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Simple Coatings to Render Polystyrene Protein Resistant

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Abstract: Non-specific protein adsorption is detrimental to the performance of many biomedical devices. Polystyrene is a commonly used material in devices and thin films. Simple reliable surface modification of polystyrene to render it protein resistant is desired in particular for device fabrication and orthogonal functionalisation schemes. This report details modifications carried out on a polystyrene surface to prevent protein adsorption. The trialed surfaces included Pluronic F127 and PLL-*g*-PEG, adsorbed on polystyrene, using a polydopamine-assisted approach. Quartz crystal microbalance with dissipation (QCM-D) results showed only short-term anti-fouling success of the polystyrene surface modified with F127, and the subsequent failure of the polydopamine intermediary layer in improving its stability. In stark contrast, QCM-D analysis proved the success of the polydopamine assisted PLL-*g*-PEG coating in preventing bovine serum albumin adsorption. This modified surface is equally as protein-rejecting after 24 h in buffer, and thus a promising simple coating for long term protein rejection of polystyrene.

Keywords: polystyrene; DOPA; polydopamine; antifouling; polyethylene glycol; Pluronic; QCM-D

1. Introduction

Preventing biological fouling is critically important to many industries. Biofouling significantly shortens the lifespan of materials in contact with biological fluids. For example, in the biomedical sector, devices such as biosensors will produce false signals when covered in proteins and filtration membranes are known to suffer, as biomolecular adhesion causes blockage of the membrane, thus preventing flow through the device [1,2]. Bio-fluids, and in particular proteins, cause this fouling phenomenon because they are charged particles that are amphiphilic and amphoteric, and therefore adsorb easily to most materials [3]. Because of the wide-reaching problems biofouling is causing, and because it is effectively halting other advances in science, particularly in biomedical engineering, a significant amount of research has been conducted to create surfaces which can reduce non-specific protein adsorption [2].

On hydrophobic surfaces, such as the commonly used polystyrene, proteins will structurally rearrange to allow the hydrophobic regions of the protein to interact with the hydrophobic surface. The denaturing exposes the hydrophobic segments (originally often on the inside of the protein to be separated from the polar solvent) to increase the hydrophobic bond with the surface, leading to a gain in entropy upon adsorption [3–5]. In the case of hydrophilic surfaces, hydration of the polar surface is very favourable and large entropies must therefore be achieved to ensure that the adsorption energy overcomes the interfering water molecules/water barrier [3–5]. Research has shown that proteins do not denature greatly on hydrophilic surfaces and thus do not spread on the surface of the substrate as readily as on hydrophobic surfaces [6]. Therefore, on hydrophilic surfaces, adsorption is mainly due to coulomb forces between charged proteins and the surface [3–5].

Molecular groups that provide the desired hydrophilic and electrically neutral characteristics, and which have therefore been used to create antifouling polymers in the past, include ethylene glycol, hydroxyl groups, and zwitterionic betaine groups [7]. Polyethyleneglycol (PEG) is one of the most commonly used anti-fouling polymers and it couples water through hydrogen bonding. More recently, zwitterions have become widely researched as they have even stronger, electrostatically induced, hydration [8]. The most common coating technique is to attach polymer 'brushes' on the surface of the substrate. These so-called brushes are polymer chains which have one end bound to the surface, while the other extends into the solution [9]. The brush formation ensures both a steric barrier to protein adsorption and an effective surface for the protective water layer [10]. Adhesion, or the physisorbed grafting-to method, is the least complicated method of surface modification and is often used to attach block copolymers to substrates. However, because this method does not involve the forming of covalent bonds between the anti-fouling polymer and the substrate, adhesion is reversible, generally leading to shorter-term anti-fouling results [11,12].

Pluronics® (BASF trademark name) are amphiphilic triblock copolymers consisting of a central hydrophobic poly(propyleneoxide) (PPO) block surrounded by hydrophilic poly(ethyleneoxide) (PEO) blocks. It is this amphiphilic behaviour that results in the differences in solubility between the blocks and, thus, in the ability of the hydrophobic block to adsorb to a hydrophobic surface (such as polystyrene) through hydrophobic interactions while the hydrophilic PEO chains extend away from the surface as well-solvated brushes, which sterically hinder protein adsorption [11–13]. Pluronics have been tested as anti-adhesion surface modifiers by many research groups [11–15]. The main characteristics of Pluronics proven to affect their anti-fouling properties are the length of their respective hydrophobic and hydrophilic blocks. An early study in 1991, by Bridgett et al. [11], tested 16 Pluronics against the adhesion of bacteria (Staphylococcus epidermidis) to polystyrene. The Pluronics differed in PPO and PEO block length and all proved successful in reducing adhesion levels by up to 97% [11]. The study demonstrated that the Pluronics' ability to adhere to the polystyrene surface via the PPO block (hydrophobic interactions) was directly related to the length of the PPO block and that the PEO chains must be long enough to be sufficiently mobile to reduce protein adhesion, while not so long as to reduce surface adhesion [11]. Although the adsorption mechanism is complex, due to the many different interactions occurring, the method to apply Pluronics to a substrate surface is simple and thus favourable. However, since the physisorption is reversible, the coating may have a limited lifespan [9,16].

Poly(L-lysine)-*graft*-poly(ethylene glycol) (PLL-*g*-PEG) was developed to provide a strong irreversible surface binding of a somewhat similar PEO construct [17,18]. The polymer contains a cationic poly(L-lysine) backbone, with grafted PEG side chains. While Pluronics interact with hydrophobic surfaces through simple adsorption, PLL-*g*-PEG was designed to bind to anionic surfaces through electrostatic interactions between the positively charged amino-terminated side chains on the PLL-backbone and anionic oxide surfaces. PLL-*g*-PEG has excellent anti-fouling properties due to its high PEG grafting density, providing the necessary steric hindrance and volume exclusion effects [17,18]. While PLL-*g*-PEG has been extensively examined as an anti-fouling surface modifier on negatively charged surfaces, it is also known to not repel proteins when deposited on hydrophobic surfaces. For example, Malmström et al. showed that PLL-*g*-PEG could not prevent adsorption of laminin, and was replaced by the adsorbing laminin during QCM-D measurement on hydrophobic gold surfaces [19]. Similarly, a study by Nonckreman et al., reported that a polystyrene surface modified with PLL-*g*-PEG could not resist human serum albumin or fibrinogen adsorption [20]. An earlier paper by Reimhult et al. seemingly contradicted these findings, stating that, while not as promising as on negatively charged surfaces, polystyrene coated in PLL-*g*-PEG showed significant protein resistance [21].

The dopamine molecule is an interesting surface modifier mimicking proteins excreted by mussels, which help them adhere to most surfaces in the marine environment [8,22]. The ability to form an adhering polydopamine film on a variety of surfaces was first demonstrated by Messersmith

et al. [22,23]. Since then, a variety of dopamine-assisted grafting techniques and DOPA modified antifouling polymers have been tested to more securely attach antifouling polymers to substrate surfaces [23–35]. The polydopamine layer not only readily adheres to most surfaces, but also provides a surface which allows various secondary reactions to take place and could therefore more easily enable the functionalization of material surfaces [22]. The reactions the catechol group of dopamine undergoes with metals, thiols, and amines has led to many research groups favouring the inclusion of dopamine when treating these surfaces [8,22,24–26,36]. While many DOPA-functionalised grafting-to methods have proved successful on the specific surfaces listed above, researchers have encountered fouling when trying the same approach for hydrophobic surfaces. It has been shown that catechol chain ends cannot sufficiently bind to hydrophobic surfaces due to the high solubility of the anti-fouling polymers to which they are bound (e.g., zwitterions) [37]. The alternative to functionalising the polymer with DOPA and thus overcoming surface adhering limitations, is to coat the entire surface in dopamine. This forms a polydopamine film, to which amine or thiol functionalised polymers can be grafted. This method has been labelled "polydopamine-assisted grafting" [22].

In this study we have chosen to investigate the use of a polydopamine-assisted binding of PLL-*g*-PEG to polystyrene. The use of PLL-*g*-PEG both allows for binding through the amine groups and provides an optimised grafting density controlled by the PLL-*g*-PEG synthetic composition. We are also comparing this approach with Pluronic F127. The results reveal that while a simple Pluronic coating of polystyrene resists protein short-term, the coating is unstable. A polydopamine-assisted PLL-*g*-PEG layer however, show promising longer term stability and excellent protein rejecting properties.

2. Materials and Methods

2.1. Materials

Silicon wafers (100, P-type, B-doped) were purchased from University Wafers (Boston, MA, USA) and divided into samples of approximately 1 cm × 1 cm. QCM-D crystals were purchased from ATA scientific (Sydney, Australia) (301), polystyrene from Polymer Source Inc. (Dorval, QC, Canada) (P3810-S, MW: 25500), PEO-PPO-PEO triblock copolymer (Pluronic F127), TRIZMA (2-Amino-2-(hydroxymethyl)-1,3-propanediol), HEPES (4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid) and NaCl was purchased from Sigma Aldrich (Auckland, New Zealand), Dopamine hydrochloride (HPLC grade) from AK Scientific (Union City, CA, USA), bovine serum albumin from MP biomedicals (Auckland, New Zealand) and PLL-*g*-PEG, (20)-[3.5]-(2) from Surface Solutions (Dübendorf, Switzerland).

The buffer used in the majority of experiments was TRIZMA 10 mM, NaCl 150 mM, pH 8.5. The only exception was PLL-*g*-PEG adsorption to bare polystyrene, which was performed in HEPES 10 mM, pH 7.4. All buffers were filtered through a 0.22 µm syringe filter and degassed by ultrasonication for 1 h.

2.2. Sample Preparation

Silicon wafers and AT-cut, quartz QCM-D crystals, were cleaned by ultrasonication in acetone and ethanol (15 min each) and dried under a stream of nitrogen. Previously used QCM-D crystals were also sonicated in toluene prior to acetone and ethanol to remove polystyrene. Clean samples were immediately coated by spin coating a solution of 3 wt % polystyrene in toluene (3000 rpm, 2 min). The polystyrene coated samples were post-baked for 5 min at 90 °C, and stored, submerged in ultrapure water, in sealed petri-dishes, until use. The samples were further ultrasonicated in ultrapure water for five minutes prior to conducting surface modifications.

2.3. Surface Modification with Polydopamine

The samples were exposed to dopamine (2 mg/mL) in TRIZMA buffer, with the samples vertically arranged, to prevent polydopamine sedimentation, and left open to the air, for 5 h at

25 °C. After coating, the samples were rinsed with ultrapure water, dried with N_2 (g) and dried in a vacuum desiccator for 24 h at room temperature.

2.4. Secondary Polymer Layer Deposition on Polydopamine

The desired polymer solutions were prepared; Pluronic F127 (2 mg/mL) and PLL-*g*-PEG (0.1 mg/mL) in TRIZMA buffer (pH 8.5), and subsequently filtered through 0.22 μm syringe filters. The polydopamine coated samples were incubated in the secondary polymer solutions, and were allowed to react with the polydopamine coated samples for 18.5 h at 50 °C. Finally, the samples were thoroughly rinsed with buffer and ultrapure water and dried under a stream of nitrogen.

2.5. QCM-D Experiments

All QCM-D experiments were carried out using the Q-Sense Analyzer from Q-Sense AB (Biolin Scientific, Gothenburg, Sweden). The temperature during all experiments was maintained at 22 °C ± 0.02 °C, and using a pump speed of 0.5, measured to correspond to approximately 0.23 mL/min. The fundamental, 3rd, 5th, 7th, 11th and 13th overtones of frequency and dissipation were recorded for each test, and at least three tests per surface type were conducted, to allow for statistical analysis.

The frequency and dissipation shifts related to mass adsorption were measured by the Q-Soft 401 software. The QCM-D data was then further assessed using the D-find program. The data from the 7th overtone was used for mass adsorption comparisons between all surfaces.

2.6. X-ray Photoelectron Spectroscopy (XPS)

XPS was performed to identify the elemental composition of the surfaces. The compositional data was obtained using a Kratos Axis Ultra-DLD spectrometer (Kratos Analytical Ltd., Manchester, UK). Spectra excitation on an analysis area of 300 by 700 μm was achieved with a 150 W X-ray power source, emitting A1 Kα X-rays (1486.7 eV) in an ultrahigh vacuum chamber (~10^{-9} torr). Survey scans (0–1300 eV) and core level scans were carried out on each surface, at pass energies of 160 eV and 20 eV, respectively. The resulting spectra were analysed with CasaXPS software (Version 2.3.14). All data was calibrated such that the C(1s) peak (corresponding to C–H/C–C energies) was situated at 285.0 eV. The Shirley model was used for peak fitting, and Gaussian-Lorentzian (30% Lorentzian) peaks were used to fit the core-level peaks.

2.7. Atomic Force Microscopy (AFM)

AFM was carried out using a Cypher ES instrument (Oxford Instruments, Asylum Research, Santa Barbara, CA, USA). Images were acquired in tapping mode with a Tap 150 Al-G cantilever (Nominal f = 150 kHz and k = 5 N/m, Budget sensors, Sofia, Bulgaria) and a free air amplitude of 1 V.

2.8. Contact Angle Goniometry

Wettability testing was carried out with a contact angle goniometer (CAM 101, KSV instruments, Helsinki, Finland). The AttensionTheta2017 software was used for acquisition of images and image analysis. All contact angles were recorded using ultrapure water and using the same drop volume (6 ± 2 μL), impact distance between the drop and surface and time before image was acquired (using automated image acquisition).

2.9. Statistics

One-way ANOVA studies were carried out on QCM-D mass adsorption data and contact angle data. From Post Hoc, Tukey analysis, probability values of <0.05 were considered to indicate significant differences. All results with more than three tests, are expressed as the mean, with standard deviations indicated. The statistical accuracy of results from AFM and XPS testing was not analysed, due to sample sizes for each surface being too small (n < 3).

3. Results and Discussion

The results from QCM-D experiments evaluating the protein fouling to the different surface coatings and the sample characterisation using AFM, XPS and contact angles are presented in the following sections.

3.1. QCM-D Analysis

QCM-D uses the piezoelectric properties of quartz and follows the frequency of a mechanical oscillation of a quartz crystal to measure the mass of adsorbed molecules in real time. The technique is also able to determine the viscoelastic properties of the adsorbed layer by recording the dissipation of the oscillation. The change in frequency observed in QCM-D is proportional to the change in mass of the oscillating crystal and adsorbed molecules (as described by the Sauerbrey equation), so long as three conditions are satisfied: the mass is distributed evenly, it obeys a no-slip boundary condition and it is sufficiently rigid, or thin, so as not to be subject to internal friction [38–41]. Most of the QCM-D results reported here show only small differences between overtones, which is why the Sauerbrey equation has been used to calculate the adsorbed mass. The mass measured by QCM-D includes everything adsorbing to the surface, including the water either hydro-dynamically coupled to the protein ad-layer or trapped within the anti-fouling film [38–40]. According to Höök et al., for globular proteins like BSA, the measured mass is around 1.75 times higher than that recorded by 'dry mass' optical methods [39].

QCM-D was carried out to observe the frequency changes associated with BSA adsorption to the different surfaces investigated. The typical frequency change over time curves, for the addition of the protein and the subsequent wash-off step on each tested surface, are shown in Figure 1A.

The majority of the surfaces show an initial rapid frequency decrease upon exposure to the protein and a subsequent short region of slower frequency shift as the system reaches saturation. Different extent of protein dissociation from the surface can be observed after rinsing with protein free buffer (indicated by black arrows in Figure 1A). The size of the frequency shifts, taken as the difference between a point immediately prior to the BSA injection, and a point 10 min into the buffer rinse, have been directly related to the mass of BSA bound to the surface, by use of the Sauerbrey equation [41]. Averages from at least three measurements of each surface type are presented in Figure 1B. From this data it is evident that polystyrene modified by adsorption of Pluronic F127 (PS + F127), and polystyrene modified with polydopamine and PLL-*g*-PEG (PS + DOPA + PLL-*g*-PEG) are the most successful coatings in terms of reducing protein adsorption to polystyrene.

The plain polystyrene coated crystal subjected to 1 mg/mL BSA shows a frequency shift, after rinsing, of around -27 Hz. This translates to 478.3 ng/cm^2 $\pm$ 8.7 ng/cm^2 BSA adsorbed, in good agreement with literature [21].

The polystyrene surface modified with Pluronic F127, presented the expected protein rejecting properties, but the Pluronic coating displayed significant instability on the surface as seen in Figure 1C. In fact, after the buffer rinse, which was done to remove any loosely bound protein, the final frequency change recorded is positive, which indicates an overall reduction of mass. This apparent reduction in mass on the crystals surface, despite BSA addition, can be clearly seen in Figure 1C, as a continuous upward drift, throughout the BSA adsorption stage and the rinse off stage. Jin et al. [42] saw a similar response in their test of Pluronic P-123 modified polystyrene, when subjecting the surface to BSA in QCM-D tests. The group attributed this drift to the release of bound water from the Pluronic brush, as BSA replaces the water molecules [42]. However, we hypothesize that desorption of the Pluronic layer from the polystyrene surface is an additional cause of the continuous upward drift. This drift highlights the issue found in literature regarding the short life-span of this protein resistant coating [14,16], which is why we are also investigating polydopamine assisted coatings.

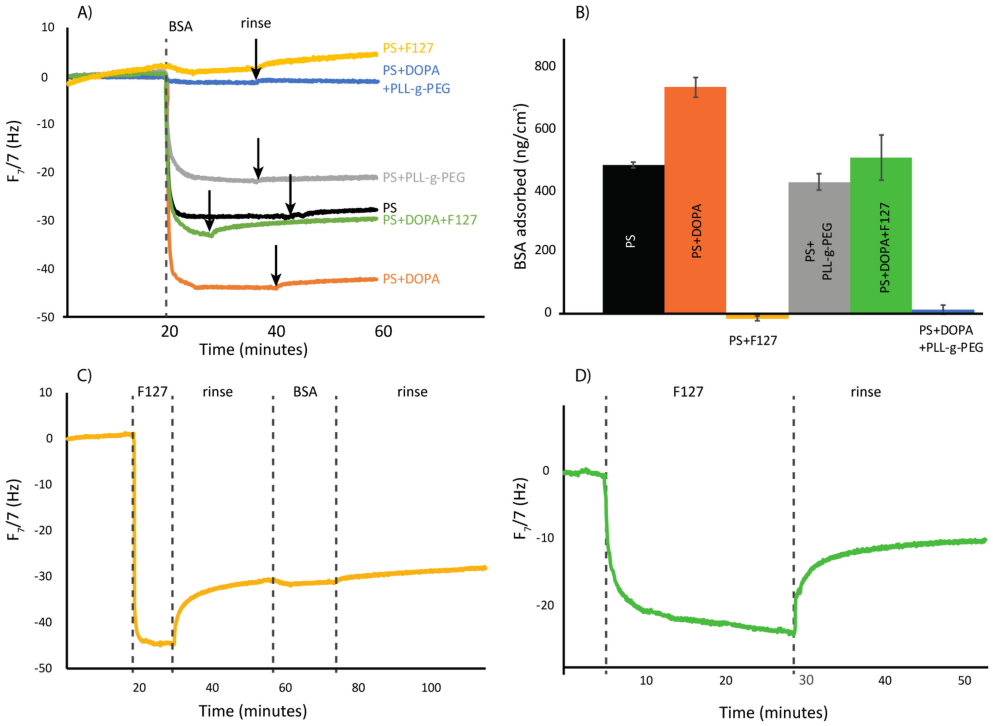

Figure 1. (**A**) Example QCM-D frequency data comparing BSA (bovine serum albumin) adsorption to the different surface chemistries investigated, namely untreated polystyrene (PS) and polystyrene treated with Pluronic F127 (PS + F127), polydopamine (PS + DOPA), PLL-*g*-PEG (PS + PLL-*g*-PEG) or PS + DOPA additionally treated with PLL-*g*-PEG (PS+DOPA + PLL-*g*-PEG) or Pluronic F127 (PS + DOPA + F127). All data has been shifted in time to start the BSA adsorption at the same time. Start of rinsing with buffer is indicated in the graph with arrows for each curve. (**B**) Average adsorbed BSA to the different surface chemistries (error bars represent standard deviation); (**C**) QCM-D frequency graph showing deposition of F127 Pluronic onto a PS coated QCM-D crystal with subsequent rinse-off and BSA adsorption step; (**D**) F127 Pluronic adsorption onto polydopamine coated PS coated crystal showing a lower adsorption of F127 compared to bare PS.

The dopamine molecule has been shown to adhere to many diverse surfaces including polystyrene. It has been found that the initial driving force for adsorption to the hydrophobic polystyrene surface is the displacement of water, followed by the strengthening of the bond through the functional group interactions [43,44]. The interactions between dopamine and polystyrene are believed to occur due to a combination of π–π overlap between the aromatic rings of polystyrene and the catechol rings, and the cation–π interactions between the protonated amine group of dopamine and the π–face of the styrene rings. These bonding theories imply that the DOPA and polystyrene aromatic rings align parallel to each other [43–47]. As there is debate about how exactly the polymer is formed and adheres to surfaces and secondary coatings [48], we tested if Pluronic F127 could in fact bind to such a polydopamine coating and create a protein rejecting interface. Interestingly, a polystyrene surface coated in polydopamine film results in the largest frequency shift of all tested surfaces, possibly due to less surface induced denaturation of BSA on this hydrophilic surface compared to the hy "glue" between polystyrene and F127, the ex situ coating method first described by Messersmith et al. [22] was employed. In Figure 1A,B it can be seen that BSA addition to such a surface resulted in a large negative frequency shift, corresponding to a mass increase of 501.8 ng/cm^2 ± 73.5 ng/cm^2. It was hoped that introducing the intermediary polydopamine layer may help the protein resistant Pluronic F127 to better adsorb to the surface. However, in light of the high mass adsorption measured, we suggest that the Pluronic either is adsorbed with its hydrophobic backbone facing the solution or that it rinses-off rapidly. Polydopamine and F127 adsorbtion onto the PS film was also explored in situ in the QCM-D,

and compared to the protein resistant polystyrene surface modified only with F127 (Figure 1C,D). The DOPA + F127 experiment carried out in the QCM-D (Figure 1D) showed that only around half as much F127 adsorbed onto the polydopamine layer, indicated by a frequency shift of -22.23 Hz $\pm$ 0.81 Hz, compared to that adsorbing on the polystyrene surface, -42.33 Hz $\pm$ 0.6 Hz. The rinsing step also shows a significant desorption of F127 from both surfaces. Such a weak binding of F127 onto polydopamine, suggests that any interactions between the polymer and the substrate, are much weaker than the interactions between the hydrophilic PEO blocks and the solvent, leading in turn to BSA adsorbing to the surface almost to the same level as to the polydopamine coating itself.

Upon failing to produce better anti-fouling results than by simply adsorbing F127 onto polystyrene, PLL-*g*-PEG was trialed, to attach the same protein resisting PEG group to the surface.

PLL-*g*-PEG has been extensively examined as an anti-fouling surface modifier on negatively charged surfaces [17,18]. Accordingly, we had reason to believe it could not attach to polystyrene without assistance. However, due to a publication citing reasonable protein resistance on polystyrene, and for comparative purposes, 0.1 mg/mL PLL-*g*-PEG was adsorbed onto the plain polystyrene surface. Figure 1 shows that upon BSA addition, a frequency drop of -23.9 Hz $\pm$ 1.5 Hz was observed, corresponding to a mass uptake of 423.2 ng/cm^2 $\pm$ 26.4 ng/cm^2. This surface modification provides near 12% protein resistance when compared to the plain polystyrene surface, and statistical analysis proved the difference to be significant ($p < 0.05$), which could explain why one group suggested the modification improved protein resistance. It is worth noting that the apparent reduction in protein adsorption could simply be due to weakly bound PLL-*g*-PEG molecules being replaced by BSA during the protein adsorption phase.

To mediate PLL-g-PEG binding to PS, a polydopamine intermediary layer was formed on the polystyrene coated crystals, upon which PLL-*g*-PEG was allowed to bind. From Figure 1B, a frequency shift of -0.8 Hz and mass of 13.8 ng/cm^2 $\pm$ 13.5 ng/cm^2 were recorded for the subsequent BSA adsorption step. These results prove the PS + DOPA + PLL-*g*-PEG modified surface was as resistant to BSA adsorption, as the well documented PS + F127 coating ($p > 0.05$), and significantly more resistant than all other trialed surfaces ($p < 0.05$). Therefore, this coating can provide a plain polystyrene surface with improved protein resistance of around 97%.

Many reports in literature have shown that thiol and amine terminated functionalized polymers could covalently bond to the dopamine molecule, and several papers also specifically used the polydopamine layer for this bonding process [8,46,49]. Messersmith et al. suggested a possible polymerisation mechanism for dopamine [22], which leads us to believe that at mildly alkaline pH the PLL-*g*-PEG ad-layer could be covalently grafted to the dopamine molecules of the polydopamine film through Michael or Schiff-base reactions. Thus, several different reaction products could be present in the final coating. The extremely low protein adsorption observed in QCM-D experiments proves that the polydopamine coating arranged on polystyrene enables the formation of a dense PLL-*g*-PEG ad-layer with PEG chains extending into the solution. It is worth noting however, that reports of PLL-*g*-PEG on negatively charged surfaces have been stated to produce protein resistance below the detection limit of the OWLS technique (<2 ng/cm^2), and while our measure is from a wet-mass technique, adjusting for the possible coupled water, does not achieve results quite as successful. Therefore, it is likely that the PLL-*g*-PEG layer formed on polydopamine is not as ordered as on negatively charged surfaces. Tsai et al. [31] published a report in 2011, in which it was stated, albeit without any supporting data, that the one-step dip coating method they had trialed, by coating a surface in a mixture of polydopamine and PLL-*g*-PEG, did not provide satisfactory cell resistant results. We show with our QCM-D results that, by first coating the surface in polydopamine and, in a second step, covalently grafting the PLL-*g*-PEG, we are able to achieve very low protein adsorption.

Finally, we investigated whether the PS + DOPA + PLL-*g*-PEG surface could provide better long-term protein resistance than the F127 modified polystyrene surface. To test the longevity of both coatings, the crystals were submerged in buffer for 24 h prior to QCM-D measurements. The response

of each soaked surface to protein exposure, along with the original QCM-D data of the successful surfaces prior to soaking, is displayed in Figure 2.

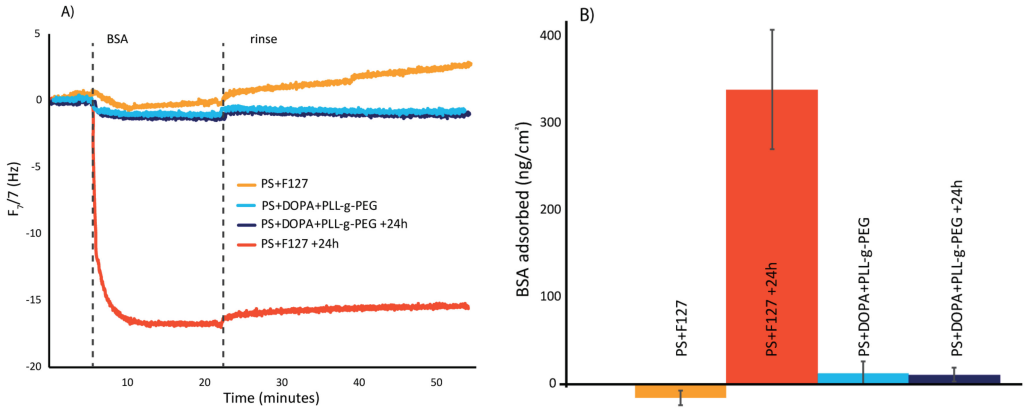

Figure 2. Testing the modified surface's response to BSA with or without pre-soaking in buffer for 24 h. Introduction of BSA and start of rinse is indicated by the dotted lines in (**A**). Average and standard deviation of adsorbed BSA mass (calculated using the Sauerbrey equation) presented in (**B**).

It is clear that F127 is no longer repelling protein after a 24 h soak, while the PLL-*g*-PEG coated polydopamine remains protein rejecting. The trend of F127 to be unstable at the surface was also evident in Figure 1, where the frequency continued to drift upward throughout the measurement. These problems have also been described in literature by several groups. For example, Marsh et al. [14] reported XPS analysis which proved that after 24 h in PBS, the Pluronic coating had washed off, and Su et al. [16] showed that after immersion in water for one week, the polystyrene originally coated in F127 had near plain polystyrene contact angles [26,28].

The frequency shift recorded for the 24 h buffer-soaked Pluronic surface, subject to BSA, was −19.2 Hz, corresponding to 340 ng/cm^2 ± 68.9 ng/cm^2. This is a significant difference to the coat spared the 24 h treatment ($p < 0.05$). Additionally, it can be seen that the buffer rinse after BSA adsorption does not cause the 24 h soaked F127 (PS+F127 +24 h in Figure 2A) surface to drift as dramatically, as it does the un-soaked coating (PS+F127 in Figure 2A), indicating that the pre-soaked surface has lost much of the anti-fouling Pluronic already. The DOPA + PLL-*g*-PEG coating however, showed no significant difference after 24 h of buffer soak ($p > 0.05$), as is clearly depicted in Figure 2. This shows that the polydopamine assisted grafting of PLL-*g*-PEG will provide the desired protein resistance for a longer time and is thereby a possible solution to overcome the life-span problems encountered by the physisorbed F127 modified surfaces. Judging by the life-span reported in literature for PLL-*g*-PEG on negatively charged surfaces, this coating could last much longer than the 24 h tested. One report shows that flowing HEPES buffer for one week, resulted in a loss of less than 5% mass of the polymer, while another group had reported PLL-*g*-PEG was stable and protein-rejecting for at least three weeks on surface-modified polystyrene microspheres [50].

3.2. Sample Characterisation

XPS allowed us to determine the chemical composition of the modified surfaces and clearly showed the expected increase in both oxygen and nitrogen after modification of the PS surface with polydopamine, in good agreement with literature, atomic percentages from quantified XPS survey spectra are presented in in Table 1 [22,51]. The presence of PLL-*g*-PEG for the polydopamine assisted graft-PLL-*g*-PEG sample is confirmed by a further increase in atomic oxygen and nitrogen percentage, which can be attributed to the protein resisting PEG chains, and the PLL-backbone, respectively [17,18,20,52]. The unmodified polystyrene coating showed unexpected oxygen and nitrogen signals, most likely due to contamination of the PS surface before the XPS was acquired (proteins or microbial contamination suspected as the sample was stored in an aqueous environment),

or possibly due to oxidation. It is worth pointing out that while polystyrene is hydrophobic, the binding mechanism of polydopamine to PS may not be the same to other hydrophobic surfaces, and the findings in this study may not apply generically to other hydrophobic materials.

Table 1. The elemental ratios present in the build-up of the PS + DOPA + PLL-*g*-PEG surface.

Surface	XPS Atomic Percent (%)					
	C(1s)	O(1s)	N(1s)	Si(2p)	Na(1s)	S(2p)
PS	94.5 ± 0.5	4.3 ± 0.3	0.8 ± 0.1	0.3 ± 0.1	–	0.1 ± 0.1
PS + DOPA	79.1 ± 0.5	15.3 ± 0.4	5.6 ± 0.1	–	–	–
PS + DOPA + PLL-*g*-PEG	73.8 ± 0.4	19.3 ± 0.3	6.4 ± 0.2	0.3 *	0.2 ± 0.0	–

* This silicon peak was only visible in one of the three scanned spots.

All surface coatings were characterized by recording the contact angles in contact with water. Figure 3, shows clearly that all surfaces containing polydopamine have a lower contact angle, representing a more "wettable" surface. More specifically, contact angle analysis shows the PS + DOPA surface to be more hydrophilic than all other coatings, bar one: PS + DOPA + PLL-*g*-PEG. This coating is the most hydrophilic surface, which helps to explain the significant protein resistance of this surface. Surprisingly, the other successful anti-fouling surface, PS + F127, does not produce the same results in this case. Values found in literature for PS + F127 however show much lower contact angles, of around 64 degrees [16]. The discrepancy may relate to rinsing protocols as F127 is not stably adsorbed on the PS surface. The PS + DOPA + F127 surface, which proved not protein resistant in QCM-D testing, is significantly more hydrophilic than PS + F127 ($p < 0.05$) most likely due to the weakly bound F127 washing off. Such wash-off may also be inferred from AFM images of the surfaces (see Figure S1 and Table S1 in Supplementary Materials). AFM also confirmed a uniform polydopamine coating on polystyrene leading to a modestly increased surface roughness, in agreement with literature [51]. The short-term successful anti-fouling PS + F127 coat appeared largely homogeneous while the dopamine assisted F127 coating, showed large areas of low-lying film which measure around 10 nm deeper than the surrounding surface. These areas could be representative of washed off F127, leaving only the polydopamine coating below. Overall, these F127 surfaces appear rougher than those modified with PLL-*g*-PEG.

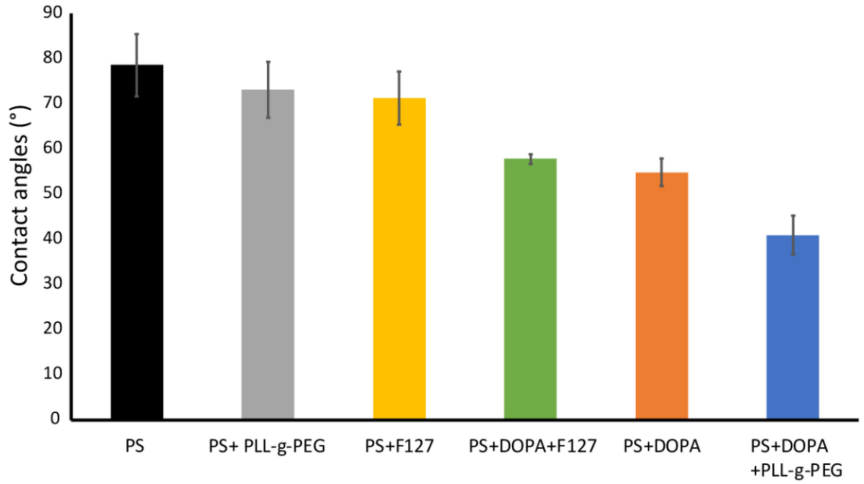

Figure 3. Average contact angles, with standard deviations (error bars), for all surface coatings investigated.

Taken together, these surface characteristics support the conclusions drawn from the QCM-D analysis, and out of these simple coating methods to modify polystyrene to render it protein rejecting, the polydopamine assisted PLL-*g*-PEG modified surface holds particular promise. Future work

evaluating this coating with other proteins and in cell culture is needed to fully confirm the long-term stability of the coating.

4. Conclusions

The main conclusions drawn from the present work are:

- F127 significantly reduces BSA adsorption in short-term studies. However, after soaking only 24 h in buffer, the resistance is significantly diminished.
- The polydopamine intermediary film produced, cannot provide the necessary attraction to bind F127 to the surface. Instead, bonds between the Pluronic and the surface are weakened, causing more protein adsorption than on unmodified polystyrene.
- By adsorbing the same "assisting" polydopamine film and subsequently grafting PLL-*g*-PEG, we can successfully prevent 97% of protein adsorption on a polystyrene substrate.
- This modified surface is equally as protein-rejecting after 24 h in buffer, and thus a promising simple coating for long term protein rejection of polystyrene.

Supplementary Materials: The following are available online at www.mdpi.com/2079-6412/8/2/55/s1, Figure S1: AFM tapping mode images; Table S1: Roughness recorded for one sample of each surface.

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References

1. Hatakeyama, E.S.; Ju, H.; Gabriel, C.J.; Lohr, J.L.; Bara, J.E.; Noble, R.D.; Freeman, B.D.; Gin, D.L. New protein-resistant coatings for water filtration membranes based on quaternary ammonium and phosphonium polymers. *J. Membr. Sci.* **2009**, *330*, 104–116. [CrossRef]
2. Lowe, S.; O'Brien-Simpson, N.M.; Connal, L.A. Antibiofouling polymer interfaces: Poly(ethylene glycol) and other promising candidates. *Polym. Chem.* **2015**, *6*, 198–212. [CrossRef]
3. Poncin-Epaillard, F.; Vrlinic, T.; Debarnot, D.; Mozetic, M.; Coudreuse, A.; Legeay, G.; El Moualij, B.; Zorzi, W. Surface treatment of polymeric materials controlling the adhesion of biomolecules. *J. Funct. Biomater.* **2012**, *3*, 528–543. [CrossRef] [PubMed]
4. Malmsten, M. Ellipsometry studies of protein layers adsorbed at hydrophobic surfaces. *J. Colloid Interface Sci.* **1994**, *166*, 333–342. [CrossRef]
5. Roach, P.; Farrar, D.; Perry, C.C. Interpretation of protein adsorption: Surface-induced conformational changes. *J. Am. Chem. Soc.* **2005**, *127*, 8168–8173. [CrossRef] [PubMed]
6. Wertz, C.F.; Santore, M.M. Effect of surface hydrophobicity on adsorption and relaxation kinetics of albumin and fibrinogen: Single-species and competitive behavior. *Langmuir* **2001**, *17*, 3006–3016. [CrossRef]
7. Chou, Y.N.; Wen, T.C.; Chang, Y. Zwitterionic surface grafting of epoxylated sulfobetaine copolymers for the development of stealth biomaterial interfaces. *Acta Biomater.* **2016**, *40*, 78–91. [CrossRef] [PubMed]
8. Sundaram, H.S.; Han, X.; Nowinski, A.K.; Ella-Menye, J.R.; Wimbish, C.; Marek, P.; Senecal, K.; Jiang, S. One-step dip coating of zwitterionic sulfobetaine polymers on hydrophobic and hydrophilic surfaces. *ACS Appl. Mater. Interfaces* **2014**, *6*, 6664–6671. [CrossRef] [PubMed]
9. Raynor, J.E.; Capadona, J.R.; Collard, D.M.; Petrie, T.A.; García, A.J. Polymer brushes and self-assembled monolayers: Versatile platforms to control cell adhesion to biomaterials Review. *Biointerphases* **2009**, *4*, FA3–FA16. [CrossRef] [PubMed]
10. Brittain, W.J.; Minko, S. A structural definition of polymer brushes. *J. Polym. Sci. Part A Polym. Chem.* **2007**, *45*, 3505–3512. [CrossRef]

11. Bridgett, M.J.; Davies, M.C.; Denyer, S.P. Control of staphylococcal adhesion to polystyrene surfaces by polymer surface modification with surfactants. *Biomaterials* **1992**, *13*, 411–416. [CrossRef]
12. Green, R.J.; Tasker, S.; Davies, J.; Davies, M.C.; Roberts, C.J.; Tendler, S.J.B. Adsorption of PEO-PPO-PEO triblock copolymers at the solid/liquid interface: A surface plasmon resonance study. *Langmuir* **1997**, *13*, 6510–6515. [CrossRef]
13. Song, X.; Zhao, S.; Fang, S.; Ma, Y.; Duan, M. Mesoscopic simulations of adsorption and association of PEO-PPO-PEO triblock copolymers on a hydrophobic surface: From mushroom hemisphere to rectangle brush. *Langmuir* **2016**, *32*, 11375–11385. [CrossRef] [PubMed]
14. Marsh, L.H.; Coke, M.; Dettmar, P.W.; Ewen, R.J.; Havler, M.; Nevell, T.G.; Smart, J.D.; Smith, J.R.; Timmins, B.; Tsibouklis, J.; et al. Adsorbed poly(ethyleneoxide)-poly(propyleneoxide) copolymers on synthetic surfaces: Spectroscopy and microscopy of polymer structures and effects on adhesion of skin-borne bacteria. *J. Biomed. Mater. Res.* **2002**, *61*, 641–652. [CrossRef] [PubMed]
15. Zheng, J.; Song, W.; Huang, H.; Chen, H. Protein adsorption and cell adhesion on polyurethane/Pluronic® surface with lotus leaf-like topography. *Colloids Surf. B Biointerfaces* **2010**, *77*, 234–239. [CrossRef] [PubMed]
16. Su, Y.; Wang, L.; Yang, X. A simple swelling and anchoring method for preparing dense and stable poly(ethylene oxide) layers on polystyrene surfaces. *Appl. Surf. Sci.* **2008**, *254*, 4606–4610. [CrossRef]
17. Huang, N.P.; Michel, R.; Voros, J.; Textor, M.; Hofer, R.; Rossi, A.; Elbert, D.L.; Hubbell, J.A.; Spencer, N.D. Poly(L-lysine)-*g*-poly(ethylene glycol) layers on metal oxide surfaces: Surface-analytical characterization and resistance to serum and fibrinogen adsorption. *Langmuir* **2001**, *17*, 489–498. [CrossRef]
18. Pasche, S.; De Paul, S.M.; Vörös, J.; Spencer, N.D.; Textor, M. Poly(L-lysine)-graft-poly(ethylene glycol) assembled monolayers on niobium oxide surfaces: A quantitative study of the influence of polymer interfacial architecture on resistance to protein adsorption by ToF-SIMS and in situ OWLS. *Langmuir* **2003**, *19*, 9216–9225. [CrossRef]
19. Malmström, J.; Agheli, H.; Kingshott, P.; Sutherland, D.S. Viscoelastic modeling of highly hydrated laminin layers at homogeneous and nanostructured surfaces: Quantification of protein layer properties using QCM-D and SPR. *Langmuir* **2007**, *23*, 9760–9768. [CrossRef] [PubMed]
20. Nonckreman, C.J.; Fleith, S.; Rouxhet, P.G.; Dupont-Gillain, C.C. Competitive adsorption of fibrinogen and albumin and blood platelet adhesion on surfaces modified with nanoparticles and/or PEO. *Colloids Surf. B Biointerfaces* **2010**, *77*, 139–149. [CrossRef] [PubMed]
21. Reimhult, K.; Petersson, K.; Krozer, A. QCM-D analysis of the performance of blocking agents on gold and polystyrene surfaces. *Langmuir* **2008**, *24*, 8695–8700. [CrossRef] [PubMed]
22. Lee, H.; Dellatore, S.M.; Miller, W.M.; Messersmith, P.B. Mussel-inspired surface chemistry for multifunctional coatings. *Science* **2007**, *318*, 426–430. [CrossRef] [PubMed]
23. Dalsin, J.L.; Hu, B.H.; Lee, B.P.; Messersmith, P.B. Mussel adhesive protein mimetic polymers for the preparation of nonfouling surfaces. *J. Am. Chem. Soc.* **2003**, *125*, 4253–4258. [CrossRef] [PubMed]
24. Barclay, T.G.; Hegab, H.M.; Clarke, S.R.; Ginic-Markovic, M. Versatile surface modification using polydopamine and related polycatecholamines: Chemistry, structure, and applications. *Adv. Mater. Interfaces* **2017**, *4*, 1601192. [CrossRef]
25. Liu, M.; Zeng, G.; Wang, K.; Wan, Q.; Tao, L.; Zhang, X.; Wei, Y. Recent developments in polydopamine: An emerging soft matter for surface modification and biomedical applications. *Nanoscale* **2016**, *8*, 16819–16840. [CrossRef] [PubMed]
26. Zhang, X.; Huang, Q.; Deng, F.; Huang, H.; Wan, Q.; Liu, M.; Wei, Y. Mussel-inspired fabrication of functional materials and their environmental applications: Progress and prospects. *Appl. Mater. Today* **2017**, *7*, 222–238. [CrossRef]
27. Chien, C.Y.; Tsai, W.B. Poly(dopamine)-assisted immobilization of Arg-Gly-Asp peptides, hydroxyapatite, and bone morphogenic protein-2 on titanium to improve the osteogenesis of bone marrow stem cells. *ACS Appl. Mater. Interfaces* **2013**, *5*, 6975–6983. [CrossRef] [PubMed]
28. Li, W.; Zheng, Y.; Zhao, X.; Ge, Y.; Chen, T.; Liu, Y.; Zhou, Y. Osteoinductive effects of free and immobilized bone forming peptide-1 on human adipose-derived stem cells. *PLoS ONE* **2016**, *11*, e0150294. [CrossRef] [PubMed]
29. Luo, R.; Wang, X.; Deng, J.; Zhang, H.; Maitz, M.F.; Yang, L.; Wang, J.; Huang, N.; Wang, Y. Dopamine-assisted deposition of poly (ethylene imine) for efficient heparinization. *Colloids Surf. B Biointerfaces* **2016**, *144*, 90–98. [CrossRef] [PubMed]

30. Pan, C.; Chen, L.; Liu, S.; Zhang, Y.; Zhang, C.; Zhu, H.; Wang, Y. Dopamine-assisted immobilization of partially hydrolyzed poly(2-methyl-2-oxazoline) for antifouling and biocompatible coating. *J. Mater. Sci.* **2016**, *51*, 2427–2442. [CrossRef]
31. Tsai, W.B.; Chien, C.Y.; Thissen, H.; Lai, J.Y. Dopamine-assisted immobilization of poly(ethylene imine) based polymers for control of cell-surface interactions. *Acta Biomater.* **2011**, *7*, 2518–2525. [CrossRef] [PubMed]
32. Yuan, S.; Li, Z.; Zhao, J.; Luan, S.; Ma, J.; Song, L.; Shi, H.; Jin, J.; Yin, J. Enhanced biocompatibility of biostable poly(styrene-b-isobutylene-b-styrene) elastomer via poly(dopamine)-assisted chitosan/hyaluronic acid immobilization. *RSC Adv.* **2014**, *4*, 31481–31488. [CrossRef]
33. Gao, C.; Li, G.; Xue, H.; Yang, W.; Zhang, F.; Jiang, S. Functionalizable and ultra-low fouling zwitterionic surfaces via adhesive mussel mimetic linkages. *Biomaterials* **2010**, *31*, 1486–1492. [CrossRef] [PubMed]
34. Kuang, J.; Messersmith, P.B. Universal surface-initiated polymerization of antifouling zwitterionic brushes using a mussel-mimetic peptide initiator. *Langmuir* **2012**, *28*, 7258–7266. [CrossRef] [PubMed]
35. Wu, J.; Zhang, L.; Wang, Y.; Long, Y.; Gao, H.; Zhang, X.; Zhao, N.; Cai, Y.; Xu, J. Mussel-inspired chemistry for robust and surface-modifiable multilayer films. *Langmuir* **2011**, *27*, 13684–13691. [CrossRef] [PubMed]
36. Kord Forooshani, P.; Lee, B.P. Recent approaches in designing bioadhesive materials inspired by mussel adhesive protein. *J. Polym. Sci. Part A Polym. Chem.* **2017**, *55*, 9–33. [CrossRef] [PubMed]
37. Sundaram, H.S.; Han, X.; Nowinski, A.K.; Brault, N.D.; Li, Y.; Ella-Menye, J.R.; Amoaka, K.A.; Cook, K.E.; Marek, P.; Senecal, K.; et al. Achieving one-step surface coating of highly hydrophilic poly(carboxybetaine methacrylate) polymers on hydrophobic and hydrophilic surfaces. *Adv. Mater. Interfaces* **2014**, *1*, 1400071. [CrossRef] [PubMed]
38. Höök, F.; Kasemo, B.; Nylander, T.; Fant, C.; Sott, K.; Elwing, H. Variations in coupled water, viscoelastic properties, and film thickness of a Mefp-1 protein film during adsorption and cross-linking: A quartz crystal microbalance with dissipation monitoring, ellipsometry, and surface plasmon resonance study. *Anal. Chem.* **2001**, *73*, 5796–5804. [CrossRef] [PubMed]
39. Höök, F.; Vörös, J.; Rodahl, M.; Kurrat, R.; Böni, P.; Ramsden, J.J.; Textor, M.; Spencer, N.D.; Tengvall, P.; Gold, J.; et al. A comparative study of protein adsorption on titanium oxide surfaces using in situ ellipsometry, optical waveguide lightmode spectroscopy, and quartz crystal microbalance/dissipation. *Colloids Surf. B Biointerfaces* **2002**, *24*, 155–170. [CrossRef]
40. Höök, F.; Rodahl, M.; Brzezinski, P.; Kasemo, B. Energy dissipation kinetics for protein and antibody-antigen adsorption under shear oscillation on a quartz crystal microbalance. *Langmuir* **1998**, *14*, 729–734. [CrossRef]
41. Sauerbrey, G. Verwendung Von Schwingquarzen Zur Wagung Dunner Schichten Und Zur Mikrowagung. *Zeitschrift Fur Physik* **1959**, *155*, 206–222. (In German) [CrossRef]
42. Jin, J.; Huang, F.; Hu, Y.; Jiang, W.; Ji, X.; Liang, H.; Yin, J. Immobilizing PEO-PPO-PEO triblock copolymers on hydrophobic surfaces and its effect on protein and platelet: A combined study using QCM-D and DPI. *Colloids Surf. B Biointerfaces* **2014**, *123*, 892–899. [CrossRef] [PubMed]
43. Baty, A.M.; Leavitt, P.K.; Siedlecki, C.A.; Tyler, B.J.; Suci, P.A.; Marchant, R.E.; Geesey, G.G. Adsorption of adhesive proteins from the marine mussel, Mytilus edulis, on polymer films in the hydrated state using angle dependent X-ray photoelectron spectroscopy and atomic force microscopy. *Langmuir* **1997**, *13*, 5702–5710. [CrossRef]
44. Baty, A.M.; Suci, P.A.; Tyler, B.J.; Geesey, G.G. Investigation of mussel adhesive protein adsorption on polystyrene and poly(octadecyl methacrylate) using angle dependent XPS, ATR-FTIR, and AFM. *J. Colloid Interface Sci.* **1996**, *177*, 307–315. [CrossRef]
45. Lee, H.; Scherer, N.F.; Messersmith, P.B. Single-molecule mechanics of mussel adhesion. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12999–13003. [CrossRef] [PubMed]
46. Moulay, S. Dopa/catechol-tethered polymers: Dioadhesives and biomimetic adhesive materials. *Polym. Rev.* **2014**, *54*, 436–513. [CrossRef]
47. Jiang, J.; Zhu, L.; Zhu, L.; Zhu, B.; Xu, Y. Surface characteristics of a self-polymerized dopamine coating deposited on hydrophobic polymer films. *Langmuir* **2011**, *27*, 14180–14187. [CrossRef] [PubMed]
48. Liebscher, J.; Mrówczyński, R.; Scheidt, H.A.; Filip, C.; Hadade, N.D.; Turcu, R.; Bende, A.; Beck, S. Structure of polydopamine: A never-ending story? *Langmuir* **2013**, *29*, 10539–10548. [CrossRef] [PubMed]
49. Mian, S.A.; Yang, L.M.; Saha, L.C.; Ahmed, E.; Ajmal, M.; Ganz, E. A fundamental understanding of catechol and water adsorption on a hydrophilic silica surface: Exploring the underwater adhesion mechanism of mussels on an atomic scale. *Langmuir* **2014**, *30*, 6906–6914. [CrossRef] [PubMed]

50. Wattendorf, U.; Kreft, O.; Textor, M.; Sukhorukov, G.B.; Merkle, H.P. Stable stealth function for hollow polyelectrolyte microcapsules through a poly(ethylene glycol) grafted polyelectrolyte adlayer. *Biomacromolecules* **2008**, *9*, 100–108. [CrossRef] [PubMed]
51. Zangmeister, R.A.; Morris, T.A.; Tarlov, M.J. Characterization of polydopamine thin films deposited at short times by autoxidation of dopamine. *Langmuir* **2013**, *29*, 8619–8628. [CrossRef] [PubMed]
52. Heuberger, R.; Sukhorukov, G.; Vörös, J.; Textor, M.; Möhwald, H. Biofunctional polyelectrolyte multilayers and microcapsules: Control of non-specific and bio-specific protein adsorption. *Adv. Funct. Mater.* **2005**, *15*, 357–366. [CrossRef]