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Alternate drop coating for forming dual biointerfaces composed of polyelectrolyte multilayers

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Abstract Two types of polyelectrolyte multilayers were formed on both sides of a quartz crystal microbalance (QCM) substrate by a novel alternate drop coating process. In this study, poly(diallyldimethylammonium chloride) (PDDA) and poly(sodium 4-styrene sulfonate) (PSS) were used as strong-strong polyelectrolytes. On the other hand, PDDA and poly(acrylic acid) (PAA) were used as strong-weak polyelectrolytes. The novel alternate drop coating process can separately fabricate each polyelectrolyte multilayer on both sides of the substrate. The substrate provides dual biointerfaces, both sides of which comprise different multilayers, by employing a combination of polymers. The formation of the multilayer by alternate drop coating was evaluated in terms of changes in the frequency of the QCM and model protein adsorption for proteins such as bovine serum albumin, and their characteristics were investigated with those of the conventional alternate adsorption process by performing dip coating. There was no significant difference between the surface properties resulting from the two formation conditions. This result strongly supported the fact that the multilayers fabricated by alternate drop coating were similar in quality to those fabricated by conventional dip coating. The resulting dual biointerfaces with polyelectrolyte multilayers provide alternative biofunctions in terms of individual protein loading. In summary, the novel alternate drop coating process for substrates is a good candidate for the preparation of dual biointerfaces in the biomedical field.

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1 Introduction

Polyelectrolyte multilayers were discovered by Decher and coworkers in the 1990s, and alternate adsorption (dip coating) is well known as a fundamental process for fabricating polymeric assemblies on a given substrate [1, 2]. The resulting ultrathin membrane has a layer-by-layer (LbL) structure. The driving force of the LbL assembly comprises intermolecular forces such as those related to electrostatic interaction, hydrogen bonding, charge transfer interaction, and stereocomplex formation; excellent reviews on the abovementioned process have been published elsewhere [3, 4]. We have studied on LbL assemblies, and one of the target applications is preparation of high-performance biomaterials [5, 6]. The alternate adsorption process provides a diversity of applications on surface modification and an alternate soaking process for hydroxyapatite formation was discovered by Taguchi et al. in 1998 [7]; moreover, an improvement to this process was recently reported [8]. Taking these reports into account, the alternate adsorption process is shown to fabricate a variety of interfaces on biomaterials.

The process of polyelectrolyte multilayer by the alternate adsorption is elaborate, and then another easier assembly technique has been introduced such as spray- and spin-assisted coating [9, 10]. Another disadvantage of the alternate adsorption process is simultaneous coating on both sides of the substrate. This problem is due to the immersion of the substrate. So far a hetero-functional

assembly has not been considered, because the alternate adsorption is carried out by the dip coating process in most cases. In the biomedical field, a hetero-functional biointerface is crucial to be advanced biomaterials. Nishikawa et al. reported that two types of cells were directly cultured on both sides of a honeycomb mesh with a stretched microporous film providing anisotropic arrays of stretched micropores [11]. In the field of colloid science, Janus particles are popular and possess two types of surface properties, morphologies, and chemical compositions [12–14]. If one could form a hetero-functional LbL assembly on each side of a substrate, the resulting polyelectrolyte multilayer is a good candidate for novel dual biointerfaces.

Recently, we have developed an alternate drop coating process for forming dual biointerfaces composed of polyelectrolyte multilayers [15]. A polyelectrolyte droplet facilitates versatile assemblies on one side of a substrate, and then the reverse side of the substrate will be independently assembled by the alternate drop coating. In the present study, we focused on the fabrication of dual biointerfaces, as shown in Fig. 1. This is the first challenge in the development of dual biointerfaces comprising polyelectrolyte multilayers on both sides of the substrate, which were prepared by the drop coating process. The most favorable characteristic of the resulting dual biointerfaces is a good bridge between hetero surfaces; for example, two types of cytokines are separately loaded on both sides of the substrate, and the loaded cytokines are released in different directions from the substrate. Therefore, cytokine adsorption and cell adhesion are independently regulated on the dual biointerfaces (Fig. 1). The final image of the dual biointerfaces will be able to be interposed between two types of tissues in vivo.

In the present study, we focused on a fundamental characterization on the dual biointerface. Particularly, surface properties on the resulting polyelectrolyte multilayers prepared by the alternate drop coating should be equivalent the surface fabricated with conventional alternate adsorption process. We have investigated the equivalent properties in terms of total amount of multilayers in each step and amount of albumin on the biointerface. Poly(diallyldimethylammonium chloride) (PDDA), poly(sodium 4-styrene sulfonate) (PSS), and poly(acrylic acid) (PAA) were selected as the typical polyelectrolytes. These polymer combinations have been employed for fabricating polyelectrolyte multilayers by many researchers [16–19]. The next subject of concern in this study is the drop coating process for the fabrication of dual biointerfaces. A droplet of the polymer solution was placed on the substrate for a while, and the droplet was removed and subsequently rinsed with buffer solution. Actually, this process is the alternate adsorption process; moreover, drop coating is shown to fabricate polyelectrolyte multilayers on both sides of the substrate. Particularly, two types of the polyelectrolyte multilayer could be fabricated in each side separately. As shown in Fig. 1, polyelectrolyte multilayers with different properties are fabricated, and the following specific surface properties are also induced. Moreover, this is a simple process, even though the morphology of the substrate is complicated. We have evaluated protein adsorption on the dual biointerfaces using bovine serum albumin as a cytokine model. The protein adsorption was performed as a model biofunction, and the total amount of protein adsorption was well regulated by the composition of each surface on the dual biointerfaces. From the present study, we have succeeded in the preparation of a variety of biointerfaces on biomaterials by using the drop coating process.

2 Materials and methods

2.1 Materials

The polyelectrolytes used in this study are shown in Fig. 2. Poly(diallyldimethylammonium chloride) (PDDA, #17338, Polyscience Inc., PA, USA; MW = 240,000 g/mol) and poly(sodium 4-styrene sulfonate) (PSS, #561959, Aldrich, MO, USA; MW = 200,000 g/mol) were used as strong polyelectrolytes. Poly(acrylic acid) (PAA, Wako Pure Chemical Industries Ltd., Osaka, Japan; MW = 250,000 g/mol) was used as a weak polyelectrolyte. Bovine serum albumin (BSA, A-8022) was purchased from Sigma, MO, USA. Tris(hydroxymethyl)aminomethane hydrochloride (Tris–HCl, Wako Pure Chemical Industries Ltd.) was used as a buffer solution. All the chemicals were used without

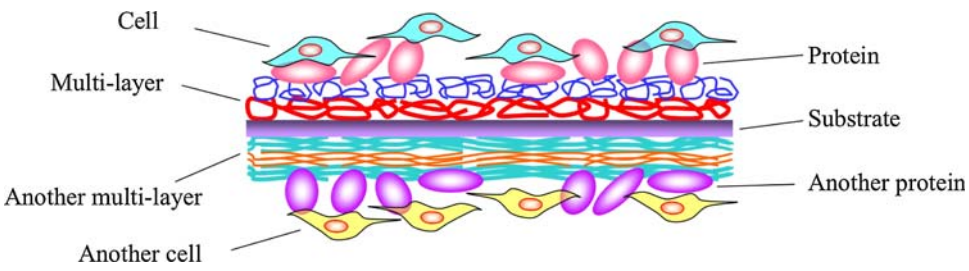

Fig. 1 Illustration of dual biointerfaces in this study. The size of each component is exaggerated

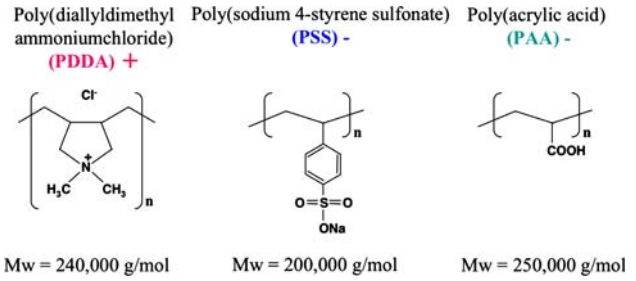

Fig. 2 Chemical structures of polyelectrolytes used in this study

further purification. Ultrapure water was used through the experiment.

2.2 Instruments

Quartz crystal microbalance (QCM) substrates were used for the preparation of dual biointerfaces. The most favorable characteristic of the QCM substrate is the instantaneous and timely detection of the total weight of the polymer assembly. Furthermore, the change in the frequency shift was correlated to the weight of the multilayer by using Sauerbrey's equation (1 Hz corresponds to 1.15 ng), and the detection limit for the weight was roughly 1 ng [20]. The QCM substrate used in the present study was an AT-cut quartz crystal with a resonant frequency of 9 MHz, and it was purchased from USI Co., Ltd., Fukuoka, Japan. AT-cut refers to orientation of the plate to the crystal structure. The crystal (diameter: 9 mm) was coated with gold electrodes (diameter: 4.5 mm) on both sides. The frequency was then monitored by a frequency counter with a 225 MHz 2-channel input (53131A, Agilent Technologies, Santa Clara, CA, USA). The leads of the QCM were sealed with a silicone rubber gel in order to protect them from corrosion during immersion in the aqueous solutions. The amount of polymer adsorption was calculated by measuring the frequency decrease in the QCM, ΔF, using Sauerbrey's equation as follows:

$$-\Delta F = \frac{2F_0^2}{A\left(\rho_q \mu_q\right)^{1/2}} \Delta m$$

where F_0 is the resonant frequency of the QCM (9×10^6 Hz); A, the electrode area (0.159 cm^2); ρ_q, the density of quartz (2.65 g/cm^3); and μ_q, the shear modulus of quartz (2.95×10^{11} dyn/cm^2). The measurements were performed after drying the QCM with N_2 gas for 3 min. The frequency was recorded after the stabilization of the counter. The measurement was performed in an air-conditioned room at 25°C. This Sauerbrey's equation is relevant when the measurements are performed in air, as described above. The mass of the solvents was never detected as a frequency shift, and the effects of the viscosity of the bound compounds can be ignored.

2.3 Alternate drop coating process for dual biointerfaces

The polyelectrolyte solutions—PDDA, PSS, and PAA—were dissolved in 50 mmol/l of Tris–HCl (pH 7.4), and the concentration was adjusted to 0.2 mg/ml. The ionic strength was adjusted to 0 or 0.15 mol/l by using NaCl. Before alternate drop coating, both sides of the QCM substrate were treated three times with a piranha solution (concentrated H_2SO_4/H_2O_2 (30 wt% aqueous solution) = 3/1, v/v) for 1 min; subsequently, it was rinsed with ultrapure water. After drying with N_2 gas, the cleaned QCM substrate was used for alternate drop coating.

Initially, the QCM substrate was fixed with a sponge to maintain a horizontal surface, as shown in Fig. 3. It is important to form dual interfaces on the QCM substrate. The PDDA aqueous solution (50 μl) was dropped on one side of the QCM substrate by a micropipette and it was allowed to remain there for 1 min. In this process, the droplet was maintained on the substrate by surface tension. The polymer solution on the surface was gently rinsed with 1 mmol/l of Tris–HCl solution. After drying with N_2 gas, the frequency was monitored as usual. The procedure was repeated using a polyanion (PSS or PAA). The resulting substrate was abbreviated as $(PDDA/PSS)_5$, which implied a 10-step multilayer obtained by the combination of PDDA and PSS. For a 9-step alternate drop coating process, the abbreviation would be $(PDDA/PSS)_4PDDA$. A specific concentration of NaCl was added to each polymer aqueous solution, thereby varying the polymer conformation and total charge. In this case, the information of the ionic strength (NaCl: 0.15 mol/l) was abbreviated as $(PDDA/PSS)_5$@0.15 M. To prepare polyelectrolyte multilayers by the conventional alternate adsorption process, the bare QCM substrate was immersed in a PDDA aqueous solution (0.2 mg/ml) for 1 min at 25°C, and the substrate was then rinsed with 1 mmol/l of Tris–HCl buffer solution to remove the surplus PDDA solution. After drying with N_2 gas, the frequency of the QCM was monitored. Subsequently, the QCM substrate was immersed in an aqueous solution of PSS or PAA (0.2 mg/ml) and then rinsed with 1 mmol/l of Tris–HCl solution. After drying with N_2 gas, its frequency was again monitored. The alternate

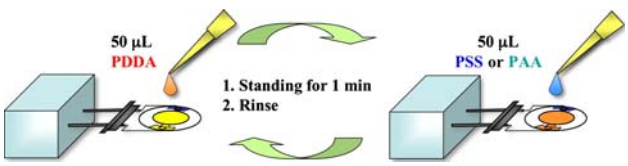

Fig. 3 Image of alternate drop coating process to fabricate dual biointerfaces

adsorption process was repeated for the preparation of the polyelectrolyte multilayers.

2.4 Protein adsorption on dual biointerfaces

Protein adsorption was evaluated by using bovine serum albumin (BSA) with a concentration of 0.45 mg/ml and 50 mmol/l of Tris–HCl buffer solution (pH 7.4); the ionic strength of the buffer solution was zero. BSA was selected as a typical model protein, and the concentration was 1% of the serum. If the concentration of BSA is much higher, multilayered protein adsorption would be occurred. In this case, the amount of BSA cannot be precisely evaluated by the QCM. In the present study, BSA as a cytokine model could adsorb on the polyelectrolyte multilayers. The bound BSA would be released from the polyelectrolyte multilayers by outer stimuli such as change in ionic strength [15]. Therefore, the polyelectrolyte multilayers would be capable of being used as protein reservoirs. The BSA adsorption was also detected by monitoring the changes in the frequency of the QCM. The BSA adsorption was carried out as follows: The QCM substrate with polymeric multilayers was incubated in the BSA solution for 1 h at 37°C. After rinsing with 1 mmol/l of Tris–HCl buffer solution, the apparent amount of BSA adsorption that was only estimated by change in the frequency, was monitored. The amount of BSA adsorption (Δm) was calculated from the decrease in the frequency (ΔF) of the QCM by using Sauerbrey's equation [20], and adsorption density ($\mu g/cm^2$) was finally calculated.

3 Results and discussion

3.1 Precise polyelectrolyte multilayer formation by alternate drop coating

Alternate drop coating was an anomalous method in the field of polyelectrolyte multilayer formation. The process has some advantages: (i) a small amount of polymer solution is sufficient for the coating, (ii) the process is easy to handle, (iii) a separately coating on both sides is achieved, (iv) this process is independent of material morphologies, and (v) a smooth coated surface is obtained. Figure 4a shows precise polyelectrolyte multilayer assemblies obtained by alternate drop coating. The drop coating was carried out for each side. The changes in the frequency were not significantly different for the two sides (surface and reverse side). Moreover, the change was compared with that for a conventional alternate adsorption process, which was considered as the control. The polyelectrolyte multilayer resulting from the conventional process was formed on both sides of the QCM substrate. By comparing the change in the

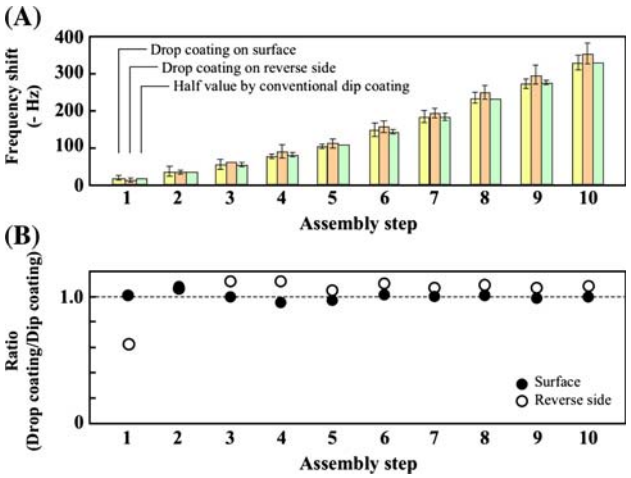

Fig. 4 Change in frequency by LbL assembly (**a**) and ratio of each frequency shift between drop coating and dip coating (**b**). Ionic strength of each polymer solution was 0.15 mol/l by NaCl. PDDA was coated in the step of odd number, and PSS was coated in the step of even number. ($n = 2$)

frequency caused by alternate drop coating with that caused by the conventional alternate adsorption process, we should divide the resulting frequency obtained by the conventional alternate adsorption process into two. From the calculations, the change in the frequency for one side by the alternate adsorption process would be obtained. The change in the frequency caused by the conventional process was fairly similar to that resulting from alternate drop coating. The difference was not significant. This result indicated that alternate drop coating could form fine polyelectrolyte multilayers on the surface and reverse side of the QCM substrate. Furthermore, the changes in the frequency in each assembly step were calculated to obtain the ratio between alternate drop coating and the conventional alternate adsorption. The resulting data are shown in Fig. 4b. The ratios on the surface and the reverse side were roughly 1.0. This result indicated that the polyelectrolyte assembly was similar in both of the coating processes. Taking this result into account, we could easily prepare a variety of multilayers on both sides of the substrate by using the drop coating process. Moreover, the resulting multilayer was similar to that obtained by the conventional process.

3.2 BSA adsorption on dual biointerfaces as protein reservoir

Our next focus was the use of dual biointerfaces as reservoirs for regulating protein adsorption. Figure 5 shows the total amount of BSA adsorption on both sides of the QCM substrate for the alternate drop coating process and the conventional alternate adsorption process. The isoelectric point of BSA is known to be pH 4.7. Therefore, BSA molecules act as charged anions in the Tris–HCl buffer

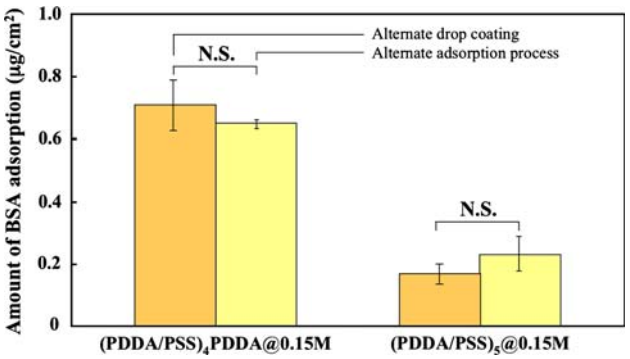

Fig. 5 Amount of BSA adsorption onto $(PDDA/PSS)_4PDDA$@ 0.15 M and $(PDDA/PSS)_5$@0.15 M. Concentration of BSA was 0.45 mg/ml. Ionic strength of protein solution was zero. ($n = 2$)

solution (pH 7.4). In the case of $(PDDA/PSS)_4PDDA$ @0.15 M, where the outermost surface was covered with PDDA, the amount of BSA adsorption was higher than that for $(PDDA/PSS)_5$@0.15 M (outermost surface: PSS). In particular, the PDDA surface in $(PDDA/PSS)_4PDDA$@ 0.15 M was compatible with the BSA molecules owing to cation-anion interactions. It is considered that the electrostatic interaction was an important factor in enhancing the BSA adsorption [16, 21]. In the case of $(PDDA/PSS)_4PDDA$@0.15 M, 0.65–0.70 μg/cm^2 of BSA adsorption was observed, and the amount of BSA adsorbed in each coating process was not significantly different. This similar protein adsorption was observed not only $(PDDA/PSS)_4PDDA$@0.15 M surface but also $(PDDA/PSS)_5$@0.15 M. This result indicated that the resulting polyelectrolyte multilayers formed during the protein adsorption were of good quality, irrespective of whether or not the multilayer was prepared by the drop coating process. From this observation, we concluded that the method of preparation of the polyelectrolyte multilayers did not affect the result. Moreover, the amount of BSA adsorption on $(PDDA/PSS)_5$@0.15 M decreased during the protein adsorption due to the surface charge (anionic surface).

Another dominant factor regarding protein adsorption is surface wettability. Generally, protein adsorption and the subsequent stabilization on a surface are also regulated by the surface hydrophobicity. In the case of hydrophobic surface, amount of adsorbed protein was much higher than that of hydrophilic surface [22]. In the present study, static contact angle measurement was carried out to evaluate surface wettability. The polyelectrolyte multilayers were prepared by the drop coating, and the resulting multilayers were $(PDDA/PSS)_4PDDA$@0.15 M and $(PDDA/PSS)_5$@ 0.15 M. The static contact angle by water was observed at 52.2° ± 1.9° and 41.4° ± 2.9°, respectively. The higher contact angle indicates more hydrophobic surface. In this meaning, $(PDDA/PSS)_4PDDA$@0.15 M was much more hydrophobic in comparison with $(PDDA/PSS)_5$@0.15 M. However, both of the contact angle gradually reduced, and roughly 10° was observed after 200 s. This observation indicated that hydrophilic segment of the polymers would be spontaneously enriched outermost surface. Thus, surface properties of the polyelectrolyte multilayer changed to hydrophilic after immersion in aqueous solution. In the protein adsorption test, the substrate with the polyelectrolyte multilayers was immersed in BSA solution for 1 h. It is enough time to rearrange hydrophilic segment on the outermost surface of the polyelectrolyte multilayers. The surface wettability was well-hydrophilic on both multilayers, $(PDDA/PSS)_4PDDA$@0.15 M and $(PDDA/PSS)_5$@ 0.15 M. Taking this result into account, the major factor of BSA adsorption was an electrostatic interaction.

3.3 Dual biointerfaces on QCM substrate by alternate drop coating

Dual biointerfaces are important for being used as a spacer between different tissues. We have demonstrated the preparation of some typical dual biointerfaces: one is composed of $(PDDA/PSS)_4PDDA$@0.15 M and $(PDDA/PSS)_5$@ 0.15 M on each side of the substrate, and the other is composed of $(PDDA/PSS)_4PDDA$@0.15 M and $(PDDA/PAA)_5$ @0 M on each side. The former substrate was different in terms of surface charge, while the latter case was different in terms of not only the surface charge but also polymer combination. Figure 6 shows the effect of the surface charge on BSA adsorption. The dual biointerfaces comprising $(PDDA/PSS)_4PDDA$@0.15 M and $(PDDA/PSS)_5$@0.15 M on each side showed roughly 0.5 μg/cm^2 of BSA adsorption. For comparison with the conventional double-sided polyelectrolyte multilayer, the amount of BSA adsorption in the conventional alternate adsorption process was calculated from the results of Fig. 5. From this result, the total amounts of BSA adsorption in $(PDDA/PSS)_4PDDA$@0.15 M and $(PDDA/PSS)_5$@0.15 M on both sides were roughly 0.65 and 0.23 μg/cm^2, respectively. Therefore, the sum of half of their values (0.44 μg/cm^2) was considered to be due to $(PDDA/PSS)_4PDDA$@0.15 M and $(PDDA/PSS)_5$@0.15 M on each side in the conventional alternate adsorption process. There was no significant difference between the actual BSA adsorption on the dual biointerfaces prepared by alternate drop coating and the calculated value for the conventional coating. Taking this into account, the dual biointerfaces prepared by the drop coating showed equivalent property in comparison with the multilayers by the conventional alternate adsorption process; particularly, amount of BSA adsorption was not significant difference. This means that the total amount of protein loading on the dual biointerfaces and the difference in the protein loading between both sides can be freely designed.

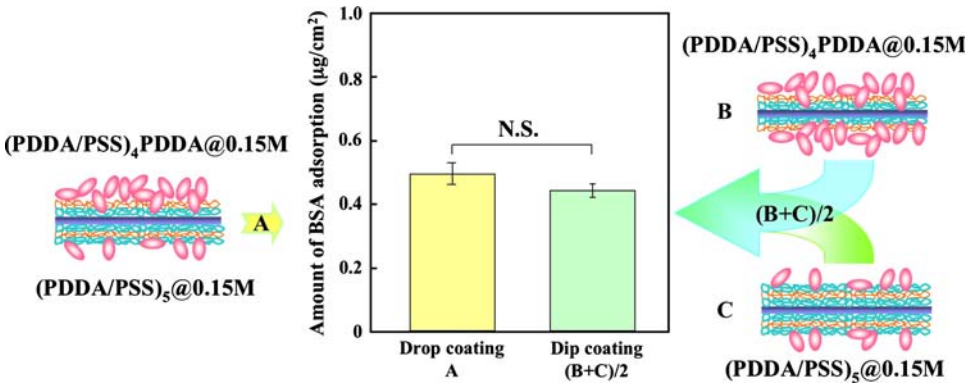

Fig. 6 Amount of BSA adsorption onto dual biointerfaces ((PDDA/PSS)$_4$PDDA@0.15 M and (PDDA/PSS)$_5$@0.15 M) ($n = 2$). The data on dip coating was calculated from total amount of BSA adsorption on (PDDA/PSS)$_4$PDDA@0.15 M ($n = 2$) and (PDDA/PSS)$_5$@0.15 M ($n = 2$)

To obtain more data on dual biointerfaces, the polymer combination along with the surface charge was changed on both sides. Figure 7 shows the (PDDA/PSS)$_4$PDDA@0.15 M and (PDDA/PAA)$_5$@0 M multilayer formed on each side of the QCM substrate by alternate drop coating. In our preliminary experiment, the BSA adsorption on (PDDA/PAA)$_5$@0 M significantly decreased during the protein adsorption in comparison with that on (PDDA/PSS)$_4$PDDA@0.15 M. Therefore, it is considered that the dual biointerfaces would show opposite protein adsorption properties on both sides. In the case of the dual biointerfaces, a total BSA adsorption of 0.2 μg/cm^2 was observed for both sides. On the other hand, (PDDA/PSS)$_4$PDDA@0.15 M and (PDDA/PAA)$_5$@0 M showed 0.65 and <0.05 μg/cm^2 of BSA adsorption, respectively. The polyelectrolyte multilayers on (PDDA/PAA)$_5$@0 M was not stable during the protein adsorption. In our preliminary test, the amount of BSA adsorption was maximum 0.05 μg/cm^2, which was evaluated by QCM measurement. In some cases, the change in the frequency increased after the BSA adsorption, reducing the total weight on the substrate. This phenomenon indicated that the polyelectrolyte multilayers might be removed from the QCM substrate. Therefore, the sum of half of their values was calculated to be 0.35 μg/cm^2, if the amount of BSA was maximum 0.05 μg/cm^2 on (PDDA/PAA)$_5$@0 M. However, the calculated value was 0.23 μg/cm^2 with large error bar. The difference between 0.35 μg/cm^2 and 0.23 μg/cm^2 is considered to be detachment of the polyelectrolyte multilayers on (PDDA/PAA)$_5$@0 M. Therefore, the experimental average value (0.23 μg/cm^2) was lower than that of calculated value (0.35 μg/cm^2). In the present study, the QCM substrate was used for the preparation of dual biointerfaces by employing alternate drop coating, and the resulting polyelectrolyte multilayer was characterized in terms of protein adsorption. However, the QCM substrate could not directly detect protein adsorption on each side. We intend to directly detect protein adsorption on each surface by using a quartz substrate and a UV–Vis spectrometer, and the results obtained will be reported in a future paper. Considering these results, a variety of multilayers on dual biointerfaces can regulate the surface protein adsorption of both sides. The designed dual biointerfaces are of great importance for the fabrication of excellent spacers owing to their ability to form strong bridges between different surfaces of the tissues.

4 Conclusions

The development of polyelectrolyte multilayers on dual biointerfaces was demonstrated by employing a novel alternate drop coating process. The protein adsorption of these interfaces was regulated by the polymer materials on

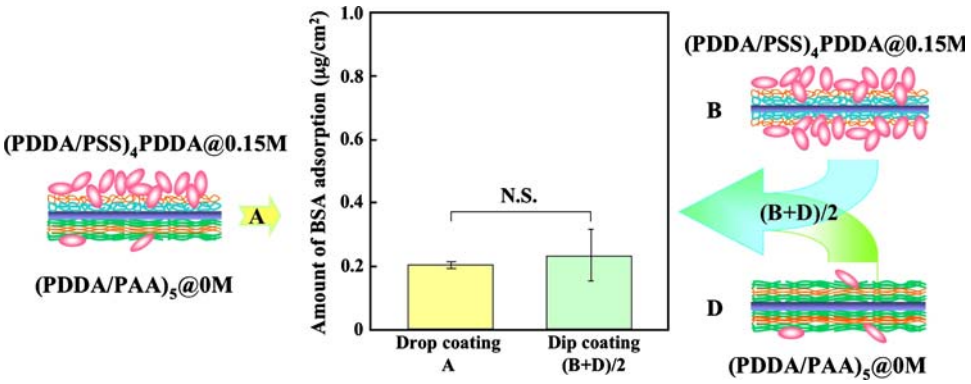

Fig. 7 Amount of BSA adsorption onto dual biointerfaces ((PDDA/PSS)$_4$PDDA@0.15 M and (PDDA/PAA)$_5$@0 M) ($n = 2$). The data on dip coating was calculated from total amount of BSA adsorption on (PDDA/PSS)$_4$PDDA@0.15 M ($n = 2$) and (PDDA/PAA)$_5$@0 M ($n = 2$)

the outermost surface. In addition, a variety of multilayers could be formed on all the materials by drop coating. The multilayers were able to adsorb serum albumin, which was used as a model protein, and the resulting biointerface is capable of using protein reservoir.

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