Insights into the Aggregation/Deposition and Structure of a Polydopamine Film

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ABSTRACT: Surface-adherent polydopamine (PDA) films as multifunctional coatings can be easily deposited onto a wide range of materials through dopamine self-polymerization. However, a lack of in-depth understanding of PDA aggregation and deposition processes and definite structure elucidation of PDA make it challenging to tailor the surface characteristic and functionality of the PDA films. Herein, we demonstrate that the surface characteristics of the PDA films can be readily tuned by controlling the competitive interplay between PDA aggregation in solution and deposition on the substrate. Moreover, a structural investigation of the PDA films using analytical tools such as X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) allows us to propose a new structure model for the PDA building block. The (DHI)₂/PCA trimer complex, which consists of two 5,6-dihydroxyindole (DHI) units and one pyrrolecarboxylic acid (PCA) moiety, is definitely identified as a primary building block of PDA, and its formation is steered by covalent interactions in the initial stages of polymerization. In latter stages, the (DHI)₂/PCA trimer complexes are further linked primarily through noncovalent interactions to build up the supramolecular structure of PDA. This study provides new insights into the mechanisms of PDA build-up.

1. INTRODUCTION

Functional modification of material surfaces plays a central role in controlling surface properties and conferring new functionalities to materials. Polydopamine (PDA) has opened a new route to the surface modification of various materials and has attracted considerable interest since its advent as a smart coating material in 2007.† Because the PDA film spontaneously formed on various substrates from buffered, aqueous dopamine solution under an ambient atmosphere, it provides an exceptionally facile route to surface modification and could easily create a conformal coating on substrates with complex geometries. Moreover, the diverse functional groups found in the PDA film allow the facile conjugation of amine- and/or thiol-containing biomolecules on surfaces.‡ The PDA film has been used successfully in many applications, especially for biotechnology and biomaterials applications.³⁻⁵

Despite the extensive use of the PDA film, the resulting uncontrollable surface characteristics, such as thickness, surface morphology, functional groups, and biocompatibility, are still major obstacles for expanding its applications. For instance, a significant drawback of the PDA film is its uncontrollable surface roughness, which can obstruct its potential applications where ultrasmooth surfaces are needed.⁵⁻⁴ In recent years, a number of publications have reported the tailoring of the surface characteristics of the PDA film through varying preparation conditions, such as the reaction temperature, solution pH,oxidant employed,³⁻⁵ initial dopamine concentrations,⁸ and so forth. Notably, Bernsmann et al. reported that the deposition kinetics of a PDA film depend markedly on the used oxidant, oxygen versus Cu²⁺, and on the nature of the buffer solution, Tris versus phosphate buffer.⁹ Recently, Kim et al. demonstrated that deposition from a dopamine solution under a pure oxygen environment led to not only a faster deposition rate but also a very homogeneous, smooth PDA film.⁵ However, the relationships between surface characteristics and the structure of the PDA film are far from being fully explored, and there seems to be no general trend to predict and engineer the PDA film with tailored characteristics.

To date, the mechanism of PDA formation and the molecular structure of PDA have yet to be clearly determined, although many efforts have been devoted to this in recent years.⁸⁻¹³ It has been suggested that the oxidation of...
dopamine followed by cyclization and rearrangement yields an important intermediate called 5,6-dihydroxyindole (DHI), which may be further oxidized to 5,6-indolequinone (IDQ). The DHI and IDQ units are connected by various covalent and/or noncovalent bindings to build up the PDA. Note that the PDA structural proposals in most previous studies were based upon the analysis of PDA aggregates collected from the dopamine solution. However, the PDA film, rather than the PDA aggregate, is directly utilized for the surface functional modification of materials. Although it is generally believed that dopamine polymerization concurrently occurs in both the aggregation process in solution and the deposition process on the substrate surface, there is no solid experimental evidence of the structural similarity between the PDA aggregate and the PDA film. Moreover, the lack of structural investigation of the PDA film itself would hinder the establishment of its characteristic—structure relationship. Thus, it is necessary to investigate the structure of the PDA film to clarify their characteristics and functionalities.

Herein, we hypothesized that the variation in the surface characteristics of PDA films synthesized under varied conditions may help elucidate the complexity of the polymerization process of PDA. In this study, the PDA films were synthesized with varied initial dopamine concentrations, i.e., 0.25−4 g/L, and their physicochemical properties were systematically characterized. A detailed structural investigation of the various PDA films has been performed using X-ray photoelectron spectroscopy (XPS), time-of-flight secondary ion mass spectrometry (ToF-SIMS), and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). The results provide new insights into the structural model of the PDA film wherein covalently bound oligomers are linked primarily through noncovalent interactions.

2. EXPERIMENTAL SECTION

Preparation of the PDA Aggregates and PDA Films. The PDA films were deposited on TiO2 substrates that were prepared by sputtering Ti on a silicon wafer with post-heat treatment to obtain well-controlled TiO2 substrate finishing and properties as previously described. The TiO2 substrates were immersed in dopamine solutions with various initial dopamine concentrations (0.25, 0.5, 1.0, 2.0, and 4.0 g/L) in 10 mM Tris(hydroxymethyl) aminomethane (Tris) buffer, (pH 8.5) at 25 °C for 12 h in an open vessel. After that, the reacted solutions were centrifuged at 4000 rpm for 10 min, and the pellet was collected and lyophilized to get a solid powder of the PDA aggregate for further analysis. Meanwhile, the PDA-coated substrates were vigorously washed with DI water and blown dry under a slight stream of nitrogen. The solution was not agitated so that the natural deposition process with a constant oxygen supply was maintained. The dopamine solution volume, substrate size, and reactor size were kept the same in all experiments. The PDA films deposited on the solid substrate and the PDA aggregates collected from dopamine solutions at different initial concentrations will be named xPDA-F and xPDA-A, respectively (where x represents the initial dopamine concentration value of 0.25, 0.5, 1.0, 2.0, or 4.0).

In complementary experiments, glass, silicon, polystyrene (PS), and poly(tetrafluoroethylene) (PTFE) were used to deposit PDA films using a 1.0 g/L dopamine solution in Tris buffer. In addition, Tris buffer was replaced by phosphate-buffered saline (PBS, pH 8.5) to deposit PDA films on TiO2 substrates using a 1.0 g/L dopamine solution.

Characterization of the PDA-A. The particle size and distribution of the PDA-A formed in dopamine solution after reaction at 25 °C for 12 h was measured using a Malvern Nano ZS90 laser particle size analyzer (Malvern, Nano ZS, U.K.). The measurement temperature was kept at 25 °C.

Characterization of the PDA-F. The thickness of deposited PDA-F was measured with a spectroscopic ellipsometer (M-2000 V, J.A. Woollam, USA). A and Ψ values measured at a wavelength of 370−1000 nm were chosen for data analysis, and the Cauchy model was used to determine the thickness of the deposited PDA-F. The surface wettability of the PDA-F was examined by water contact angle (WCA) measurements with a contact angle instrument (Digidrop, France) and applying the sessile drop method. The surface topography of the PDA-F was analyzed by an atomic force microscope (AFM, NanoScope IIIa/Dimension 3100, Digital Instruments, CA). The AFM images were obtained in tapping mode, and the root mean square (rms) was used to evaluate the surface roughness of the PDA-F on the basis of a 20 μm × 20 μm scan area. The surface morphology of the PDA-F was observed by scanning electron microscope (SEM, JSM-6700F, JEOL, Japan).

XPS Analysis. The surface chemical composition of the PDA-F was measured using XPS (Kratos, Axis Ultra DLD, UK). A monochromatic Al Kα X-ray was used as an excitation source (hv = 1486.6 eV) running at 15 kV and 150 W. For each sample, a survey scan (0−1400 eV) was taken with a pass energy of 160 eV, followed by high-resolution spectra of Ti 2p, C 1s, N 1s, and O 1s with a pass energy of 20 eV. The atomic percentages of the various elements were derived from the high-resolution spectra using the sensitivity factors provided by the manufacturer. The neutral C 1s peak (C−C(H), set at 285.0 eV) was used as a reference for charge correction.

ToF-SIMS Analysis. Static ToF-SIMS spectra were obtained from a ToF-SIMS V spectrometer (ION-TOF GmbH, Münster, Germany). Freshly prepared PDA-F was bombarded with Bi3+ primary ions, which were accelerated at 25 kV with an average pulsed current of 0.3 pA. The raster area was 200 μm × 200 μm, and the acquisition time for each spectrum was 40 s. This resulted in an ion flux dosage of less than 3 × 1011 ions cm−2, meeting static SIMS conditions. Charge compensation was realized using a low-energy flood gun. Three positive and negative spectra were recorded for each specimen at different locations. The spectra were calibrated before the extraction of ion intensity data using IonSpec software. Three positive masses (H, Cl2, and CH3O+) were also chosen for the calibration of positive ion spectra. Mass calibration errors were maintained below 20 ppm. The mass resolution (M/ΔM) at m/z 29 (C3H4+) was about 6000 for all of the PDA films. In addition, the ToF-SIMS spectra of pure dopamine monomer and Tris buffer were also obtained as references using the dopamine HCl powder pressed on an indium substrate and the 10 mM Tris buffer (pH 8.5) solution dropped onto the TiO2 substrate, respectively.

MALDI-MS Analysis. The MALDI-MS measurements were performed with a MALDI micro MX time-of-flight mass spectrometer (Waters, Milford, MA) operated in reflectron mode. The MALDI source is equipped with a 337 nm N2 laser operated with a 4 ns-pulse duration pulse. The laser pulse energy was set as 200 arbitrary units primary ions, which were accelerated at 25 kV with an average pulsed current of 0.3 pA. Three positive and negative spectra were recorded for each specimen at different locations. The spectra were calibrated before the extraction of ion intensity data using IonSpec software. Three positive masses (H, Cl2, and CH3O+) were also chosen for the calibration of positive ion spectra. Mass calibration errors were maintained below 20 ppm. The mass resolution (M/ΔM) at m/z 29 (C3H4+) was about 6000 for all of the PDA films. In addition, the ToF-SIMS spectra of pure dopamine monomer and Tris buffer were also obtained as references using the dopamine HCl powder pressed on an indium substrate and the 10 mM Tris buffer (pH 8.5) solution dropped onto the TiO2 substrate, respectively.

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3. RESULTS AND DISCUSSION

PDA-A in Solution. During the oxidation and self-polymerization reactions of dopamine, the color of the solution gradually changed from colorless to dark brown and then to opaque. Quite interestingly, the different color of the solutions with various initial concentrations was observed after 12 h of
reaction (Figure 1A): the color of the solutions became darker as the initial concentration increased from 0.25 to 1.0 g/L but turned light yellow when the initial concentration further increased to 2.0 and 4.0 g/L. It is also worth noting that the black precipitation at the bottom of the vessel was observed only for solutions with initial concentrations of 2.0 and 4.0 g/L on standing.

Moreover, the particle size of the PDA-A formed in dopamine solutions with different concentrations (shaken afterward) was measured (Figure 1B,C). The average size of the PDA-A showed a linear increase from $\sim 60$ nm at 0.25PDA-A to $\sim 255$ nm at 1.0PDA-A and then suddenly jumped to $\sim 1510$ nm at 2.0PDA-A and $\sim 1690$ nm at 4.0PDA-A. It seems that precipitation occurs when the growing polymer size reaches $\sim 1510$ nm or above because the precipitation was observed only in solutions of 2.0 and 4.0 g/L. These observations are quite consistent with the results obtained in another study in which it was reported that the maximum apparent size attainable by PDA-A before precipitation was $\sim 1200$ nm.

**Thickness and Morphology of the PDA-F.** Interestingly, the thickness evolution of the deposited PDA-F revealed a biphasic trend (Figure 2): the thickness proportionally increased with initial concentration from 0.25 to 1 g/L but started to decrease when the concentration was higher than 1 g/L. The maximum thickness of $20 \pm 2$ nm was obtained for 1.0PDA-F. The water contact angle (WCA) measurement showed that PDA deposition slightly increased the surface hydrophobicity compared to that of the pristine TiO$_2$ surface, and all WCA values of PDA-Fs were centralized at $\sim 56-70^\circ$.
Moreover, the surface morphology and surface roughness of various PDA-Fs were revealed by AFM and SEM (Figure 3A,B). The root-mean-square (rms) roughness displayed a biphasic dependence on the initial dopamine concentration: compared to $2.7 \pm 0.2$ nm for the pristine TiO$_2$ surface, the rms slightly increased on 0.25PDA-F and 0.5PDA-F and strikingly increased to $18.0 \pm 2.0$ nm on 1.0PDA-F but started to decrease on 2.0PDA-F and 4.0PDA-F. Both AFM and SEM images showed the appearance of PDA aggregates on top of the uniform PDA layer. The quantity of PDA aggregates appearing on surfaces is compliant with the biphasic trend of surface roughness, suggesting that the conjugation of PDA aggregates on the substrate may contribute to an increase in the surface roughness of PDA-Fs.

**XPS Analysis.** XPS survey spectra showed the signatures originating from C, N, O atoms and suggested the successful deposition of all the PDA-Fs on the substrate as shown in our previous report.\textsuperscript{17} Ti was undetected by XPS on PDA-Fs, except for 0.25PDA-F and 4.0PDA-F. This suggested that the 0.25PDA-F and 4.0PDA-F were thinner than others, which was consistent with the results determined by ellipsometry (Figure 2).

The atomic concentrations and functional group distributions were further analyzed quantitatively. The atomic concentrations and ratios were calculated and listed in Table 1. It can be seen that the N/C and O/C ratios obtained on the PDA-Fs are $\sim 0.1$ and $\sim 0.28$ respectively, which are close to the stoichiometric values of dopamine (N/C = 0.125, O/C = 0.25). These results indicate that the PDA-Fs have a chemical composition similar to that of dopamine. Also, little variation in the N/C and O/C ratios was observed among the various PDA-Fs, suggesting that their compositions are similar.

To further determine the concentrations of functional groups for the various PDA-Fs, the high-resolution spectra of C 1s, O 1s, and N 1s were curve-fitted (Figure 4). The peak positions (listed values $\pm 0.2$ eV) and atomic concentrations of the functional groups are listed in Table 2. The atomic concentration of each functional group was obtained by

<table>
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<th>TiO$_2$</th>
<th>0.25PDA-F</th>
<th>0.5PDA-F</th>
<th>1.0PDA-F</th>
<th>2.0PDA-F</th>
<th>4.0PDA-F</th>
<th>dopamine (theoretical)</th>
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<td>72.0</td>
<td>71.7</td>
<td>73.3</td>
<td>72.1</td>
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<td>O</td>
<td>22.1</td>
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<td>20.7</td>
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<td>Ti</td>
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<td></td>
<td></td>
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<td>0.105</td>
<td>0.098</td>
<td>0.103</td>
<td>0.125</td>
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<tr>
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<td>0.289</td>
<td>0.266</td>
<td>0.277</td>
<td>0.250</td>
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<tr>
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<td></td>
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<td>14.800</td>
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multiplying the percentage of the functional group obtained from curve fitting by the atomic concentration of the element. The C 1s spectra were fitted with five components assigned to C−C(H) at 285.0 eV, C−OH/C−N at ∼286.4 eV, C≡O at ∼288.0 eV, O−C≡O at ∼289.0 eV, and the π → π* shakeup.18

The N 1s spectra were fitted with three peaks assigned to amines (−C−NH) at ∼400.3 eV, aromatic N at ∼399.5 eV, and protonated N (C−NH₃⁺) at ∼402.0 eV.18 The major contribution to N 1s came from amine groups −C−NH, not from the other two components (aromatic N and C−NH₃⁺).

Figure 4. High-resolution XPS spectra of C 1s, N 1s, and O 1s regions for the various PDA-Fs.
This implies that the cyclization reaction occurs during PDA formation and a few open-chain dopamine units remain on the PDA-F. The possible production of C=NH$_2^+$ might be ascribed to the spontaneous proton transfer from the acid (catechol, C=OH) to the amine (C=NH$_2$). The O 1s spectra were fitted with two major peaks at 531.5 and 533.0 eV. The peak at 531.5 eV is assigned to oxygen in C=O, which comes from quinone and the carbonyl oxygen in O=C–O as indicated in C 1s curve fitting. The peak at 533.0 eV is assigned to oxygen in C–O, which is mainly due to catechol C=OH and to a lesser extent the oxygen in O=C–O groups. It is interesting that the amount of C–O was much higher than that of C=O on all PDA-Fs, suggesting that catechol C=OH is the major oxygen functional groups on the PDA-Fs. In addition, the O 1s spectra of the 0.25PDA-F and 4.0PDA-F exhibited an additional peak at 529.7 eV, which was attributed to the TiO$_2$ substrate. The visibility of this TiO$_2$ oxygen peak in 0.25PDA and 4.0PDA (3.4 and 0.3%, respectively) indicates that the film thickness is below or close to the XPS probing depth of ∼9 nm, which is also consistent with the results determined by ellipsometry (Figure 2). From Table 2, little variation in the concentrations of functional groups was observed among the various PDA-Fs, suggesting that the molecular structures of these films are similar.

In addition, XPS analysis of the PDA-Fs deposited onto various types of substrates, i.e., glass, silicon, PS, and PTFE, and formed in PBS revealed similar signals of C 1s, N 1s, and O 1s from the PDA (Supporting Information, Figures S1 and S2). Little variation in the atomic compositions of the PDA-Fs on various substrates and formed in different buffer solutions suggested that the composition of the PDA-Fs was independent of the substrate compositions and buffer solutions (Supporting Information, Table S1).

**ToF-SIMS Analysis.** For comparison, both the dopamine HCl and the Tris buffer were analyzed by ToF-SIMS. The positive spectrum of dopamine HCl reveals significant peaks at $m/z$ 30 (CH$_2$N$^+$), 91 (C$_5$H$_7$), 137 (C$_6$H$_5$NO$^+$), 154 (C$_7$H$_7$NO$^+$), and 307 (C$_8$H$_7$N$_2$O$_2$), which validate the existence of dopamine monomers (Figure 5A). The positive spectrum of Tris suggests the intact Tris molecule with peaks at $m/z$ 122 (C$_3$H$_8$N$^+$) (Figure 5B). There is no distinguishable peak detected above $m/z$ 400 for either dopamine or Tris. 0.25PDA-F, 1.0PDA-F, and 4.0PDA-F had similar characteristic ToF-SIMS spectra (Figure S3). The positive spectra of the various PDA-Fs reveal several common peaks at $m/z$ 30 (CH$_2$N$^+$), 41 (C$_6$H$_7$), 55 (C$_8$H$_7$), 77 (C$_9$H$_7$), 91 (C$_9$H$_7$), 115 (C$_{10}$H$_{11}$N$^+$), 130 (C$_{10}$H$_{11}$NO$^+$), 149 (C$_{11}$H$_{11}$NO$^+$), and 402. The peak at $m/z$ 149 corresponds to an intact DHI unit, and the peak at $m/z$ 130 is due to the liberation of a hydroxyl group from a DHI unit. However, no significant peak originating from either dopamine or Tris was observed in the spectra. These observations indicate that cyclized indole units (DHI-like) are more likely to exist on the surface of PDA-F rather than uncyclized dopamine-like units or Tris. More interestingly, an intense peak at $m/z$ 402 was observed in the high-mass region ($m/z > 200$), which may originate from a stable building block of the PDA structure on the trimer level.

In the negative spectra of dopamine HCl and Tris (Figure 5A,B), the same molecular ions of dopamine and Tris as in the positive spectra were observed. In line with the results from positive spectra, the negative spectra of the PDA-Fs (Figure 5C) did not show any distinct signatures of dopamine or Tris, which further confirms that neither dopamine nor Tris existed at a significant level on the surface of the PDA-Fs. The low-mass region ($m/z < 100$) is dominated by N-containing fragments. In addition to the nonspecific fragments such as CN$^−$ and CNO$^−$, it is interesting to observe fragments such as C$_5$N$^−$, C$_6$N$^−$, and C$_7$N$^−$. These ions are mainly derived from cyclic nitrogen, e.g., DHI. In the mass range of $m/z$ 100−200, interestingly, two intense peak clusters around $m/z$ 110 and 122 were detected on the PDA-Fs, which might suggest the existence of pyrrolocarboxylic acid (PCA) moieties derived from the oxidative degradation of indole units.

In addition, experiments showed that the PDA-Fs deposited onto various types of substrates, i.e., glass, silicon, PS, and PTFE, and the PDA-Fs formed in PBS had similar signature of mass spectra (Supporting Information, Figures S4 and S5). These data suggest that the structure of PDA-F seems to be independent of substrates and buffer solutions.

Interestingly, it was found that the relative intensity of the ion at $m/z$ 402 with respect to the total positive ions varied on different PDA-Fs (Figure 6). It decreased with increasing initial dopamine concentration; in particularly, the relative intensity decreased by ∼71% from 0.25PDA-F to 4.0PDA-F. This indicates that it is increasingly difficult to obtain the ion fragmentation at $m/z$ 402 from ToF-SIMS as the initial dopamine concentration increased.

To the best of our knowledge, ToF-SIMS investigation of the PDA-Fs has been quite limited. Messersmith et al. reported

### Table 2. XPS Atomic Concentration of Functional Groups in All Atoms of the Various PDA-Fs

<table>
<thead>
<tr>
<th></th>
<th>0.25PDA-F</th>
<th>0.5PDA-F</th>
<th>1.0PDA-F</th>
<th>2.0PDA-F</th>
<th>4.0PDA-F</th>
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<tr>
<td>C 1s</td>
<td>285.0 (C=C, C–C, CH$_3$)</td>
<td>31.8</td>
<td>35.1</td>
<td>34.6</td>
<td>37.2</td>
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<td>N 1s</td>
<td>286.4 (C–OH and C–N)</td>
<td>20.6</td>
<td>24.0</td>
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<tr>
<td>O 1s</td>
<td>288.0 (C=O)</td>
<td>7.1</td>
<td>7.0</td>
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<td>6.4</td>
</tr>
<tr>
<td>N 1s</td>
<td>289.0 (O–C=O)</td>
<td>3.5</td>
<td>3.6</td>
<td>3.9</td>
<td>3.8</td>
</tr>
<tr>
<td>O 1s</td>
<td>290–291 (π → σ* shake up)</td>
<td>2.7</td>
<td>2.3</td>
<td>3.6</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*The atomic concentration of each functional group is the percentage of each functional group in corresponding element × the atomic concentration of the corresponding element in all atoms.*
only the positive ToF-SIMS spectrum with a limited mass range, which revealed a peak at $m/z$ 445 originating from a trimer of DHI together with a characteristic pattern of fragmentation. However, the peak at $m/z$ 445 is not present in the spectra obtained in this study or in other published literature. It is worth noting that the ToF-SIMS spectra of PDA-Fs from two other reports confirmed the existence of the intense peak at $m/z$ 402 as we observed here, but they did not assign it to any specific structure.

Figure 5. ToF-SIMS ion spectra of (A) dopamine hydrochloride, (B) Tris buffer, and (C) 1.0PDA-F. Left: positive ion spectrum. Right: negative ion spectrum.
MALDI-MS Analysis. Compared to ToF-SIMS, which is a very surface-sensitive technique (probing depth <1 nm), MALDI-MS is capable of analyzing the volume of a sample and large ions of up to several 100 kDa molecular mass produced by relatively soft ionization. To get more structural information from the whole volume of the PDA-Fs, we carried out a structural investigation of representative PDA-Fs using MALDI-MS. The spectra of both dopamine HCl and Tris buffer showed unique peaks corresponding to an intact dopamine molecule and a Tris molecule, respectively, along with some peaks resulting from the matrix (Figure 7A,B).

The MALDI spectra of 0.25PDA-F, 1.0PDA-F, and 4.0PDA-F showed similar peak patterns (Figure 7C and Supporting Information, Figure S6). Notably, the spectra of all the PDA-Fs showed a common intense peak at around m/z 402.1, which is quite consistent with what we observed from the ToF-SIMS spectra. However, the spectra of the PDA-Fs did not contain any ionic species corresponding to a single DHI unit, as observed in ToF-SIMS spectra. This discrepancy may be ascribed to the relatively soft ionization of MALDI-MS with respect to the ToF-SIMS, so that the DHI-containing oligomers were not able to be fragmented to get single DHI units. This also provides evidence that the DHI units observed in the ToF-SIMS spectra may, at least partially, originate from the fragmentation of the oligomer at m/z 402 because there was no other distinguishable peak observed in the relatively high m/z region. It is also worth noting that the peak at m/z 154.1 corresponding to the dopamine monomer was very weak or almost invisible in the spectra of 0.25PDA-F and 1.0PDA-F (Figure 7C), but it became more prominent in the spectra of 4.0PDA-F. However, there was no apparent signature of dopamine on the top surface of the PDA-Fs on the basis of the ToF-SIMS spectra. One possible reason is that unpolymerized dopamine was physically trapped within PDA-F during polymerization, and it became more significant at higher dopamine concentration, giving a larger amount of dopamine trapped within 4.0PDA-F. It is worth mentioning that the lower
cell density on 4.0PDA-F compared to that on other PDA-Fs synthesized at lower dopamine concentrations was demonstrated in our previous study, which may result from a larger release of trapped dopamine within 4.0PDA-F.10

In addition, the 1.0PDA-A collected from the dopamine solution was also investigated by MALDI-MS (Figure 7D). It is noticeable that the spectra of 1.0PDA-A showed significant peaks at m/z 122.0 and 154.0 originating from the Tris and dopamine monomers, respectively. However, these two peaks were not observed on their counterpart films, suggesting that the Tris and dopamine molecules are more easily trapped within PDA-A than within PDA-F. More importantly, the spectra of 1.0PDA-A showed the same potent peak at m/z 402.2 as for the spectra of 1.0PDA-F, suggesting that PDA-A and PDA-F shared a common building block. To the best of our knowledge, this is the first time that solid experimental evidence of a certain structural similarity between the PDA-A in solution and the PDA-F on substrate has been provided; most previous studies on PDA structure investigation were based only on the analysis of PDA-A collected from the solution.10–13

Model of Competitive Processes of Aggregation and Deposition. It has been believed that the aggregation in solution and the deposition on the substrate occur concurrently during the polymerization of dopamine.8 In this study, we further proved that the aggregation and deposition processes produce structurally similar PDA through MALDI-MS analysis, so they may involve similar polymerization processes. We believe that the aggregation and deposition are two competitive processes in terms of consuming the dopamine monomers and/or DHI units in the solution. Moreover, it is intriguing to find the biphasic dependence of the PDA-F thickness and surface roughness on the initial dopamine concentrations.

On the basis of these results, we propose a model of the competitive process for the evolution of PDA aggregation and deposition rates as a function of initial dopamine concentrations, as depicted in Scheme 1. The evolution of PDA aggregation and deposition rates is divided into two regimes.

Scheme 1. Evolution of the Aggregation Rate in Solution and the Deposition Rate on the Substrate As the Function of the Initial Dopamine Concentration during PDA Polymerization

In regime I, i.e., 0.25–1.0 g L\(^{-1}\), the initial increase in the dopamine concentration in solution results in an elevated deposition rate of PDA-F on the substrate, whereas the aggregation rate in solution might remain at a relatively low level because of the large mean free path between dopamine and/or DHI units. Moreover, the small PDA-A formed in solution is able to incorporate into the deposited PDA-F. Thus, the PDA-F thickness and surface roughness increase with increasing initial dopamine concentration.

In regime II, i.e., 1.0–4.0 g L\(^{-1}\), the further increase in the dopamine concentration dramatically reduces their mean free path, which substantially accelerates the aggregation rate in solution against the deposition rate on the substrate. More dopamine and/or DHI are involved in aggregation than in deposition. Meanwhile, the large PDA-A formed in solution is not capable of contributing to the deposition of PDA-F. Thus, further increases in the initial dopamine concentration result in decreases in PDA-F thickness and surface roughness.

It is worthy noting that our model is supported by a previous finding that the PDA-F growth rate on the substrate reached a plateau value for initial concentrations greater than 1.0 g L\(^{-1}\).8,25 Recently, Ball et al. demonstrated that the surface roughness of PDA-F scaled linearly with the film thickness, which is also in agreement with our findings (Figures 2 and 3A). However, they observed a linear increase in film thickness with the initial concentrations, whereas we did not (Figure 2). The discrepancy might result from different oxygen concentrations supplied in dopamine solutions because the solution was vigorously agitated to increase the oxygen concentration in their study whereas we did not. Interestingly, Kim et al. found that the higher oxygen concentration in solutions would accelerate the deposition rate of the PDA-F.3 The finding might reveal that the biphasic evolution of PDA-F thickness with increasing initial concentrations results from not only the competition between aggregation and deposition in consuming the dopamine and/or DHI units but also the limited oxygen supply in solutions. Nevertheless, further study of the effects of oxygen concentration is necessary to achieve a better understanding of the dynamic processes of PDA formation.

New Insights into the Structural Model of PDA. To date, five typical models of the PDA structure have been proposed, as shown in Scheme 2. On the basis of the revealed experimental results here, we find that some previously proposed PDA models are less likely to elucidate the PDA-F formation studied in this work. For example, the polycatecholamine model (Scheme 2A) is questionable because we have not observed apparent features of uncyclized dopamine units from mass spectra. Also, the eumelanin-type (polyindole) model (Scheme 2B) cannot be experimentally supported by our results because if the PDA were the homopolymer of indoles, such as DHI and its dione derivatives, we would expect to observe significant quantities of dimer and trimer repeat units. In fact, we have not observed significant features of the dimer, trimer, or any other low oligomers of DHI units from the mass spectra of PDA-F. As previously mentioned, Messersmith et al. proposed this model based on their observation of the DHI trimer at m/z 445 from the positive ToF-SIMS spectrum.1 However, the peak at m/z 445 is not present in the ToF-SIMS spectra obtained in this study or in any other published literature.22,23 On the basis of the same arguments, the quinhydrone model (Scheme 2C) is also very unlikely. According to this model, PDA is not a covalent polymer but instead is a supramolecular aggregate of DHI and its dione derivative that were held together through a combination of charge transfer, \(\pi\)-stacking, and hydrogen-bonding interactions.13 Moreover, the recently identified physical trimer model of \((\text{dopamine})_2/{\text{DHI}}\) (Scheme 2D) should not exist at a significant level in the PDA-Fs prepared in this study because if it did then a large proportion of uncyclized dopamine units rather than cyclized DHI units should be present in the films.
Similarly, the failure to detect significant levels of uncyclized dopamine units also contradicts the recently proposed poly(indole-dopamine) model (Scheme 2E), wherein indole units with different degrees of (un)saturation and dopamine units are covalently linked by C−C bonds. Notably, Della Vecchia and coworkers have recently identified a novel pyrrolecarboxylic acid (PCA) moiety in their solid-state NMR analysis and proposed it as one of the pathways for PDA buildup. Inspired by their findings, a tentative assignment of the main peaks in the ToF-SIMS and MALDI spectra of the PDA-Fs in this study is shown in Scheme 3. Hypothesized structure \((\text{DHI})_2/\text{PCA}\), corresponding to the major peak at \(m/z\) 402, consists of two kinds of units, i.e., two DHI units and one PCA moiety. The two DHI units contribute to the large proportions of fragments at \(m/z\) 149 (DHI repeat unit ion) and 130 (DHI losing an OH) shown in the positive ion spectra of ToF-SIMS. The PCA moiety accounts for the intense cluster ions around \(m/z\) 110 and 122 in the negative ion spectra of ToF-SIMS. It is also noticeable that no significant peak above \(m/z\) 402 was detected from either ToF-SIMS or MALDI-MS spectra, which was consistent with previous mass spectrometric data of DHI polymers. This observation suggests that the \((\text{DHI})_2/\text{PCA}\) trimer complexes, as the major building blocks of PDA, were unlikely to be covalently bonded to build up PDA. If they were, then we would expect to observe significant levels of \([((\text{DHI})_2/\text{PCA})_2\] and \([((\text{DHI})_2/\text{PCA})_3\] at \(m/z\) of around 804 and 1206, respectively, in the MALDI mass spectra. However, these ions were not detected in our MALDI spectra. Herein, we propose that PDA is more likely to be a supramolecular aggregate of \((\text{DHI})_2/\text{PCA}\) trimer complexes that are held together primarily through noncovalent interactions. It must be emphasized that the supramolecular structure of PDA has also been suggested in several recent studies, although there is no consensus on the molecular structure of the building unit. In addition, the decrease in the relative intensity of the \((\text{DHI})_2/\text{PCA}\) trimer complex with increasing dopamine concentration observed in the ToF-SIMS also supports the supramolecular structure of PDA. This is because the increase in the initial dopamine concentration would increase the intermolecular interaction among the \((\text{DHI})_2/\text{PCA}\) trimer complexes (as evidenced by the increase in their aggregate size); consequently, it would become more difficult to get the \((\text{DHI})_2/\text{PCA}\) trimer ion in ToF-SIMS. This is somewhat similar to a

**Scheme 2. Traditional and Recent Models of the PDA Structure Proposed in the Literature**

**Scheme 3. Tentative Structures Assigned to the Molecular Species Detected in the Mass Spectra of PDA-F and PDA-A**
well-known phenomenon observed for cross-linked polymers: the polymer ion intensity drops substantially after cross-linking.

Altogether, the data described above provided new evidence and further expanded the new scenarios in the PDA buildup presented in the latest studies.\textsuperscript{13,16} Our results suggested that there were two distinct stages in the PDA buildup with regard to the relative contribution of covalent versus noncovalent interactions to polymerization. In initial stages, the covalent interactions dominate the monomer–monomer and/or monomer–low oligomer coupling processes, producing large amount of low oligomers, such as (DH\textsubscript{2})\textsubscript{2}/PCA trimer complexes as uncyclized catecholamine/quinones and Tris buffer small entities that then gradually grow up to large supramolecular aggregates.

In addition, Della Vecchia and coworkers proposed that both uncyclized catecholamine/quinones and Tris buffer were covalently incorporated into the PDA structure.\textsuperscript{13} An important remark is that their findings were based on the analysis of the PDA-A collected from the solution. In this study, indeed both the uncyclized dopamine monomer and Tris buffer are present in the PDA-A, as evidenced by MALDI-MS analysis (Figure 7D). However, these molecules are hardly detected on PDA-F using MALDI-MS, and only the 4.0PDA-F shows a modest signal of these molecules in MALDI-MS. Moreover, these molecules have never been detected on the top surface of PDA-Fs using ToF-SIMS. This suggests that the top surface of PDA-F is free of dopamine monomer and Tris buffer, which is understandable because the PDA-F has been extensively washed with DI water. All of these results lead us to believe that the dopamine and Tris molecules detected in the aggregates are due to physical trapping that occurs rather easily during PDA aggregation in solution. This also points out the fact that the direct analysis of deposited PDA-Fs provides more meaningful information on the PDA structure. From a practical point of view, it is the functional groups present on the surface of PDA-F that will be useful for their applications.

4. CONCLUSIONS

We demonstrated that the surface characteristics of PDA-F can be readily tuned by varying the initial dopamine concentrations. Specifically, the thickness and surface roughness of PDA-F showed a biphasic dependence on initial dopamine concentrations. These tunable surface characteristics result from two competitive processes of PDA aggregation in solution and PDA deposition on a substrate. Furthermore, the structural investigation of PDA-F opens new scenarios in the mechanisms of PDA buildup that were contributed by both covalent and noncovalent interactions. The covalent interactions steer the formation of the (DH\textsubscript{2})\textsubscript{2}/PCA trimer complex that is identified to be the major building block of the PDA in this study. The (DH\textsubscript{2})\textsubscript{2}/PCA building blocks are further linked primarily through noncovalent interactions to build up the supramolecular structure of PDA-F. Therefore, important progress in the elucidation and understanding of the characteristics and structure of PDA-F has been achieved. The new insights acquired here shed light on the characteristic–structure relationships and better tailoring functionality of PDA-F.

ASSOCIATED CONTENT

Supporting Information

XPS wide spectra of PDA-Fs deposited on various substrates and prepared in PBS. ToF-SIMS ion spectra of 0.25PDA-F, 4.0PDA-F, and 4.0PDA-F deposited on various substrates and prepared in PBS. MALDI spectra of 0.25PDA-F and 4.0PDA-F and a table of atomic concentrations and ratios of PDA-Fs deposited on various substrates and PDA-F prepared in PBS. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Laura Yu Cao in the mass spectrometry laboratory of the department of chemistry at The Hong Kong University of Science and Technology (HKUST) for assistance and helpful discussions about MALDI-MS experiments and Mr. Nick Ho of the Materials Characterization and Preparation Facility at HKUST for assistance with XPS measurements. This work was financially supported by a grant from HKUST (FSGRF13EG58), the Program for New Century Excellent Talents in University (NCET-10-0704), and the Sichuan Youth Science Technology Foundation (2011JQ0010).

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