screened the full 55-member monomer library, then selected the low mucus adhesive surfaces after 1 hr mucus adsorption (non-equilibrium), and followed

this with an equilibrium adsorption study (24 hr exposure to mucus) at different mucus concentrations to obtain Langmuir-type isotherms for 5 “winner” from each monomer class. Next, we interpreted the results using Hansen solubility parameters and suggest that the reduction of mucus adsorption is related to the surface-water interactions.

Synthesizing, testing and selecting low mucus adhesion surfaces: Non-equilibrium adsorption for 1 hr

In Fig.2, the normalized amount of mucus adsorbed onto each of the 55 modified surfaces was compared with that on the unmodified PES membrane surface (control). Modified surfaces with $q < 1$ exhibited reduced mucus adsorption. For hydroxyl monomers (#s 1-4), monomer #3 showed significantly reduced mucus adsorption (~30% below the control). This seems reasonable because of its three exposed hydroxyl groups and strong hydrophilic character. This is consistent with the behavior of sugar functionalized self-assembled monolayers (SAMs) containing hydrogen-donors (–OH groups) that reduce fibrinogen adsorption [18]. For the surfaces grafted with the PEG monomers (#s 5-11), the structural difference between these monomers was the number of the ethylene glycol (EG) repeat units. The surfaces grafted with the repeated 8 (#8), 22 (#9) and 9 (#11) EG units showed ~20-30% less mucus adsorption than the control. The surfaces grafted with acid monomers (#s 13-23), all except monomer #22, exhibited reduced mucus adsorption by ~10-50% compared to the control. These results are explained as followed:

- i. The improved hydrophilic or swelling character of the surface after grafting. For example, when the solution at pH 7.4 was above the pKa (4.28) of poly (acrylic acid) (PAA), the carboxyl groups of PAA have strong interactions with water [28]. This resulted in greater swelling of the grafted PAA [29]
- ii. The most dominant component of mucus is mucin [19], which is negatively charged at pH 7.4 [20, 21]. Hence, the repulsion between the negatively charged surfaces and the negatively charged mucus were electrostatic in nature.

PES membrane surfaces grafted with most amine monomers showed reduced mucus adhesion. About 30-50% of mucus adsorption was below the control for monomer #s 23, 26, 28, 29, 34, 35 and 36. Except for monomer #42, all the basic & zwitterionic monomers resulted in reduced mucus adsorption (~25-30% below the control). These results are consistent with previous results indicating that zwitterionic SAM surfaces reduced protein adsorption [22-27].

As expected, membrane surfaces grafted with the hydrophobic methacrylates (#44-45) showed high amounts of mucus adsorption. The high level of mucus adsorption with monomer #51 (contains a thiol group) possibly resulted from a thiol–disulfide interchange reaction between the surface and the mucus [28, 29].

Characterization of selected monomers: Non-equilibrium adsorption for 1 hr

The adsorption ratio after 1 hr of exposure to mucus (i.e. non-equilibrium measurements), degree of grafting and solubility parameters of the five best performing modified membrane surfaces and the two worst performing modified surfaces are shown in Table 1. The five best surfaces from each class that exhibited reduced mucus adhesion (~ 40-60% below the control) with the small DG values (~0.1-0.2) were selected for further analysis. Our previous work suggested that the interactions (polar and hydrogen-bonding) of monomers with water play an important role in protein-resistance [17]. Such interactions can be characterized with the Hansen solubility parameters (HSP). Here, monomer #s 3, 9, 16 and 28 display higher values of both δ_p and δ_h or higher values of δ than that of the monomer #s 51 and 53. Hence, the low amount of mucus adsorption on a surface could be attributed to

the strong surface-water interactions. Much research has suggested that protein resistance is mainly due to surface-water interactions [30-33]. To further understand the mechanism of reduction of mucus adsorption, δ_d vs. $(\delta_p^2 + \delta_h^2)^{1/2}$ for monomers (except zwitterionic #s 37-43, as literature values are not available) and water is plotted in Fig. 3. Details are discussed below.

Mucus adsorption isotherms: Equilibrium adsorption for 24 hr

In Fig. 4, adsorption isotherms at $22 \pm 1^\circ\text{C}$ for the five best performing low mucus adhesion surfaces exposed to the mucus solution for 24 hr were reported. All these surfaces exhibited reduced mucus adsorption when compared with the control surface at a mucus concentration of 0.5-30 mg/ml. The equilibrium mucus adsorption on the surfaces was in the order of Control > #9 > #28 $\approx$ #16 > #3 > #43.

Discussion

Most surfaces grafted with hydrophilic monomers showed lower mucus adsorption than those grafted with the hydrophobic methacrylates and the control surfaces (Fig. 2). PES membranes are relatively hydrophobic with a sessile contact angle of $\sim 52^\circ$ [17] and mucus contains hydrophobic protein domains [1, 3], an increase in polar character (hydrophilicity) of most modified PES surfaces is responsible for the reduction of adsorbed mucus. These results are consistent with a number of the previous reports in which hydrophilicity is mainly considered to be the cause of reduced protein adsorption [34-38].

The hydrophobic methacrylates (#44-55) showed the largest distance from the water coordinate among the monomers (Fig. 3). The closer to the water coordinate for the other monomers resulted in reduced mucus adsorption. Hence, the weakest interactions with water for the methacrylate-modified surfaces correlates with their high mucus adsorption, and increased surface-water interactions resulted in reduced mucus adhesion. In Fig. 2, the amount of mucus adsorption showed a dependence on size of PEG monomers (#s 5-9). This connects with the intensity of PEG-water interactions (Fig. 3). As PEG monomers have weak interactions with water (slightly higher than methacrylates but much lower than hydroxyls), we speculate that the helical structure and flexibility of the grafted PEG chains possibly explain their reduced mucus adsorption amount [39, 40].

Except for the above five best listed surfaces (marked by circles in Fig. 3), the other surfaces with comparable q which were grafted with monomer #s 12, 14, 21, 23, 26 and 35 (marked by hexagons in Fig. 3) had high δ values of 0.55, 0.52, 0.46, 0.84, 0.44, and 0.54, respectively. These values are higher than maximum δ value (0.39 for #44) among the hydrophobic methacrylates. Though there are no referenced HSP values for quaternary amine within basic&zwitterionic monomers (#s 37-43) which had values of $q < 1$ (except #42), we expect that their HSP and δ values would be large and would contribute to strong surface-water interactions. Therefore, HSP characterization of selected monomers demonstrates the importance of surface-water interactions for reducing mucus adhesion [30, 31]. The hydration layer generated by surface-water interactions prevents mucus from approaching the surface [33].

We plotted the adsorption ratio, q , against d_{M-H_2O} for five classes of monomers (PEG acid, hydrophobic, amine and hydroxyls) and found excellent correlation for only the PEG data (Fig. 4). Although the correlations were poor for the other classes, the data for the amines and the acids increased with positive slopes. Too few data were available for the hydroxyls (only 4 data points) to observe a trend and, not unexpectedly, the hydrophobic data were clumped without a clear trend.”

By plotting q^{-1} (cm^2/mg) versus $[C]^{-1}$, the values of q_s and K were obtained from the intercept and slope of the linear curve, respectively. At low mucus concentration, $K[C]$ is much less than one in the denominator. Hence, q is linearly related with $[C]$. The initial slope of adsorption isotherms (Fig. 5), $q_s K$ (ml/cm^2), indicates the potential resistance of the surfaces to mucus adsorption. In Table 2, the resistance ($q_s K$) decreased in the order of #43 > #3 > #28 $\approx$ #9 > #16 > control. Furthermore, all these five surfaces had lower binding affinity with mucus than with the control surface (Table 2). The surfaces grafted with monomer #43 had the lowest binding constant, K and the lowest q_s . The surface grafted with monomer #3 had the slightly higher values of K and q_s . The five best monomer modified surfaces and the control surface showed lower q_s than that at the mucus concentration of 30 mg/ml (Table 2). Since the values of q_s from the Langmuir model which assumes a monolayer adsorption, is lower in all cases than the amount adsorbed (q) from solution (Table 2), one can assume that multilayer adsorption of mucus occurred during 24 hrs exposure on the polymer surfaces.

Conclusions

A series of new polymer surfaces that exhibited minimum mucus adhesion were selected by high throughput UV-induced graft polymerization method. The grafted polymeric surfaces that exhibited the low porcine mucus adhesion were screened and tested with the HTP-PGP method. Hydrophilic surfaces grafted with PEGs (#s 8, 9 and 11), basic & zwitterionic (#s 37-41 and 43), hydroxyls (#3), acids (#s 13-23) and amines (#s 23, 26, 28, 29, 34, 35 and 36) displayed lower mucus adsorption than the control surface. After exposure to 0.5-30 mg/ml of mucus for 24 h, the five best surfaces exhibited significantly lower binding affinity with mucus than the control surface. The surface grafted with monomer [2-(acryloyloxy)ethyl]trimethyl-ammonium chloride(#43) exhibited both the lowest binding affinity with the mucus and the lowest amount of saturated mucus adsorption. Another alternate monomer with excellent anti-mucus properties is N-[tris(hydroxymethyl)methyl]acrylamide (#3). The structure-properties relationships from the Hansen solubility parameters indicate that reduced mucus adhesion is mainly due to surface-water interactions (polar and hydrogen-bonding) and possibly the flexibility of graft polymer chains. The amount of adsorbed mucus showed an excellent linear correlation with distance d_{M-H_2O} ($R^2 = 0.998$) and hence the intensity of surface-water interactions for PEG modified surfaces.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
Monomer library for HTP UV-induced graft polymerization. 55 monomers from 6 classes are shown.

Fig. 2.

Normalized adsorbed amount of mucus ($q = q_{\text{mucus mod}}/q_{\text{mucus control}}$) for a 1 hr mucus adsorption period for different modified PES membranes in a 96-well filter plate.

$q_{\text{mucus control}} = 0.46 \pm 0.03 \text{ mg/cm}^2$. $T = 22 \pm 1^\circ\text{C}$. The as-received PES membrane was used as the control. Four replicates in average for were used for each sample. The error bar is the standard deviation for each sample.

Fig. 3.

Hansen solubility parameters (HSP) of δ_d vs. $(\delta_p^2 + \delta_h^2)^{1/2}$ of tested monomers and water. HSP of monomers were calculated using group contribution method. HSP of water was derived from a correlation of total miscibility of the given solvents with water, and was adopted from Hansen [41].

Fig. 4.

Distance, d_{M-H_2O} , from a PEG point to the water point shown in Fig. 3. Here,

$$d_{M-H_2O} = \{[(\delta_{P,M}^2 + \delta_{h,M}^2)^{0.5} - (\delta_{P,H_2O}^2 + \delta_{h,H_2O}^2)^{0.5}]^2 + (\delta_{d,M} - \delta_{d,H_2O})^2\}^{0.5}$$
, where, $\delta_{d,M}$, $\delta_{p,M}$ and $\delta_{h,M}$ are the non-polar, polar and hydrogen bonding components of PEG monomer's HSP, respectively; δ_{d,H_2O} , δ_{p,H_2O} and δ_{h,H_2O} are the non-polar, polar and hydrogen bonding components of water's HSP, respectively. Fouling index versus d_{M-H_2O} was fit with an exponential curve: $y = -0.88 - 0.13 \times x$, $R^2 = 0.998$, $p = 3.21 \times 10^{-7}$. Inserted figure is a blow-up for PEG monomer #s 6 and 7. The error bar is the standard deviation for each sample.

Fig. 5. Adsorption isotherm of five selected best surfaces and the control surface for 24 hr mucus adsorption, at $T = 22 \pm 1^\circ\text{C}$. Four replicates in average for were used for each sample. The error bar is the standard deviation for each sample.

Adsorption ratio and degree of grafting of modified PES membranes and Hansen Solubility Parameters (HSP) of selected monomers.

Table 1

Water and monomers	Adsorption ratio, q (-)	Degree of grafting, DG (-)	HSP (MPa ^{1/2}) ⁽¹⁾			
			Dispersion, δ_d	Polar, δ_p	Hydrogen bonding, δ_h	δ^2
H ₂ O	-	-	18.1	17.1	16.9	1
#3	0.70 ± 0.06	0.08 ± 0.02	9.9	11.6	20.6	0.98
#9	0.67 ± 0.09	0.21 ± 0.03	15.8	8.81	8.5	0.49
#16	0.50 ± 0.06	0.16 ± 0.02	13.9	7.67	16.2,	0.75
#28	0.47 ± 0.08	0.12 ± 0.02	16.6	6.2	7.5A	0.40
#43	0.69 ± 0.04	0.08 ± 0.01	-	-	-	-
#51	1.19 ± 0.08	0.14 ± 0.01	16.6	2.8	6.3	0.29
#53	1.10 ± 0.09	0.14 ± 0.01	14.5	3.2	6.7	0.30

(1) The HSP of water was cited from [42], and those of the monomer #s 3, 9, 16, 28, 51 and 53 were estimated using the group contribution method [43].

$$\bar{\delta} = \frac{(\sqrt{\delta_p^2 + \delta_h^2})_{Monomer}}{(\sqrt{\delta_p^2 + \delta_h^2})_{Water}}$$

(2)

Table 2

Fitting parameters for a Langmuir adsorption isotherm

Monomer	Adsorption density, q (mg/cm ²) ^a	Saturated adsorption density, q_s (mg/cm ²) ^b	Binding constant, K (ml/mg) ^b	$q_s K$ (ml/cm ²) ^b	R^2 ^c
Control	1.99 ± 0.10	0.81	2.53	2.05	0.97
#3	0.79 ± 0.09	0.59	0.61	0.36	0.98
#9	1.41 ± 0.07	0.63	1.00	0.63	0.95
#16	1.17 ± 0.09	0.79	1.08	0.85	0.97
#28	1.21 ± 0.05	0.78	0.77	0.60	0.96
#43	0.66 ± 0.14	0.50	0.24	0.12	0.99

^a q values were taken at $C = 30$ mg/ml mucus adsorption for 24 h.

^b The estimated error is ± 5%.

^c R^2 values obtained from a linear fit of q^{-1} versus $[C]^{-1}$.