Bioinspired Anti-fouling Coating on Hydrophobic Surfaces

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Abstract

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Anti-biofouling is very important in many applications ranging from marine coatings to biomedical devices. The poor bio-compatibility of medical devices with human body causes undesired effects. Therefore, to achieve long-term stability of implanted medical devices the task is to modify the surfaces of medical devices so as to resist protein and make them compatible. In this thesis, the objective is to study a universal dip-coating method based on a zwitterionic polymer-DOPA conjugate for its anti-biofouling performance.

Contents

1 Introduction

1.1 Anti-fouling background

Anti-biofouling is very important in many applications ranging from marine coatings to biomedical devices. Implantable medical devices, such as man-made blood vessels, heart stents and artificial lungs, have saved millions of lives. [1-3] Unfortunately, their poor bio-compatibility with human body causes undesired effects. For example, cells and proteins in human body may attach to the surfaces of these medical devices, causing thrombosis and bacterial colonies. Therefore, the task is to modify these surfaces so as to resist protein adsorption and platelet adhesion and achieve the long-term stability of implanted medical devices.

Several materials can achieve protein resistance via different hydration mechanisms. Polyethylene glycol (PEG) is a neutral and hydrophilic polymer and widely used for coating biomaterials, which binds to water through hydrogen bonding. Zwitterionic polymers have a positively and a negatively charged group placed on the same repeating unit and have even stronger hydration than PEG. They bind to water through electrostatically induced hydration. They are shown to be ultra-low fouling even when in contact with complex media such as undiluted human plasma or serum. [4]

1.2 Surface modification methods

Typical zwitterionic polymers such as poly(carboxybetaine) (PCB) and poly(sulfobetaine) (PSB) have been applied to a broad range of biomedical and

engineering fields. They are shown to form a hydration layer as a coating material, which prevents nonspecific protein or cell fouling and tremendously reduces the undesired bio-fouling on medical devices. [4] Thus, it is ideal to introduce a PCB or PSB layer onto target surfaces.

Surfaces coated with zwitterionic materials can achieve significant antifouling performance by electrostatically induced hydration on the top of targeted surfaces. However, PCB and PSB chains are extremely hydrophilic and hence have high solubility under aqueous working conditions. Thus, it is challenging to effectively attach zwitterionic chains onto target surfaces. In general, there are two strategies to attach a polymer chain onto a surface, known as “graft from” and “graft to” methods. [4]

The graft-from surface modification method firstly modifies a surface with suitable initiating groups and then grows polymer chains from the modified surface. This method allows polymer chains to stand up as brushes due to their special restrictions on the surface, achieving coatings with very high graft density. This graft-from method can utilize atom transfer radical polymerization (ATRP), radical addition fragmentation chain transfer (RAFT), and other living polymerization to produce precisely controlled polymer chain lengths or film thicknesses. However, these protocols often require oxygen-free conditions. Hence, it is not suitable for practical large-scale applications. Instead, a simple and robust method such as dip coating is highly desirable.

The graft-to surface modification method is to graft a pre-made polymer,

consisting one functional moiety and one surface-anchoring moiety, to a surface. As compared to the graft-from method, the graft-to method is more convenient and suitable for modifying large surface areas in one step and can achieve relatively high surface packing density under optimized assembly conditions. This thesis will focus on this graft-to surface modification method [5].

Applying this graft-to method to surfaces requires a well-designed molecule that has both the function of anti-fouling and the ability to anchor surfaces, forming a compact antifouling layer with high packing density.

1.3 Research of bioinspired binding group

Surface modification methods highly depend on the properties of target surfaces. Different surfaces often require different surface-binding groups. For example, thiol group work for the gold surface and saline group work for silica and glass surfaces. [6, 7] Therefore, it would be more convenient if there is a universal surface modification method that can be easily applied to all kinds of surfaces, regardless of different surface chemistries.

For this purpose, a nature-inspired approach based on L-3,4-dihydroxyphenylalanine (DOPA) is promising. DOPA is found rich in the mussel foot protein. It helps mussel to attach themselves tightly on the surface of rocks or boat husk against the scour of ocean wave. [8]

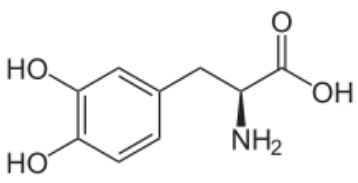

Figure 1. Molecular structure of DOPA

The habitat offers mussels lots of attractive resources such as aerated seawater for respiratory gas exchange, rapid waste removal, rich nutrient supply, and the security of a highly interactive community. The habitat also exacts energy from mussel, mainly from the holdfast termed the byssus (**Figure 2a**). It is very important to make and maintain a byssus, which is absolutely necessary adaptations to resist the lift and drag of waves in the intertidal zone and cost between 8% and 12% of the total metabolic energy of a mussel. [9, 10]

The structure of byssus is a bundle of threads and made of four parts: plaques, threads (including distal and proximal portions), stem, and root. The plaques at the distal thread ends attach to a foreign surface and the proximal ends radiate from the stem, which connect the living tissues of the mussel by the mussel root (**Figure 2b**). [5]

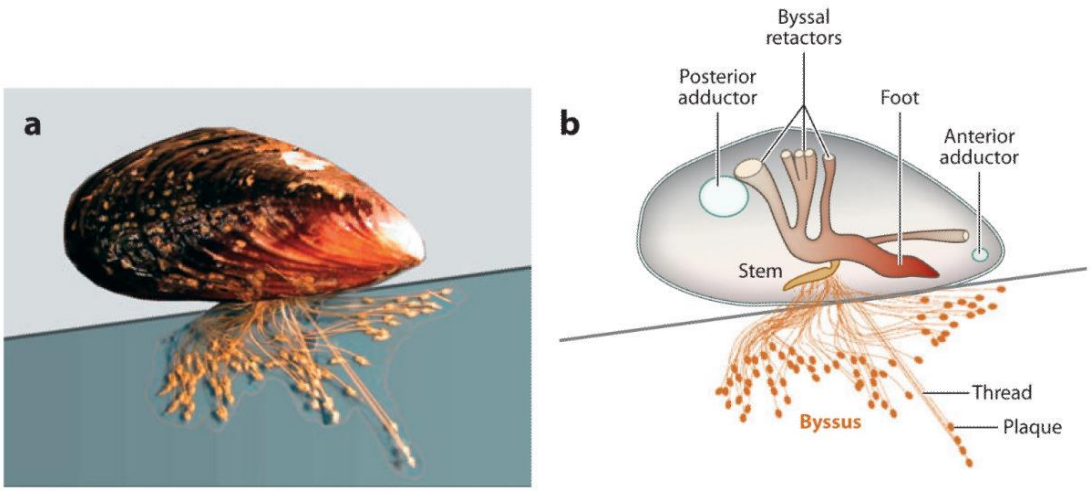

Figure 2. Adhesion in the marine mussel.

(a) Adult mussel (5 cm length) shows an extensive byssus attaching to a mica surface.

(b) Schematic mussel on a halfshell. [7]

The secret of mussel's attachment depends on all portions of the mussel byssus. Byssal plaques are the most important and special part contributing adhesions. It is investigated that byssus contains around 25~30 kinds of proteins. Around 7~8 of these are present in the plaque and only 5 are unique to plaque. It is found that all mussel foot proteins contain the post-translationally modified amino acid L-DOPA. [5]

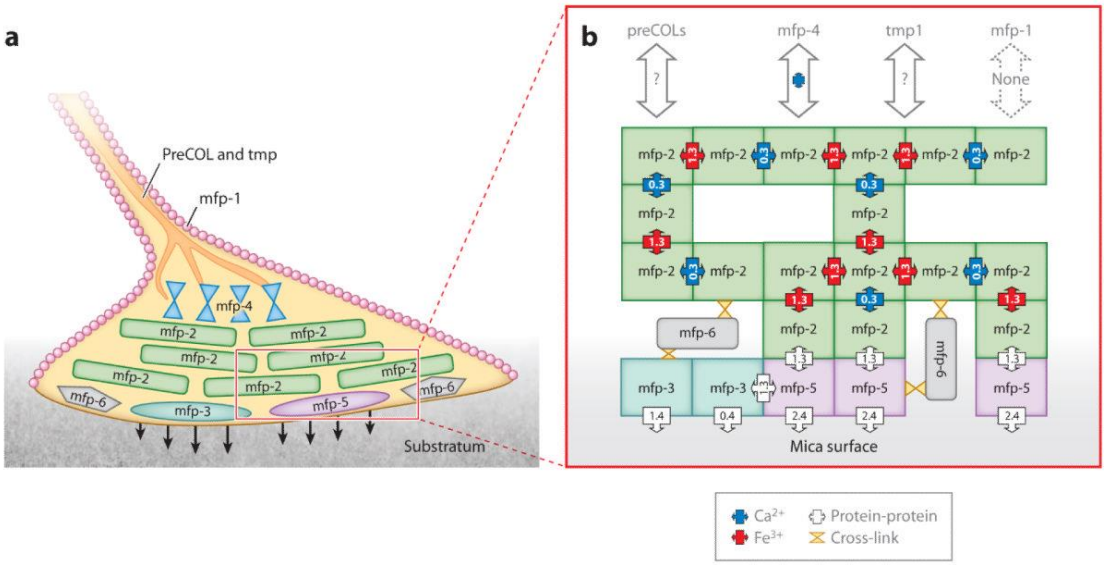

Figure 3. Schematic view of mussel foot proteins (mfps) in a byssal plaque.

(a) Mfp-3 and mfp-5, which attach to surface directly, are the main anchoring protein. Mfp-2 is the most protein in each plaque.

(b) Schematic view of all mfp interactions as determined by the surface forces apparatus. [5]

1.4 Development of DOPA-Zwitterionic polymer conjugates

The strong surface adhesion ability of DOPA offers many opportunities for the development of novel polymer coatings in a variety of ways. Depending on chemical structure, the catechol group may serve as an anchor in immobilizing polymer chains on the surface.

Therefore, DOPA was applied to our polymer system for its ability to attach to different kinds of surfaces which are made of diverse chemical compositions. The adhesive moiety DOPA and non-fouling material PCB or PSB constitute a stable anti-fouling coating system. DOPA-PCB and DOPA-PSB conjugates can be synthesized by using DOPA-Br initiator and then followed by ATRP [11, 12]. The synthesis processes developed in our group are shown in **Figures 4 and 5**. These DOPA-zwitterionic polymer conjugates can then be easily attached to many surfaces, producing a relatively high packing density and good anti-fouling performance via graft to or graft from method as discussed previously. Graft-to is a desirable method for this work, as shown in **Figure 6.**

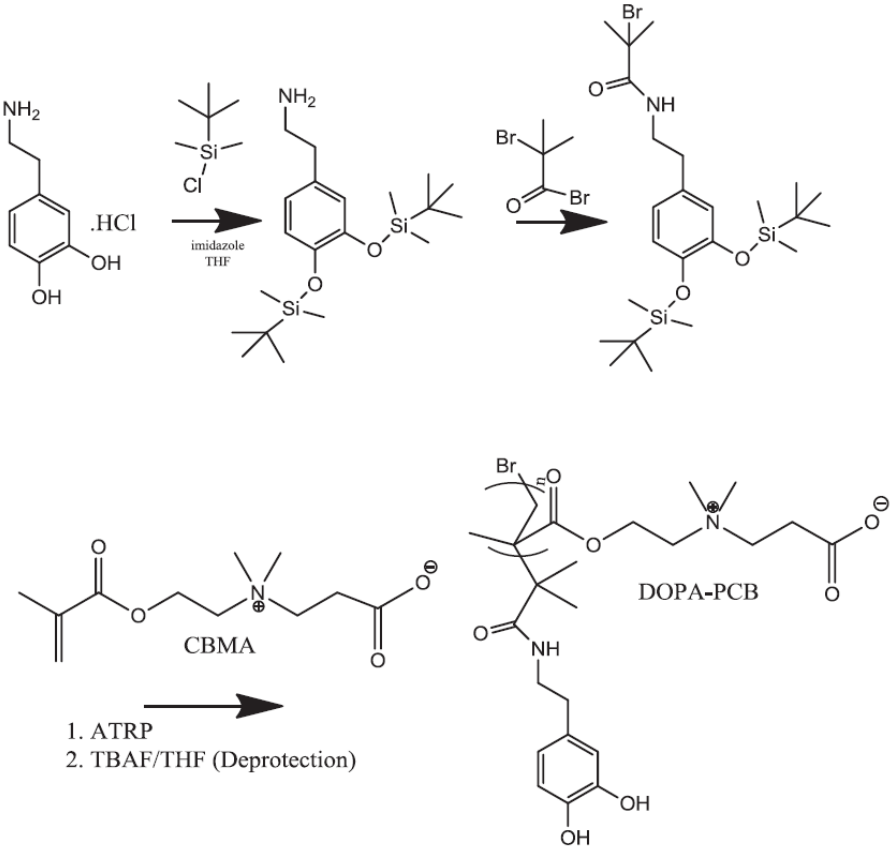

Figure 4. Synthesis of DOPA-PCB from DOPA-Br initiator via ATRP. [11]

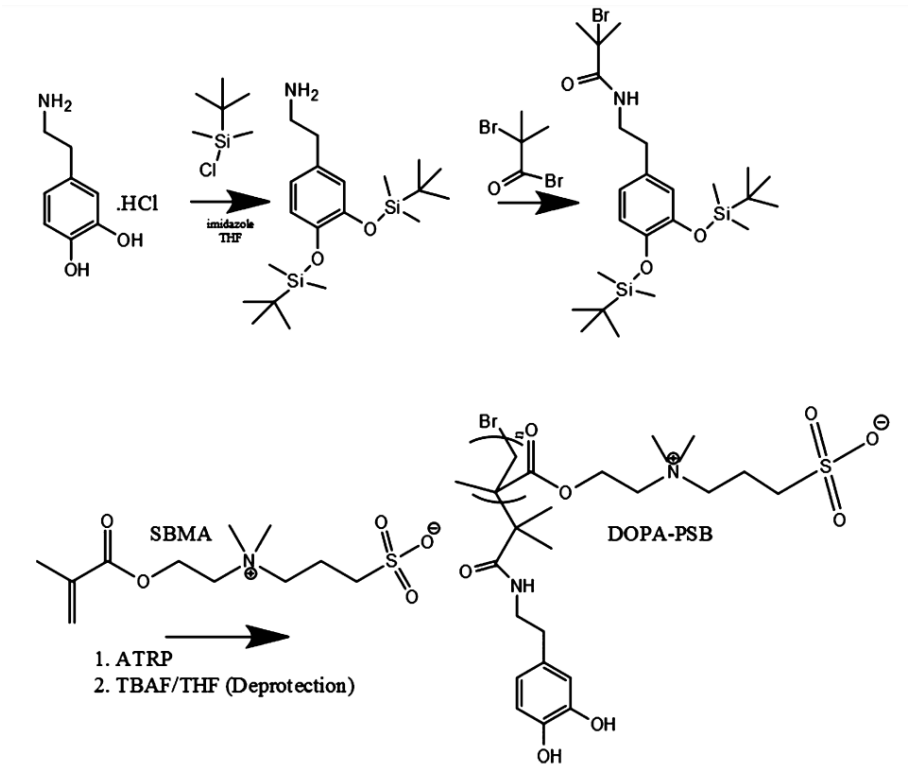

Figure 5. Synthesis of DOPA-PSB from DOPA-Br initiator by ATRP. [12]

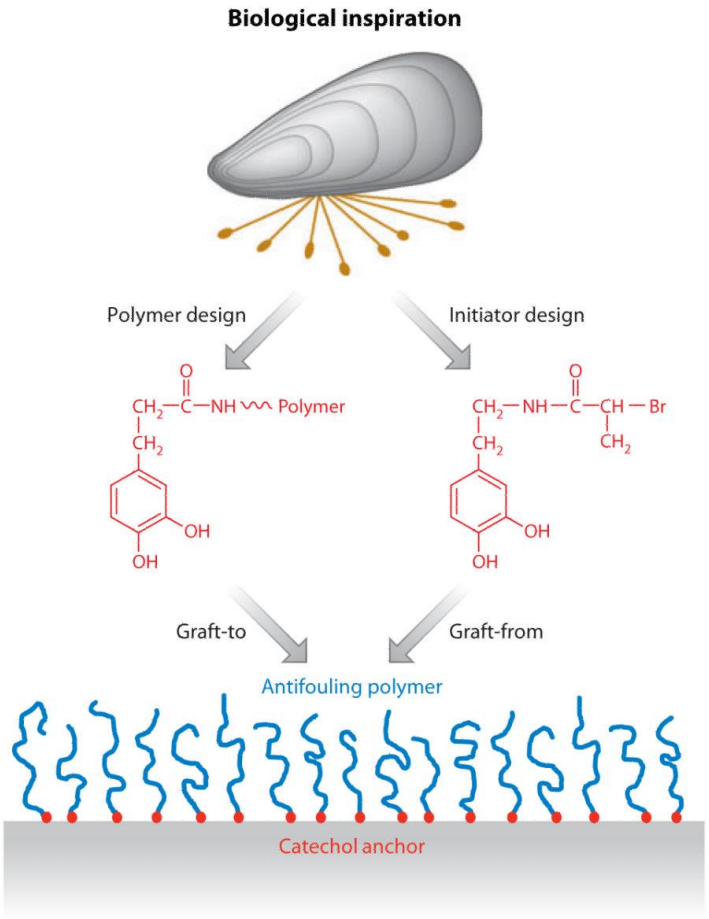

Figure 6. Schematic illustration of bioinspired methods for grafting antifouling polymers onto surfaces. Catechol groups may be chemically connected to an antifouling polymer and attached onto a surface ("graft-to" method) or to an initiator that is used for surface-initiated polymerization ("graft-from" method). [5]

In this work, we aim to perform strong catechol surface binding via physical or chemical forces and develop a simple and universal coating graft-to method, which is suitable for all surfaces, especially hydrophobic surfaces, and maintain stability of super-hydrophilic zwitterionic polymers in aqueous solutions. In order to achieve a universal surface modification method, it is necessary to have a fundamental understanding of the key factors governing the attachment of polymers to surfaces. Thus, several tasks need to be fulfilled.

Firstly, the relationship between catechol surface adhesion and zwitterionic polymer solubility for binding should be clearly understood. We can hypothesize that certain factors such as polymer chain length, the number of binding groups, and polymer type have effects on surface binding as shown in **Figure** 7).

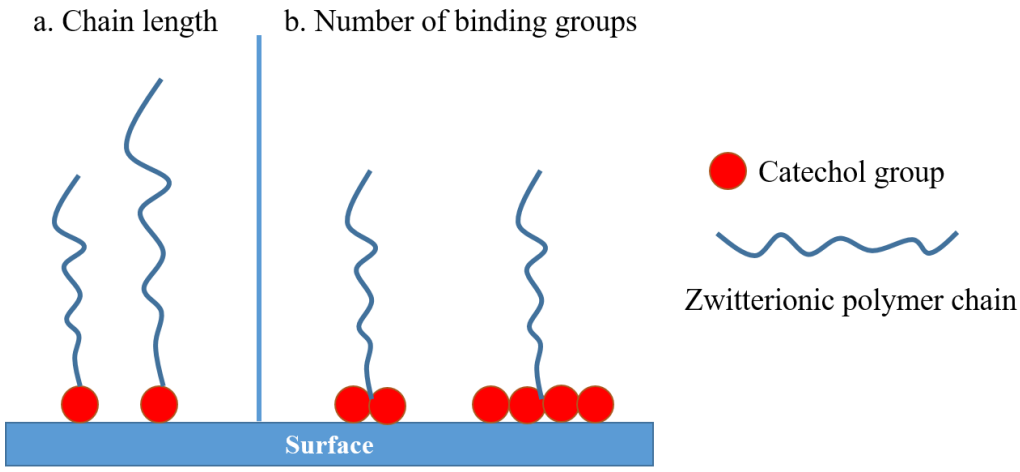

Figure 7. Binding onto a surface against the dissolution of zwitterionic polymers: (a) dependence of chain lengths; (b) dependence of catechol numbers.

It is known that higher molecular weight polymers occupy more volume in solvent, retain more water and are more flexible, all of which can improve non-fouling performance. However, at the same time, being more hydrophilic, these polymers are vulnerable for dissolution from the surface. But it can be compensated with strong surface binding.

While DOPA-PEG conjugate are used on titanium surfaces., DOPA-PCB and DOPA-PSB conjugates are shown to work well on many more surfaces. [11] In our lab, DOPA-PCB and DOPA-PSB with various repeating units were synthesized, coated and tested on PDMS. ELISA results show a tremendous decrease in fibrinogen

adsorption as compared to control surface. These conjugates of zwitterionic polymer chains and L-DOPA binding groups are ideal candidates for graft-to methods. The DOPA-zwitterionic polymer conjugate with one catechol group significantly decreases protein adsorption. How about increasing the catechol groups to two or four?

Different DOPA-PCB polymers such as DOPA-PCB (one catechol group and one PCB chain), $(DOPA)_2$-PCB (two catechol groups and one PCB chain) and $(DOPA)_2$-$(PCB)_2$ (two catechol groups and two PCB chains), respectively, were tested. For low fouling coatings, the surface packing density of the polymer chains is essential. $(DOPA)_2$-$(PCB)_2$ showed the best non-fouling properties on silica due to its strong surface binding and increased surface coverage. [13]

Becker et al. investigated the relationship between the numbers of binding groups with the binding ability. Osteogenic growth peptide (OGP) domain and surface-binding catechol domains were obtained through solid phase synthesis. The **Figure 8** shows the structure of OGP-$(Cat)_n$ (n=1, 2, 4). It is demonstrated that the binding force of polymer with four DOPA groups can reach 184 times force as compared with that of polymer with one DOPA group on titanium oxide surface. This is essential in keeping the highly soluble zwitterionic polymers firmly attached to the surface. On a surface favorable for catechol binding, one catechol may be enough to keep the polymer chain intact. However, for polypropylene (PP) and PDMS, many catechol groups are required to keep the polymer chains attach on the surface. The binding strength of these polymers on different surfaces will be studied. [14]

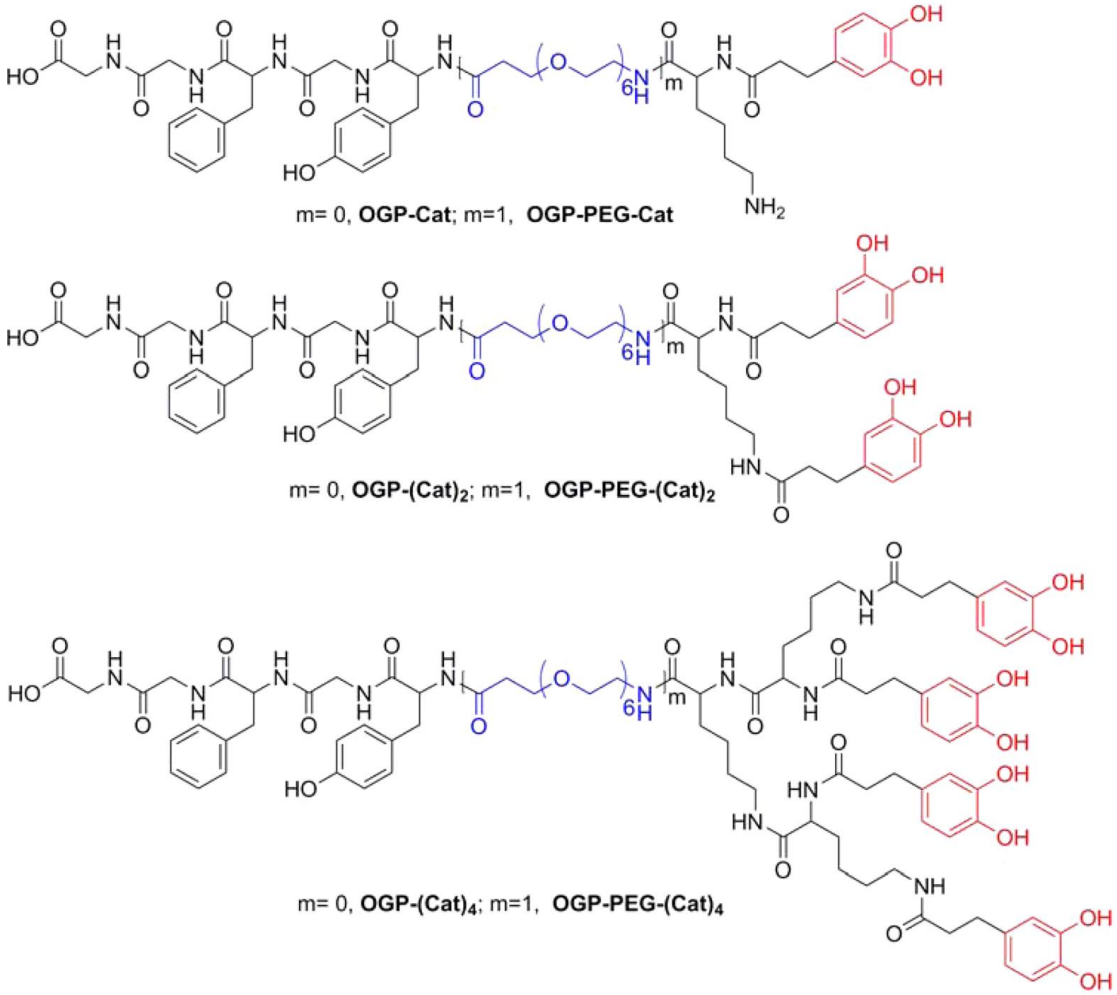

Figure 8. Molecular Structures of OGP-$(Cat)_n$ and OGP-PEG-$(Cat)_n$, n = 1, 2, 4 [14]

Besides the structure effects, different self-assembly conditions that affect the coating properties also need to be investigated. For example, catechol groups are known to attach to surfaces with various chemical compositions, but the mechanisms are different and unclear. For metal oxide like TiO_2, catechol can form H-bonding or coordinate bonds. This requires acid or reduced conditions. While on organic and hydrophobic surfaces, hydrogen bond and coordinate bond are difficult to form, which weaken the binding strength between catechol group and surfaces. Unfavorable surfaces, such as PP and PDMS, lack function groups and as a result are difficult to

functionalize. Thus, it is challenging to coat highly hydrophilic PCB onto these hydrophobic surfaces via a simple “graft to” method.

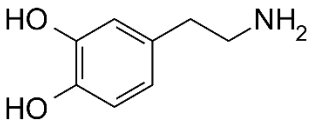

Figure 9. Molecule Structure of Dopamine

In previous experiments, dopamine is used to assist DOPA-PCB to coat onto hydrophobic surfaces to compensate the high solubility of the polymer. Dopamine is a small molecule containing a catechol group and an amine on the same molecule (shown as **Figure 9**). It has been found to polymerize by itself. It is well known that the catechol group upon oxidation to a quinone can react with amine groups forming poly(dopamine) which enhances surface attachment. The polymerization process of dopamine is shown in **Figure 10**. Therefore, when DOPA-PCB is mixed with dopamine, a cross-linking structure is formed with the existence of dopamine. Messersmith et al. attached an ATRP initiator which is a mixture of a dopamine derived ATRP initiator (DOPA-Br) and dopamine on the surface and then grew polymer chains from the initiator via ATRP. DOPA-PCB was used with dopamine, resulting in the direct attachment of PCB to the surface in one step. Furthermore, adding methanol to the solvent can improve the wettability of hydrophobic surfaces and form a more uniform coating on certain hydrophobic surfaces. [11]

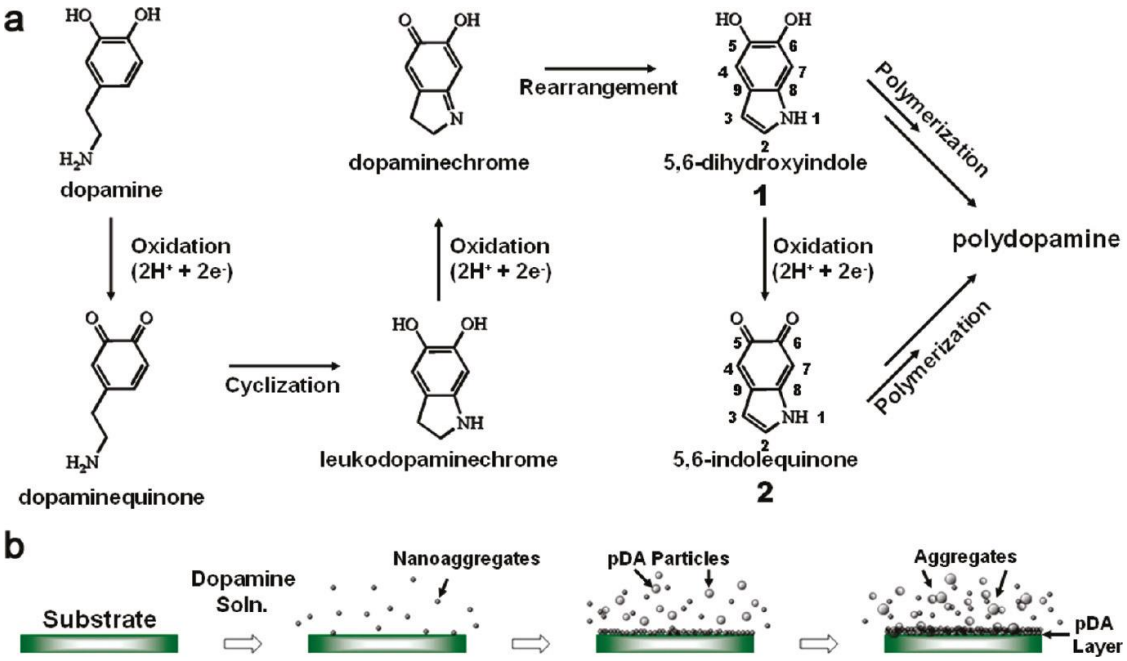

Figure 10. (a) Oxidation process of dopamine in aqueous solution. (b) Possible deposition process of poly(dopamine) on the substrate surface. [15]

Due to the oxidation of dopamine in alkaline condition, a thick layer of "glue-like" coatings is formed on sample surfaces and amine groups are introduced on these sample surfaces for subsequent surface modification. This method has been shown to work for a wide range of materials and surfaces. However, glue-like coatings with uncontrolled morphologies are formed on the material surfaces, which normally turn black due to oxidation, requiring further surface modification to bring desired functionalities [15]. The conjugation of dopamine and DOPA to functional polymers changes the properties of these respective molecules. In order to prevent the "glue-like" dopamine coating, we can introduce a free amine to the DOPA binding groups.

1.5 Application of anti-fouling coating

One of the important applications of DOPA-zwitterionic polymer conjugates is to coat the surface of artificial lungs. Though the fibers are made of hydrophobic polymer to reduce blood plasma leakage into the fiber wall, phospholipids, lipoproteins and proteins from the plasma inevitably penetrate the submicrometer pores over time, reducing membrane gas permeability, which can cause device failure within days. Microchannels may be fabricated from poly(dimethylsiloxane) (PDMS), a silicone material which is common used in microfluidic devices. PDMS performs well in blood-contact applications. It is highly gas-permeable, cost-efficient and easy to handle, which make it a suitable material for the fabrication of blood-flowing microchannels. [16] **Figure 11** shows the gas transfer system of an artificially lung. Oxygen is introduced intraluminally through the fibers and diffused into venous blood flowing around the fibers.

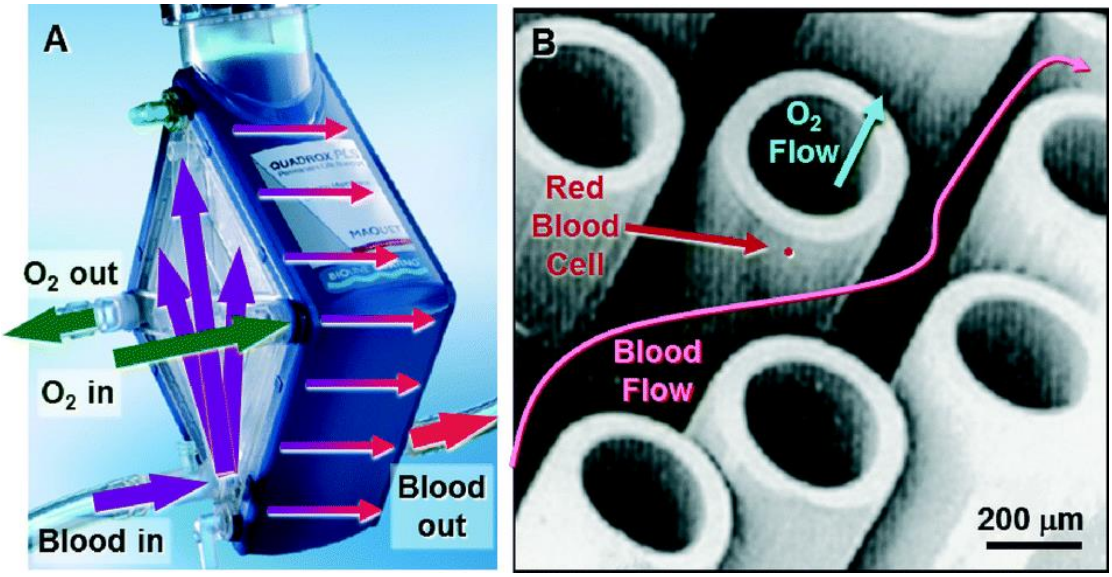

Figure 11. Current hollow-fiber artificial lung technology. [17]

2 Experimental details

2.1 Materials

Solvents and chemicals:

Dichloromethane (DCM, Fisher Scientific); Hexanes (Certified ACS, Fisher Scientific); Tetrahydrofuran (THF, Fisher Scientific); Methanol (Reagent grade, Fisher Scientific), acetone (Certified ACS, Fisher Scientific); Tetra-n-butylammonium fluoride (TBAF, 98%, Acros Organics); Trifluoroacetic acid (TFA, 99%, Sigma-Aldrich); Copper (I) bromide (98%, Acros Organics); N,N,N',N'',N''-pentamethyldiethylenetriamine (99%, Sigma-Aldrich); CBMA monomer was synthesized as reported. 2-amino-2-hydroxymethyl-propane-1,3-diol (TRIS, Fisher Scientific); 1 N hydrochloric acid (HCl, Fisher Scientific); Phosphate buffered saline (PBS) solution (0.01 M, pH 7.4) was made from PBS powder purchased from Sigma Aldrich. Horeseradish peroxidase (HRP)-conjugated goat anti-human IgG was purchased from Alpha Diagnostics.

Zwitterionic materals:

1DOPA-PCBMA$_{100}$: Poly(carboxybetaine) with a hundred repeating units and one catechol group, no free amine group.

1DOPA-PSBMA$_{100}$: Poly(sulfobetaine) with a hundred repeating units and one catechol group, no free amine group.

4DOPA-PCBMA$_{100}$: Poly(carboxybetaine) with a hundred repeating units and four catechol groups.

4DOPA-PSBMA$_{100}$: Poly(sulfobetaine) with a hundred repeating units and four

catechol groups.

2.2 Dip-coating protocol of 1DOPA-PCBMA$_{100}$ and 1DOPA-PSBMA$_{100}$

Prepare:

(1) 1DOPA-PCBMA$_{100}$ solution in TRIS buffer (pH 8.5, 10 mM, no NaCl) with 20% methanol at 2 mg/mL.

(2) 1DOPA-PCBMA$_{100}$ solution in TRIS buffer and 20% methanol at 2 mg/mL, add Dopamine and the mass ratio of polymer to Dopamine is 8:1.

(3) 1DOPA-PSBMA$_{100}$ solution in TRIS buffer and 20% methanol at 2 mg/mL.

Prepare samples of PDMS fiber in the same size. Test the weight of each sample for future calculation.

Take 2 ml plastic vial and place the sample into it. Transfer 2 ml solution into each plastic vial rapidly. Coat the samples in solution (1) and (3) overnight. Coat the samples in solution (2) for 2.5 hours. The color turns black because of dopamine. Keep the samples on the rotator in order to coat the entire surface well.

Pour out of the polymer solution after required time. Clean the samples by PBS buffer for 5 times then do the Elisa test.

2.3 Synthesis and test of 4DOPA-PCBMA$_{100}$, 4DOPA-PCBMA$_{200}$

Zwitterionic polymers with four catechol groups are successfully synthesized in our group. More binding groups bring stronger surface binding ability for zwitterionic polymer-DOPA conjugates. Free amine groups help cross-linking between polymer molecules. Nuclear magnetic resonance (NMR) was used to prove that 4DOPA-Br initiators are synthesized successfully. The NMR result and the final structure of

4DOPA-PCBMA$_{100}$ are as below:

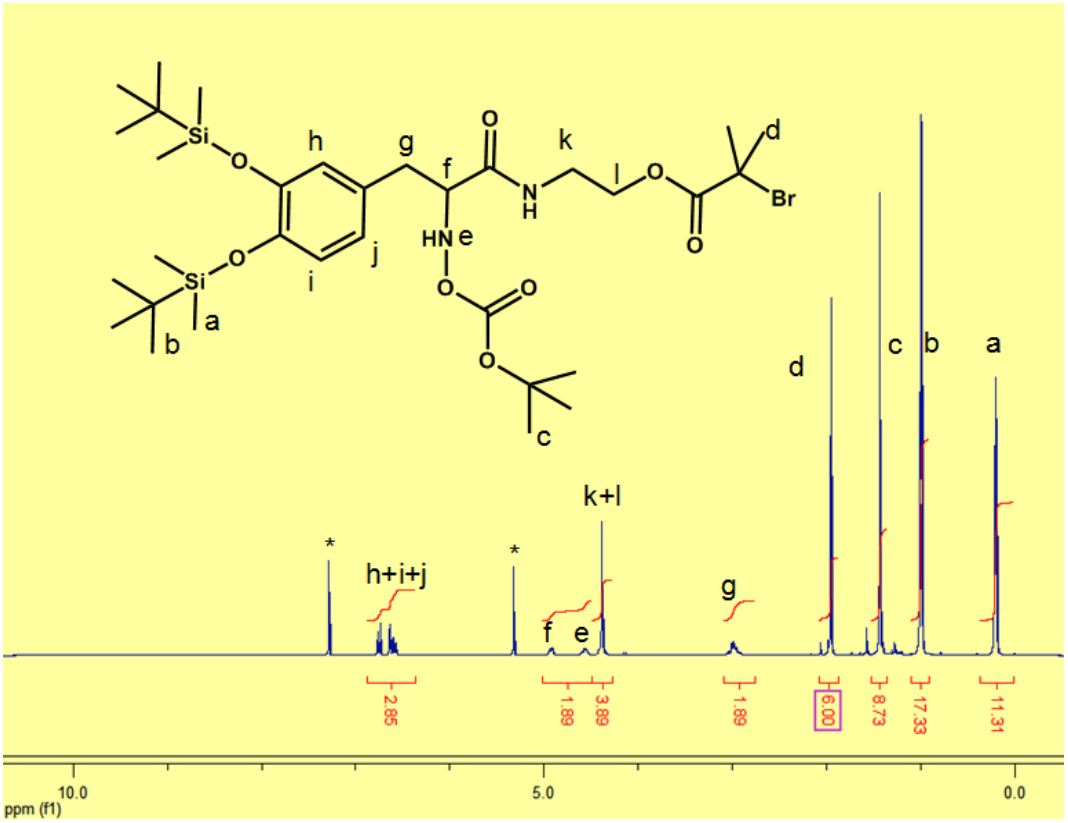

Figure 12. NMR of 4DOPA-Br initiator

After synthesizing the final initiator, polymer chains are grown by atom transfer radical polymerization (ATRP), which is an example of a reversible-deactivation radical polymerization and forms a carbon-carbon bond through a transition metal catalyst. The atom transfer step is the key step in the reaction responsible for uniform polymer chain growth. **Figure 13** presents a typical ATRP reaction.

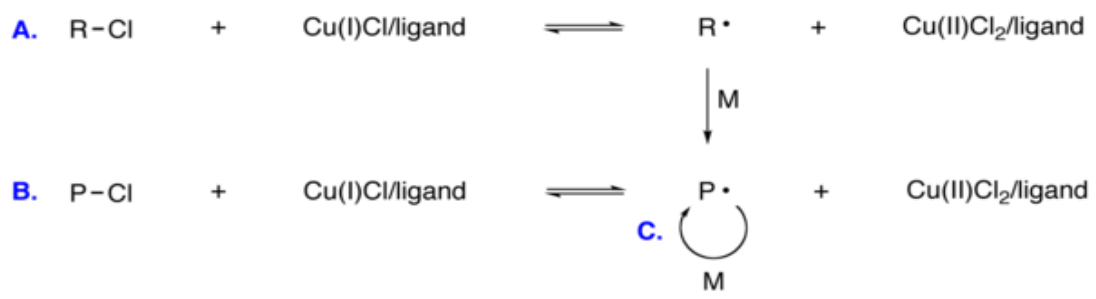

Figure 13. General ATRP reaction. A. initiating. B. Equilibrium with dormant species. C. Propagation. [18]

ATRP usually employs a transition metal complex as the catalyst with an alkyl halide as the initiator (R-X). Various transition metal complexes, such as Cu, Fe, Ru, Ni, Os, etc., have been employed as catalysts for ATRP. In an ATRP process, the dormant species is activated by the transition metal complex to generate radicals via one electron transfer process. At the same time, the transition metal is oxidized to higher oxidation state. This reversible process rapidly establishes an equilibrium that is predominately shifted to the side with very low radical concentrations. The number of polymer chains depends on the number of initiators. Each growing chain has the same possibility to propagate with monomers to form living/dormant polymer chains (R-Pn-X). As a result, polymers with similar molecular weights and narrow molecular weight distribution can be prepared. [18]

ATRP reactions are very robust in that they are tolerant of many functional groups like allyl, amino, epoxy, hydroxy and vinyl groups present in either the monomer or the initiator. ATRP methods are also advantageous such as easy to prepare, commercially available and inexpensive catalysts (copper complexes),

pyridine based ligands and initiators (alkyl halides). [18]

Synthesis protocol of 4DOPA-PCBMA100:

(1) Polymerization

Add the CBMA monomer (2.27g, 10 mmol), the initiator (257 mg, 0.10 mmol), CuBr (14.3 mg, 0.10 mmol) To a Schlenck flask equipped with a magnetic stirring bar. Dissolve the mixture in methanol (10 mL), and then remove the oxygen inside the Schlenck flask by four vacuum nitrogen cycles.

Add PMDETA (17.4 mg, 0.10 mmol) at the end of third vacuum nitrogen cycles. Conduct polymerizations at 25 °C and stir 24 h.

At the end of the polymerization, unseal the Schlenck flask to stop reaction. Get 4DOPA-PCBMA100 product by dialysis against DI water to remove copper and dried by lyophilization.

(2) Deprotection

Add TBAF (0.5g, 1.91 mmol) and 4DOPA-PCBMA100 (1g, 0.019 mmol) and 10 mL anhydrous THF sequentially and precisely into a round bottom flask (50 mL) equipped with a magnetic stirring bar. Conduct the reaction at 25 °C and stir 24 h. Purify the insoluble polymer product by centrifuge at 2000 rpm for 1 min and wash the product with THF 3 times and vacuum dry the product for 3 h.

Add TFA (0.5ml, 6.53 mmol) and polymer product (1g, 0.019 mmol) and 5mL anhydrous THF sequentially and precisely into a round bottom flask (50 mL) equipped with a magnetic stirring bar. Conduct the reaction at 25 °C and stir 24 h.

Purify the insoluble polymer product by centrifuge at 2000 rpm for 1 min and

wash the product by THF 3 times and use vacuum dry the product for 12 h.

Synthesis protocol of 4DOPA-PCBMA200:

(1) Polymerization

Add the CBMA monomer (4.54g, 20 mmol), the initiator (257 mg, 0.10 mmol), CuBr (14.3 mg, 0.10 mmol) To a Schlenck flask equipped with a magnetic stirring bar. Dissolve the mixture in methanol (10 mL), and then remove the oxygen inside the Schlenck flask by four vacuum nitrogen cycles.

Add PMDETA (17.4 mg, 0.10 mmol) at the end of third vacuum nitrogen cycles. Conduct polymerizations at 25 °C and stir 24 h.

At the end of the polymerization, unseal the Schlenck flask to stop reaction. Get 4DOPA-PCBMA200 product by dialysis against DI water to remove copper and dried by lyophilization.

(2) Deprotection

Add TBAF (0.5g, 1.91 mmol) and 4DOPA-PCBMA200 (1g, 0.009 mmol) and 10 mL anhydrous THF sequentially and precisely into a round bottom flask (50 mL) equipped with a magnetic stirring bar. Conduct the reaction at 25 °C and stir 24 h. Purify the insoluble polymer product by centrifuge at 2000 rpm for 1 min and wash the product with THF 3 times and vacuum dry the product for 3 h.

Add TFA (0.5ml, 6.53 mmol) and polymer product (1g, 0.009 mmol) and 5mL anhydrous THF sequentially and precisely into a round bottom flask (50 mL) equipped with a magnetic stirring bar. Conduct the reaction at 25 °C and stir 24 h. Purify the insoluble polymer product by centrifuge at 2000 rpm for 1 min and wash

the product by THF 3 times and use vacuum dry the product for 12 h.

Dip-coating protocol:

For testing the anti-fouling performance, prepare: (1) 4DOPA-PCBMA$_{100}$ solution; (2) 4DOPA-PCBMA$_{200}$ solution solution in TRIS buffer with 20% methanol at 2 mg/mL.

Prepare PDMS (fiber) samples in the same size. Test the weight of each sample for future calculation.

Take 2 ml plastic vial and place the sample into it. Transfer 2 ml solution into each plastic vial rapidly. Coat the samples in each solution overnight. Keep the samples on the rotator in order to coat the entire surface well.

Pour out of the polymer solution after required time. Clean the samples by PBS buffer for 5 times then do ELISA test.

2.4 Dip-coating protocol of 1DOPA-SBMA$_{100}$ and 4DOPA-CBMA$_{100}$ with dopamine

The cross linking between free dopamine and the binding group at the end of zwitterionic chain significantly decreases protein adsorption onto the surface. 1DOPA-SBMA$_{100}$ and 4DOPA-CBMA$_{100}$ have already performed good anti-fouling performance by themselves. The effect of adding dopamine to the coating system worth investigation.

Dip-coating protocol:

Prepare:

(1) 1DOPA-PSBMA$_{100}$ solution in TRIS buffer and 20% methanol at 2 mg/mL, add

dopamine and the mass ratio of polymer to dopamine is 8:1.

(2) 4DOPA-PCBMA$_{100}$ solution in TRIS buffer and 20% methanol at 2 mg/mL, add dopamine and the mass ratio of polymer to dopamine is 8:1.

Prepare PDMS (fiber) samples in the same size. Test the weight of each sample for future calculation.

Take 2 ml plastic vial and place the sample into it. Transfer 2 ml solution into each plastic vial rapidly. Coat the samples in each solution overnight. Keep the samples on the rotator in order to coat the entire surface well.

Pour out of the polymer solution after required time. Clean the samples by PBS buffer for 5 times then do the Elisa test.

2.5 Investigate the effect of oxidation process to polymer's anti-fouling performance

pH-induced polymerization of dopamine and its derivatives are used for surfaces modification. Dopamine can form a thin, surface-adherent polymer film on almost all materials surfaces. However, the spontaneous polymerization of dopamine must be under alkaline condition. Dopamine is oxidized to dopaminequinone firstly; then the intramolecular cyclization of the dopaminequinone via 1,4-Michael addition leads to the leucodopaminechrome; and then leucodopaminechrome is oxidized to dopaminechrome; and the dopaminechrome polymerizes to polydopamine at last. [15]

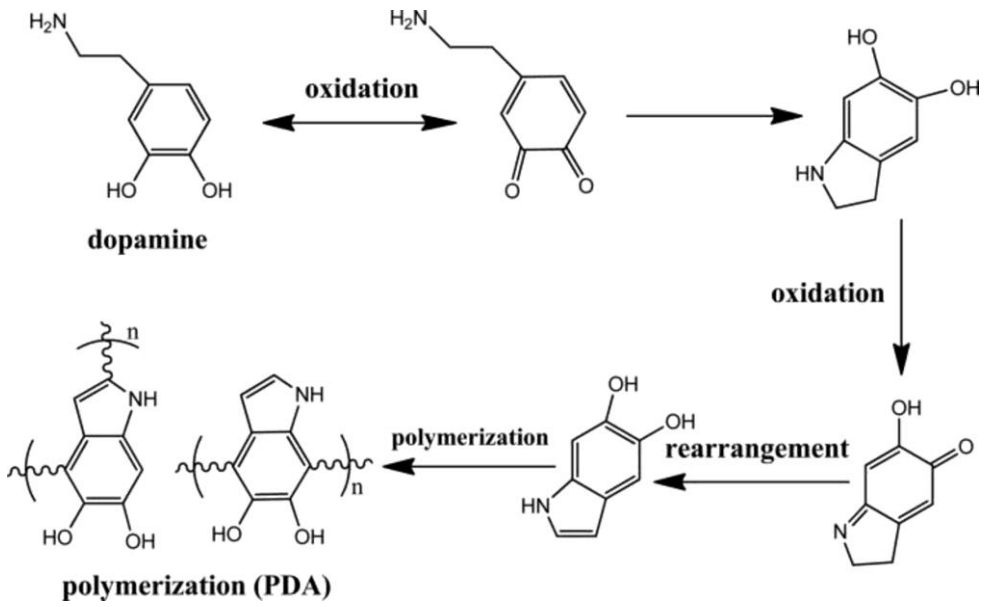

Figure 14. Dopamine polymerization process [19]

In neutral or acidic condition, however, the polymerization is not spontaneous and would need the help of oxidant. Zhao et al. group investigates the effect of adding oxidant, ammonium persulfate into the non-alkaline and alkaline media, respectively. The results show that dopamine can hardly to polymerize without oxidant but can polymerize when oxidant added at pH 5.5 or pH 7.0. At pH 8.5, dopamine can polymerize either with or without oxidant, but the reaction speed of the system with oxidant is much faster than without oxidant. [20]

Ultraviolet–visible spectroscopy (UV-Vis) refers to absorption spectroscopy or reflectance spectroscopy in the ultraviolet-visible spectral region. It uses light in the visible and adjacent ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In a study by Prof. Choi's group, a perfluorinated dopamine derivative (f-DOPA) was designed to make surfaces superhydrophobic in order to achieve self-cleaning functions. This molecule can

undergo oxidative polymerization, forming a rough film of extremely low surface energy on various substrates. The experiments also use oxidant to improve oxidation in alkaline condition. The oxidation process of f-DOPA was investigated by UV-Vis spectroscopy. The first peak shown around 283 nm is recognized as the symmetry-forbidden transition (L_a–L_b) of the catechol moiety in f-DOPA. After adding the oxidant NaIO4 to the solution, the peak at 283 nm decreased and a new peak around 330 nm was observed, which is the representative peak of O-quinone, indicating that the catechol group is oxidized to O-quinone. [21]

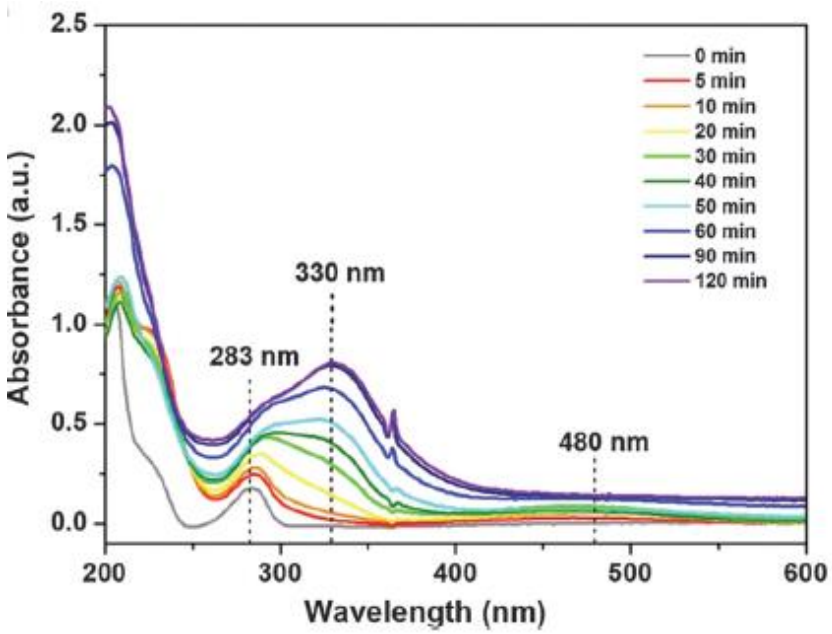

Figure 15. UV-Vis spectra of the acetonitrile solution of f-DOPA with NaIO4-induced oxidation. [21]

O-quinone is a hydrophobic group, which should perform stronger binding ability than catechol group. Furthermore, it is also a premise of forming cross-linking system between molecules. We designed an experiment to monitor the speed of forming o-quinone by adding sodium periodate. Prepare 0.1 mg/mL dopamine solution in

water (neutral condition). Add around 2 mM sodium periodate inside.

2.6 Dip-coat zwitterionic polymer-DOPA conjugates on PP substrate

PP is a very common hydrophobic material. It has a very stable polymer structure, which is difficult to modify and coat with general anti-fouling materials. The new zwitterionic polymer-DOPA conjugate, 4DOPA-$CBMA_{100}$, brings a possible method to solve this problem.

Dip-coating protocol:

Prepare 4DOPA-$PCBMA_{100}$ solution in TRIS buffer with 20% methanol at 2 mg/ml. Prepare PP samples in the same size. Test the weight of each sample for future calculation.

Take 2 ml plastic vial and place one sample into each vial. Transfer 2 ml solution into plastic vial. Coat the samples in each solution overnight. Keep the samples on the rotator in order to coat the entire surface well.

Pour out of the polymer solution after required time. Clean the samples by PBS buffer for 5 times. Keep 3 samples into the PBS buffer.

Prepare sodium periodate solution at 2 mM. Place 3 samples into the plastic vial with 2 ml sodium periodate solution inside. After 2 hours, pour out of the oxidant solution and clean the samples by PBS buffer for 5 times and do ELISA test for all the samples.

2.7 Enzyme-Linked ImmunoSorbent Assay (ELISA) protocol

ELISA is a test that uses antibodies and color change to identify a substance. In the assay, the antigen of interest is immobilized by direct adsorption to the assay plate or

by first attaching a capture antibody to the plate surface. Detection of the antigen can then be performed using an enzyme-conjugated primary antibody (direct detection) or a matched set of unlabeled primary and conjugated secondary antibodies (indirect detection). [22]

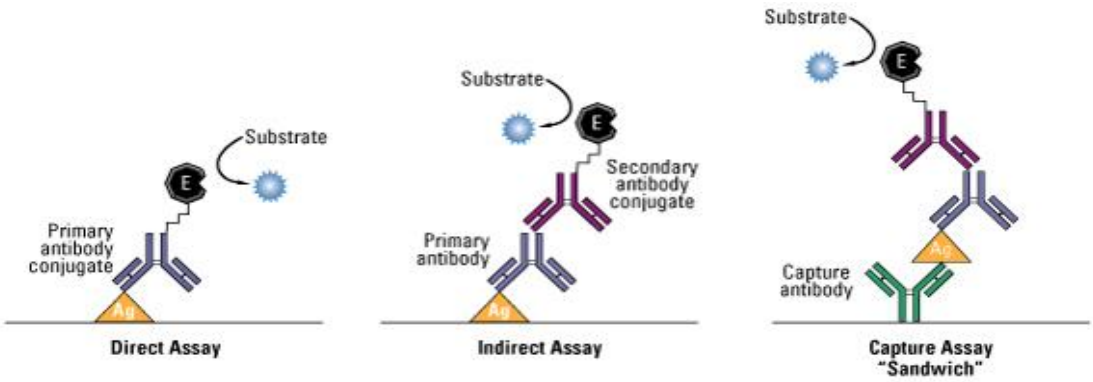

Figure 16. Diagram of common ELISA formats [22]

In our experiments, we mostly use direct ELISA to test protein fouling for all the samples, which is quick because only one protein solution and fewer steps are used. The protocol is as below:

(1) Thaw Fibrinogen-HRP for 20 minutes.

(2) Place samples and controls of similar size in a 24-well plate and submerge each in PBS for a few minutes. If hydrophobic samples float, try wetting them first with ethanol.

(3) Prepare a solution of 20 µl Fibrinogen-HRP in 15 ml PBS in a centrifuge tube. Mix gently and thoroughly.

(4) Remove PBS from wells by pipette and deliver 1 ml Fibrinogen-HRP solution to each well. Treat 30 minutes.

(5) Thaw O-phenylenediamine dihydrochloride (OPD).

(6) After 30 minutes, rinse substrates 5 times with PBS and transfer each one to a fresh well. Ideally soaking for a few minutes between each rinse.

(7) Prepare 1 mg/ ml OPD solution in a centrifuge tube: 12.6 ml water + 30 µl H_2O_2 + 1.4 ml pH 5.5 citrate phosphate buffer + 14 mg OPD.

(8) Shield OPD tube from light with OPD tube from light with foil and mix it on the rotator for a few minutes

(9) Add 600 µl OPD solution to each well. Incubate 30 minutes on the tabletop shaker.

(10) Add 600 µl 1 N HCl solution to quench reaction. Incubate around 5 minutes.

(11) Take 600 µl resulting solution to a new well and test with spectrophotometry at the wavelength of 492 nm.

3 Results and Discussions

3.1 Anti-fouling performance of 1DOPA-PCBMA$_{100}$ and 1DOPA-PSBMA$_{100}$

The results of anti-fouling test are as below:

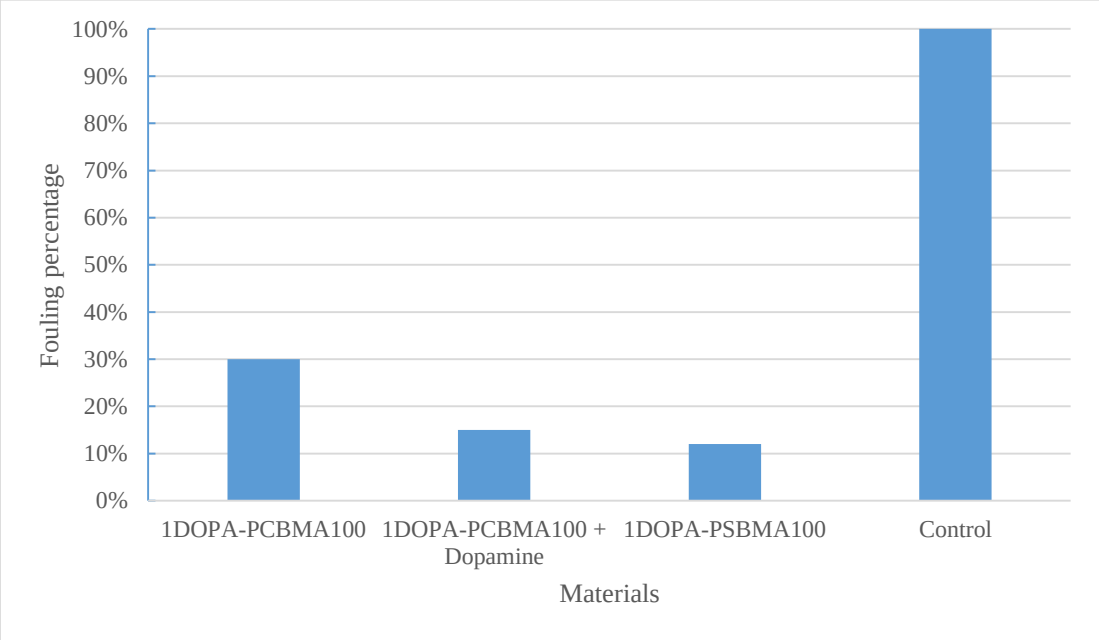

Figure 17. Anti-fouling performance of 1DOPA-PCBMA$_{100}$ and 1DOPA-PSBMA$_{100}$.

From the results, it can be concluded that:

(1) 1DOPA-PSBMA$_{100}$ shows better anti-fouling performance than 1DOPA-PCBMA$_{100}$ without adding dopamine. Both carboxybetaine and sulfobetaine can bind water strongly via electrostatically induced hydration. Carboxybetaine, however, is more soluble than sulfobetaine, which results in lower surface packing density of 1DOPA-PCBMA$_{100}$.

(2) The existence of dopamine helps zwitterionic polymer-DOPA conjugates to resist protein adsorption. The oxidation product of catechol group, quinone, can react

with oxidant product of dopamine, forming a cross-linking structure to bind strongly on the surface.

3.2 Anti-fouling performance of 4DOPA-PCBMA$_{100}$ and 4DOPA-PCBMA$_{200}$

The structure of 4DOPA-PCBAA$_{100}$ synthesized by ATRP is as below:

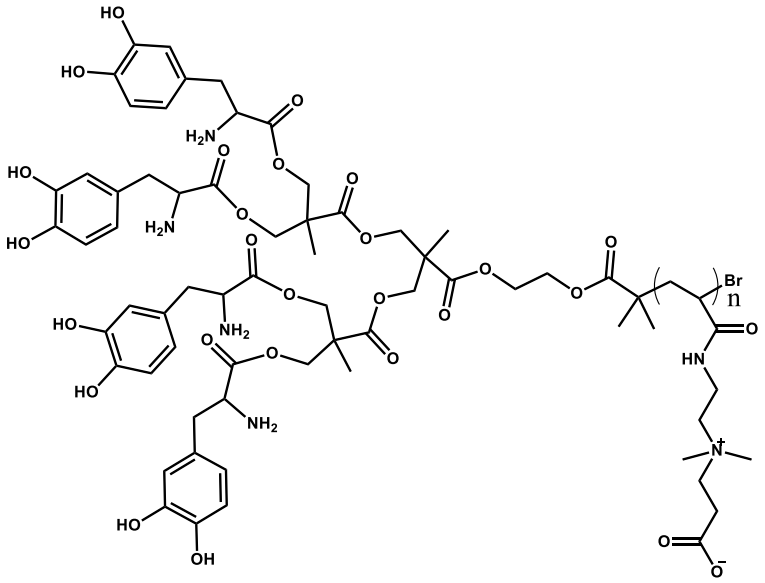

Figure 18. Structure of 4DOPA-PCBAA$_{100}$

The results of anti-fouling test are as below:

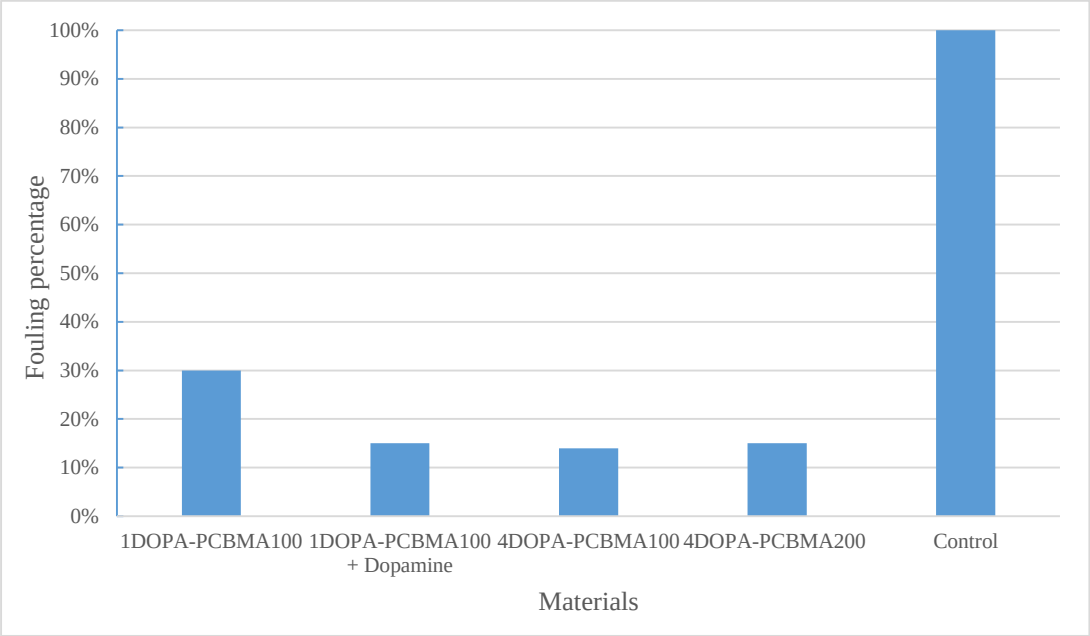

Figure 19. Anti-fouling performance of zwitterionic polymer with 4 binding groups

From the results as above, 4DOPA-CBMA$_{100}$ and 4DOPA-CBMA$_{200}$ show better anti-fouling performance than 1DOPA-CBMA$_{100}$. Four catechol groups are demonstrated to provide much stronger binding force than one catechol group, which can result in higher surface packing ability and resist more protein finally. 4DOPA-CBMA$_{100}$ shows similar anti-fouling performance with 1DOPA-CBMA$_{100}$ with the existence of dopamine. However, adding dopamine into the coating system changes the color of surface to black. Only the zwitterionic-DOPA can keep the surface clear and transparent.

3.3 Anti-fouling performance of 1DOPA-SBMA$_{100}$ and 4DOPA-CBMA$_{100}$ with dopamine

The anti-fouling results of the samples are as below:

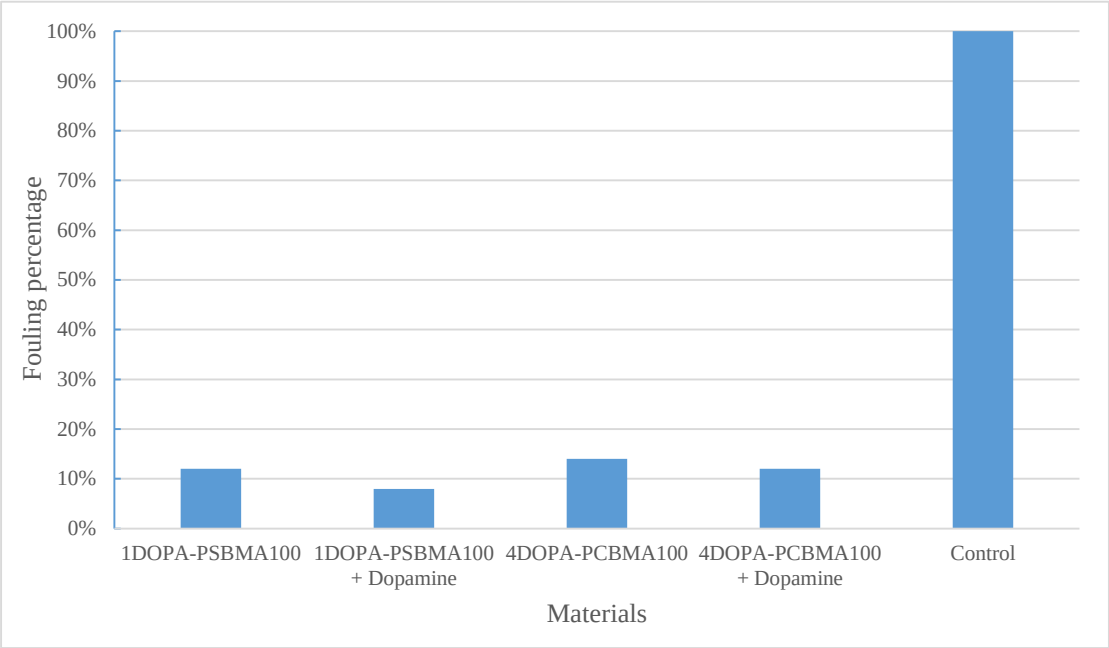

Figure 20. Anti-fouling performance when mixing dopamine with 1DOPA-SBMA$_{100}$ and 4DOPA-CBMA$_{100}$

As above, two zwitterinoic-DOPA materials show better anti-fouling performance with adding dopamine, but not significantly. PSB chain develops higher surface packing density than PCB chain. Therefore, dopamine plays a more important role in 1DOPA-PCBMA$_{100}$ system than 1DOPA-PSBMA$_{100}$ system. For 4DOPA-PCBMA$_{100}$, four catechol groups in the polymer chain end form a stronger binding force than one group, which also keep a high surface packing density. Furthermore, amine groups from the polymer chain ends can play the role of dopamine to develop cross-linking structure on the surface.

3.4 Effects of oxidation process to anti-fouling performance

Test UV-vis of 0.1 mg/mL dopamine with oxidant at different time.

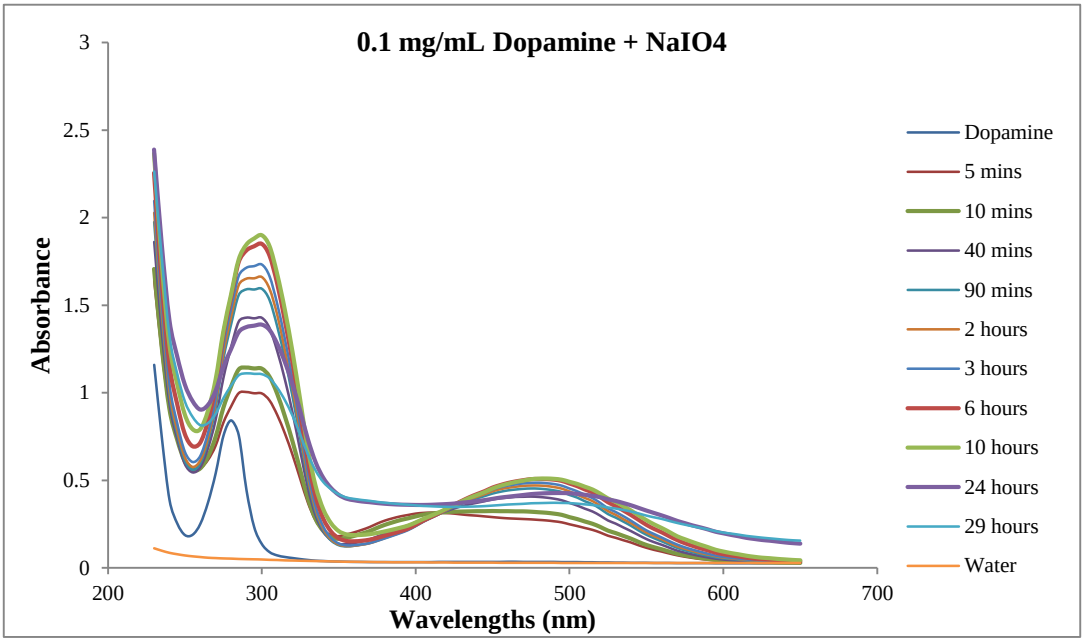

Figure 21. UV spectra of 0.1 mg/mL Dopamine with NaIO4 induced oxidation

From **Figure 21**, two peaks can be found from 250 to 350 nm. The 280 nm is from catechol group and the 310 nm is from o-quinone. After 5 minutes adding sodium periodate, the peak from quinone appears around 310 nm. In this neutral condition, it is obvious that dopamine is oxidized rapidly after adding sodium periodate, which demonstrates that the existence of oxidant assist dopamine to form a fine coating system to target surfaces in a quick method. Furtgermore adding oxidants to a 4DOPA-zwitterinoic polymer system can avoid blackening the surface compared with adding dopamine, both of which play a similar role in assisting cross-linking.

3.5 Anti-fouling performance of Zwitterionic polymer-DOPA conjugates on PP substrate

The results of anti-fouling test on PP surface are as below:

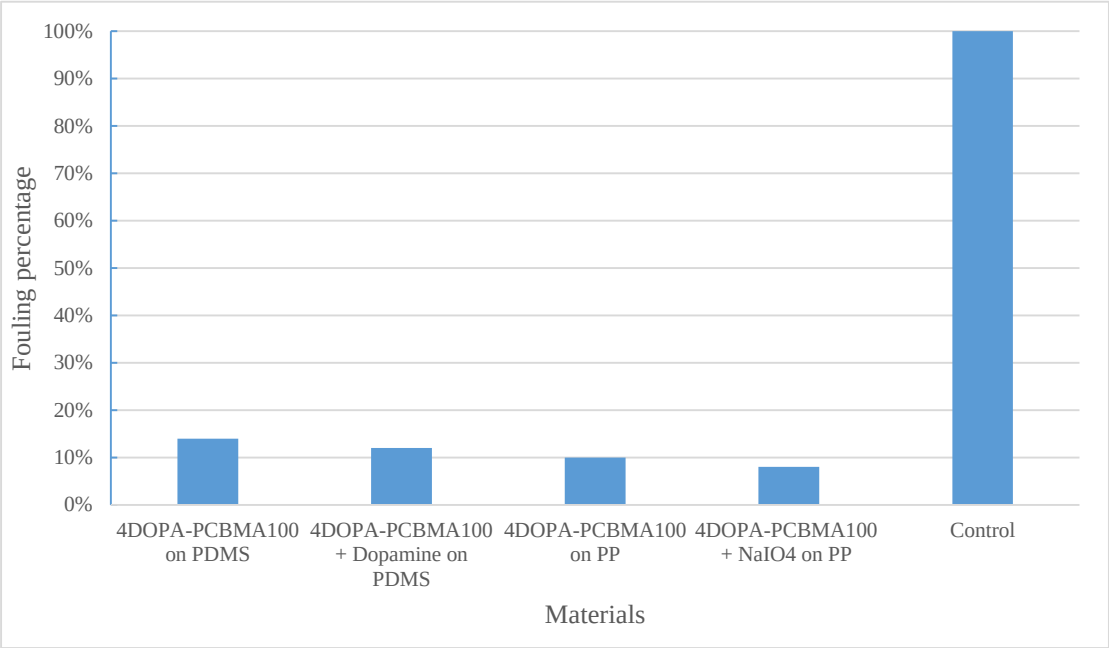

Figure 22. Antifouling performance of 4DOPA-CBMA$_{100}$ on PDMS and PP surfaces in different conditions

From the results above, PP also shows good anti-fouling performance in the 4DOPA-zwitterinoic polymer system. Adding oxidant did not show significant decrease in protein adsorption, which is similar with adding dopamine to this system. The results of adding dopamine to 4DOPA-CBMA$_{100}$ solution also perform similar fouling with single zwitterionic coating.

4 Conclusions

4DOPA-PCBMA100 has shown better anti-fouling performance than 1DOPA-PCBMA100 on hydrophobic surfaces such as PDMS and PP and has similar performance as 1DOPA-PCBMA100 coating with dopamine. Because of more binding groups, 4DOPA-PCBMA100 can bind target surfaces stronger than 1DOPA-PCBMA100. Furthermore, the free amine groups of 4DOPA-PCBMA100 can perform cross-linking among molecules, which can act as dopamine to form a stable surface binding system. The effect of adding oxidant to the surface binding system is still under investigation.

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