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## COMMUNICATION

# Mussel-inspired Antifouling Coatings bearing Polymer Loops

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This work reports the preparation of antifouling coatings bearing polymer loops using a mussel-inspired *ABA* triblock copolymer by simple drop coating method. With similar end graft density, the loops-bearing surfaces show an enhanced protein-reduction performance than the brushes-bearing surfaces.

Nonspecific protein adsorption to material surfaces is generally considered as the first stage in a "successional" process of fouling community development, which would facilitate the subsequential adsorption of bacteria and cells to form biofouling films, hindering the effectiveness of various medical devices and inducing further inflammatory responses and infection.<sup>1</sup> Therefore, improving the antifouling properties of material surfaces is of great significance to biomedical applications. Various strategies have been developed for surface modification, among which antifouling polymer coatings have been extensively studied because the easy control of their chemistry and architecture can provide great mechanical and chemical robustness with desired long-term stability.<sup>2</sup> Self-assembly, grafting-to method via physical adsorption or chemical linkage, and grafting-from method via surface-initiated polymerization have been commonly applied for preparing antifouling polymer coatings.<sup>3</sup> The latter method allows a closer control of the architecture of the coated polymer layer; however, the multi-step treatment involving surface pretreatment, immobilization of surface initiators and controlled polymerization normally leads to increased production cost. On this aspect, grafting-to method enjoys great superiority due to its ease of operation, especially for antifouling polymers conjugated with stable and generally applicable surface anchors.

Inspired by mussel's remarkable underwater adhesion capability to various surfaces by secreting adhesive proteins in which 3,4-Dihydroxyphenyl-L-alanine (DOPA) plays a significant role,<sup>4</sup> much effort has been dedicated to the preparation of antifouling polymers conjugated to musselmimetic polymeric anchors.<sup>5</sup> End-tethered to target surfaces through stable anchoring, the polymer chains tend to be extended and partially oriented to avoid excluded volume effects, forming a layer of polymer brushes. Due to the strong hydration and steric repulsion of polymer chains, the surfaces bearing polymer brushes generally exhibit considerable antifouling performance. When polymers possessing adhesive anchors on both chain ends come into contact with a surface, loops are expected to be formed. However, very limited research has been conducted on polymer loops, among which emphasis has been placed on understanding loop formation process through experiments or theoretical modelling.<sup>6</sup> To the best of our knowledge, no experimental research has been reported on studying the antifouling performance of surface bearing polymer loops, especially the ones formed using mussel-mimetic adhesive anchors, nor has the comparison of antifouling performance between loops-bearing surface and brushes-bearing surface been studied.



**Fig. 1** (a) Chemical structure of the triblock copolymer PDN-PEG-PDN and the diblock copolymer PDN-PEG. (b) Schematics of the preparation of surfaces bearing polymer brushes and polymer loops using drop coating method.

Herein we report the preparation of antifouling coatings bearing polymer loops using an *ABA* triblock copolymer by simple drop coating method. Mussel-inspired catecholfunctionalized poly(*N*,*N*-dimethylacrylamide) and poly(ethylene glycol) (PEG) were selected as the adhesive *A* block and antifouling *B* block, respectively. This copolymer poly[(*N*,*N*-dimethylacrylamide)<sub>15</sub>-*co*-(*N*-3,4dihydroxyphenethyl acrylamine)<sub>2</sub>]-*b*-poly(ethylene glycol)<sub>90</sub>-*b*-poly[(*N*,*N*-dimethylacrylamide)<sub>15</sub>-*co*-(*N*-3,4-

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dihydroxyphenethyl acrylamine)<sub>2</sub>] (PDN-PEG-PDN, Fig.1) was synthesized by reversible addditional fragment transfer (RAFT) polymerization (See Fig.S1-S3 in ESI† for detailed synthesis process and characterizations). By simple drop coating, this triblock copolymer can form a layer of loops onto substrate surface with the assistance of two adhesive anchoring blocks, which is compared with a layer of brushes prepared by dropcoating a diblock copolymer poly[(*N*,*N*-dimethylacrylamide)<sub>15</sub>*co*-(*N*-3,4-dihydroxyphenethyl acrylamine)<sub>2</sub>]-*b*-poly(ethylene glycol)<sub>45</sub> (PDN-PEG, Fig.1) with the same anchoring block and half of the middle PEG chain length.

Mica surfaces coated with PDN-PEG and PDN-PEG-PDN were imaged with atomic force microscope (AFM) and their surface morphologies are shown in Fig. 2b and 2d. Both surfaces were rather smooth with a root-mean-square (rms) roughness of ~0.2 nm and ~0.3 nm, respectively. The Flory radius ( $R_F$ ) of PEG chain for PDN-PEG was calculated using equation (1),where *a* is the monomer length (0.35 nm),<sup>7</sup> N is the number of monomers per polymer chain and *v* is 0.6 for a good solvent.<sup>8</sup> The average distance between grafted sites *S* was calculated using a dry film thickness measured by the surface forces apparatus (SFA) through equation (2),<sup>9</sup> where  $\sigma$  is the graft density,  $\rho$  is the polymer density (~1.2 g/cm<sup>3</sup>), *l* is the dry film thickness (3.3 nm),  $N_A$  is the Avogadro's constant and *M* is the molecular weight of the polymer. As  $R_F$  (3.4 nm) was larger than *S* (1.3 nm), the coated PDN-PEG was in a brush regime.<sup>9</sup>



Fig. 2 (a) Force-distance profiles measured between symmetric surfaces coated with PDN-PEG in 1mM NaCl solution and (b) AFM topographic image showing the surface morphology of PEG-PDN film. (c) Force-distance profiles measured between symmetric surfaces coated with PDN-PEG-PDN in 1mM NaCl solution and (d) surface morphology of the PDN-PEG-PDN film.

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SFA has been widely used for direct force measurements of numerous material surfaces as a function of the absolute surface separation distance D as determined using an optical technique called multiple beam interferometry by employing fringes of equal chromatic order (FECO).<sup>10</sup> In this study, forcedistance profiles measured between symmetric surfaces coated with PDN-PEG (Fig. 2a) and with PDN-PEG-PDN (Fig. 2c) in 1mM NaCl solution were obtained using SFA in experimental configurations shown in Fig. 3, which were also used to confirm the successful formation of polymer loops. For a typical force measurement, the two surfaces were first brought together to reach a "hard wall", and after contacting for a certain period of time, the two surfaces were separated apart. Here "hard wall" refers to a confined distance between two mica surfaces which did not change significantly with increasing normal load. The reference distance (D=0) was set as the adhesive contact between two bare mica surfaces in air. As shown in Fig.2a and 2c, for both PDN-PEG and PDN-PEG-PDN surfaces, pure repulsive forces were measured and the force curves obtained during approach and separation almost overlapped, showing no adhesion hysteresis.<sup>11</sup> This interaction behaviour could be explained by the interpenetration hindrance due to large excluded volume of the hydrated PEG chains which led to the steric repulsion between opposing swollen PEG chains. Previous studies showed that if the doubly bound chain ends were not strongly adsorbed to the same substrate, the formation of bridges across the layer gap would be favoured due to the entropic advantage, thus attraction would be measured during the loops-loops interaction.<sup>12</sup> However it was also proposed that by applying triblocks with longer or more strongly adsorbed end blocks, the formation of bridges could be avoided.<sup>13</sup> In the present work, the mussel-mimetic adhesive anchors can hold sufficiently fast to the mica surfaces so no bridging occurred, resulting in the pure repulsion measured. It is noted that for the opposing loops-bearing surfaces of PDN-PEG-PDN, if thick coatings were prepared by spin coating using relatively high concentration of polymer solution, there would inevitably be free adhesive anchors that led to strong adhesion measured during separation (Fig.S4, ESI<sup>+</sup>). Therefore, for all the surface forces measurements shown in the main text, single-layered coating was achieved by drop coating method using a relatively low concentration of polymer (1mg/ml) in acetate buffer solution (as further discussed below).



Fig.3 Simplified schematics of experimental configurations for opposing polymer layers in SFA measurements: (a) brushes formed by PDN-PEG, (b) loops formed by PDN-PEG-PDN, (c) combination of brushes and loops with free adhesive anchors formed by PDN-PEG-PDN.

The chain length of the extended PEG part for PDN-PEG was calculated as 15.8 nm using equation  $L=N\times a$ , where N is the number of monomers per polymer chain and  $a\approx 0.35$  nm<sup>7</sup> is the effective monomer length. For interactions measured between symmetric surfaces bearing polymer brushes (Fig. 2a), repulsion started from a distance of around 30 nm which was

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roughly twice of the extended PEG chain length (15.8 nm), proving that the coated PDN-PEG were single layered. For interactions measured between symmetric surfaces bearing polymer loops (Fig. 2c), if the two mussel-mimetic adhesive anchors (shown as red dots in Fig. 3) were not tethered to the same mica surface (in other words there were free adhesive anchors swaying as illustrated in Fig. 3c), adhesive force caused by the bridging effect would be measured as aforementioned or the repulsion would appear at a much longer distance than 30 nm if only one free adhesive anchor was fixed on the substrate surface. However, in Fig. 2c only pure repulsion starting at ~30 nm was measured, confirming the successful formation of a single layered polymer loops with PDN-PEG-PDN.

The dry film thickness of PDN-PEG-PDN and PDN-PEG layer were both measured as 3.3 nm by SFA, and the graft density of PDN-PEG-PDN chains was calculated using equation (2) and found to be half of the value of PDN-PEG (~0. 6 chains/nm<sup>2</sup>) due to the doubled molecular weight of PDN-PEG-PDN. When the graft density of chain ends (or so-called end graft density) was referred to, the prepared loops-bearing coating and brushes-bearing coating had the same end graft density. With identical end graft density, a layer of loops could be viewed as closely equivalent to a layer of regular brushes with half of the loop chain length, if seen by "cutting" the loops at their midpoint.<sup>14</sup> Thusly the loops-bearing coating formed by PDN-PEG, facilitating the further meaningful comparison of their antifouling performance.



Fig.4 (a) Changes in frequency and dissipation associated with the adsorption of protein BSA on bare silica sensor, silica sensors coated with PDN-PEG brushes and PDN-PEG-PDN loops using a QCM-D. (b) Protein adsorption on the three substrate surfaces  $(ng/cm^2)$  before and after rinsing with PBS buffer.

Polymers were then drop coated to silica sensors to study the antifouling performance of different coatings against bovine serum albumin (BSA) using a Quartz Crystal Microbalance with Dissipation (QCM-D). As shown in Fig.4a, for bare silica or silica sensors coated with different polymers, a strong negative frequency shift and a positive dissipation shift were observed upon the introduction of a 5wt% BSA solution in

phosphate buffered saline (PBS) into the QCM-D chamber, indicating the adsorption of BSA on all tested surfaces. After stable frequency and dissipation shift curves were attained, PBS was introduced into the chamber to remove the loosely-bound proteins, resulting in an increase in frequency and a decrease in dissipation. The QCM-D data were fitted using the Voigt viscoelastic model to obtain the mass of proteins adsorbed on the different surfaces before and after rinsing with PBS. As shown in Fig. 4b, less proteins were absorbed on the polymer brushes-coated sensor  $(1980.0 \pm 210.6 \text{ ng/cm}^2)$  than on bare silica surface  $(3775.2 \pm 326.4 \text{ ng/cm}^2)$ , while the protein adsorption was the least on polymer loops-coated sensor (567.6  $\pm$  61.6 ng/cm<sup>2</sup>). After rinsed with PBS, a significant portion of the BSA proteins was washed off and the remanent BSA on the loops-coated and brushes-coated sensor were  $31.7 \pm 2.9$  ng/cm<sup>2</sup> and  $277.2 \pm 32.4 \text{ ng/cm}^2$ , respectively; while for the uncoated silica sensor, the BSA remained on the surface was  $937.2 \pm$  $82.3 \text{ ng/cm}^2$ . The dry film thickness of the polymer coatings on silica sensors was measured to be 3.3 nm using ellipsometry, which is consistent with the thickness determined using SFA. This result is expected as silica possesses very similar surface chemistry as mica and the surface coatings for SFA and QCM-D experiments were prepared under the same conditions. Therefore, the end graft density of PDN-PEG-PDN and PDN-PEG was very similar on silica, and the protein adsorption results indicate that the loops-coated surface shows better protein reduction (or antifouling) performance (94.4%) than the brushes-coated sensor (86.0%). It is expected that optimizing the graft density would further enhance the protein-reduction performance of the loops-coated surfaces.

Polyethylene glycol monomethylether  $PEG_{45}$  and polyethylene glycol  $PEG_{90}$  were also coated onto silica sensors (mainly through hydrogen bonding) for comparison. Evaluated following the same QCM-D measurements for protein adsorption as that in Fig. 4a, the physisorbed PEG layers only demonstrated slightly enhanced antifouling properties as compared to the bare silica surfaces, showing weaker antifouling performance than that of brushes-coated and loops-coated surfaces (Fig. S5, ESI†) which was most likely attributed to the random conformation of the physisorbed PEG chains with smaller excluded volume, and less steric hindrance as compared to the chemically grafted PDN-PEG brushes and PDN-PEG-PDN loops assisted by the PDN adhesive anchors.



Fig.5 (a) Force-distance profile between a PDN-PEG-PDN film and a BSA film in 1 mM NaCl solution, (b) Topographic AFM image of BSA absorbed on mica surface.

The interactions between a PDN-PEG-PDN loop layer and a BSA film coated on mica were measured in 1 mM NaCl solution using the SFA (Fig. 5). As illustrated in Fig. 5a, the hard wall separation was found to be 10 nm, which was close to that observed in the symmetric loops-loops interaction, indicating the successful deposition of BSA on mica as also confirmed by AFM imaging shown in Fig. 5b. Previous studies have shown that weak attraction could be measured when proteins were pressed into a layer of polymer brushes at relatively high load, most likely due to the rearrangements in polymer conformation and protein penetration into the polymer core.<sup>15</sup> Fig. 5 shows that pure repulsive forces were measured between PDN-PEG-PDN loops and BSA during both approach and separation and no adhesion hysteresis was detected. These results indicate that the polymer loops could better adapt external compression and reduce the protein penetration, exhibiting great potential in antifouling applications. The interactions between symmetric loops-bearing surfaces in aqueous solutions of different salt concentrations (0.001 M, 0.01 M and 0.1 M NaCl) were also measured and similar forcedistance profiles were obtained (Fig. S6, ESI<sup>+</sup>), which indicates that the PDN-PEG-PDN loop coating was neutrally charged and the repulsive forces between the loops-bearing coating and proteins arose from the steric hindrance of the extended PEG loops.

In summary, a mussel-inspired ABA triblock copolymer PDN-PEG-PDN was synthesized and used to prepare surfaces bearing polymer loops. By direct drop coating, this triblock copolymer can form a layer of loops onto substrate surfaces with the assistance of two adhesive anchoring blocks. The mussel-inspired adhesive anchors can provide stable anchoring points and facilitate the grafting of PEG chains to achieve large excluded volume. A diblock copolymer PDN-PEG was also synthesized which could form a brush layer on substrate surfaces by drop coating. The QCM-D protein adsorption tests demonstrate that the loops-coated surfaces show stronger protein-reduction performance over the brushes-coated surface with similar end graft density. The superior antifouling properties of PDN-PEG-PDN loops is mainly attributed to the strong steric hindrance of the neutrally charged polymer loops as confirmed by direct force measurements.

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### Notes and references

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