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Design of Peptoid-Based Coating to Reduce Biofouling in Gas Exchange Devices

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Design of Peptoid-Based Coating to Reduce Biofouling in Gas Exchange Devices

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by

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Abstract

In recent decades, membrane technology has been used commonly in biomedical area. However, membrane fouling is a widespread problem in different applications. One method to minimize fouling is through surface modification of membranes. My research explores a novel polymer to minimize nonspecific protein adsorption in biomedical applications.

It firstly focuses on grafting the electrically neutral NMEG peptoid, containing 2-methoxyethyl side chains, to polysulfone (PSU) membrane via polydopamine. Contact angle measurements indicated that the hydrophilicity of the peptoid-grafted membranes was significantly improved while the pore size and strength of the membranes remained unchanged. The modified membranes showed an improved fouling resistance when tested with bovine serum albumin, lysozyme and fibrinogen proteins. To further investigate the low fouling surfaces, peptoid length was varied length of peptoids (NMEG5, NMEG10, NMEG15 and NMEG20). The effect of peptoid length and grafting density on fouling resistance of the membranes was studied. Static adsorption experiments with bovine serum albumin revealed that there is an optimal grafting density to improve fouling resistance of peptoid modified membranes, which was dependent on the length and amount of the grafted peptoids.

To evaluate the application of modified hollow fibers in the biomedical field, a gas exchange system was designed and built. The peptoid-grafted hollow fiber membranes could preserve an excellent oxygen gas transmission compared with PSU membranes after exposure to bovine serum solution (35 mg/ml in PBS). To expand the understanding about dynamic fouling resistance of peptoid grafted surfaces, cross-flow filtration tests using bovine serum solution as the feed, was designed and built. According to the cross-flow filtration results, NMEG modified

membranes showed a significant improvement in antifouling ability. Furthermore, flux recovery ratios obtained from NMEG modified membranes were much higher than unmodified membranes. The outcome of this study suggests that peptoids are a promising material for fouling-resistant membrane surface modification.

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Dedications

This dissertation titled Design of peptoid-based coating to reduce biofouling in gas exchange devices is dedicated to my parents and my husband Mojtaba.

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List of Published Papers

The chapter three has been published in colloids and Surfaces B: Biointerfaces as PEG-Mimetic Peptoid Reduces Protein Fouling of Polysulfone Hollow Fibers “Mahmoudi, N., et al., PEG-mimetic peptoid reduces protein fouling of polysulfone hollow fibers. colloids and Surfaces B: Biointerfaces, 2017. 149: p. 23-29”.

1. Chapter 1 Introduction

1.1 Membrane Oxygenators

Several hundred thousand Americans are suffering chronic and acute lung diseases and despite advances in biotechnology, almost 350,000 Americans die because of these diseases [4, 5]. The respiratory system is responsible to oxygenate blood and release unneeded carbon dioxide from the blood (Figure 1.1). Moreover, the number of people who need a lung transplant is increasing [6]. Although extracorporeal and mechanical ventilation can aid to transplant success, both have limitations [7]. There are two pathways for lung replacement: transplantation of a viable a lung from a donor to another patient, or implantation of an artificial lung. Since the number of people who need a lung is much larger than the numbers of donors, and transplant wait-time for lung is almost two years, the mortality rate of people who are on the lung transplant wait list is over 20%. Therefore, the development of an artificial lung is a potential solution for this problem [8, 9]. Additionally, a suitable artificial lung can be a supplement to mechanical ventilation or a support device after transplant [6].

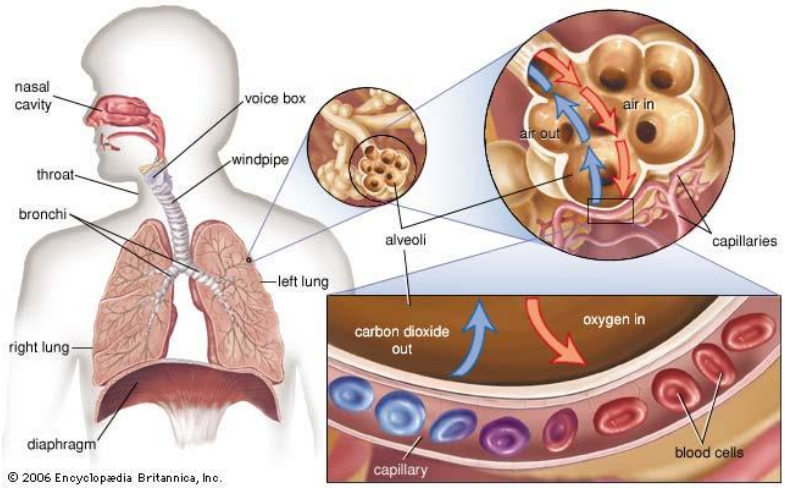

Figure 1.1.A schematic of ventilation system, including lungs, trachea, alveoli and bronchioles [2].

Extracorporeal membrane oxygenators are commonly known as artificial lungs which oxygenate the blood and remove carbon dioxide from the blood without the need for functioning lungs [10, 11]. Oxygenator devices can help patients to survive and heal from cardiopulmonary surgery and using as a bridge to lung transplant [12]. Extracorporeal membrane oxygenators technology has significant progresses since the earliest advancement on artificial lungs began in the 1930s [13], including development in tubing, blood pumps, gas exchangers. Figure 1.2 shows an example of extracorporeal membrane oxygenators which is currently in hospitals. Membrane oxygenators devices can be used for both heart and lungs; however, since they undergoes fouling they are not capable to use for long term by patients [14]. Membrane oxygenators commonly composed of hollow fiber membranes. The function of artificial lungs is to oxygenate blood and remove

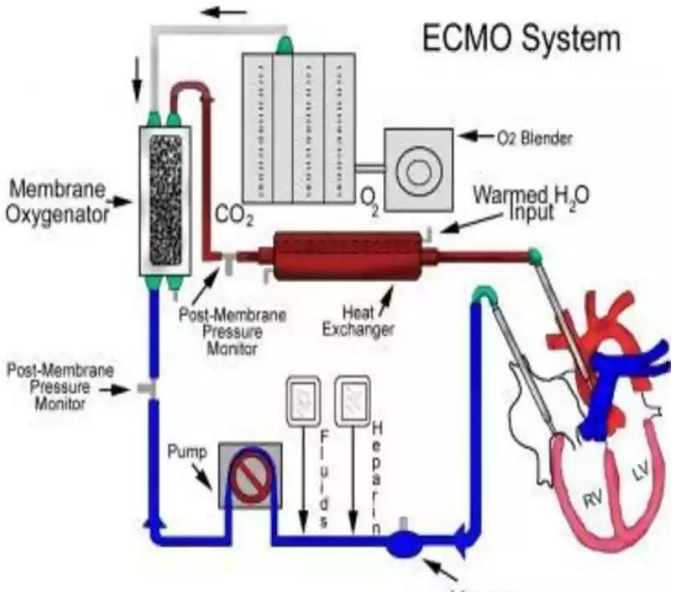

Figure 1.2. A typical example of membrane oxygenator [1]

carbon dioxide from the blood, where blood flows outside of the hollow fibers, and oxygen (O_2) passes through the inside of hollow fibers in the artificial lung. Based on the concentration gradient, oxygen diffuses across the wall pores into blood, and carbon dioxide diffuses from the

blood into the fibers. However, there are some problems in artificial lung technology, including low rate of gas exchange and insufficiency and lack of biocompatibility for long-time periods.

Significant efforts have been reported since 1970 to design, assemble, and test an ideal implantable artificial lung [15]. However, work is still needed to design an ideal artificial lung with long-term performance by improving biocompatibility to minimize thrombotic deposition and increasing gas transfer efficiency.

1.2 Polysulfone Membranes

Polysulfone (PSU) (Figure 1.3) is used in different applications, such as gas separation, hemodialysis, nanofiltration, and wastewater treatment [16-19]. PSU is one of the most common polymers for biomedical membrane applications due to its high chemical, physical, and thermal stability [20-22].. PSU is also highly porous and can be sterilized via different methods, such as e-beam, ethylene-oxide β-/γ- ray, and steam [22]. PSU can be easily prepared via a phase inversion method with high permeability [22]. These properties introduce PSU as an appropriate material for medical application [23]. Despite the advantages of this polymer, biological fluids, proteins, and other materials can adsorb to the PSU membrane surface and. within its pores. These cases are referred to as membrane fouling [20, 21, 24, 25]. The hydrophobic character and low surface energy of PSU can cause membrane fouling and fail to provide good hemo and/or bio-compatibility. Moreover, the adsorption of protein and formation of a protein layer onto the surfaces of medical implants can create a bio-film, which has a harmful effect on biomedical device performance [26]. The fouling of membranes leads to a decrease in flux across the membrane, coagulation, increased energy consumption, and increased operational cost [25, 27]. The biocompatibility of PSU membranes must be improved to be more viable for use in biomedical devices [22, 25]. In order to improve the biocompatibility, PSU membranes must be

modified to alter the surface properties and fouling [20, 28]. Membrane fouling occurs due to hydrophobic interactions between the membrane surface and biological foulants, van der Waals interactions, etc [25, 29, 30].

In order to have biocompatible polymers, developments can be categorized in three ways: (1) physicochemical characteristics control on material surface, (2) surface modification via biomolecules, and (3) development of biomimetic membrane surface [31]. One strategy to reduce fouling is to modify the surface properties such that the hydrophobicity is decreased. Research suggests that effective, non-fouling surfaces should be (i) hydrophilic, (ii) electrically neutral, and (iii) free of hydrogen bond donors with hydrogen bond acceptors.

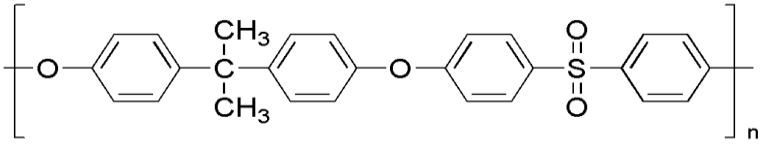

Figure 1.3. Polysulfone (PSU) structure

1.3 Peptoid

Poly-N-substituted glycines, or peptoid, can be named as effective antifouling polymers without any biodegradability problems. Peptoids are a class of biomimetic polymers that have a protein-like backbone with the side chains attached to the amide nitrogen, rather than the α-carbon (see Figure 1.4A) [32]. This change in side chain position leads to several backbone alterations that allow peptoids to resist protease degradation and increase biostability compared to peptides [33, 34]. Peptoids do not have hydrogen bond donors in the backbone unlike their peptide counterparts [35]. Peptoids are synthesized in a sequence-specific manner following a submonomer protocol that allows for the addition of diverse side chain variety [36]. The submonomer protocol is based on a two-stage monomer addition cycle: acylation of a secondary

amine and replacement of primary amine. In the first stage, acylation of a secondary amine on the resin occurs with bromoacetic acid. In this step an SN_2 reaction substrate leaves. In the second stage, the primary amine is displaced with bromide. These steps are repeated until the desired sequence is obtained (Figure 1.4B) [37]. After synthesis is completed, peptoids are cleaved from the resin using trifluoroacetic acid.

Statz et al. studied peptide-peptoid hybrids composed of PEG-like side chains (NMEG) and a mussel adhesive-inspired DOPA-Lys peptide. The peptide-peptoid hybrids anchor to TiO2 surfaces (via DOPA-Lys) and prevent cell and protein adhesion [38]. This research was extended to study three different peptoid side chains (2-methoxyethyl (NMEG), 2-hydroxyethyl, and 2-hydroxypropyl) [39]. The peptoid-modified TiO_2 surfaces resisted adsorption of proteins including fibrinogen, lysozyme, and serum proteins. However, NMEG-coated surfaces exhibited improved long-term fouling resistance during *in vitro* cell attachment studies for up to six weeks. The decrease in protein adsorption onto NMEG-coated surfaces with time is likely due to the absence of hydroxyl functional groups, which are present in both of the other side chains. Studies of self-assembled monolayers showed that presence of hydrogen bond donors in the hydroxyl group. increases the adsorption of protein [39, 40]. It was also shown that the length of the NMEG (n = 10 to 50 for a coating thickness ranging from 2.8 to 4.2 nm) had statistically no effect on protein fouling but at least 15-mer peptoid length is needed for long-term fouling resistance [41]. In 2011, Liu and Jia introduced new peptoid side chains (N-ethyl-β-alanine and N-methyl-β-alanine) and grafted the poly(β-peptoid)s to gold surfaces via terminal thiol groups. Fouling was evaluated by surface plasmon resonance over ten minutes with single proteins (fibrinogen, bovine serum albumin, and lysozyme). The data showed that while the poly(β-peptoid) coatings have good protein resistance, oxidation of the thiol groups to form sulfonate

groups causes the adhesion to gold to weaken with time [42, 43]. Therefore, thiol terminated polymers are not suitable materials to resist fouling for long-term use [43].

However, PSU hollow fibers do not have reactive sites on the surface. Therefore, a suitable functional step has to be carried out. There have been many physical and chemical methods to functionalize the surface, such as γ-irradiation treatment, plasma discharge, and plasma polymerization. These methods have been used to activate the PSU surface [44]. Nevertheless, each of these methods often have various limitations, such as change in pore size distribution, change in membrane structure, reduction in mechanical strength, and permeability [45]. For practical applications, the use of novel methods__which are simpler and more convenient__ would be desired to graft peptoid polymers onto a PSU surface. Covalent attachment of biomolecules is able to provide a stable and long-term performance. Furthermore, covalent attachment often creates different functional groups, which introduce reaction sites to graft other biomolecules [46].

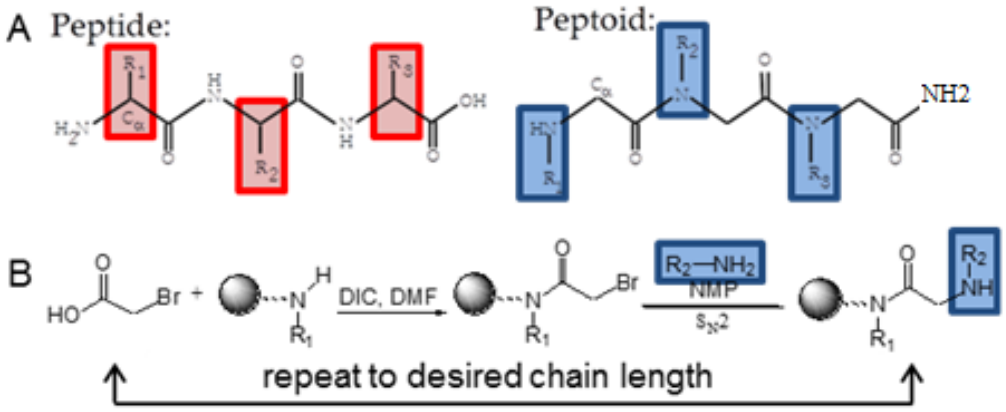

Figure1.4. (A) Peptide and peptoid backbone structures. (B) Peptoid submonomer synthesis protocol.

1.4 Application of PDA to Membranes

Recently, surface modification using materials inspired by the adhesive secretions of mussels and other sessile marine organism have been attracted lots of interests [47-49]. Mussel adhesive proteins (MAPs) create strong water-resistant adhesion to materials in wet environments. MAPs are rich in L-3,4-dihydroxyphenylalanine DOPA, and lysine amino acids, which play a crucial role for strong attachment onto the substrates [50]. Dopamine, which is found in MAPs, contains catechol and amine functional groups. Messersmith et al. demonstrated that covalent conjunction of DOPA groups to peptoid was able to modify the titanium surface into fouling resisting ones [38]. Moreover, mussel-inspired polydopamine (PDA) modification showed that they did not have any toxic effect to cells after applied to a variety of surfaces [51]. In 2007 Messersmith et al. [52] reported a facile and versatile aqueous surface modification technique using dopamine which undergoes self-polymerization in aqueous solution and create a tightly adherent PDA layer to the surface. This method can be applied to virtually any solid materials, including polymers and ceramics, and PDA can serve as useful platforms for secondary reactions and surface functionalization under mild conditions [52, 53]. A number of studies have been reported the wide use of this biocompatible materials in water purification, sensing, biomedical and energy [3, 54, 55].

PDA has some advantages over other traditional methods for surface modification. For example, there is no need of special reaction between the membrane surface and the PDA coating to deposit PDA onto the surface; while, many grafting process depend on the presence of specific moiety on the surface of membranes [56, 57]. Additionally, modification of surface using PDA occurs under aqueous and mild conditions, and the underlying membrane does not suffer degradation; while, degradation of membrane happens in the modification of membrane using

irradiation [58, 59] or plasma based modification [60, 61]. Furthermore, PDA modification happens in aqueous solution in which membrane remains wet during the whole modification steps. In contrast modification of membranes by some other methods such as plasma treatment, drying is required; during drying process pore collapse induced by strong capillary forces which can decrease membrane permeability [62, 63]. Another problem of using chemical grafting methods is that they needs to be activated by plasma, UV, ozone or chemical agents in order to graft PEG onto the surface and for some cases it is complicated, expensive, and not applicable to diverse polymer materials with complex shapes processing [64]. A common problem with coating methods to modify membrane surface is the decrease in membrane permeability associated with the coating. Even though this problem cannot be removed in all cased in PDA modification surfaces, it can be avoided by controlling the thickness of PDA deposition. By changing the concentration of PDA in the solution and deposition time of PDA, the PDA thickness can be controlled, .and thin PDA thickness can remain membrane permeability[65-67]. Finally, complexity or specificity of many membrane surface strategies limit the application of them in industry, PDA may be a useful method to modify membrane surface [68].

Many membranes successfully have been modified using PDA and generally PDA coated membranes are rinsed with an organic solvent such as methanol to remove weakly or un-bound PDA. Furthermore, Messersmith and co-workers developed a two-step method for surface modification without the need for catechol conjugated organic synthesis molecules [47]. A thin layer of PDA film is first deposited onto a surface by immersion with an alkaline aqueous dopamine solution, followed by immobilization of biomolecules onto the PDA coatings which exhibit latent reactivity toward amine and thiol groups [69]. In the second step of the approach, biomolecules were immobilized onto the surface via a reaction between nucleophiles and the

PDA surface. In a mechanistic point of view, some reaction intermediates were formed, such as indole species, 5,6-hydroxyindole, and 5,6-indolequinone, via oxidation and rearrangement [28, 70]. PDA nanoaggregates with free catechol groups are formed via covalent binding and/or a physical assemble of intermediates reactions [71, 72] and formed a PDA layer onto the surface [73]. Nevertheless, the PDA layer can bind biomolecules via Michael addition or Schiff base substitution reaction between the PDA surface and nucleophiles such as thiols and amines [47]. Although the detailed dopamine polymerization mechanism is still under investigation [3], proposed structures of polymerized dopamine are shown in Figure 1.5. In the case of using attachment of hydrophilic polymer onto membranes, Li et al. [74] used PDA to graft PEG onto PES flat sheets. They found that in comparison to the unmodified and PDA modified PES membranes, PEG modified membranes adsorbed less BSA (~ 9, 5 and 4 μg/cm^2 for PES, PDA modified and PEG modified surfaces, respectively) under the same condition (1mg/ml BSA in PBS solution, 24 hr) [74].

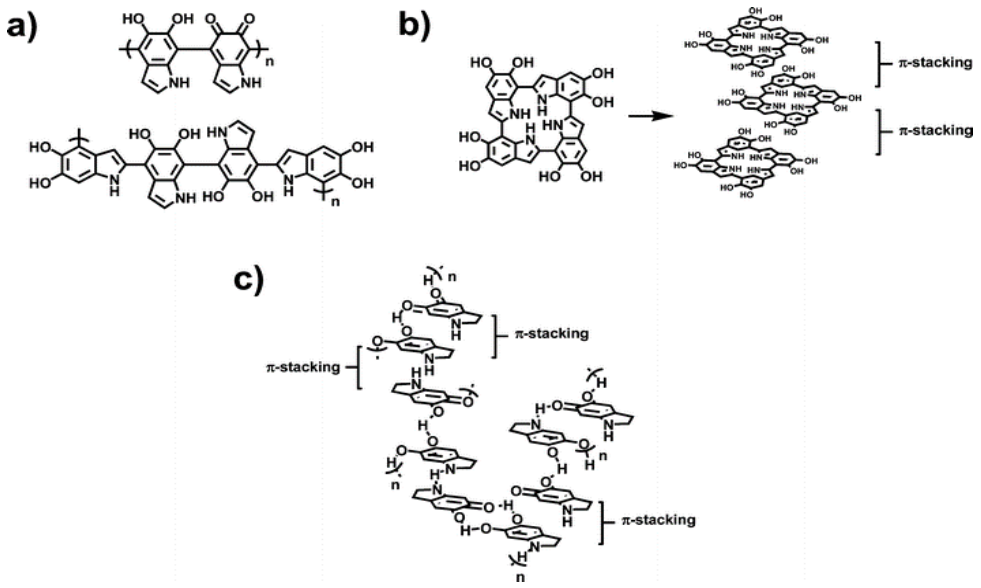

Figure 1.5. Proposed structure of PDA including a) covalent linkage of monomers, b) combination of supramolecular and covalent linkages, or c) supramolecular bonding interactions [3].

1.5 Purpose and Significance of Research

In the medical applications, proteins, platelet and cells can strongly adhere to surfaces, changing performance of the device with harmful outcomes. Therefore, biofouling must be minimized or controlled to maintain safety and performance of medical devices. A common strategy to minimize fouling is to attach an antifouling material to a surface. Important parameters of the modified material include chemical characteristic, flexibility, molecular weight and the method by which the antifouling material is attached to the surface. One of the antifouling materials is peptoid with 2-methoxyethyl side chains (NMEG), a water-soluble polymer with low toxicity, flexible backbone and a history of use in medical application. NMEG peptoid can be synthesized easily and grafted onto surfaces to reduce the nonspecific adsorption of proteins and cells. The main purpose of the work conducted in this dissertation is to find an innovative method to minimize biofouling onto PSU polymers and maintain membrane performance.

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2. Chapter 2. Surface Modification of Membranes in Biomedical Areas

2.1 Introduction

Synthetic polymers have been commonly used in medical therapy, such as implantable medical devices, modulation of wound healing, artificial organs, dentistry, bone repair, prostheses, drug delivery system and ophthalmology [1]. Polymeric materials display advantages including the ability to manufacture various shapes at reasonable cost, desirable physical and mechanical properties [1]. Most membranes such as polysulfone (PSU), polyether sulfone (PES), polyvinylidene fluoride (PVDF), polypropylene (PP), polyacrylonitrile (PAN), and polyamides (PA) are hydrophobic since the hydrophobicity of membrane material is useful to maintain structural integrity while the membranes are used in aqueous environments [2]. However, proteins have a higher tendency to adsorb to hydrophobic surfaces than hydrophilic surfaces. Many studies reviewed different factors contributing to membrane fouling and the mechanisms by which foulant accumulation proceeds [2-5]. Protein adsorption from blood and tissue on surface of membranes is a rapid phenomena and denaturation of proteins may happen, resulting in platelet adhesion and aggregation, leading to subsequent blood coagulation and thrombosis formation (Figure 2.1) [6]. Therefore, a membrane with low biocompatibility limits the use of these them in biomedical areas [1, 4, 7-10]. For this reason, many studies have been implementing different methods to improve biocompatibility of hydrophobic membranes using increasing hydrophilicity of surfaces [11-23]. It is suggested that hydrophilic surfaces tightly bind a layer of water, which would decrease the adsorption of proteins from blood on to the surface of the membrane. In this way, hydrophilic-hydrophobic interaction between proteins and membranes are mitigated [2].

Dr. Belford et al. studied the fouling behavior of over 66 monomers grafted onto ultrafiltration membranes [2, 4, 7, 24, 25]. The results showed that the most resistant monomers to protein adhesion were hydrophilic, contain hydrogen bond acceptors, no hydrogen bond donors and electrically neutral, in agreement with findings from Whiteside's group [26]. Additionally, studies show that hydrophobic, rough, and charged membrane surfaces are susceptible to protein adsorption, and it is hypothesized that hydrophilic, smooth and electrically neutral membranes may foul less [2]. The amount of protein adsorbed onto membranes depends on the various interaction types between membranes surface and proteins, such as hydrophobic interaction, electrostatic interaction, hydrogen bonding, van der Waals interaction and dipole–dipole interaction [27]. To minimize fouling impact on membrane efficiency or prevent protein adsorption many strategies based on the nature of the membrane material have been applied [28]. One of the main factors with a significant effect on extent of protein adsorption is to minimize hydrophobic interaction which decreases as hydrophilicity of membrane increases [29]. The hydrophilicity of membranes can be improved by modifying the hydrophobic membrane surface using hydrophilic antifouling polymers [30].

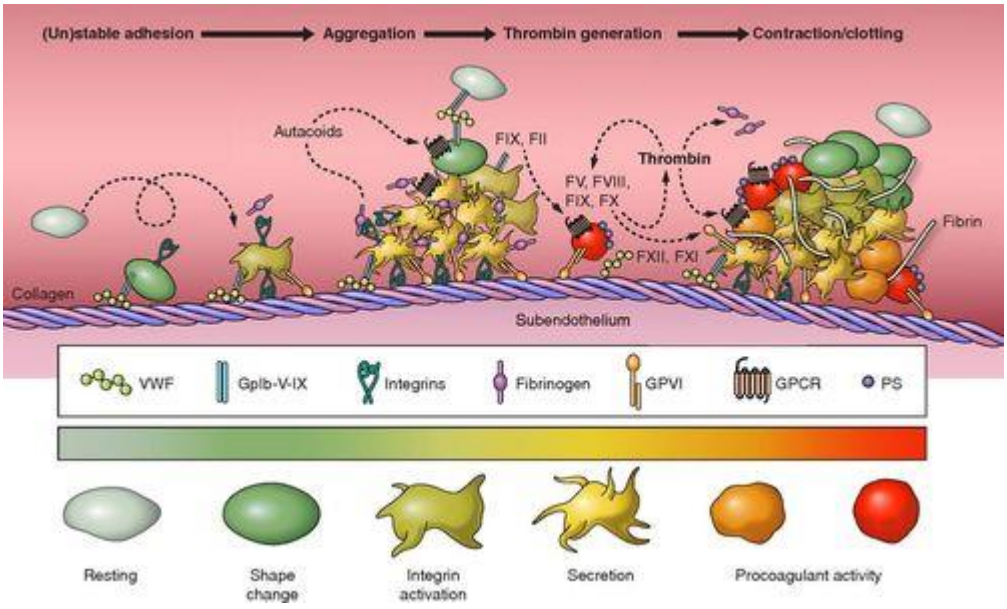

Figure 2.1. The pathway of platelet activation and thrombus formation [6].

Modification of surfaces using antifouling polymers has been a significant focus in efforts to prepare biocompatible membranes. Many techniques have been used to create fouling resistant membranes such as additive blending (where one or more antifouling macromolecules are incorporated into a polymer to cast the membranes) [31], chemical treatment [23], plasma treatment [32], UV irradiation [14]. Chemical, plasma and UV irradiation treatment methods may be applied alone or with other methods. For example, exposure of the membranes to plasma can make surface more hydrophilic with antifouling properties. Plasma treatment may also be applied to activate the membrane surface for further modification such as immobilizing of fouling resistant macromolecules to the surface [32]. Moreover, anti-fouling polymers may be coated into surfaces by dipping the membrane in a solution containing the anti-fouling polymer, known as coating technique [33].

Membrane modifications explored to date have some limitations. For example, hydrophilicity of membranes increased by directly blending hydrophilic polymers additives such as polyethylene

glycol (PEG) or polyvinyl pyrrolidine (PVP) into membrane bulk [34-37]. However, these polymers are water-soluble and can be leached out from hydrophobic membranes during membrane preparation. UV and plasma treatments also can bring some disadvantages to membranes such as change the membrane structure and are difficult to control although hydrophilicity of membranes can be improved [17]. Photo-grafting method includes formation of radicals on membrane backbone; however, this technique does not have the same efficiency for all membranes materials [38]. For example, when membranes are PA, PVDF and PTFE radicals are not formed onto their surface under UV irritation. Moreover, some techniques such as plasma treatment and multi-step organic reactions may be expensive or difficult to apply in membranes [39]. Covalent grafting or cross-linking with additives has been proposed to solve the leaching out issue [40, 41]. The grafting method divide into two groups of "grafting-to" and grafting-from" methods. When polymer chains with reactive groups at the sides or ends are covalently couple to the membrane surface is known as "grafting-to" process while in "grafting-from" method uses the active sites existing on the membrane surfaces to initiate the monomer polymerization from the surface towards the outside bulk phase. "Grafting-from" technique has some advantages such as grafting chains with a high density and exact localization can be applied controllably and easily [42]. Finally, recently modification of surface via polydopamine (PDA) has been used as a developed surface modification technique. Formation of PDA is an aqueous-based method that can be applied on almost any surfaces. PDA coated surfaces become hydrophilic, but the PDA coating layer is conformal and thin; therefore, surface geometry is unaltered [43]. PDA chemistry is still unknown, thus a literature review that describes PDA chemical structure and catecholamine compounds is also included, mainly as it may relate to the improvement and development of membrane.

This review surveys the latest efforts in exploring the antifouling materials and methods to improve membrane biocompatibility in biomedical applications, as well as the current status and future prospects for antifouling membranes, including advanced antifouling polymers and advanced antifouling strategies for fabrication biocompatible membranes. It should be noted that the references provide in this review are not comprehensive but may help as a starting point to know more detailed studies. Additionally, there are numbers of excellent review papers on biomedical, fouling release coating and marine biofouling application which suggest vital guidelines on antifouling strategies, preparation methods to antifouling membranes and fouling mechanism in this review [2, 44-50]. In addition, since fouling mechanism, design and fabrication methods for materials working in biological environment is nearly the same for most antifouling materials used in aqueous environment, marine coating, heat exchangers and the like, this review paper may have great suggestions for other applications.

2.2 Oligo and Polyethylene Oxides/glycols-based Materials

Oligo ethylene oxide (OEO), polyethylene oxide (PEO), polyethylene glycol (PEG) constructs and their derivatives with many different molecular weights have been commonly studied as the most investigated/employed class of antifouling coating materials over the years [51-53]. Imparting these constructs has been commonly employed due to their low toxicity, nonimmunogenic and super low fouling ability to decrease protein adsorption and cell adhesion on a variety of surfaces [19, 54]. Moreover, PEG polymers do not harm active proteins or cells even when they interact directly with biological matters [55]. In terms of surface modification, Whiteside's group first reported that OEG and PEG based materials were effective protein resistant coating, and suppress platelet adhesion in-vivo and vitro, resulting in reduced risk of tissue damage, thrombus formation, and other cytotoxic effects [56]. The fouling resistant

property of PEG polymers is due to their hydrophilicity, unique coordination with surrounding water molecules in aqueous solution, large excluded volume, steric hindrance effects and high mobility [53, 57].

2.2.1. Grafting Method

There are various methods to modify membrane surfaces using PEG including simple physical adsorption, blending, and graft polymerization. We begin this subsection with PEGylating membranes with the work of Higuchi at el [58], who used physical method to attach PEO terminated polymer and use a Pluronic surfactant to form a more stable adsorbed layer on the polysulfone (PSU) surface [59]. The membranes were exposed to the mixed protein solution of human serum albumin (4 mg/ml, 37 ºC, 2h), human γ-globulin (1mg/ml, 37 ºC, 2h) and human fibrinogen (0.3 mg/ml, 37 ºC, 2h). There was no reduction of albumin and γ-globulin by Pluronict-coated PSU membranes in comparison to unmodified membranes; however, the adsorption of fibrinogen decreased 90% after exposure to the mixed protein solution. It has been reported that bioinert property of PEO segment in the Pluronic surfactant can considerably reduce the adsorption of plasma proteins and platelets on the coated membranes [58]. However, in physical grafting or surface coating, PEGylated polymer, can be easily washed away during application and the adsorbed polymer may increase the resistance of membranes and then flux may drop [60]. PEGylation via physical adsorption lead to an unstable surface coating and since long-term stability of PEG on the surface is needed PEGylating membranes via grafting methods can address this problem, wherein in this method monomers are covalently bonded. Grafting techniques include click chemistry [18], radiation [38], plasma- induced methods [61], and chemical agents [62] in order to graft PEG materials onto the polymeric surfaces. Ulbricht et al.[38] in 1996 for the first time photo-grafted PEG methacrylates (PEGMA) with different

molecular weights onto poly acrylonitrile (PAN) ultrafiltration membranes to study their antifouling properties [38]. The study concluded that the amount of adsorbed protein on PAN-g-PEGMA526 (MW: 526, graft polymerization: 500 µg/cm^2) after exposure to bovine serum albumin solution (pH= 4.7, 10 mg/ml) for 2 hours was estimated to be 0.2 µg/cm^2, a value slightly lower that recorded on unmodified PAN (6.6 µg/cm2) [38]. After successfully grafted PEG onto PAN, different studies grafted PEG-based chains on to membranes via UV-induced graft polymerization method and improved antifouling ability of modified surfaces [63]. However, photo/UV grafted PEG has some disadvantages including only photosensitive polymers can be used and severe degradation of the pore structure with loss of membrane function can happen during UV irradiation grafting method; therefore, photo/UV grafted PEG is not a suitable method for all type of membranes [64-66].

Another way to graft PEG onto membranes is using chemical agents to introduce chemical groups and then PEG can react covalently with reactive groups of the surface. Tipathi et al. [41] prepared antifouling membranes by covalent cross-linking of sulfonated PES with amino functionalized PEG (Figure 2.2). The PEG cross-linked membranes showed antifouling ability in comparison to the unmodified membranes. The BSA protein adsorption (1mg/ml, room temperature, 4 hr) on the membrane surfaces was about 75 µg/cm^2 whereas on PEG modified membranes was about 7.5µg/cm^2 [41]. Moreover, PEG can be grafted to microporous polyacrylonitrile-co-maleic acid hollow fiber membrane with reactive carboxyl groups through chemical grafting (esterification reaction) method. It was found that after tethering PEG (MW:400), the protein adsorption reduced from 14 mg/g to 3.2 mg/g and platelet adhesion (20 ml fresh PRP, 37 ºC for 30 min) on the membrane`s surface was obviously suppressed [67]. However, the problem of using chemical agents is that side chain reactions may occur and the

reaction is not homogenous [68]. Moreover, some changes in membrane properties such as change in glass transition temperature of membranes after using chemical agents may happen [69].

Nevertheless, these modification methods have some major challenges, including chemical grafting needs to be activated by plasma, UV, ozone or chemical agents in order to graft PEG onto the surface and for some cases it is complicated, expensive, and not applicable to diverse polymer materials with complex shapes processing [60]. Moreover, damage to the bulk properties and membrane structures under polymerization conditions can happen [66, 70]. Messersmith et al. [71] developed a facile and versatile aqueous surface modification technique using dopamine which undergoes self-polymerization in aqueous solution and create a tightly adherent polydopamine (PDA) layer to the surface. This method can be applied to virtually any solid material and PDA can serve as useful platforms for secondary reactions and surface functionalization under mild conditions [71, 72]. Li et al. [62] used PDA to graft PEG onto PES flat sheets and they found that in comparison to the unmodified and PDA modified PES membranes, PEG modified membranes adsorbed less BSA (~ 9, 5 and 4 $\mu g/cm^2$ for PES, PDA modified and PEG modified surfaces, respectively) under the same condition (1mg/ml BSA in PBS solution, 24 hr) [62].

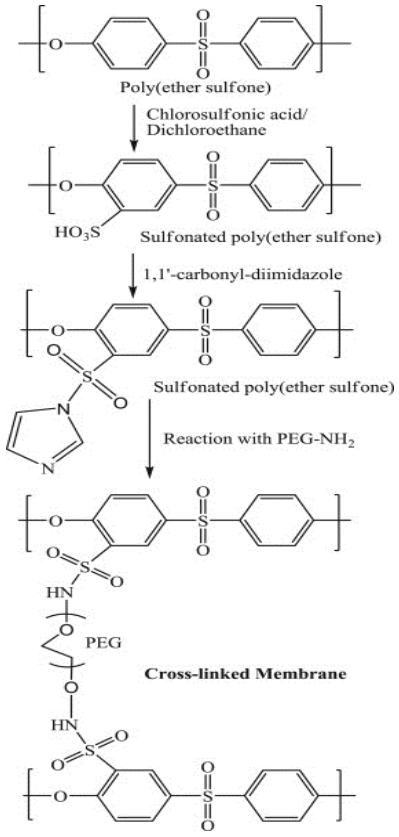

Figure 2.2. Reaction scheme covalent cross-linking of PEG on PES membranes [41]

2.2.2. Blending Method

Although grafting method can create a strong attachment of PEG onto polymeric materials it can just modify membrane surface. In the case that modification of whole membrane bulk is needed PEGylation of membranes can perform using blending method. PEG has been commonly used to blend with membranes as pore-forming additives [73-75]. However, PEG is not stable and could be easily washed away by water due to their linear structure and the incompatibility with hydrophobic membranes [76]. After Mayes et al. [77] studied the preparation of protein resistance of polyvinylidene fluoride (PVDF) surfaces using amphiphilic comb-like copolymer (polymethyl methacrylate-r-polyethylene glycol methyl ether methacrylate) as membrane additives in 1999, a great amount of research performed on amphiphilic copolymers and their applications to improve antifouling resistance of membranes [77]. The use of some amphiphilic

copolymers might prevent washing away of hydrophilic polymer problem and the topological structures of these polymers such as linear, comb-like, and hyperbranched-star play an important role in the properties of membranes [78]. Generally, amphiphilic copolymers have both hydrophilic and hydrophobic chain segments, hydrophilic side chain segments would segregate and enrich onto the membrane surface and hydrophobic chains have a good compatibility with hydrophobic membranes via surface segregation self-organization effect during the phase inversion process [76]. The surface segregation and hydration of amphiphilic copolymer (polymethacrylate-r-polyethylene glycol methyl ether methacrylate) is presented in Figure 2.3.

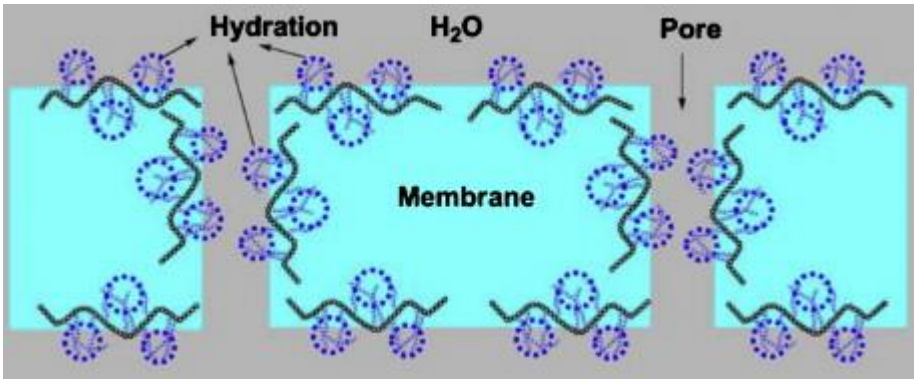

Figure 2.3. The surface segregation and hydration of (polymethacrylate-r-polyethylene glycol methyl ether methacrylate) during the PVDF membrane phase inversion [76].

A linear PEG with a molecular weight of more than 20,000 is a non-biodegradable polymer that must be eliminated by the kidney to prevent accumulation inside the body. Moreover, in order to keep the hydration and mechanical properties of copolymers, the use of high molecular weight of PEG is needed. Studies showed that star-shaped PEG has a smaller hydrodynamic radius than the corresponding linear 2-armed PEG [79]. Nagahama et al. [79] designed a biocompatible PEG-poly L-lactide block copolymers using the star-shaped PEG (8-armed PEG, Mw :10 000 and 35 000) and investigated their properties as soft, biodegrable biomaterials. Membranes were exposed to albumin (4 mg/ml, 37 ºC, 2 h), fibrinogen (3 mg/ml, 37 ºC, 2 h) and fibrinectin (0.5

mg/ml, 37 ºC, 2 h). The result showed that the 8-armed PEG3k-b-poly L-lactide 37k films suppressed protein adsorption to 0.9 μg/cm^2 (albumin), 0.4 μg/cm^2 (fibrinoctein) and 0.6 μg/cm^2 (fibrinogen) while it was 1.7 μg/cm^2 (albumin), 1.5 μg/cm^2 (fibrinoctein) and 1.35 μg/cm^2 (fibrinogen) on linear 2-armed PEG10K-b-poly L-lactide A33K [79]. Moreover, amphiphilic hyperbranched-star copolymers (hyperbranched polyester-g-methoxy PEG) with a highly branched structure and a large number of terminal functional groups have been synthesized by grafting methoxy PEG to a hydroxyl-terminated aliphatic hyperbranched polyester (HPE) (Figure 2.4) and blended with PVDF to fabricated porous membranes through a typical phase inversion route. The 3% HPE-g-MPEG b-PVDF membranes showed a decrease in BSA adsorption (when exposed to 1mg/ml BSA, 30 ºC, 24 hr with a shaking speed of 150 rpm) from 78 μg BSA/mg on PVDF membranes to 20 μg BSA/mg [80]. Additionally, the effect of MPEG arms in hyperbranched-star polymer was evaluated and various molecular masses (Mn = 350, 750 and 2000) of PEG were selected. It was found that the MPEG arms in hyperbranched-star polymer could improve hydrophilicity of membranes with increasing MPEG arm length. Membrane fouling resistance was tested using BSA as protein model (various concentrations, 30 ºC, 24 hr with a shaking speed of 150 rpm). An effective reduction in protein adsorption was achieved with the increase of the MPEG arm length (30, 15 μg BSA/mg for the membranes of PVDF/HPE-g-MPEG750 and PVDF/HPE-g-MPEG2000, respectively) while protein adsorption of PVDF membranes was 65 μg BSA/mg [81].

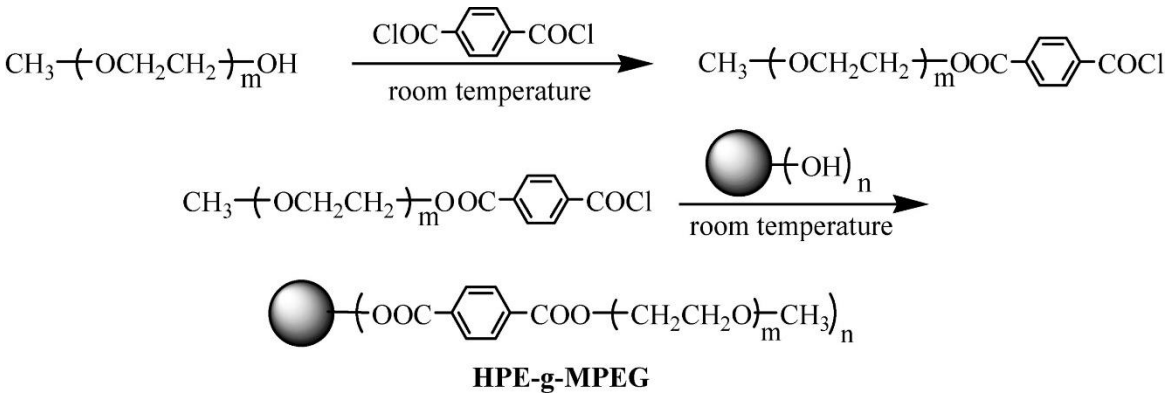

Figure 2.4. Synthesis of the amphiphilic hyperbranched-star polymer [79]

Although hyperbranched-star polymer can improve fouling resistance of membranes many studies evaluated fouling behavior of amphiphilic brush like copolymers [76]. Amphiphilic brush-like copolymer (polymethacrylate-r-PEG methyl ether methacrylate) could be synthesized by the radical polymerization method. Then blended with PVDF hollow fiber membranes by the phase inversion method. The protein adsorption decreased with increasing content of (polymethacrylate-r-PEG methyl ether methacrylate) when exposed to BSA solution (1mg/ml, 25 ºC, 24 hr) [76]. Although significant progress in blending has been made *via in-situ* modification using water insoluble copolymers it limits the possibilities of application of copolymers with other polymers. Wet-immersion using water as non-solvent is usually used to prepare an antifouling membrane and generally lead to finger like structure and presenting a skin layer less or more porous. Vapor-induced phase separation is a useful method to address this problem to form an antifouling membrane. Moreover, Tri-block copolymers with one anchor hydrophobic block and two hydrophilic blocks can probably show better antifouling resistance than di-block copolymers having only one single hydrophilic block [20]. Carretier et al. [20] formed PVDF using vapor-induced phase separation and modified with a tri-copolymer of polystyrene and PEG methacrylate moieties (PEGMA124-b-PS54-b-PEGMA124). The hydrophilic capacity of membranes was increased by 90 percent, leading to severe drop of BSA

(1mg/ml, 25 C, 2 hr), lysozyme (1mg/ml, 25 C, 2 hr) and fibrinogen adsorption, up to 85-90 percent from a 4wt% copolymer content (in the initial casting solution) [20].

2.2.3. Relation between PEG Surface Coverage and Fouling

After talking about different modification methods to have PEGylation membranes, one important challenge is verifying complete and uniform surface coverage of PEG based materials to reduce protein adsorption [53]. Many studies have been done with varying grafting conditions such as the effect of molecular weight, chain length, density of PEG based materials and various grafting methods to determine which factors and structures of PEG coatings are the most effective factors to reduce fouling [17, 53]. In 2001, Kingshott and co-workers [53] grafted methoxy-terminated aldehyde-PEG (MW 5000) and dialdehyde-PEG (MW 3400) onto two surfaces of different amine group densities using radio frequency glow discharge (r.f.g.d.) deposition of n-heptylamine (low density) or allylamine (high density). PEG coatings were exposed to a multicomponent protein solution consisting of IgG, lysozyme, lacoferrin and albumin (0.5 mg/ml, 1–1.5 h). The PEO binding was optimal at cloud-point conditions and found that optimization of PEO chain density is the key factor to have minimal protein adsorption. Moreover, if the initial functional group density was too low, longer PEG chains could improve antifouling properties and there was no need of high-density amine surfaces for longer PEG chains [53]. The antifouling performance of PEG modified surfaces improved with increasing chain length and density in the surface-grafted film [42, 82].

To evaluate the effect of molecular weight of PEO polymer on fouling performance, Hou and coworkers [17] grafted different molecular weights of PEO (Mw: 120, 350, 550) onto carboxylated cardopoly aryl ether ketone via EDC/NHS methodology. Static protein adsorption was tested using FITC-labeled BSA (1mg/ml, room temperature, 8 hr) and no protein adsorption

was observed for PEO modified membranes with molecular weight of 350 and 550. The improved biocompatibility can be attributed to the reduced electronegativity and increased hydrophilicity of carboxylated cardopoly aryl ether ketone membrane surface with higher molecular weights [17]. The relation between degrees of hydration (defined as the difference in weight between the hydrated poly PEGMA modified membranes and hydrated polytetrafluoroethylene (PTFE) membrane divided by the weight of the hydrated PTFE membrane) and antifouling behavior of modified surfaces was evaluated by Chang et al. [83]. They modified PTFE membranes by grafting poly PEGMA via surface-activated plasma treatment and following thermally induced graft copolymerization. The authors studied biocompatibility of membranes by incubation of them in a platelet rich plasma solution (1000 µL solution, 37 ºC, 2 hr), and single protein adsorption (1mg/mL fibrinogen γ globulin, albumin solutions) then the amount of adsorbed protein was estimated by ELISA. The result indicated that membranes with highest PEGMA grafting density (25-wt % PEGMA) had the lowest amount of protein adsorption (70% of γglobulin, 92% fibrinogen and 98% albumin). Moreover, the number of the adhered platelets decreased from 1.1×103 (cells/cm^2) for the unmodified membranes to no platelet adhesion on PEGMA modified membranes with any surface coverages. The same group [84] also modified PVDF membranes with PEGMA by ozone treatment and subsequent thermally induced graft copolymerization. They controlled the PEGMA grafting density on PVDF microfiltration membranes by different macromonomer concentrations in the reaction solution. The platelet adhesion tested by incubation membranes in a platelet rich plasma solution (200 µL solution, 37 ºC, 2 hr) and the relative protein adsorption of albumin, fibrinogen and γ-globulin from platelet rich plasma solution on the membranes was evaluated using ELISA (500 µL of 100% PRP solution, 37 ºC, 3 hr). The platelet adhesion was remarkably suppressed

on PEGMA grafted membranes, as the adhered platelets was about 3.3×105 cells/cm^2 for the PVDF membranes and almost no platelet adhered to the PEGMA modified PVDF membranes was observed even with a low surface coverage of PEGMA polymer on the membranes.

The effect of plasma treatment time on protein adsorption was also evaluated. The grafting density of the PEGylated layers on PTFE membranes was found to increase with plasma treatment time (0s-120 s), eventually leading to a maximum value of 0.145 mg/cm^2 and fibrinogen adsorption (1mg/ml, 37 ºC, 2 hr) was reduced by 82% at highest grafting density [28]. It was also interesting to note that the relative protein adsorption was effectively decreased with increasing amount of the PEGMA polymer chain grafted on PVDF the membrane surface. Poly PEGMA modified membranes were found to form a uniform polymer hydrogen-like layer and showed antifouling properties [84]. Therefore, the reduction of protein adsorption on the surface coverage of PEGylated membranes by varying PEG grafting amounts and while the surface grafting of PEG layer is fully covered, PEGylated membranes have good fouling resistance [83, 84].

Additionally, since surface modification and the structure of PEG polymer can affect the PEG surface grafting and membrane's antifouling ability, Chang et al.[85] used different surface modification methods, including thermal-induced radical polymerization, surface-initiated atom transfer radical polymerization (ATRP), and low pressure plasma-induced graft polymerization to control PEGMA surface grafting on PVDF membranes. They grafted two different structures of PEGMA (network and brush-like structures) layers on PVDF. Brush-like PEGMA on the PVDF surface membranes was prepared using surface-initiated thermal polymerization, and surface-initiated ATRP and the network-like PEGMA structure was prepared via plasma-induced graft-polymerization at low pressure. The surface grafting result (the grafting weight (mg/cm^2)

was defined as the difference in weight between the modified PVDF membrane and the unmodified PVDF membrane divided by the total surface area of the PVDF membrane) showed that surface modification via plasma treatment could provide high grafting efficiency at a short grafting time. Moreover, the hydration capacity (the difference in wet weight between the PEGMA grafted PVDF membrane and the unmodified PVDF membrane divided by the total surface area of the unmodified PVDF membrane) of network like structure PEGMA surface which was prepared via low pressure by plasma induced grafting copolymerization is the highest in comparison with other two methods (brush-like PEGMA). Although network-like PEGMA on PVDF surface had a highest hydration capacity, brush-like PEGMA on the PVDF membrane showed the lowest protein adsorption (decreased from 58 $\mu g/cm^2$ on unmodified PVDF to 12 and 34 $\mu g/cm^2$ on network-like and brush-like PEGMA, respectively), while membranes were incubated in BSA solution (1mg/ml, 37 C, 24 hr). The result suggesting that not only hydration capacity and hydrophilicity of membranes are important to reduce protein adsorption but also the surface grafting structure of the prepared PVDF is a key factor to reduce BSA fouling [85].

In order to evaluate the effect of grafting density in blending method, Venault et al. evaluated the effect of additive concentrations on biocompatibility of PVDF membranes [60]. They blended PVDF membranes with polyethylene oxide-polypropylene oxide-polyethylene oxide triblock copolymer as additive and prepared membranes by vapor induced phase separation. The adsorption of BSA (1mg/ml, 25 C, 2 hr), lysozyme (1mg/ml, 25 C, 2 hr), and fibrinogen (1 mg/ml, 27 C 2hr) onto PEGylated copolymers was studied as the copolymer additive content was changed. It was shown that amphiphilic additive permitted to a reduction of BSA by 65%, lysozyme by 95% and the worse ones were obtained using fibrinogen (35% reduction) for the PEGylated membrane containing 5-wt% additive. Therefore, It was shown that in blending

method similar to grafting PEG onto the surface, antifouling behavior of modified membranes improved by increasing the additive content of amphiphilic copolymers [60]. In 2015, the same group by inspiration from the first work evaluated the effect of additive concentration of polystyrene-b-PEG methacrylate (PS-b-PEGMA) on blood compatibility of PVDF membranes. A similar result was obtained, the maximum reduction (1mg/ml, 37 C, 2hr of 65% ɤ-globin, 71% serum albumin and 81 % of fibrinogen adsorption were reached using membrane containing highest additive (5wt% Ps-b-PEGMA), compared to unmodified PVDF membranes [86].

Therefore, the reports demonstrated molecular weight, chain density, chain length, modification method, and chain conformation of grafted hydrophilic PEG-based polymer on the surface are the determining factors that associated with the surface fouling behavior. Despite many studies reporting the reduction of protein adsorption on PEGylated membranes, PEG can decompose in the presence of transition metal ions and oxygen found in biologic solutions especially at elevated temperatures, or in vivo in the presence of enzymes which becomes critical in long-term operations [19]. Additionally, cleavage of PEG chain may occur even in aqueous systems and PEG grafted surfaces may lose their antifouling ability at temperature above 35 °C [87, 88]. In addition, the terminal hydroxyl group of PEGs may be oxidized to an aldehyde by alcohol dehydrogenase, then this aldehyde can reaction with proteins or other molecules with amine groups. The aldehyde undergoes further oxidized by aldehyde dehydrogenase [89]. Therefore, identifying alternatives to PEG constructs have attracted much attention, and we will discuss about the other antifouling materials in fallowing sections.

2.3 Zwitterionic Modified Membranes

PEG-based polymers may be insufficient in long-term applications, inspired by delicate structure and composition of most outer cell membrane (Figure 2.5), the zwitterionic polymers have been

recognized and as a promising alternatives antifouling material. Zwitterionic molecules have also drawn a great attention as a new generation of antifouling materials in recent years [11, 30, 90-92]. Zwitterionic monomers have both negative and positive charged on the same monomer units but are overall electrically neutral, were effective in preventing protein adhesion [93]. Zwitterionic polymers are further classified into three different major groups such as sulfobetaine (SB), phosphorycholine (PC) and carboxybetain (CB) [94]. They have the high capacity to generate a strong and stable hydration layer on the surface of membranes owing to their strong electrostatic interaction rather than hydrogen bonding with water molecules [95-97]. More studies showed that zwitterionic unites such as SB not only can bind with about 7-8 water molecules per SB unit but also can keep more mobility on the first hydration layer for unbound water molecules [98]. Therefore, SB modified membranes can result in a strong repulsive force to protein and without a significant conformation change make the protein contact with the surface in a reverse manner [99].This is the reason that why zwitterionic molecules show higher antifouling performance compared to PEG-based materials. Surface modified with zwitterionic groups shows more stability to oxidation over those based on PEG layers [100]. Chen et al. [101] reported strong antifouling property of zwitterionic PC. They used both molecular simulation and experimental methods to evaluate key factors of the protein resistance of zwitterionic materials. PC head groups shows the similar packing densities to membrane lipids favor an antiparallel orientation for the minimization of dipole.

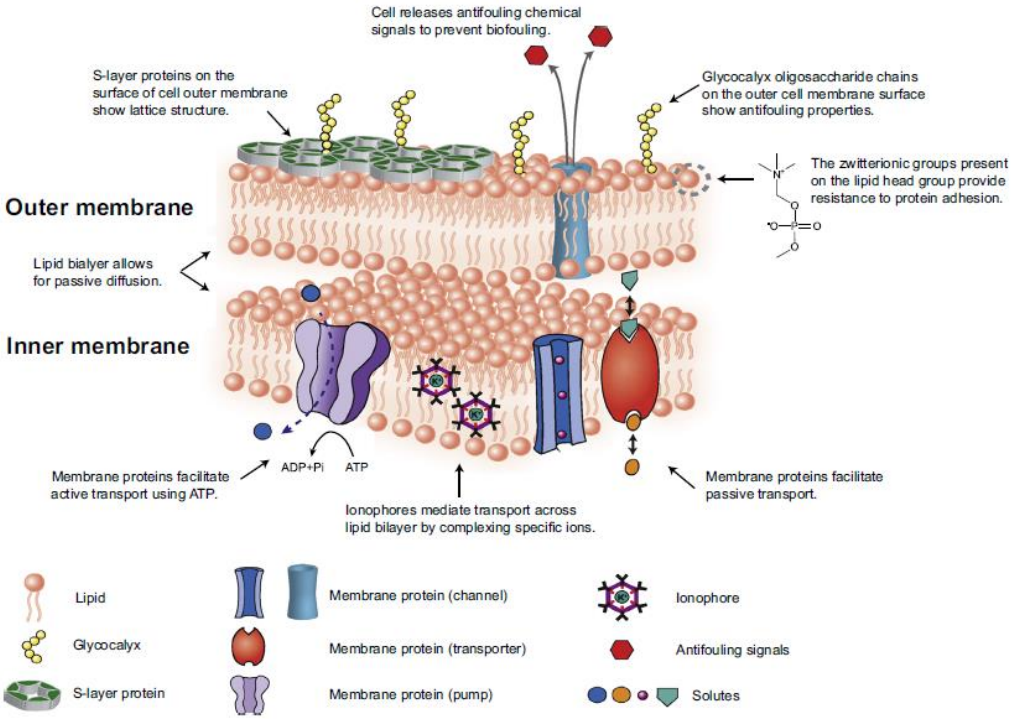

Figure 2.5. Biological membrane separation and antifouling strategies for an example of a gram-negative bacterial organism [102]

2.3.1. Grafting Method

Zwitterionic polymers are commonly introduced into membranes by a variety of strategies including blending [103, 104] and covalently grafting method (like as O_2 plasma surface grafting [105], surface initiated atom transfer radical polymerization ATRP [106-108], atmospheric plasma induced surface copolymerization [109], chemical agents [110] and surface coating (such as chemical vapor deposition [111], self-assembling [112] and biomimetic adhesion [113], etc). Zhao group introduced sulfobetaine-based material onto the surface of polypropylene non-woven fabric membranes by means of oxygen plasma pretreatment UV-induced graft technique [105]. They immobilized varied grafting amounts of zwitterionic polymer, 3-(methacryloylamino) propyl-dimethyl (3-sulfopropyl) ammonium hydroxide (MPDSAH), onto membranes. BSA was selected as a model protein and membranes were immersed into BSA solution containing 1 and 2 mg/ml BSA in PBS (37 ºC, 2h). Moreover, in order to determine potential biocompatibility of

the membranes platelet adhesion studies were carried out by exposing the membranes to platelet rich plasma (20µl of fresh PRP, 37 ºC, 1h). The amount of BSA adsorption was 12.5 µg/cm^2 and 14.8 µg/cm^2 on unmodified membranes where decreased to 2 mg/cm^2 and 2.4 mg/cm^2 on poly (MPDSAH) modified membranes with zwitterionic polymer`s highest grafting density (grafting density = 327.7 µg/cm2) in BSA of 1 mg/mL and 2 mg/mL, respectively. The amount of BSA adsorption among all different grafting densities of zwitterionic modified membranes, which had above 80% reduction compared to unmodified membranes. The platelet adhesion revealed that there are large amount of platelets aggregated and adhered on the surface of unmodified membranes, whereas poly (MPDSAH)-modified membranes possessed excellent resistance to platelet adhesion [105]. However, plasma treatment generally leads to the chemical degradation of grafted polymers due to the high energy of ion bombardment or UV radiation [57, 109]. Additionally, these methods are rather chemistry-intensive and are not easy to apply on the delicate structure of polyamide RO membranes [114]. To overcome this problem l-DOPA from its alkaline solution was used to attach zwitterionic materials onto the surface on reverse osmosis (RO) membranes to improve their organic fouling resistance. [12, 115].

To modify PVDF membrane surface with zwitterionic polymers different methods, including alkaline treatment, ozone method, plasma treatment can be used. However, low grafting yield and long modification time were required and these methods were mostly compatible on flat sheet membranes [109]. Furthermore, the alkaline treatment damages membranes and decrease its strength [116]. Zhang and coworkers grafted polySBMA on PVDF via ATRP and used as amphiphilic copolymer additive in preparation of PVDF membranes by immersion precipitation process [103]. The static fouling experiment were performed with BSA solutions in PBS (500 µg/ml, 1000 µg/ml, 1500 µg/ml and 2000 µg/ml) at 30 °C. For all membranes with increasing

BSA concentration, adsorption of BSA increased, too. Under the same protein concentration, the BSA amount adhered on the membrane surfaces decreased linearly with increasing the ratio of amphiphilic copolymer in cast polymer. For example, the BSA adsorption in 0.5 mg/ml BSA concentration was 109 μg/cm^2 on the unmodified membranes, where reduced 92 μg/cm^2, 60 μg/cm^2 and 29 μg/cm^2 for different ratio of amphiphilic polymer additive polySBMA grafted on PVDF. The adsorption trends for all other BSA concentrations was the same to that in 0.5 mg/ml. Therefore, amphiphilic polymer additive polySBMA grafted on PVDF could effectively reduce protein adsorption on the PVDF membrane surfaces [103]. Moreover, Wang et al. could successfully have grafted a high density of a zwitterionic polymer, poly(3-(methacryloylamino) propyl-dimethyl-(3-sulfopropyl) ammonium hydroxide), on the surface of PVDF hollow fiber membranes [106]. To evaluate fouling behavior of unmodified and modified PVDF membranes, the membranes were incubated in BSA and lysozyme solutions (1 mg/ml, 24 hr, 37 °C). The BSA and lysozyme adsorption of unmodified membrane was 21 and 17 μg/cm^2 respectively. In contrast after modification of surface by poly(3-(methacryloylamino) propyl-dimethyl-(3-sulfopropyl) ammonium hydroxide), protein adsorption reduced with increasing grafting amount of zwitterionic polymer and when grafting amount was around 513 μg/cm^2 , BSA and lysozyme adsorption were negligible [106].

2.3.2. Blending Method

Many literatures have been reported many successes in improving the fouling resistance of polymeric membranes using grafting methods. However, there exist some limitations in using these methods especially in industry. For example, modification using UV-treatment only photosensitive polymers can be used. The substrate materials may damage membrane structure because of its high energy at low wavelengths [65]. Moreover, grafting modification only permit

to modify the top-layers of the membrane [20] and difficult to scaleup due to the complicated process and rigorous conditions [117]. Blending method is simple but to avoid migration of hydrophilic compounds during preparation of membrane, in-situ cross-linking polymerization can be used [118]. In recent years, A novel zwitterionic glycosyl modified PES membranes were prepared using *in-situ* cross-linking polymerization (epoxy group decorated PES) coupled with phase inversion method [118]. The hydrophobic interaction between PES and protein molecules led to high BSA and fibrinogen adsorption of 5.3 and 5.8 µg/cm2, respectively. After modification of membranes the BSA adsorption amount drop to 0.6 µg/cm^2 and fibrinogen amount to 0.37 µg/cm^2 [118].

2.3.3. Relation Between Zwitterionic Materials Surface Coverage and Fouling

It is always challenging to control the surface grafting of highly polar zwitterionic polymers onto the hydrophobic and chemically inert membrane surfaces [109]. Moreover, studies demonstrated that to minimize the electrostatic interaction with plasma protein and blood cells, the charge of grafted polymer should be neutral [109]. The effect of grafting weight of zwitterionic polymer by changing the atom transfer radical polymerization (ATRP) time on PVDF membranes have been developed by Chiang et al.[119]. PVDF ultrafiltration membranes was modified through surface grafting sulfobetaine methacrylate polymer (SBMA) via ozone surface activation and ATRP onto membrane surface. They tested static fouling performance of different grafted membranes by exposing them to BSA (1mg/ml, 37º C) and ɤ-globulin (1mg/ml, 37° C). The result showed that the BSA and ɤ-globulin adsorption reduced linearly with grafting weight and the slope were almost the same. When polySBMA grafting weight was at 0.4 mg/cm^2 the BSA adsorption was at the lowest amount of 4 µg/cm^2 while unmodified was at 24 µg/cm^2. Moreover, polySBMA at 0.35 mg/cm^2 had a lowest ɤ-globulin adsorption decreased from 38 µg/cm^2 on unmodified PVDF

to 11 μg/cm^2 on polySBMA modified membranes [119]. However, this approach has a lack efficient grafting control due to low grafting yield and long modification time in order to process the surface copolymerization of zwitterionic monomers onto PVDF surfaces [109].

Another group used a new interfacial process of atmospheric plasma-induced surface copolymerization to control grafting of zwitterionic polySBMA polymer [109]. They evaluated the effect of plasma treatment time and grafting densities of polySBMA polymer on the electrical neutrality, grafting morphology, hydration capacity, hydrophilicity and blood compatibility of zwitterionic modified membranes [109]. Human fibrinogen was selected, and membranes were incubated in 500 μL of fibrinogen solution with a concentration of 1 mg/ml for 2 hr. They concluded that fibrinogen adsorption reduces with the increase in thickness of polySBMA layer. When plasma treatment time of grafted polySBMA was 90 s, the fibrinogen adsorption was at lowest amount (reduced to ~10% of that on unmodified membranes), while a plasma treatment time of 120 s showed relative less protein resistance by ~35%. It may be associated with chemical degradation of grafted zwitterionic layer on the membrane [109]. Platelet adhesion test showed the formation of thrombosis on the unmodified membranes; however, there was no platelets adhered on the polySBMA membranes surface with overall electric neutrality [109].

Yue and coworkers grafted the same polymer, polySBMA, onto PSU membranes using SI-ATRP. Firstly, chloromethylation PSU was synthesized using phase separation method then SBMA was immobilized the membrane surface via living polymerization Figure 2.6. The grafting amount increased linearly with increasing of the reaction time, and when the reaction time was about 150 min, the greatest grafting amount of 2.5 mg/cm^2 was achieved. Protein adsorption was carried out with BSA and fibrinogen solutions as model proteins. Membranes

were incubated in protein with concentration of 1mg/ml for 2hr at 37 °C. They found when the grafting amount increased, there was a small difference in protein adsorption; while a significant difference of protein adsorption has been observed after surface modification (BSA and fibrinogen adsorption dropped from 18 μg/cm^2 and 17 μg/cm^2 on unmodified to ~ 2.5 μg/cm^2 and 2.2 μg/cm^2 on poly SBMA modified membranes) [107]. Here, they found grafting amount of polySBMA zwitterionic polymer did not have a significant effect on protein adsorption while surface hydrophilicity of surfaces might be the key factor to reduce protein fouling [107].

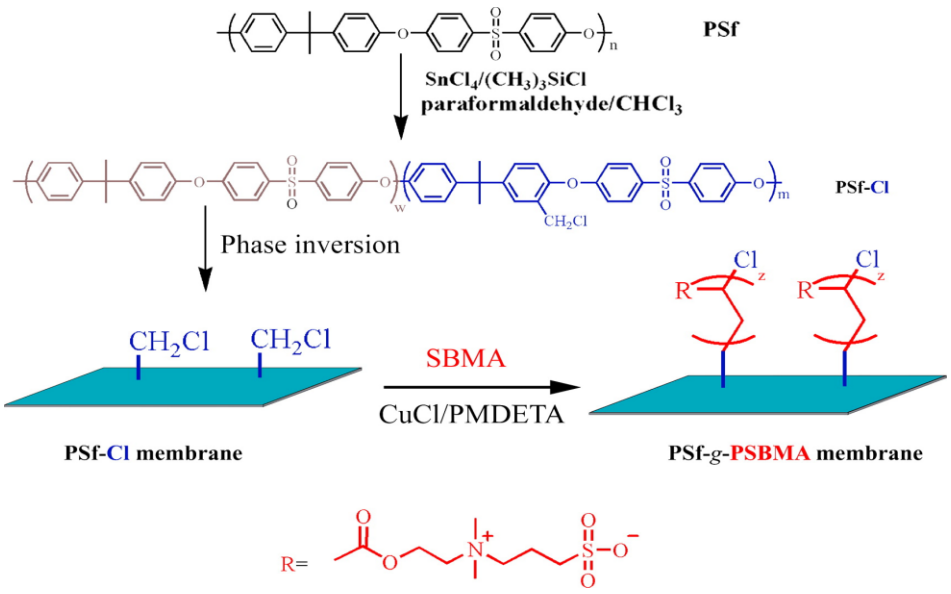

Figure 2.6. Preparation of PSBMA grafted PSU membrane [107].

In general studies exhibited fouling behavior of zwitterionic materials strongly depended on surface hydrophilicity and charge-bias of zwitterionic modified membranes [109]. Therefore, a nanometer scale homogenous, neural surface from zwitterionic groups can provide excellent hemocompatibility behavior [109].

Despite all excellent antifouling behavior of zwitterionic materials, there is a fatal limit for the application of zwitterionic polymer to modify polymeric membranes since the super ion

hydration capacity of zwitterionic polymer make it insoluble in the organic solvent which is needed to prepare polymeric membranes. Furthermore, harsh precursor, multistep process and harsh reaction condition to synthesize and modify polymeric membranes cause the reported applications of zwitterionic materials in membranes field [120]. Therefore, large scale preparation of zwitterionic antifouling membranes is yet a great challenge [120]

2.4 Other Surface-Grafted Polymers

Polymers other than PEG and zwitterionic molecules have been explored for biomedical applications. Regarding blood compatible materials, heparin seems to be one of the effective way to improve biocompatibility of surfaces and number of ways to surface immobilization of heparin have been studied [121]. Heparin is a mixture of linear anionic polysaccharide having 2-acetamido-2-deoxy-α-D-glucose, β-D-glucuronic acid, 2-deoxy-2-sulfamino-6-O-sulfo-α-D-glucose, 2-Osulfo-α-L-iduronic acid, and α-L-iduronic acid as major saccharide units can be counted as an efficient and confessed agent in curtailing thrombosis [121-123]. Furthermore, heparin is a hydrophilic polymer with a number of chemically reactive functional groups [123]. Heparin immobilized surfaces show decreased loss of blood cells, increased plasma decalcification time, decreased platelet adhesion and increased activated partial thromboplastin time, lead to improve biocompatibility without compromising thrombo-resistant capabilities [123]. Heparinized surfaces through antithrombin III mediated pathway also prevents the initial contact activation coagulation enzymes and show anticoagulant properties [123]. Therefore, incorporation of heparin is regarded as an most popular technique for preventing the thrombogenicity of materials and heparin modified surfaces have anticoagulant properties that prolong blood clotting time [109, 122].

Marconi et al. [124] covalently grafted heparin (0.1 and 1% heparin concentration in the reaction) onto an ethylene-vinyl alcohol copolymer (3:7 molar ethylene –vinylalcohol ratio). Afterward, the total amount of heparin onto the surface was calculated. The anticoagulant activity test was evaluated using measuring activated partial thromboplastin time following contact with plasma and a correlation between activated partial thromboplastin time and the heparin content was observed [124]. Kang et al. [125] heparinized a polyurethanes through plasma glow discharge method. Afterwards either an amino or a carboxyl group was introducing to the surface for the linking of heparin to the surface. The amount of heparin grafted by the amino groups was higher than that by carboxyl group. However, the stability of heparin-immobilized surfaces was found not sufficient for biomedical applications [125].

Immobilization of heparin onto dense polyurethanes and ethylene-vinyl alcohol copolymer membrane films has been investigated by Kang [125] and coworkers and Marconi et al [126], respectively. However, the heparin-grafting yield was at a relatively low level because these films were nonporous [127].

To increase the grafting of heparin, Lin et al. [127] used porous PVDF membranes with very different surface porosity to evaluate their anticoagulation capabilities with respect to platelet rich plasma. They grafted heparin onto surface by introduction of PAA as an inter-linkage between PVDF and heparin, in order to graft PAA on PVDF membrane, plasma induced polymerization was used [127]. They could reach the highest grafting yield of heparin (0.68 mg/cm^2). Blood compatibility was tested via platelet adhesion test. Membranes immersed in human PRP where membranes immersed in PRP for 60 minutes and heparin modified membranes could inhibit platelet adhesion on membranes. Moreover, they found the grafting yield of heparin increased as the following preparation parameters increased [127]. Although

heparin modified membrane can prevent platelet adhesion, it does not decrease protein adsorption [128] and continuous exposure may cause some problems for patients, including significant risk of catastrophic bleeding, responsible for significant patient mortality [129].

2.5 Conclusions and Perspectives

Many kinds of antifouling polymers have been developed for medical devices, including zwitterionic materials, PEG/OEG-based materials, heparin polymers and etc. although the outlook for using antifouling polymers is positive, much efforts is still needed. Moreover, as modification of membranes can be quite complex based on the type of membrane and application, no single antifouling material is universally suitable for all membranes. In the future, the following aspects should be studied in order to do the research on modification of membranes in biomedical area using antifouling polymers. At first, more studies should be focused to explore the advantages of mixing different types of antifouling materials. We believe numerous research efforts exist to develop new antifouling polymers based on peptoids. Moreover, the development of more stable antifouling polymers with better attachment onto the surface should be explored since stability of antifouling polymers in medical devices is crucial. Furthermore, more efforts should be done on an easy and cheap grafting/surface anchoring strategy to have more uniform grafting density of antifouling polymers on the surface of membranes.

Table 2.1. Some polymeric materials commonly employed for biomedical application

Polymer	**Membrane Application**	**Ref**
PAN (Polyacrylonitrile)	Ultrafiltration membranes, dialysis, enzyme-immobilization, pervaporation, water/wastewater treatment, support the attachment of hepatocytes in an artificial liver support system [67], reverse osmosis pretreatment [130] biopharmaceutical recovery and food and dairy processing, bioartificial organs [131].	[38, 130-133]
PES (Polyethersulfone)	Ultrafiltration, , protein separation and purification [134], water purification technologies, downstream processing in biotechnology [135]	[37, 41, 62, 134-141]
PET (polyethylene terephthalate	Blood vessel, [142], polymeric matrixes and supports for the immobilization of cells and biomolecules [143], packaging material for drinking water [144], packaging for food, decorative coatings, capacitors and magnetic tape [145].	[142, 144-146]
PLLA (Poly L-lactide)	Used as an implantable material including tissue treatments such as bone plates, rods, and screws [79]	[79]
PSU (Polysulfone)	Ultrafiltration, water treatment, food processing, and biotechnology [147], a supporting layer for pervaporation membranes [148], water/wastewater treatment and water reclamation [14] hemodialysis, apheresis [40], reverse osmosis pretreatment, separations process [130], bioartificial organs [131], dialyzer [149]	[40, 58, 150-155]
PP (Polypropylene)	Microfiltration, blood oxygenators [91], wastewater treatment, separation process, hemodialysis, plasmapheresis, leukodepletion process [156], medical materials and medical packaging [157]	[18, 105, 157, 158]
PTFE (Polytetrafluoroethylene)	Zeparation processes [159], membrane distillation processes, wastewater treatment applications[28]	[28, 83]
PVDF (Polyvinylidene fluoride)	Microfiltration, ultrafiltration, nanofiltration (NF)[84], membrane bioreactor, membrane, distillation, gas separation, water purification, separator for lithium ion battery recovery of biofuels, ion exchange process [160], aqueous solution separation [161]	[20, 21, 57, 60, 76, 80, 81, 85, 86, 109, 119, 161-163]

Table 2.2. Comparison of various antifouling materials presented in this paper

Antifouling Material	Advantage	Disadvantages	Ref
PEG	low toxicity, nonimmunogenic electrically neutral no antigenicity	Can decompose in the presence of transition metal ions and oxygen [19]	[38]
Zwitterionic		Zwitterionic polymer can be dissolved in water, but the super ion hydration capacity makes it insoluble in the organic solvent used for the preparation of polymeric membranes and harsh reactions and multistep process are needed to graft them zwitterionic materials onto surfaces. are needed[120, 164, 165]	[105, 109, 119]
Heparin	Hydrophilic, antigualent polymer [128].	It does not reduce protein adsorption [128] and continuous exposure places patients at significant risk of catastrophic bleeding, responsible for significant patient mortality [129].	[124-126]

Table 2.3. Comparison of various modification methods together

Modification Method	Advantage	Disadvantages	Ref
UV/ozone treatment	Easy, fast, and low-cost, increasing the surface hydrophilicity [29]	Only photosensitive polymers can be used. The substrate materials. may lead to the damage of membrane substrates because of its high energy at low wavelengths [65]. only permit to modify the top-layers of the membrane [20]. difficult to scaleup due to the complicated process and rigorous conditions [117].	[29]
Radio frequency glow discharge	Produce thin uniform coatings with a range of densities, strong adhesion of r.f.g.d. deposited polymeric coatings on a variety of substrates [53]	Only permit to modify the top-layers of the membrane. [20]. Process is usually complicated and time-consuming[57], and needs extensive use of organic solvents and monomers [57]	[53]
Surface coating	Simple and cheap [87]	Unstable and might be easily eroded during the operation process [57, 76]. only permit to modify the top-layers of the membrane.[20]. Poor reliability and durability of modified surface [57].	
Surface grafting using Plasma treatment	Clean and pollution-free [151]	Needs an extra step to modify the surface chemistry of the membrane, not suitable for an industrial scale production and high cost [76, 86]. Only permit to modify the top-layers of the membrane.[20]. time dependency of the induced changes [136]. results in the chemical degradation of grafted polymer [57]	[83, 84, 109, 151]
Surface-initiated atom transfer radical polymerization	Graft density, chain length, and chemical composition can be controlled [107]	Usually required pre-treatment of the surface to attach suitable initiator moieties and the polymerization step needs to be carried out under an inert atmosphere, making this method unpractical for large and intricate shapes [166].	
Blending	Single-step method [76], simple and effective to maintain surface and pore structure [118, 167]	Difficulty to find a common solvent for the polymer and the polymer additive leading to an homogeneous blend [28]. Deterioration in membrane mechanical properties [117].	[20, 28, 60, 76, 86, 162]
Polydopamine	Universally applicable surface grafting method, easy, low cost. [65]	May drop membrane permeability	[62, 65]

Table 2.4. Reduction of fouling by modification of surface by OEG/PEO/PEG constructs

Substrate	**Antifouling polymer**	**Reduction in adsorbed proteins (%)**			**Platelet adhesion**	**Surface Properties**		**Ref**
		BSA	Fbg	Lys		Contact Angle	Ra (nm)	
PAN	PEGMA	97			No	Un:50 Mo:40		[38]
PES	PEG	90			No	Un: 88 Mo:52		[41]
PES	PEG	55			No	Un:44Mo: 25	Un:6 Mo:19	[62]
PSU	(PEO)–polypropylene oxide (PPO)–PEO	90			Yes	Un:40 Mo:21		[58]
Poly (an-co-maleic acid)	PEGs (various kD)	77			Yes	Un:67 Mo:33		[67]
poly(L-lactide)	8-armed PEG3k-b-poly(L-lactide)37k	47	70		No	Un:70 Mo:50		[79]
PVDF	hyperbranched-star PEG in casting solution	76			No	Un:90 Mo:40	17	[81]
PVDF	PEG	74			No	Un:90 Mo:49		[80]
PVDF	30wt% PEGMA	89	92		Yes	Un:120 Mo:60	Un:163 Mo:21	[84]
PTFE	20wt% PEGMA	92	98		Yes	Un:110 Mo:58		[83]
PVDF	PEGMA	79			No	Un:80 Mo:50	Un:39.4 Mo:96.1	[85]
PVDF	polyethylene oxide–polypropylene	65	23	95	No	Un:132 Mo :41	Un:60 Mo:65	[60]
PES	PEG (nucleophilic addition method)	93	12		Yes	Un:44 Mo:30		[168]
Polytetrafluoroethylene	PEG (atmospheric plasma-induced)		82		No	Un:105 Mo: 9	Un:248 Mo:319	[28]
PVDF	polystyrene-b-polyethyleneglycol methacrylate	72	82		Yes		Un:143 Mo:139	[86]
PVDF	4wt% PEGMA124-b-PS54-b-PEGMA124	90	85	90	No	Un:126 Mo:109	Un:282 Mo:273	[20]

Table 2.5. Reduction of fouling by modification of surface by zwitterionic materials

Substrate	**Antifouling materials**	**Reduction in adsorbed proteins %**			**Platelet adhesion**	**Contact Angle**	**Ref**
		BSA	Fbg	Lys			
PVDF	grafted zwitterionic sulfobetaine methacrylate via ozone surface activation and ATRP	83			No	Un 82 Mo: 52	[119]
Polypropylene non-woven fabric	zwitterionic polymer, [3-(methacryloylamino) propyl]-dimethyl (3-sulfopropyl) ammonium hydroxide	82			Yes	Un:120 Mo:30	[105]
PVDF	surface-grafted with the zwitterionic PSBMA via atmospheric plasma-induced surface copolymerization		70		Yes	Un:103 Mo:28	[109]
PVDF	poly(3-(methacryloylamino) propyl-dimethyl-(3-sulfopropyl) ammonium hydroxide) via a two-step polymerization	100		100	No	Un: 87.5 Mo:22.1	[106]
Composite polyamide	incorporation of redox functional amino acid 3-(3,4-dihydroxyphenyl)-lalanine (l-DOPA)	50			No	Un: 55 Mo:20	[115]
Polyethylene terephthalate	zwitterionic cysteine immobolized using polydopamine	50			Yes	Un: 70 Mo: 15	[12]
PSU	Poly (sulfobetaine methacrylate) was grafted *via* Surface-initiated atom transfer radical polymerization	86	85			Un:78 Mo:25	[107]
PES	zwitterionic glycosyl vi in-situ crosslinking	88	93			Un:71 Mo:42	[118]

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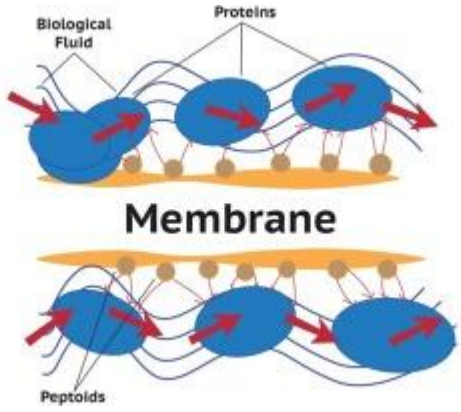
3. Chapter 3. PEG-Mimetic Peptoid Reduces Protein Fouling of Polysulfone Hollow Fibers

Abstract

Biofouling is a persistent problem for membranes exposed to blood or other complex biological fluids, affecting surface structure and hindering performance. In this study, a peptoid with 2-methoxyethyl (NMEG5) side chains was immobilized on polysulfone hollow fiber membranes to prevent protein fouling. The successful attachment of NMEG5 to the polysulfone surface was confirmed by X-ray photoelectron spectroscopy and an increase in hydrophilicity was confirmed by contact angle analysis. The NMEG5-modified surface was found to resist fouling with bovine serum albumin, fibrinogen, and lysozyme. The NMEG5 coated membranes adsorbed significantly less fibrinogen as compared with other published low-fouling surfaces. Due to the low fouling nature and increased biocompatibility of the NMEG5 coated membranes, they have potential applicability in numerous biomedical applications including artificial lungs and hemodialysis.



3.1. Introduction

Membranes are widely used in medical devices including oxygenators, cardiovascular implants, hemodialysis, and diagnostic devices [1, 2]. Polysulfone (PSU) is one of the most common

polymers for biomedical membrane applications due to its high chemical, physical, and thermal stability, as well as high porosity [3-5]. However, proteins and other materials adsorb to the PSU membrane surface and within its pores, referred to as membrane fouling or biofouling [1-4]. This results in coagulation at the surface that leads to a decrease in flux across the membrane, substantial energy consumption, and a significant increase in operational cost [1, 6]. The biocompatibility of PSU membranes must be improved to be more viable for use in biomedical devices [1, 5].

Membrane fouling occurs due to hydrogen bonding, hydrophobic, electrostatic and van der Waals interactions between the membrane surface and biological foulants [1, 7] and is driven by the interaction of foulants with the surface which are largely affected by surface properties including wettability, surface free energy, surface charge, and roughness [8-11]. Research suggests that effective non-fouling surfaces should be (i) hydrophilic, (ii) electrically neutral, (iii) free of hydrogen bond donors, and (iv) contain hydrogen bond acceptors [12, 13]. Hydrophilicity, or wettability, of the surface affects protein adsorption, electrically neutral surfaces minimize electrostatic interactions, and elimination of hydrogen bond donors minimizes hydrogen bonding [14]. Therefore, a hydrophilic and electrically neutral surface with the absence of hydrogen bond donor groups is preferred for ultra-low fouling applications.

One approach to improve the biocompatibility and reduce fouling of PSU membranes is to alter the surface properties to decrease hydrophobicity [3, 15]. This has previously been achieved by surface immobilization of self-assembled monolayers and antifouling polymers [16, 17] including poly-ethylene-glycol (PEG), oligo-ethylene-glycol (OEG), and their derivatives [18, 19]. Messersmith and co-workers used 3,4-dihydroxyphenylalanine (DOPA) to attach PEG to TiO2 substrate. These PEG modified surfaces were found to decrease cell adhesion by 98%

compared to control surfaces up to two weeks [19]. However, PEG and OEG are susceptible to oxidative degradation in vivo that limits long-term use in physiological environments [20-24]. Alternatives to PEG include carbohydrate derivatives [25], poly(2-methyl-2-oxazoline) [26], zwitterionic polymers [27], glycomimetics [28], and poly-N-subsituted glycines (peptoids) [13, 22, 29, 30]. Each of these coatings exhibit antifouling properties and have different advantages that can be leveraged for various applications. Here we have chosen to use peptoid sequences demonstrated to have long-term antifouling properties in biological environments [22, 29].

Peptoids are a class of biomimetic polymers that have a protein-like backbone with the side chains attached to the amide nitrogen rather than the α-carbon [31]. Furthermore, peptoids lack hydrogen bond donors in the backbone, unlike their peptide counterparts [32]. These changes to the backbone structure allow peptoids to resist protease degradation and ultimately have increased biostability as compared to peptides [33]. Peptoids are synthesized in a sequence-specific manner following a submonomer protocol that allows for the addition of a diverse variety of side chain chemistries [34]. These characteristics combined show that peptoid-coated membranes have promise for use in biomedical applications.

Peptoids containing the PEG-mimetic side chain, NMEG, have been shown to resist fouling [13] and are promising for use in vivo due to low immunogenicity and protease resistance [29]. NMEG is polar, uncharged, hydrophilic, has no hydrogen bond donors, and contains hydrogen-bond acceptors. Statz et al. studied peptide-peptoid hybrids composed of PEG-like side chains (NMEG) and a mussel adhesive-inspired DOPA-Lys peptide. The peptide-peptoid hybrids anchor to TiO2 surfaces (via DOPA-Lys) and prevent cell and protein adhesion [13]. This research was extended to study three different peptoid side chains (2-methoxyethyl (NMEG), 2-hydroxyethyl, and 2-hydroxypropyl) [29]. The peptoid-modified TiO_2 surfaces resisted

adsorption of proteins including fibrinogen, lysozyme, and serum proteins. However, NMEG-coated surfaces exhibited improved long-term fouling resistance during *in vitro* cell attachment studies for up to six weeks. The decrease in protein adsorption onto NMEG-coated surfaces with time is likely due to the absence of hydroxyl functional groups, which are present in both of the other side chains. Studies of self-assembled monolayers showed that presence of hydrogen bond donors in the hydroxyl group increases the adsorption of protein [29, 35]. It was also shown that the length of the NMEG (n = 10 to 50, for a coating thickness ranging from 2.8 to 4.2 nm) had statistically no effect on protein fouling [30]. In 2011, Liu and Jia introduced new peptoid side chains (N-ethyl-β-alanine and N-methyl-β-alanine) and grafted the poly(β-peptoid)s to gold surfaces via terminal thiol groups. Fouling was evaluated by surface plasmon resonance over 10 minutes with single proteins (fibrinogen, bovine serum albumin, and lysozyme). The data showed that while the poly(β-peptoid) coatings have good protein resistance, oxidation of the thiol groups to form sulfonate groups causes the adhesion to gold to weaken with time [22, 36]. Therefore, thiol terminated polymers are not suitable materials to resist fouling for long-term use [36].

In this study, PSU hollow fiber membranes were coated with an NMEG peptoid to decrease protein fouling and improve transport properties in biomedical applications. Attachment of peptoids to the fibers was confirmed by X-ray photoelectron spectroscopy and surface hydrophilicity was evaluated by contact angle analysis. Protein adsorption to the unmodified and modified fibers was evaluated by UV absorbance at λ=280 nm. To our knowledge, this is the first time low fouling peptoids have been used to improve the biocompatibility of hollow fiber membranes.

3.2. Materials and Methods

3.2.1. Materials

Piperidine, bovine serum albumin, lysozyme from chicken egg-white, fibrinogen from human plasma, and 3,4-dihydroxyphenethylamine (dopamine) hydrochloride were purchased from Sigma-Aldrich (Milwaukee, WI). PSU pellets (average MW ~35,000) were obtained from Nanostone (Oceanside, CA). MBHA rink amide resin was purchased from NovaBiochem (Gibbstown, NJ). Epoxy Epon Resin 828 and Epikure glue 3030 were purchased from Hexian (Houston, TX). All other reagents were purchased from VWR and used without further modification, unless otherwise indicated. Ultrapure water used for experiments was purified with a minimum resistivity of 18.2 MΩ cm, using a NANOpure Diamond^{TM} Life Time system (Barnstead/Thermo scientific, Essex, United Kingdom).

3.2.2. Peptoid Synthesis and Purification

A 5-mer NMEG peptoid (NMEG5; Figure 3.1) was synthesized via a submonomer protocol on rink amide resin as previously described [37]. NMEG5 is polar, uncharged, hydrophilic, has no hydrogen bond donors, and contains hydrogen-bond acceptors. In addition, it has a flexible backbone and high water solubility, which help to reduce fouling [12, 13, 35]. Briefly, rink amide resin was swelled with dimethylformamide (DMF) and the Fmoc protecting group was removed by incubation in 20% piperdine in DMF. The backbone secondary amine was acylated by adding 1 M bromoacetic acid in DMF. Side chains were appended by incubation with 0.5 M amine in N-methylpyrrilidone (NMP) for 20 minutes. The peptoid was cleaved from the resin by bathing in a mixture of 95% trifluoroacetic acid (TFA), 2.5% triisoproylsilane, and 2.5% water for ten minutes. The acid was removed using a Heidolph Laborota 4001 rotating evaporator (Elk

Grove Village, IL) and the peptoid was diluted in a 25:75 solution of acetonitrile: water to a final concentration of ~3 mg/ml.

The peptoid was purified by preparative high-performance liquid chromatography (HPLC; Waters Delta 600, Milford, MA) with a Duragel G C18 150 × 20 mm column (Peeke Scientific, Novato, CA). Gradients were run at ~1% per minute using solvent B in A (solvent A: water, 0.1% TFA; solvent B: acetonitrile, 5% water, 0.1% TFA) at room temperature. Peptoid purity was confirmed to be >98% by analytical HPLC (Waters Alliance) with a Duragel G C18 150 × 2.1 mm column (Peeke Scientific) using a linear gradient of 5 to 95% solvent D in C (solvent D: acetonitrile, 0.1% TFA; solvent C: water, 0.1% TFA) over 30 minutes. The molecular weight of the peptoid was confirmed using MALDI-TOF mass spectrometry (Arkansas Statewide Mass Spectrometry Facility) and compared to the desired value calculated with ChemSketch (ACD/Labs, Toronto, ON). Purified peptoid solutions were dried to powder using a Labconco lyophilizer (Kansas City, MO) and stored at −20°C.

3.2.3. Preparation of PSU Porous Hollow Fibers

PSU hollow fiber membranes were fabricated using a conventional hollow fiber membrane-spinning device. Nitrogen gas pressurized a spinneret with 0.8 mm inner and 1.6 mm outer diameters to push dope and bore solutions. The dope solution was 17.8% (v/v) of PSU in NMP and the bore solution was 15% (v/v) NMP in water. The solutions were extruded through the spinneret into the water bath at 23°C and phase inversion occurred to form the hollow fiber membranes. The air gap between the water and the spinneret was set to 8 cm. The fibers were pulled under dowels, immersed in the water, and rolled onto a draw wheel at an uptake speed of 2 m/min. The PSU fibers were first stored in DI water for 3 days with daily water changes to wash away extra solvent. The fibers were then stored at 5ºC in 0.25% (v/v) sodium benzoate.

3.2.4. Surface Modification of PSU Hollow Fiber Membrane

NMEG5 was attached to PSU fibers via polydopamine (PDA), which undergoes oxidation and contains an equilibrium of quinone and catechol groups [38]. Catechol groups are reactive toward nucleophiles, such as the peptoid amine terminus. This reactivity was leveraged to covalently attach the peptoid to PSU fibers. A schematic for hollow fiber coating with PDA and peptoid is shown in Figure 3.1. Dopamine hydrochloride was dissolved in Tris-HCl (10 mM, pH 8.5) at 0.5 mg/ml to prepare the PDA solution. The fresh PDA solution was shaken at room temperature in continuous contact with atmospheric oxygen to prevent the formation of large PDA aggregates [39]. PSU hollow fibers were soaked in ethanol for 30 minutes and washed with ultrapure water. Hollow fibers were shaken vigorously in fresh PDA solution at room temperature for 1 to 24 hours, washed with ultrapure water to remove unattached PDA, and dried with nitrogen gas.

The PDA-modified hollow fibers (PSU-PDA) were incubated with 0.5 mg/ml peptoid in phosphate buffered saline (PBS, pH 7.4) at 60ºC for 1 to 24 hours. The peptoid-modified hollow fibers (PSU-PDA-NMEG5) were washed with ultrapure water to remove unreacted peptoid and dried with nitrogen gas before storage.

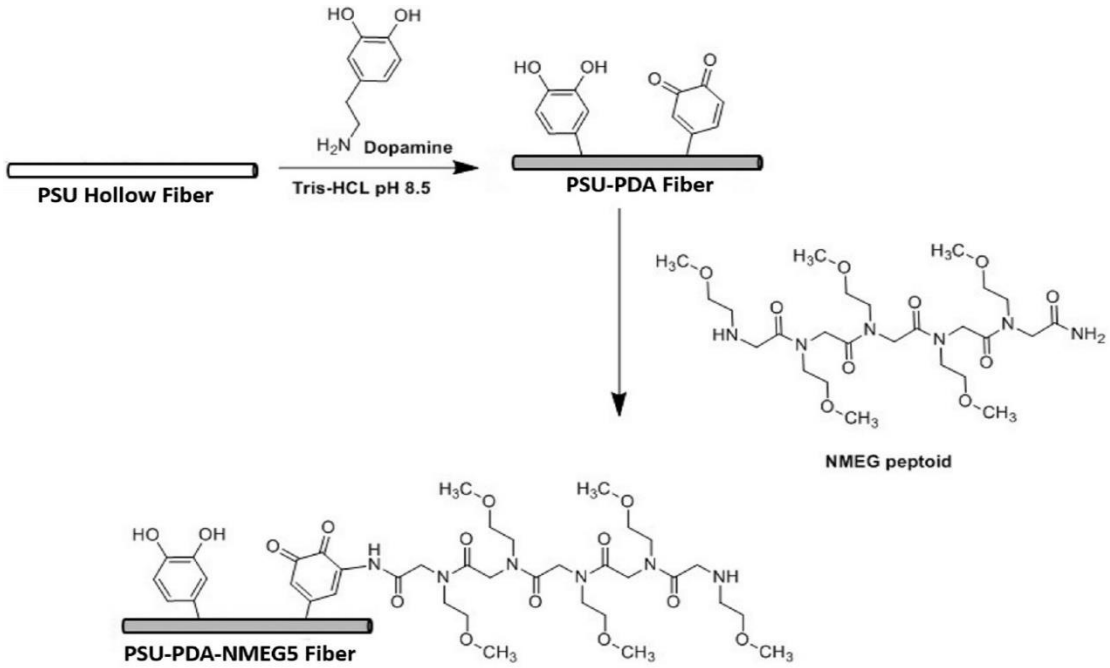


Figure 3.1.Schematic of hollow fiber coating process. PSU hollow fibers are immersed in dopamine (0.5 mg/ml in TRIS-HCl, pH. 8.5) for 3 hours at room temperature. PSU-PDA fibers are immersed in NMEG5 (0.5 mg/mL in PBS) for 24 hours at T=60ºC

3.2.5. X-Ray Photoelectron Spectroscopy (XPS) Measurements

XPS (PHI 5000 VersaProbe, ULVAC-PHI, Kanagawa, Japan) was used to confirm modification of the fiber surface. Prior to XPS measurement, the fibers were washed with methanol and ultrapure water then dried under nitrogen gas. Initial survey scans (0-1000 eV binding energy, 45º) were followed by detailed scans for oxygen (527-541 eV). The elemental composition from the peak areas was calculated using PHI MultiPack data analysis software.

3.2.6. Water contact angle measurements

The surface hydrophilicity of the fibers was measured by water contact angle (OCA 15, DataPhysics Instruments GmbH, Filderstadt, Germany). Static contact angles were determined using the sessile drop method, as previously described [36]. Briefly, a 1 µL deionized water drop was formed at the tip of a needle and lowered to the fiber surface. The contact angle was calculated using DataPhysics SCA software. Contact angles were measured 10 times across the

fiber surface. All water contact measurements were performed at ambient laboratory conditions (25ºC and 50% relative humidity).

3.2.7. Pore Size Measurements

Pore diameter and pore distribution were measured by evapoporometry, as previously described [40]. The evapoporometry method, developed by Krantz et al. [41], relates pore diameter to evaporation rate of isopropyl alcohol using the Kelvin equation [40]. The equation describing pore radius (r) as a function of instantaneous evaporation rate can be derived from $r = -\frac{2\sigma V}{RT\cos\theta \ln\left(\frac{W'}{W^{\circ}}\right)}$, where σ, V, R, T, Θ, W´, and W° are surface tension, liquid molar volume, gas constant, absolute temperature, contact angle, instantaneous evaporation rate and normal evaporation rate of the free standing liquid layer found before the volatile liquid begins evaporating from the membrane, respectively [41].

Fibers were glued onto a plexiglass sample chamber with 2:1 epoxy Epon Resin 828 and Epikure glue 3030. The fibers were soaked in isopropyl alcohol for 2 hours to ensure saturation of the fibers. Isopropyl alcohol was added to completely fill the chamber and placed on a microbalance (Mettler Toledo AB104-S/FACT, Columbus, OH). The change in mass per time was measured as the isopropyl alcohol evaporated. The program logged the mass every 30 seconds until the isopropyl alcohol completely evaporated.

3.2.8. Protein Adsorption Assay on the Membranes

Bovine serum albumin, lysozyme, and fibrinogen were selected as model proteins to assess protein adsorption on hollow fibers based on previous studies [22, 29, 30]. Fibers were cut to 2 cm length and 15 fibers were immersed in a 1 mg/ml protein solution for 24 hours at 37ºC. Samples were taken at 1, 2, 3, 5, 12, and 24 hours, and between each incubation time the fibers

were washed with PBS to remove any unattached proteins from fibers, and the fibers were dried with nitrogen gas. Additionally, a control experiment with a vial that contained 1 mg/mL protein solution with no fibers was run with the same conditions.

The concentration of protein in solution was measured by UV absorbance at λ=280 nm (NanoDrop 1000 Spectrophotometer, Thermo Fisher Scientific, Wilmington, DE). The amount of adsorbed protein on the fibers was calculated by subtracting the concentration of protein after incubation with fibers from the protein concentration in the control vial. The total protein adsorbed was divided by the linear surface area of fibers to give the reported data.

3.3. Results

3.3.1. XPS Spectral Analysis

Attachment of PDA and NMEG5 to the PSU fibers was confirmed by XPS (Table 3.1). XPS survey scan and oxygen core-level spectra show that unmodified PSU fibers have 2.1% sulfur, 1.2% nitrogen, and 0% C-O-C bonds, consistent with the chemical structure of PSU. The sulfur content decreased to 1.4% and the nitrogen content increased to 3.9% following incubation with PDA, consistent with the addition of PDA to the surface. The addition of PDA to the surface masks the sulfur groups in PSU, as well as adding nitrogen groups to the surface from the PDA backbone. After incubation with NMEG5 the sulfur content was further reduced to 0.2%, the nitrogen content was further increased to 8.4%, and the C-O-C bond content was increased to 11.6%. These results are consistent with NMEG5 attaching to the PDA surface further masking the sulfur groups in PSU, adding additional nitrogen groups in the backbone, and introducing side chains that contain C-O-C bonds. These results confirm that PDA and NMEG5 were successfully attached to the PSU fiber surface.

Table 3.1 .XPS data providing surface elemental composition of PSU, PSU-PDA, and PSU-PDA-NMEG5 fibers.

Sample	Elemental percentage (atom %)						
	C=O	C-OH	C-O-C	C1s	N1s	O1s	S2p
PSU	85.5	14.5	0	73.6	1.2	23.1	2.1
PDA	97.5	2.5	0	70.1	3.9	24.6	1.4
NMEG5	88	0.4	11.6	64.6	8.4	26.8	0.2

3.3.2. Contact Angle Measurements

Protein adsorption is reduced on hydrophilic surfaces due to the interaction of water molecules that forms strong repulsive hydration forces [42-44]. The hydrophilicity of modified and unmodified fibers was evaluated by measuring static water contact angle (Figure 3.2). The coating times for PDA and NMEG5 were varied from 1 to 24 hours to determine the optimal coating time for each to increase hydrophilicity.

Unmodified PSU fibers were found to have a water contact angle of 98° [45]. Incubation with PDA resulted in a rapid decrease in contact angle to 75° over 3 hours and a slow decrease to 58° over the next 21 hours. This is consistent with previous results [46, 47] and is likely due to the addition of hydroxyl, carboxylic acid, and amine groups to the surface [48-50]. These results indicate that PDA formed a near complete monolayer on the surface after 3 hours [15, 48, 51], therefore all future studies were performed with PSU-PDA fibers that were coated for 3 hours. Incubation of the PSU-PDA fibers (initial contact angle of 75°) with NMEG5 resulted in a steady

decrease in contact angle to 30° over 12 hours and a minimal decrease to 25° over the next 12 hours. This is expected due to the hydrophilic nature of the NMEG side chain [29, 52]. These

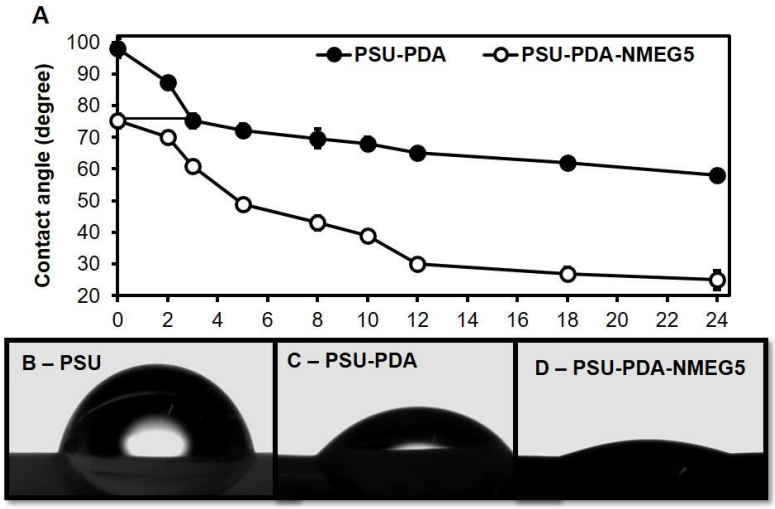


Figure 3.2. (A) Contact angle measurements as a function of time for PSU, PSU-PDA, and PSU-PDA-NMEG5 fibers. Images of water drops on PSU (B), PSU-PDA after 3 hours (C), and PSU-PDA-NMEG5 after 3 hours in PDA and 24 hours in NMEG5 (D). Data are expressed as the means ± standard deviation of 10 independent measurements of three fibers

results indicate that NMEG5 attaches to the PSU-PDA fibers and greatly increases the surface wettability. Additionally, the PSU-PDA-NMEG5 fibers have higher hydrophilicity, up to 24-hour incubation times, as compared to both PSU and PSU-PDA fibers.

3.3.3. Pore Size

Membrane pore size is important because it effects the permeability and mechanical strength of membranes [53]. Evapoporometry was performed to confirm that attachment of PDA and NMEG5 to the PSU fiber surface does not affect pore size (Figure 3.3). The data show that the pore size distribution for PSU, PSU-PDA, and PSU-PDA-NMEG5 fibers is similar, with an average pore size of 6-7 nm. The PSU membranes made for this study have a small, but significant, number of pores >10 nm. The presence of pores larger than the protein increases the total surface area available for protein fouling. However, protein adsorption in these larges pores is unlikely to affect gas exchange. The mean pore size for PSU fibers is 6 ± 7.5 nm, while the

pore size for PSU-PDA and PSU-PDA-NMEG5 fibers is 7 ± 13 nm and 7 ± 10 nm, respectively. Overall, the attachment of PDA and NMEG5 to the surface maintains the porosity of the PSU fibers.

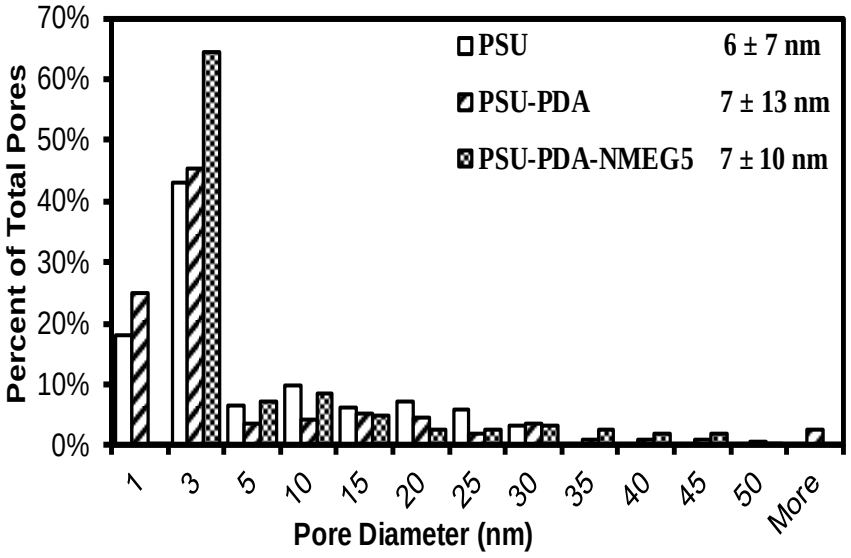


Figure 3.3. Pore distribution of PSU (open), PSU-PDA (stripes), and PSU-PDA-NMEG5 (checkered) fibers as determined by evapoporometry. Each data point represents one measurement of three fibers.

3.3.4. Protein Adsorption on Hollow Fiber Membranes

Adsorption of single proteins on materials is commonly used to evaluate blood compatibility [54, 55]. However, assessment of only one protein can be misleading since varied protein properties greatly affect surface interactions. For these reasons three proteins, bovine serum albumin, lysozyme, and fibrinogen, with different properties including molecular weight (MW) and isoelectronic point (pI) were selected for these studies. Albumin (MW 67,000, dimension 90 × 50 × 50 Aº and pI 4.8) is the most abundant protein in human blood, with a concentration of 35-50 g/L in plasma. Lysozyme (MW 14,400, dimension 46 × 30 × 30 Aº and pI 12) was selected for due to its small size, providing information regarding the density of the NMEG5 layer [29]. Fibrinogen (MW 340,000, dimension 450 × 90 × 90 Aº and pI 6) is present in plasma protein at a

concentration of 1.5-4 g/L and is part of the clotting cascade. Even low amounts of fibrinogen adsorbed to a surface can lead to high fouling due to platelet adhesion [56]. The results of static fouling studies on PSU, PSU-PDA, and PSU-PDA-NMEG5 fibers with bovine serum albumin, lysozyme, and fibrinogen are shown in Figure 3.4. The unmodified PSU fibers rapidly adsorbed all three proteins, reaching a plateau after 2-3 hours. Specifically, the unmodified PSU surfaces adsorbed 4.3 ± 1.2 μg/cm^2 of bovine serum albumin, 4.9 ± 1.3 μg/cm^2 of fibrinogen, and 14 ± 0.5 μg/cm^2 of lysozyme after 24 hours. The PSU-PDA fibers did not have a significant difference in the amount of protein adsorbed as compared to unmodified fibers for any of the proteins, and also reached a plateau within 2-3 hours.

The addition of NMEG5 to the fiber surface resulted in a significant decrease in protein adsorption after 3 hours for all three proteins tested. Incubation of the PSU-PDA-NMEG5 fibers with protein resulted in an initial rapid increase in adsorbed protein with a plateau after 1-2 hours. The amount of protein adsorbed to the NMEG5-coated fibers was significantly less, with 2.2 ± 0.7 μg/cm^2 bovine serum albumin, 1.39 ± 0.5 μg/cm^2 fibrinogen, and 6.4 ± 0.8 μg/cm^2 lysozyme adsorbed after 24 hours. In order to better compare these data with other surfaces the data was normalized to the amount of protein adsorbed to the unmodified PSU fibers in the plateau region. This analysis revealed that compared to the unmodified fibers the PSU-PDA-NMEG5 fibers adsorbed ~60% of the bovine serum albumin, ~45% of the lysozyme, and ~34% of the fibrinogen. The low fouling properties of the PSU-PDA-NMEG5 fibers can be attributed to increased hydrophilicity and coordination with surrounding water molecules [57, 58].

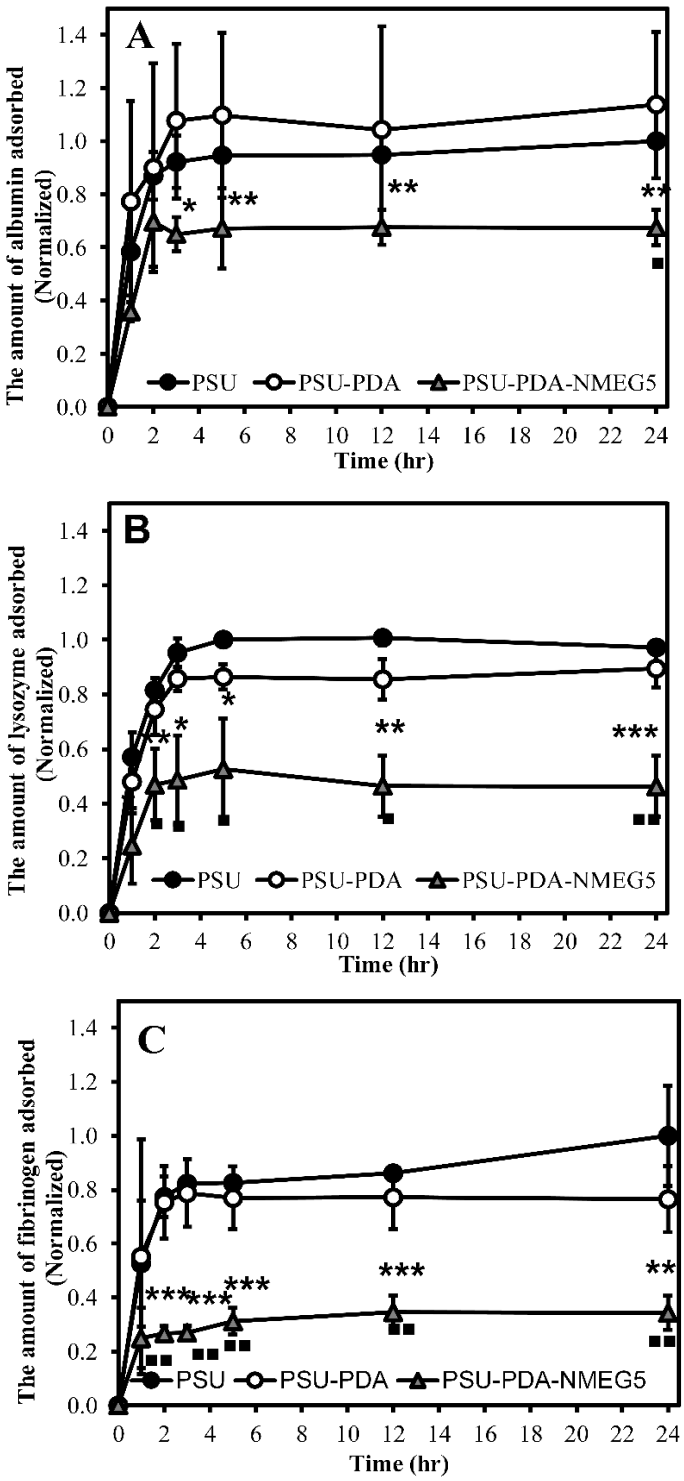


Figure 3.4. Adsorption of bovine serum albumin (A), lysozyme (B), or fibrinogen (C) on PSU (closed circle), PSU-PDA (open circle), and PSU-PDA-NMEG5 (open triangle) fibers. Data are expressed as the mean ± standard deviation of three independent measurements. $p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ for PSU-PDA-NMEG5 vs. PSU and ■$p < 0.05$, ■■$p < 0.01$, and ■■■$p < 0.001$ for PSA-PDA-NMEG5 vs. PSU-PDA.

3.4. Conclusion

In this study, for the first time, PSU hollow fiber membranes were coated with a peptoid (NMEG5) to reduce protein fouling. Previous research suggests that increasing surface hydrophilicity leads to a decrease in protein fouling [59]. Thus, the peptoid NMEG5 was designed to increase the hydrophilicity of the PSU membranes based on previous studies in the Messersmith lab [13, 29, 30]. The NMEG side chain has the desired properties to create a low-fouling surface including hydrophilicity, hydrogen bond acceptors, no hydrogen bond donors, and no charge. Additionally, the protease resistance of peptoids makes them ideal for long-term use *in vivo* [13, 29].

In this study, NMEG5 was attached to PSU fibers via reaction with catechol groups in a PDA layer. The presence of the PDA layer and subsequent immobilization of NMEG5 was confirmed by XPS. Contact angle measurements showed that hydrophilicity increased with longer coating times of PDA and NMEG5. PSU fibers were incubated with PDA for 3 hours and PSU-PDA fibers were incubated with NMEG5 for 24 hours. The NMEG5 coated fibers decreased fouling compared to unmodified fibers by 40% for bovine serum albumin, 55% for lysozyme, and 66% for fibrinogen. Protein fouling was time dependent; a rapid increase in adsorption was observed over the first 1-3 hours followed by a plateau that extended to 24 hours.

Table 3.2 contains static fouling data for various coated hollow fiber membranes. The fibers are composed of PSU, polyethersulfone (PES), or polyvinylidene fluoride (PVDF) and coated with poly(vinylpyrrolidone) (PVP) [4], poly(sulfobetaine methacrylate) (PSBMA) [5], poly(ethersulfune)/poly(vinylpyrrolidinone) nanoparticles (M15) [10], poly(acrylonitrile-co-acrylic acid) (PAN-AA) [60], poly(styrene-b-poly(ethylene glycol) methacrylate) (Ps-b-

PEGMA) [61], and/or heparin [62]. All data are normalized to protein adsorption on the unmodified membrane. It should be noted that the unmodified surface was not the same in all cases. Modification of the membrane surfaces resulted in reduced protein adsorption for all coatings.

The hydrophilic PVP coating, which contains no ionic groups, was best at reducing adsorption of BSA with 91% reduction compared to the unmodified surface after 2 hours. The amphiphilic PS-b-PEGMA coating was best at decreasing lysozyme adsorption with 88% reduction in adsorption compared to the unmodified surface after 2 hours. The coating introduced in this paper, NMEG5, was best able to reduce fouling by fibrinogen with a 66% reduction in adsorption as compared to the unmodified surface after 2 hours. While the NMEG5 coating was not the best coating for all proteins tested, it did reduce fouling in all cases. The data in Table 3.2 provides guidance on how to better design future coating to resist fouling by multiple proteins.

Although long-term fouling performance in complex solutions is vital for biocompatible materials, most studies use short incubation times and purified protein samples. In this study, protein fouling was assessed for up to 24 hours using three purified proteins. The NMEG5 modified surface significantly reduced the adsorption of bovine serum albumin, lysozyme, and fibrinogen over 24 hours, with very little difference observed after 3 hours. Further studies must be performed both for longer times, as well as with complex biological solutions to characterize the surface. Based on previous literature, it is expected that the surfaces will be less effective at preventing biofouling in the presence of complex biological solutions [22, 29, 56, 63]. Previous studies with an NMEG 20mer showed that the surface absorbed 7 ng/cm^2 of fibrinogen versus 15 ng/cm^2 of human serum [30]. Peptoid sequence will continue to be optimized to improve the ability to prevent long-term fouling in complex biological solutions.

Table 3.2. Comparison of low fouling coatings on hollow fiber membranes.

	Bovine Serum Albumin (hr)		Lysozyme (hr)	Fibrinogen (hr)	
	1	2	2	1	2
Uncoated membrane	1	1	1	1	1
PSU-PDA	1.32	1.03	0.91	1.04	0.97
PSU-PDA-NMEG5	0.61	0.8	0.57	0.47	0.34
PSU-PVP-2000-12 [4]		0.09			0.37
PSU-g-PSBMA [5]	0.13				
PES-M15 [10]	0.1				
PES/PAN-AA 16/0.4 [60]		0.44			0.81
PVDF-Ps-b-PEGMA-5wt% [61]		0.27	0.12		
PSU-PDA-Heparin [62]	0.71				

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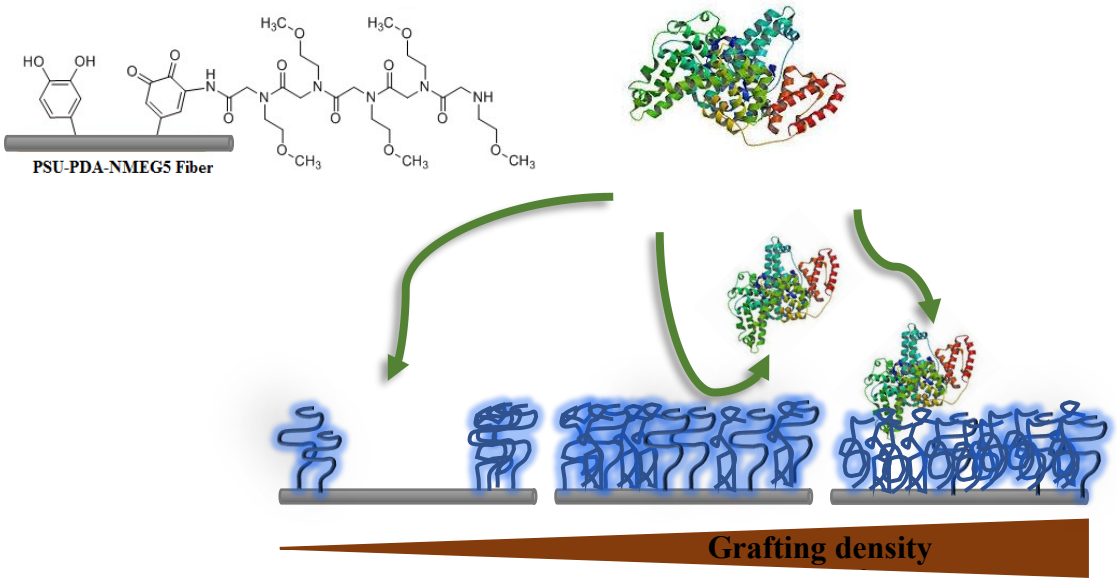
4. Chapter 4 Peptoid Grafting on Polysulfone Hollow Fiber Membrane to Increase Antifouling Characteristics – Effect of Grafting Density and Chain Length

Abstract

The development of antifouling membranes to minimize nonspecific protein adsorption has relevance in various biomedical applications. In this project, electrically neutral NMEG peptoids containing 2-methoxyethyl side chains were attached to polysulfone (PSU) hollow fiber membranes via polydopamine. NMEG peptoids with varying length (NMEG5, NMEG10, NMEG15, and NMEG20) were synthesized and attached to PSU membranes and antifouling performance was assessed. NMEG peptoids presented a high hydrophilicity as compared to unmodified PSU membranes. The long-term stability of the peptoid coating was confirmed over five months. The antifouling performance of the membranes was evaluated using a bovine serum albumin and platelet adhesion experiments. Additionally, the effect of side chain length and grafting density on protein adsorption was evaluated. It was determined that there is an optimal grafting density for reduction of protein adsorption, which was dependent on the length and grafting density of the peptoids. This study provides a convenient strategy to improve antifouling, hydrophilicity and hemocompatibility of PSU membranes for use in biomedical and blood-contacting applications.



4.1. Introduction

There has been an increasing need for synthesis of biomaterials to use them in different fields, such as biosensors, implants and artificial organs due to an interest in human life expectancy [1]. Synthetic polymers such as cellulose acetate, polymethylmethacrylate, ethylenevinyl alcohol copolymer, polyethylene, polypropylene, polyacrylonitrile, polyvinyl alcohol, polyethersulfone and polysulfone (PSU) are widely used in blood contacting devices because of the ease in controlling their structure, compositions and properties [2]. Among them, PSU polymer has been widely used in different biomaterial fields for example blood purification [3] and clinical hemodialysis [4] due to its mechanical strength, chemical inertness, thermal stability [5]. PSU can be easily prepared via phase inversion method into porous membrane with excellent permeability [5]. However, the hydrophobic nature of PSU polymers limits its application in biomedical areas since adsorption of unwanted biological matter happens after contacting with blood and often result in the serious side effects such as infection, thrombosis and other

complications [6, 7]. Therefore, modification of PSU membranes is desired to improve their hemocompatibility.

Several studies have been conducted to create PSU surfaces with low protein adsorption tendency [5, 8]. For example, Higuchi et al, used physical method to at first attach PEO terminated polymer, then used a Pluronic surfactant to form a more stable adsorbed layer on the PSU surface. The membranes were exposed to the mixed protein solution of human serum albumin (4 mg/ml, 37 ºC, 2h), human γ-globulin (1mg/ml, 37 ºC, 2h) and human fibrinogen (0.3 mg/ml, 37 ºC, 2h). There was no reduction of albumin and γ-globulin by Pluronict-coated PSU membranes in comparison to unmodified membranes; however, the adsorption of fibrinogen decreased by 90% after exposure to the mixed protein solution [6]. Zhao et al. immobilized zwitterionic polymer of poly sulfobetaine methacrylate onto PSU membrane using surface-initiated atom transfer radical polymerization. Biocompatibility experiments were performed with bovine serum albumin solution and platelet adhesion tests. Membrane exposed to bovine serum albumin solution (1mg/ml) and platelet rich plasma at 37 ºC for 2 h. The polysulfobetaine methacrylate grafted membranes showed 77% reduction in protein adsorption and no platelet adhesion compared to unmodified membranes when the grafting density of polysulfobetaine methacrylate onto PSU surface was 120 (μg/cm^2) [9]. Recently, Zheng et al. grafted poly ethylene glycol (PEG) and heparin on PSU to improve membrane hemocompatibility. Membranes were exposed to bovine serum albumin (0.1 mg/ml, 1h, 37 ºC), fibrinogen (0.1 mg/ml, 1h, 37 ºC) and platelet rich plasma (100 μl, 2 h, under 5% CO_2 in air). The modified membranes demonstrated prominent blood compatibility than unmodified PSU (45% in bovine serum albumin, 58% in fibrinogen and a significant reduction in platelet adhesion compared to PSU membranes) [10]. Therefore, researchers have shown that one strategy to improve

biocompatibility of membranes is modification of the surface via physical treatment, coating, or grafting methods [11, 12]. Among antifouling polymers, PEG-based materials have been widely used to modify the surface to resist fouling in biomedical fields. However, PEG undergoes autoxidation when exposed to oxygen and transition metal ions, in the blood [13]. Another common antifouling polymer to reduce thrombus formation is heparization of surfaces. Although grafted heparin onto surfaces can effectively decrease blood coagulation, it can cause hemorrhagic complications in patients at high risk of bleeding. Additionally, heparinization of surface cannot decrease protein adsorption of the membranes [14]. To overcome these difficulties, we investigated the use of a novel, protease-resistant, PEG-like peptoid as a coating on PSU hollow fibers. Peptoids have a peptide-like backbone, but with increased resistance to protease degradation and low immunogenicity making them ideal candidates to use in biomaterials. Peptoids have a similar backbone to peptides but the side chains attached to the amide-nitrogen rather than the alpha-carbon [15]. This small backbone change imparts peptoids to resist protease degradation and ultimately increases biostability compared to peptides [16]. Peptoids with 2-methoxyethyl (NMEG) have previously been shown reduce biofouling [17-20]. NMEG is uncharged, polar, has no hydrogen donors, and contains hydrogen bond acceptors, properties that make it promising as an antifouling coating [21]. We have previously attached a NMEG5 peptoid to PSU hollow fiber membranes via polydopamine (PDA) to reduce biofouling [17]. Our studies showed that the NMEG5-modified PSU hollow fibers had a significant reduction in protein adsorption as compared to unmodified PSU hollow fibers, with a 40% reduction of fouling by bovine serum albumin, 55% by lysozyme, and 66% by fibrinogen [17]. However, we are seeking lower antifouling surfaces.

Various studies have investigated protein resistance of different antifouling polymers with respect to polymer grafting density and chain length to reach the lowest amount of protein adsorption. Sofia et al. found that polyethylene oxide grafted silicon surfaces reached the lowest adsorption at the highest grafting density [22]. Feng et al. studied the effect of graft density and chain length of PEG and phosphorylcholine polymers on silicon wafers after contacting to the adsorption of fibrinogen. They controlled the grafting density using surface density of atom transfer radical polymerization initiator and chain length was controlled through the ratio of monomer to initiators. The result showed that the adsorption of fibrinogen on both grafted surfaces decreased as grafting density and chain length increased [23]. The same group also found that although the adsorption of fibrinogen was influenced by both graft density and chain length, it showed a stronger dependence on graft density than on chain length. Moreover, protein adsorption began to increase above a certain graft density due to inability of polymer chains to hydrate [24]. Lau and coworkers grafted polysarcosine (the elementary peptoid) onto TiO_2 by a mussel adhesive- inspired DOPA-Lys pentapeptide. The result showed that fibrinogen adsorption decreased with increasing grafting density of polysarcosine, and fibrinogen was the lowest above certain critical chain density [25]. In 2009, Kizhakkedathu et al. evaluated the effect of PEG-based N-substituted acrylamide macromonomers chain length and monomer concentrations on graft density of polymer chains on the surface. The grafted surface density of polymer chains increased with increasing monomer concentrations [26]. It has also been argued that the macromonomer chain length affected the chains surface grafted density due to limited accessibility and steric influence of larger side chains. Moreover, studies on whole blood protein adsorption showed that grafted antifouling layers decreased protein adsorption as a function of graft density of chains on the surface [26]. Wang et al. evaluated the effect of grafting density

and pendant length of methoxypolyethylene glycol brushes on permeability and fouling behavior of membranes. The results showed that surface modified with longer pendent length and higher grafting density led to in reduction of normalized fluxes of tap water and bovine serum albumin solution, while membrane antifouling properties improved. They found pendent length and grafting density played equally important role in membrane fouling, and pendent length role was more significant in membrane permeability [27]. According to these studies, we hypothesize that antifouling property of NMEG peptoid coatings will achieve significantly lower fouling at some optimal surface coverage.

The aim of this work was to optimize the NMEG peptoid grafting condition to obtain a modified membrane with the lowest protein adsorption and platelet adhesion amount. We employed different peptoid concentrations, reaction times and side chain lengths to identify the optimal grafting density required to prevent protein adsorption. We have shown that the amounts of adsorbed protein from bovine serum albumin on NMEG grafted membranes depended both on the peptoid grafting density and peptoid length.

4.2. Materials and Methods

4.2.1. Materials

MBHA rink amide resin was purchased from NovaBiochem (Gibbstown, NJ). Piperdine, bovine serum albumin, fibrinogen, FITC-bovine serum albumin, and dihydroxyphenethylamine (dopamine) hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). PSU pellets (average MW ~35,000) were obtained from Nanostone (Oceanside, CA). Fresh porcine venous blood was purchased from Lampire biological laboratories (Pipersville, PA). All other reagents and materials were purchased from VWR. Ultrapure water was purified with a minimum

resistivity of 18.2 MΩ cm, using a NANOpure DiamondTM Life Time system (Barnstead/Thermo scientific, Essex, United Kingdom). All reagents were of analytical grade and used without further purification.

4.2.2. Peptoid Synthesis and Purification

Peptoids were synthesized via a submonomer protocol [15] on rink amide resin, as previously described [17]. Briefly, the resin was swelled with dimethylformamide (DMF) and the Fmoc protecting group was removed using 20% piperidine in DMF. The submonomer cycle begins with addition of 1.2 M bromoacetic acid in DMF in the presence of N, N'-diisopropylcarboniimide at a ratio of 4.3:1. NMEG side chains were added by incubation with 0.5 M methoxyethylamine in N-methylpyrrilidone (NMP) for 5 minutes. This cycle was repeated until the desired sequence was achieved. Peptoid were cleaved from the resin using a mixture of 95% trifluoroacetic acid (TFA), 2.5% water and 2.5% triisoproylsilane for five minutes. The resin was filtered from the peptoid solution, TFA was removed using a Heidolph Laborota 4001 rotating evaporator (Elk Grove Village, IL), and the peptoid was diluted to a final concentration of ~3 mg/ml in a 25:75 solution of acetonitrile: water.

The peptoids were purified by preparative reversed-phase high performance liquid chromatography (HPLC; Waters Delta 600, Milford, MA) with a Duragel G C18 150 × 20 mm column (Peeke Scientific, Novato, CA) using a linear gradient of 0-65% solvent B in A (solvent A: water, 0.1% TFA; solvent B: acetonitrile, 5% water, 0.1% TFA) at room temperature over 60 minutes. Final peptoid purity was confirmed to be >98% by analytical reversed-phase HPLC (Waters 2695 separations module) with a Duragel G C18 150 × 2.1 mm column (Peeke Scientific) and a linear gradient of 5 to 95% solvent D in C (solvent C: water, 0.1% TFA, solvent D: acetonitrile, 0.1% TFA) over 30 minutes at room temperature. The molecular weight of the

peptoids were confirmed by matrix-assisted laser desorption/ionization mass spectrometry. Purified peptoid solutions were lyophilized (Labconco lyophilizer, Kansas City, MO) and stored at -20 °C prior to use.

4.2.3. Preparation of PSU Porous Hollow Fibers

A conventional hollow fiber membrane-spinning device was used to prepare PSU hollow fiber membranes, as previously described [17]. Briefly, a dope solution (17.8 wt% PSU in NMP) and bore solution of (15 vol% NMP in water) fed into a spinneret with 0.8 mm inner and 1.6 mm outer diameters under pressurized nitrogen gas. The solutions were extruded through the spinneret into a water bath at 23 °C with an 8 cm air gap between the bath and the spinneret, and phase inversion occurred to form PSU hollow fiber membranes. The fibers were pulled under dowels, immersed in the water, and rolled onto a draw wheel at an uptake speed of 2 m/min. The fibers were washed in DI water for 3 days with daily water changes to remove extra solvent. The fibers were stored at 5 ºC in 0.25% (v/v) sodium benzoate in water prior to modification.

4.2.4. Surface Modification of PSU Hollow Fiber Membrane

NMEG peptoids were attached to PSU fibers via PDA as previously described [17]. PSU membranes were immersed in ethanol for 30mintures and rinsed with ultrapure water. Then, membranes immersed in fresh PDA solution at room temperature in the presence of oxygen for 3 hours. PSU-PDA membranes washed with ultrapure water and incubated with varied peptoid concentrations in phosphate buffered saline (PBS, pH=7.4) at 60 ºC for 1-48 h. The peptoid modified membranes (PSU-PDA-NMEGs) were washed with ultrapure water to remove any un reacted peptoid and dried with nitrogen gas before storage.

4.2.5. Grafting Density Measurements

The grafting density of peptoid onto the PDA-modified fibers was measured by careful weighing of the fibers with a Mettler Toledo microbalance (readability of 0.1 mg, sensitivity offset of 4×10^{-6} sample weight; Columbus, OH) before and after incubation with peptoids. Prior to each measurement the membranes were washed with water for 24 hours and the solvent was removed by lyophilization. The peptoid grafting density was calculated using equation (4.1) , as previously described [27]:

$$\text{Grafting density} = \frac{(m_1 - m_0)}{A \times Mw} \times N_A \tag{4.1}$$

where grafting density represents the number of peptoid chains immobilized per area, m_1 is the mass of the peptoid-modified hollow fibers, and A is the linear surface area of the hollow fibers (inner and outer). M_W is the molecular weight of the peptoid, and N_A is Avogadro's number.

4.2.6. Water contact angle measurements

The static contact angle of water on unmodified and modified hollow fibers was measured at room temperature using a contact angle goniometer (OCA 15, DataPhysics Instruments GmbH, Filderstadt, Germany). Sessile drop technique was used to obtain static contact angles, as previously described [28]. Briefly, a 0.5 µL deionized water drop was formed at the tip of a needle and lowered to the hollow fiber surface. Contact angle was calculated using Data Physics surface contact angle (SCA) software. Contact angle was measured 9 times across three hollow fiber surfaces. All water contact angle measurements were performed at ambient laboratory conditions (25 ºC and 50% relative humidity).

4.2.7. Surface Morphology

The surface and cross-sectional membrane morphologies were observed using scanning electron microscopy (SEM, Nova Nanolab 200, 15 kV) and (SEM; FEI, Hillsboro, OR), respectively. For cross-sectional observation, liquid nitrogen was used to freeze the samples before fracturing.

The surface morphology of the membranes was observed using scanning electron microscope (SEM; FEI, Hillsboro, OR). Afterward, the surface of membranes was sputter with gold prior to prevent charging before examination.

4.2.8. Protein Adsorption

The ability of the peptoid-modified PSU hollow fibers to prevent fouling was evaluated by incubation with bovine serum albumin as previously described [17]. The proteins were dissolved in phosphate buffered saline (PBS, pH 7.4) to a final concentration of 1 mg/ml. The hollow fibers were placed vertically into 1ml polypropylene nonstick vials at 37 °C with sufficient protein solution to cover the fibers. The protein concentration of the incubated solutions was measured using a Pierce BCA protein kit with a bovine serum albumin standard curve and the amount of protein adsorbed was calculated using equation (4.2):

$$protein\ adsorption = \frac{C_0 - C_1}{A} \times V \tag{4.2}$$

where C_0 is the solution protein concentration in the control vial, C_1 is the solution protein concentration after incubation with hollow fibers, A is the linear surface area of the inside and outside of the hollow fibers, and V is the volume of solution.

4.2.9. Fluorescent Staining Measurements

Fluorescently labeled bovine serum albumin was used to visually assess protein adsorption on the hollow fiber surfaces. Unmodified and modified PSU hollow fibers were incubated for 2 h in a 1 mg/mL solution of FITC-bovine serum albumin (PBS, pH 7.4) and washed with PBS to remove unbound proteins. Fluorescence images of the fibers were obtained using a Nikon Eclipse CI microscope with 200 ms exposure time. Color intensity was measured using ImageJ software and an average value was calculated for three images.

4.2.10. Platelet Adhesion Test

Platelet rich plasma experiment was used to study platelet adhesion on the membranes. Healthy porcine blood with anticoagulant ratio of 2%NaEDTA to whole blood was purchased from Lampire biological laboratories (Pipersville, PA). Platelet rich plasma was obtained after centrifuging the whole blood at 1000 rpm for 15 min. According to method reported previously [9, 29], unmodified and modified membranes with the surface area of (2 cm^2) were immersed in PBS and equilibrated at 37 ºC for 1 h. After equilibration, membranes were placed in 0.6 ml centrifuge vials, 500 µl fresh PRP was introduced and incubated at 37 ºC for 3h under static condition. After being rinsed membranes three times with PBS, the membranes were treated with 2.5 wt% glutaraldehyde in PBS at 4 ºC for 2 days to fix adhered platelets. Thereafter, the samples were washed with PBS and subjected to a drying process by passing them through a series of graded ethanol-PBS solutions (v/v) (25%, 50%, 75% and 100 %) and isoamyl acetate-ethanol (25%,50%, 75% and 100%) for 15 min each time. After rinsing sufficiently with distilled water, then membranes dried in a freeze-dried (Labconco lyophilizer, Kansas City, MO) overnight and coated with a gold layer. Finally, the morphology of the adhered platelet on the membranes was observed by a scanning electron microscope (SEM; FEI, Hillsboro, OR).

4.2.11. Statistical Analysis

Data were expressed as mean ± SD and analyzed using a one-way analysis of variance (ANOVA) test followed by a Tukey posthoc test. Polyplex size data was analyzed using a t-test, and the results are presented as mean ± SD. Single, double, and triple asterisks represent $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively. Differences were considered statistically significant when $p < 0.05$.

4.3. Results

4.3.1. Immobilization of NMEG to PSU Membranes

Nonspecific adsorption to implanted medical devices can lead to deleterious biological responses, such as platelet adhesion, aggregation, bacterial infection, blood clot formation and even failure of medical devices [30]. Thus, developing materials that can resist protein adsorption is critical for biomedical applications. The peptoids with 5-20 NMEG side chains (Table 4.1), which have been shown to exhibit high biocompatibility, were synthesized to improve biocompatibility of PSU membranes [18-20]. Peptoids were immobilized to the hollow fiber surfaces via PDA, which attaches strongly to a variety of wet surfaces, as previously described [17, 31, 32]. We previously showed that NMEG5 was successfully attached onto PSU hollow fibers via PDA and reduced fouling by bovine serum albumin, lysozyme, and fibrinogen [17].

Table 4.1. The average molecular weights of NMEGs

Polymer	M_w
NMEG5	592.68
NMEG10	1168.1
NMEG15	1743.5
NMEG20	2319

4.3.2. Membrane Surface Morphology

Surface modification may affect the membrane surface morphology. SEM was used to evaluate the changes in surface morphology of unmodified and modified PSU hollow fibers, which is shown in Figure 3.1. The SEM images of cross section of PDA coated and peptoid modified membranes show that PDA and peptoids molecules did not penetrate membrane pore size. SEM images of surfaces also reveal that the membrane pore size and porosity did not show any discernible difference after modification of surface with PDA and NMEG5 peptoid which is consistent with our previous work via evapoporometery technique [17]. Moreover, compared with PSU and PSU-PDA membranes, the surfaces are much smoother after grafting peptoid onto the surface.

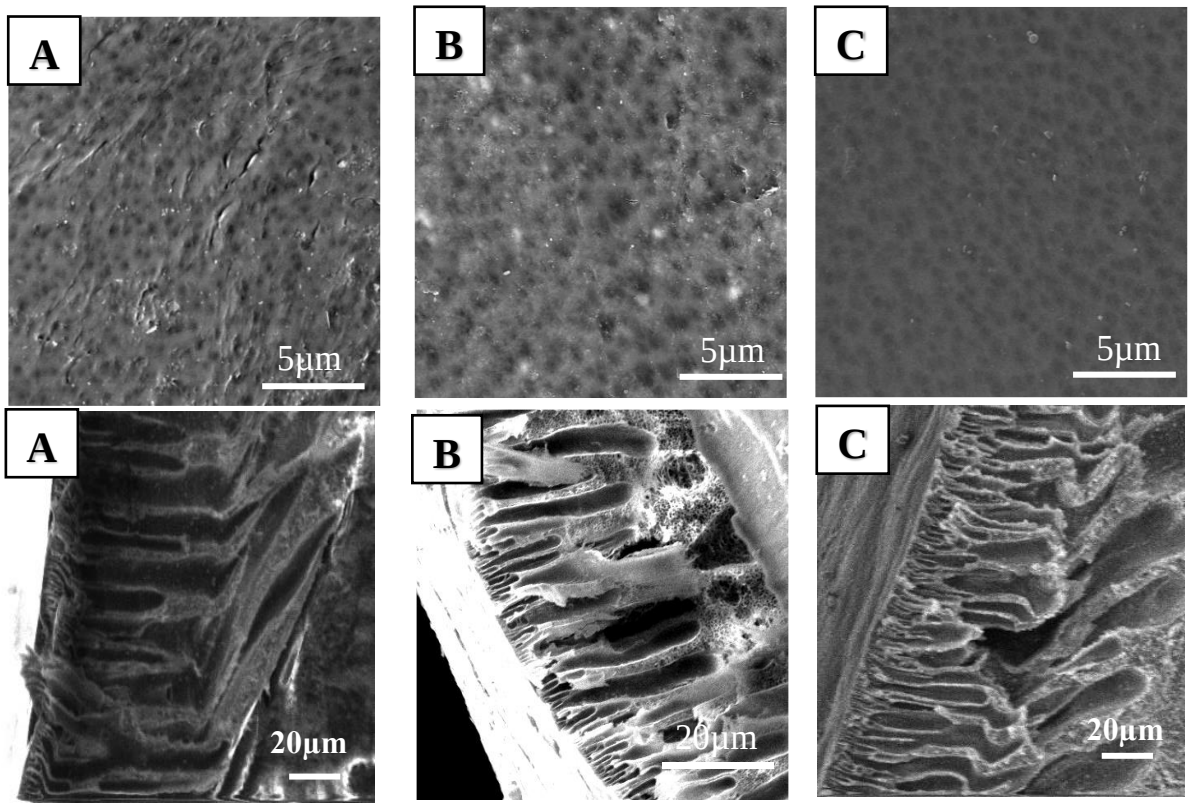


Figure 4.1. Surface and cross-section SEM images of membranes: (A) PSU; (B) PSU-PDA; (C) PSU-PDA-NMEG5

4.3.3. Effect of NMEG Incubation Time, Reaction Time and Chain Length on the Grafting Density

Side chain length (molecular weight) and grafting density (number of chains per surface area) are determining factors in membrane antifouling characteristics and permeability [24, 27]. Theoretical and experimental studies show there is a strong relation between protein adsorption and grafting density due to the steric repulsion caused by the compression of stretched chains [26, 33]. The grafting density of NMEGs on the hollow fibers with varied reaction times is presented in Figure 4.2. A. Results indicate that the grafting density of NMEG quickly increases with reaction time and then plateaus. This can be attributed to the saturation of immobilized

NMEGs molecules on the surface. At the beginning of this reaction, the $–NH_2$ (–NH–) functional group in NMEGs chain could easily reach the active sites in the PDA layer due to the Brownian motion [34]. However, when reaction time increases, the mobility of immobilized molecules and steric hindrance hinder the reactive substances from contacting; therefore, the total reaction rate dramatically decreases. It is also very interesting to observe that the grafting density of NMEG5 is remarkably higher than that of NMEG20 on the PSU-PDA surface. NMEG brushes can get access to the reaction sites with the decrease of NMEG chain length to higher grafting density under the same peptoid reaction time because of limited accessibility and steric hindrance of larger side chains.

Moreover, the NMEG concentration was varied from 0.01 to 3 mg/ml to investigate the variation of NMEG concentrations with grafting density (Figure 4.2. B). NMEGs show a rapid rise in grafting density at low peptoid concentration, with a leveling off when a maximum grafting is attained. The dependence of grafting density on peptoid concentration can be explained by the strong interactions of peptoid molecules with water molecules in aqueous solution. Studies have shown that hydrated hydrophilic molecules prevented overlapping in aqueous solution since this can disrupt the interaction of water polymers. However, hydrophilic molecules do overlap at high solution concentration [22]. Therefore, when the peptoid concentration is low enough peptoid chains do not overlap, and bound peptoid chains on a surface deprive other chains from that occupied space. As peptoid concentration increases, overlapping of peptoid chains occurs and peptoid chains can bind to the surface. When solution concentration is higher, the density of peptoid chains grafted to the surface becomes high enough and the additional peptoid chains cannot penetrate the grafted layer to bind to the PSU-PDA surfaces. Therefore, when surface is

saturated by peptoid, the effect of steric hindrance result in an increasing resistance for additional peptoid chains to diffuse the immobilized peptoid layer and bind to the PSU-PDA surfaces.

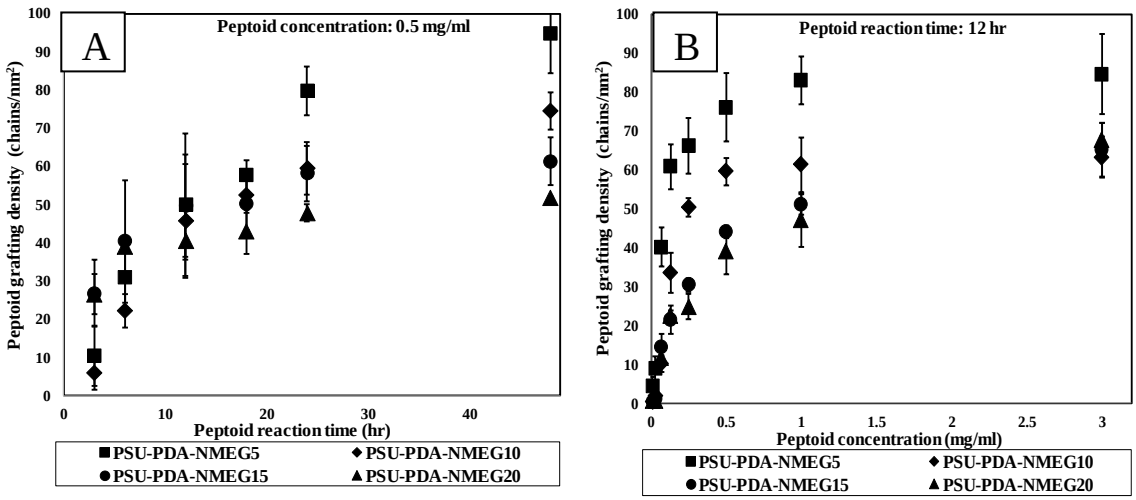


Figure 4.2. The surface weight of grafted peptoid chains measured by weighing after lyophilized membranes. A wide range of surface grafting density was achieved by solution grafting of peptoids from (A) varied peptoid reaction time; (B) peptoid concentration

4.3.4. Effect of NMEG Grafting Density on Surface Hydrophilicity

Water contact angle is related to the chemical composition of the surface, porosity, pore size, and roughness [27]. The initial contact angle of PSU was 72º ± 5° and decreased to 61º ± 6º following coating with PDA. The high-water contact angle on PSU is due to the nonpolar C-C/C-H groups on the surface [35], and the decrease in contact angle following modification with PDA is due to the introduction of hydrophilic groups such as $–NH_2$, –COOH, and –OH [34]. Figure 4.3 shows that contact angle of PSU-PDA hollow fibers decreases to 28 °±7.9° when the NMEG5 grafting density is (80-100) chains/nm^2 and increased gradually after increasing side chain length of NMEGs.

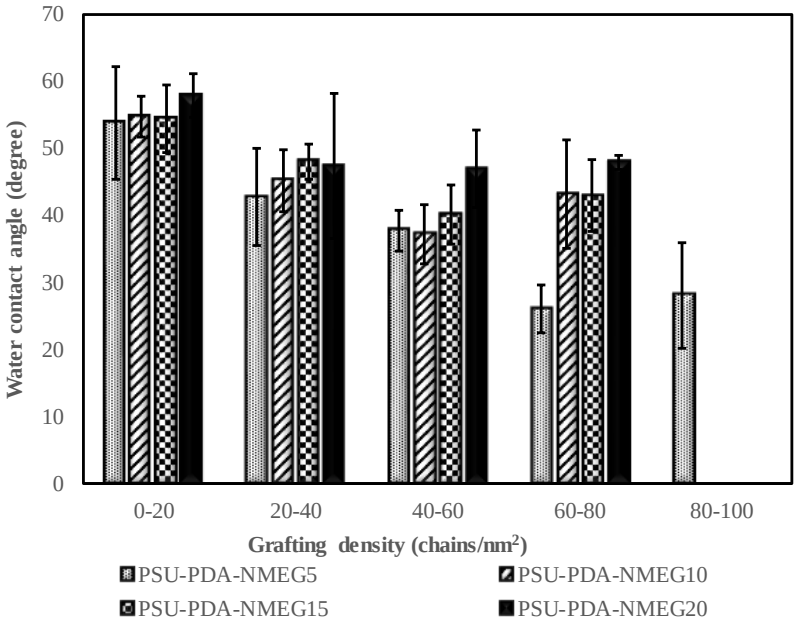


Figure 4.3. Water contact angles as a function of NMEGs grafting density

4.3.5. Protein Adsorption

The adsorption of protein on the membrane surface was a significant factor causing membrane fouling [36]. In theory, NMEG peptoids satisfy the general concepts of a protein-resistant surface: they are all hydrophilic, electrically neutral, do not have hydrogen bod donors and contain hydrogen bond acceptors [18-20], however their efficiency also depends on surface coverage. investigate antifouling capacity of PSU-NMEG modified membranes, bovine serum albumin was selected as a model protein and static protein adsorption experiments were conducted. The ability of NMEG modified surfaces with varied grafting densities to repel in bovine serum albumin protein was assessed by BCA kit. The amount of adsorbed protein on the various membranes surface were shown in Table 4.2. The hydrophobic interaction of bovine serum albumin with the PSU membranes surface led to large amount of protein adsorption on unmodified membranes (3.9±0.9 μg/cm^2 after 12 hr incubation time) [36]. After NMEGs attached to the surface, consisted with our previous study [17], less bovine serum albumin was

adsorbed to the NMEG modified membranes than to the PSU and PSU-PDA coated membranes. The protein resistance of NMEG surfaces is related to its excellent flexibility, hydrophilicity, and unique coordination with surrounding water molecules in aqueous solutions [18-20].

In this work, protein adsorption of NMEG grafted surfaces depended on the peptoid grafting density. It appears that an increase in NMEG grafting density, causes a decrease in protein adsorption. However, as grafting density becomes too high, NMEG groups are tightly packed on the surface, and they may lose their ability to maintain a stable hydration and result in protein adsorption. All four different peptoid side chain lengths showed the same behavior. It is seen that in each NMEG side chain there was a maximum in resistance lead to that there is an optimal chain density for protein resistance due to uniform surface coverage of NMEGs on the membrane surface. For example, the optimal grafting density for PSU-PDA-NMEG5 is at (60-80 (chains/nm^2), for NMEG10, NMEG15 and NMEG20 is (40-60 (chains/nm^2) while above each protein resistance decreased. Moreover, the static fouling experimental results demonstrated that the minimum BSA adsorption after 12 h incubation time could be achieved when (4-8) $\mu g/cm^2$, (8-12) $\mu g/cm^2$, (8-16) $\mu g/cm2$ and (12-16) $\mu g/cm^2$ amount of NMEG5, NMEG10, NMEG15 and NMEG20, respectively immobilized onto the PDA coated surfaces.

Additionally, Figure 4.4 shows all four NMEG chain lengths at optimal condition show an excellent protein resistance and the protein adsorption decreased by 74%, 61%, 66% and 56% for NMEG5, NMEG10, NMEG20 and NMEG20, respectively after 12-hour protein incubation time. The decrease protein adsorption can be ascribed to the uniform surface coverage of NMEGs on the membrane surface. The data indicated that grafting density is a key factor in the prevention

of protein adsorption. Additionally, as grafting density is associated with chain conformation and hydration, these properties may also play a role in decreasing protein adsorption [37].

Table 4. 2. Protein adsorption on PSU-PDA-NMEG with varied peptoid grafting densities

Substrate	Peptoid grafting density (chains/nm^2)	Bovine serum adsorption amount (μg/cm^2)		
		3h	5h	12h
PSU		3±0.9	3.5±0.9	3.9±0.9
PSU-PDA		3.8±1.4	4.4±1.5	4.6±1.3
PSU-PDA-NMEG5	(0-20)	3.6±0.6	3.6±.8	3.6±0.8
PSU-PDA-NMEG5	(20-40)	2.5±0.4	2.7±0.3	2.7±0.2
PSU-PDA-NMEG5	(40-60)	2±1.1	2±1	2±1
PSU-PDA-NMEG5	(60-80)	0.5±0.2	1.1±0.6	1±0.54
PSU-PDA-NMEG5	(80-100)	2.2±0.8	2.1±1.6	2.4±1.3
PSU-PDA-NMEG10	(0-20)	2.9±0.6	3.2±0.1	3.24±0.2
PSU-PDA-NMEG10	(20-40)	2.5±0.4	2.7±0.3	2.7±0.2
PSU-PDA-NMEG10	(40-60)	1.5±0.7	1.8±0.4	1.5±0.5
PSU-PDA-NMEG10	(60-80)	2.4±.4	2.5±0.3	2.4±0.4
PSU-PDA-NMEG15	(0-20)	2.1±0.2	2.4±0.3	2.8±0.1
PSU-PDA-NMEG15	(20-40)	1.3±0.4	2.25±0.7	2.2±0.9
PSU-PDA-NMEG15	(40-60)	0.7±0.4	1.1±0.2	1.3±0.04
PSU-PDA-NMEG15	(60-80)	0.9±0.4	1.4±0.4	1.6±0.3
PSU-PDA-NMEG20	(0-20)	2.55±0.1	2.8±0.4	3±0.3
PSU-PDA-NMEG20	(20-40)	1.2±.3	1.2±0.2	1.9±0.4
PSU-PDA-NMEG20	(40-60)	1.4±0.04	1.4±0.6	1.7±0.3
PSU-PDA-NMEG20	(60-80)	1.9±0.9	2.32±0.9	2±0.6

4.3.6. Platelet Adhesion

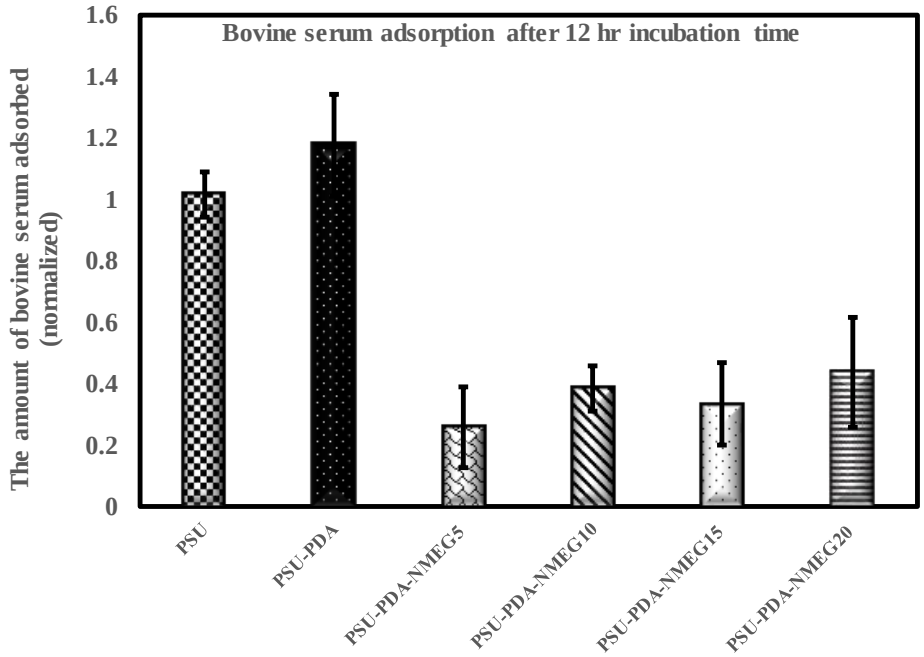


Figure 4.4. Reduction in bovine serum albumin adsorption to PSU surfaces modified with varied NMEG side chain length.

The practical application of modified PSU membranes could be in blood contact devices. In this respect, membranes have to show blood biocompatibility. Surface properties and the non-specific protein adsorption such as fibrinogen have a close relationship with the blood compatibility of blood-contacting materials [38]. Both PDA and peptoids may affect the surface nature of PSU hollow fiber membranes. As is known, one of the important factors in blood compatibility is blood coagulation which greatly depended on platelet adhesion and the activation of coagulation pathway [34]. Therefore, in assessing the hemocompatibility of peptoid modified PSU hollow fibers, we examined the amount and morphology of platelet adhered when in contact with peptoid modified hollow fibers relative to the unmodified PSU and PDA coated hollow fiber membranes.

Representative SEM images show platelet adhesion and spread on the unmodified hollow fibers Figure 4.5 (A). On the surface of PSU hollow fibers, there were numerous platelet performing as adhesion, outspread and aggregates. This aggregated tent to form clusters and has pseudopodium morphology. The pseudopodium morphologies and irregular shape also revealed the activation and deformation of platelets. After PDA modification, less platelets were found on the membranes which are consistent with other studies.[34]. Although separated and spherical morphologies of platelet observed distinctly, the transmutation and pseudopodia of platelet still existed. In contrast to the unmodified PSU hollow fibers, platelet adhered on the NMEGs peptoid modified surfaces (optimal condition) at very low attachment and displayed a round morphology Figure 4.5 (C, D, E and F). The single and spherical platelets with sparse pseudopodium and without pseudopodium showed the activation and deformation of platelets were reduced significantly. This observable rounded morphology upon contact to the NMEG modified PSU hollow fibers suggests that the platelets are not activated [39]. These results demonstrated that, the adhesion, activation and transmutation of platelets were significantly decreased by modification of surface using peptoids.

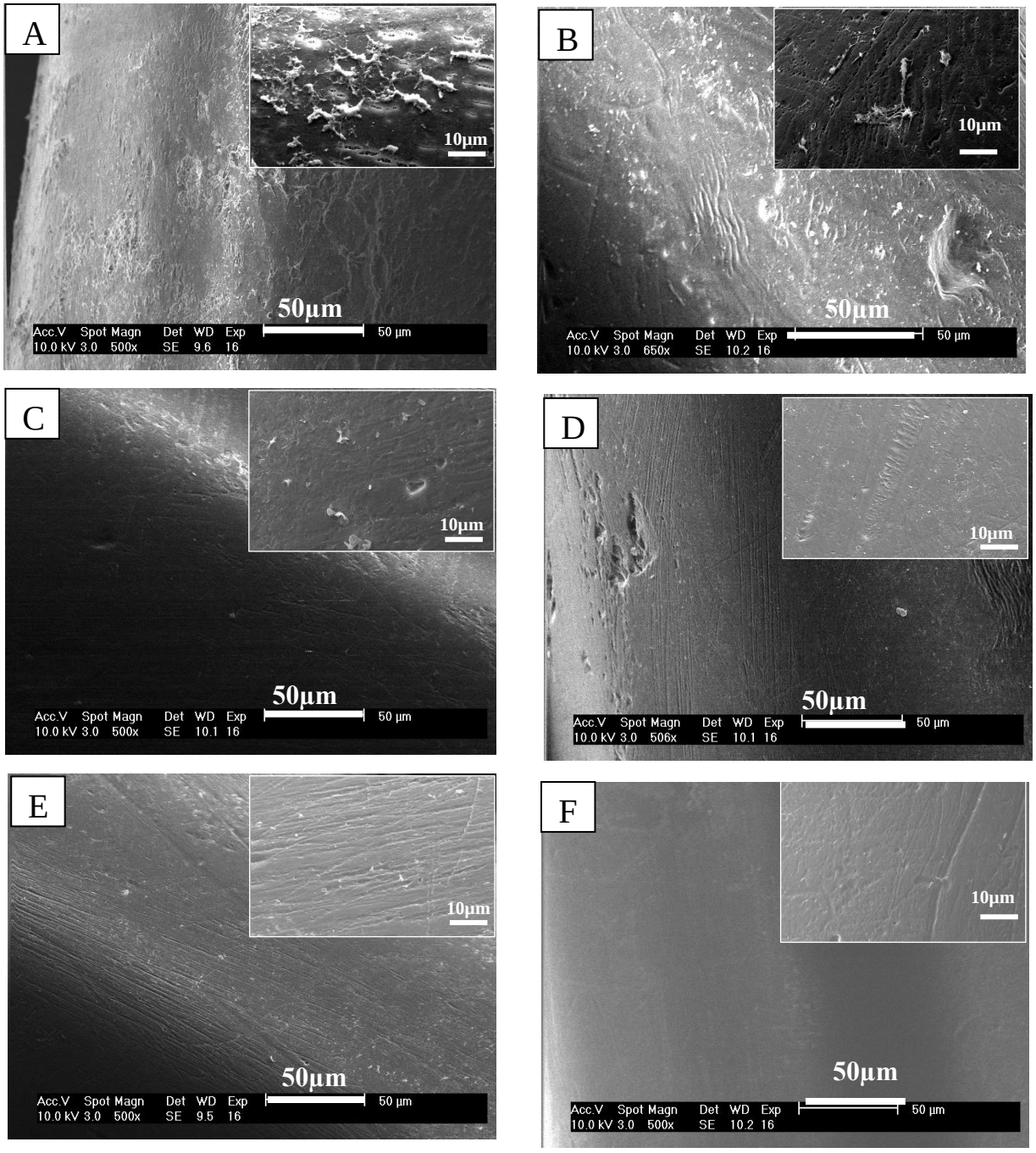


Figure 4.5. Scanning electron micrographs of hollow fiber surfaces after contact with porcine blood for 3 h at 37 °C (A) PSU; (B) PSU-PDA; (C)PSU-PDA-NMEG5; (D) PSU-PDA-NMEG10; (E) PSU-PDA-NMEG15; (F) PSU-PDA-NMEG20

4.3.7. FITC-Bovine Serum Albumin Fouling Behavior

The fouled membranes could be observed by a fluorescent microscope. All fouled membranes have particularly fluorescently bright areas, which indicates the deposition of FITC−bovine serum albumin and suggests that a cake layer of the foulants formed. Figure 4.6. shows fluorescence microscopy images of PSU and PSU-PDA-NMEG15 membranes upon exposure to FITC-BSA for 2 hours. Figure 4.6 shows a summary of the FITC-bovine serum albumin emission intensity measure at PSU, PSU-PDA, and PSU-PDA-NMEGs at different side chain length. The emission is the highest for unmodified PSU and is reduced by 77% For PSU-PDA-NMEG15 under the experimental conditions.

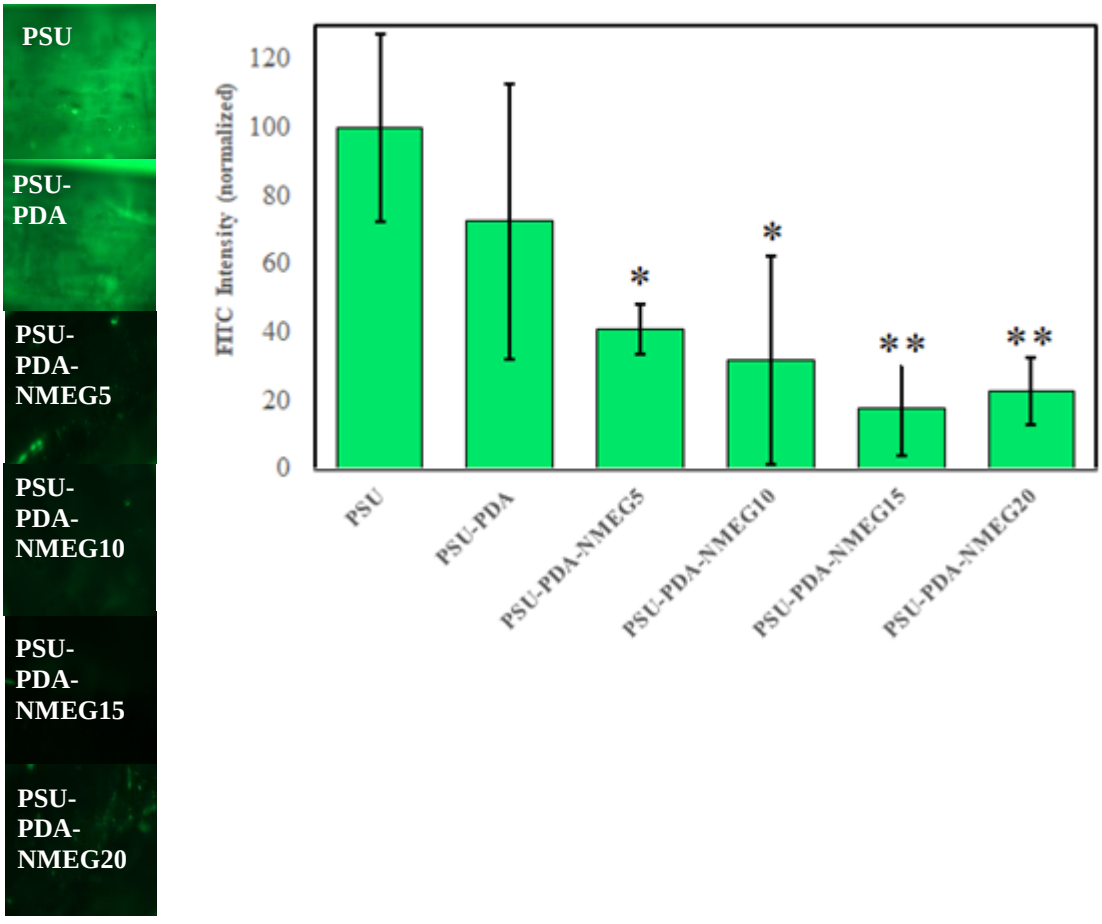


Figure 4.6. Fluorescence images of PSU hollow fibers and PSU-PDA-NMEG15-24, after incubation in FITC-Bovine serum solution (image width = 308 μm). The region that was not modified with NMEG appears brighter in the image, thus indicating greater BSA adsorption;

4.3.8. Durability of Peptoid Polymers on the Surface

Moreover, the stability of the modified layers onto membranes is always important subject to control membrane fouling. To achieve a high performance biocompatible membrane, it is important to sustain the hydrophilicity during the practical application. The robustness and stability of modified layers (NMEG and PDA-coated) on PSU hollow fibers were carried out. The modified membranes were immersed in ultra-pure water and incubated into water bath at 37 ºC for different time spans. The change in initial contact angle, which is tabulated in Table 4.3, was used to monitor stability of modified layers and hydrophilicity of PSU membranes. Additionally, PDA absorb UV light at 280 nm wavelength. The peak at 280 nm is linear respect to concentration of PDA. The absorbance provides an easy way to track the leaching amount of PDA and NMEG from membranes. Figure 4.7 presents the PDA and NMEG leaching from PSU surface. Approximately 0.3-0.5 μg/cm^2 is detected as leaching number of modified layers. Therefore, after 5 months of membrane rinsing leaching of PDA is undetectable. The result from contact angle and UV absorbance at 280 nm show that the membranes exhibit a goof long-term durability.

Table 4. 3. Effect of long time washing on contact angle of modified membranes

Sample	Incubation time (Month)				
	0	1	2	3	4
PSU	72.2±3		71.8±1.8	68.8±2.3	75.1±7.1
PSU-PDA	61.6 ± 6.1	50.5 ± 4.5	58.45±10	52.7±6.3	61±10.1
PSU-PDA-NMEG5	30.53±10.4	20.5±4.5	36.7±3.7	35.6±6.2	41.6±3
PSU-PDA-NMEG10	44.25±7.65	38±4.5	44.6± 6.6	46.7±6.7	45±7.2
PSU-PDA-NMEG15	46.05±12.5	35.5±3.5	45±5.8	48.3±4.6	53.3±9.2
PSU-PDA-NMEG20	47.1 ±3.95	45.5 ± .5	47.3±3.6	48.5±9.5	50.8±4.7

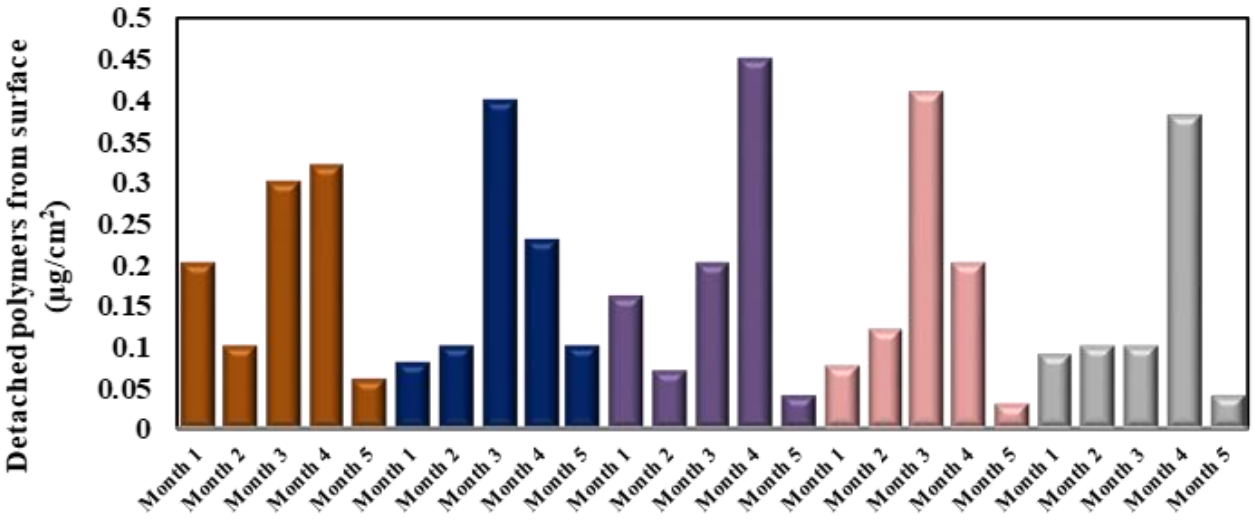


Figure 4.7. The amount of polymers detached from surface (µg/cm2), PSU-PDA (dark brown), PSU-PDA-NMEG5 (dark blue), PSU-PDA-NMEG10 (purple), PSU-PDA-NMEG15 (pink) and PSU-PDA-NMEG20 (gray).

4.4. Conclusion

In this work, we used self-polymerized 3,4-dihydroxyphenethylamine (dopamine) to form a surface-adherent polydopamine layer onto polysulfone (PSU) followed by covalent grafting of a peptoid with methoxyethyl (NMEG) side chains to reduce protein fouling. The modified surfaces were characterized to determine grafting density, morphology and contact angle. The long-term stability of the peptoid-modified hollow fibers was evaluated over five months and shown to be consistent. Peptoid-grafted membranes showed improvement in hydrophilicity compared to unmodified PSU surfaces. The adsorption amount of BSA decreased initially with increased peptoid grafting density until (60-80) chains/nm^2 for NMEG5 and (40-60) chains/nm^2 for other NMEGs, and then slightly increased on the (80-100) chains/nm^2 NMEG5 and (60-80) chains/nm^2 other NMEGs. Here for the first time, it was shown that the key factor to have minimum protein adsorption is finding optimum grafting density of NMEG peptoids on the PSU hollow fiber

membranes where the optimal surface coverage of peptoid was dependent on the length and grafting density of the peptoids. Here in this study, the result demonstrated that although the longer brush lengths generally confer higher protein resistance, the hydration behavior is the determining factor to reduce fouling (by increasing the hydrophilicity of surfaces protein adsorption decreased).

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5. Chapter 5. Improved the biocompatibility of Membrane Oxygenators *Via* Peptoid Immobilization

Abstract

The biocompatibility of polysulfone (PSU) hollow fiber membranes was improved using surface modification for membrane oxygenator application. The modification was performed by grafting NMEG peptoid on PSU hollow fiber using polydopamine. Crystal Microbalance with Dissipation Monitoring (QCM-D) was conducted to confirm successful grafting of peptoid during the modification. Tensile strength data showed no significant changes for modified membranes compared to unmodified PSU membranes. The biocompatibility of PSU membranes was analyzed through protein adsorption using QCM-D method. Oxygen transfer rate was determined to evaluate the mass transfer properties of PSU hollow fibers. Results revealed that peptoid-grafted hollow fibers have higher oxygen transfer coefficient and less fouling compared to unmodified PSU hollow fibers. Oxygen flux was stable on peptoid modified surfaces even after 6 hours exposure to bovine serum solution where dropped around zero on unmodified surfaces. Overall, these findings suggest that the peptoid-grafted modified membranes can meet the requirement in membrane oxygenator devices.

5.1. Introduction

Lung transplantation represents the fastest growing category of organ transplantation, with a 44.6% increase in organs transplanted between 2006 and 2015 [1]. The lung waiting list has a median wait time greater than three months, and in 2015 12% of patients removed from list had died before a lung could be acquired [2]. Therefore, there is an essential need to use membrane oxygenators or artificial lungs. Polymeric materials are commonly used in biomedical areas such as artificial organs or medical devices [3, 4]. Bundles of polymeric materials such as porous

hollow fiber membranes composed of polysulfone (PSU) or polypropylene are commonly used in membrane oxygenators [3, 5]. In order to obtain sufficient CO_2 and O_2 gas exchange using membrane oxygenators, a large surface area of fibers is needed (roughly a membrane surface area of 2-4 m2 to 136-340 ml of blood) [6, 7].

However, putting these porous membrane surfaces in contact with blood for extended times allows for the build-up of protein and platelets, referred to as biofouling, which ultimately leads to a decrease in gas permeability and frequent replacement of the membranes [3, 8]. Therefore, extracorporeal membrane oxygenator is expensive and is generally used for short times, up to two weeks [8]. One commonly used method to decrease the formation of thrombus is heparization, which reduces blood coagulation [3]. While administration of heparin prevents platelet adhesion, it does not reduce protein adsorption [3] and continuous exposure places patients at significant risk of catastrophic bleeding, responsible for significant patient mortality [9].

A wide variety of surface modification methods have been used to reduce biofouling of membranes; however, there have been relatively few studies with hollow fiber membranes for gas exchange performance [3, 6]. Studies showed that covalently immobilization of carbonic anhydrase or grafted siloxane onto hollow fiber surfaces via plasma polymerization technique improved the biofouling of hollow fiber [6, 10, 11]. However, modification of surfaces by plasma treatment may be harmful to membrane and difficult to control [12]. Another method to improve biocompatibility of hollow fibers is to coat with a cell outer membrane mimetic 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymers containing 3-(Tri- methoxysilyl) propyl methacrylate (TSMA) and/or n-butyl methacrylate (BMA) units, poly(MPC-co-BMA-co-TSMA) and poly(MPC-co-BMA). They have improved hemocompatibility of hollow fibers

through cosslinkable phospholipid polymer coating with no negative effect on oxygen transfer efficiency [3]. Another group used a simple thiol-end radical polymerization and a reversible addition−fragmentation chain transfer polymerization technique to modify hollow fibers surface by covalent coupling of carboxyl functionalized zwitterionic without detrimental effects on gas transfer capacity. The study showed that modified membranes effectively improved thromboresistance and maintained a high gas exchange rate in bovine blood compared to unmodified membranes [13]. Therefore, an effective strategy to improve biocompatibility of membrane surface is surface modification using an antifouling polymer. Antifouling polymers can be introduced via different methods including physical attachments or covalent bonding interactions. In physical grafting or surface coating antifouling polymeric coatings may be easily washed away during application and the adsorbed polymer may increase the resistance of membranes and then oxygen permeability may drop [14]. Although grafting methods may creat a robust coating of antifouling polymer to the membrane surface generally, grafting antifouling polymers may take place under severe condition such as alkaline treatment, plasma treatment, ultraviolet (UV) irradiation and O_3/O_2 pretreatment. All these chemical modifications may change the membrane surface properties permeability and stability [15, 16].

Most antifouling polymers are extremely hydrophilic and have high solubility in water, making it difficult to attach them to a surface. Unfortunately, most polymeric membranes have inert surfaces and are difficult to be modified. The lack of an efficient and inexpensive attachment method has been a major problem for practical implementation of low fouling coatings [17]. Messersmith et al found that dopamine under alkane conditions spontaneously self-polymerizes to form a polydopamine (PDA) coating on nearly any substrate [18]. Previously we attached a peptoid with 2 methoxyethyl (NMEG5) side chains onto PSU hollow fiber via PDA molecules.

PDA underwent oxidation under alkane condition; afterward peptoid amine terminus covalently reacted with catechol groups of PDA and attached to PSU fibers. Static fouling tests revealed that NMEG5 modified membranes improved biocompatibility of hollow fiber membranes compared with PSU and PSU-PDA coated surfaces. Due to NMEG5 modified membranes showed the low fouling nature and improvement in biocompatibility of membranes, they have the potential applicability in biomedical fields for artificial organs such as membrane oxygenators [19]. Herein, we attached NMEG5 to PSU hollow fiber surfaces to improve biocompatibility of membrane oxygenators. The antifouling performance of the unmodified and modified membranes were evaluated in dynamic environments. The effect of PDA and NMEG5 peptoid modified hollow fiber surfaces on oxygen exchange and biocompatibility of PSU hollow fibers was investigated while exposed bovine serum albumin.

5.2. Materials and Methods

5.2.1. Materials

MBHA rink amide resin was purchased from NovaBiochem (Gibbstown, NJ). Piperidine, bovine serum albumin, N,N-dimethyacetamide (DMAc, ≥99%), Trizma base (primary standard and buffer, ≥99.9% [titration]), and dihydroxyphenethylamine (dopamine) hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). Ammonium hydroxide (ACS, 28.0-30.0% NH_3) was purchased from Alfa Aesar (City, State). Hydrogen peroxide (30%) is purchased from BDH chemicals. Phosphate Buffered Saline (PBS, 100ml) tablets and bovine serum albumin were purchased from Amresco. Hydrochloric acid (HCl, certified ACS Plus, 36.5 to 38.0% w/w) and the quartz crystal sensors (Q-sense, QSX 301, Gold) were purchased from Fisher-Scientific and Biolin Scientific, respectively. PSU pellets (average MW ~35,000) were obtained from Nanostone (Oceanside, CA). Epoxy Epon Resin 828 and Epikure glue 3030 were purchased

from Hexian (Houston, TX). All other reagents and materials were purchased from VWR. Ultrapure water was purified with a minimum resistivity of 18.2 MΩ cm, using a NANOpure Diamond™ Life Time system (Barnstead/Thermo scientific, Essex, United Kingdom). All reagents were of analytical grade and used without further purification unless otherwise noted.

5.2.2. Membrane Fabrication

PSU hollow fiber membranes were fabricated using a ubiquitous dry-jet wet spinning process and a custom-built spinning apparatus (Figure 5.1), as previously described [19]. Briefly, PSU pellets (35 kDa) were dissolved in N-methyl-2-pyrrolidone (NMP) for three days at ambient temperature to form a homogenous PSU solution. Subject to nitrogen gas pressure, the PSU dope solution (17.8 wt% PSU in NMP) and a bore solution (15 vol% NMP in water) were extruded through a stainless-steel spinneret (AEI, Inc.; City, State) with 0.8 mm inner diameter and 1.6 mm outer diameter. Contact of nascent dope and bore solutions with a water bath resulted in precipitation of the membranes by nonsolvent-induced phase separation. The condition to prepare PSU hollow fiber is presented in Table 5.1. After solidification, fibers were removed from the coagulation bath and stored for three days in 5 w/v % aqueous NaCl, with solution exchange once per day. To prevent bacterial growth, hollow fibers were stored in 25 w/v% glycerol with 0.2 v/v% sodium benzoate at 5 °C.

Table 5. 1 Spinning conditions for preparation of PSU hollow fibers

PSF dope solution (wt.%)	PSF/NMP (17.8/82.2)
Bore fluid (v.%)	NMP/Water (15/85)
Dope extrusion pressure (PSI)	1
Bore extrusion pressure (PSI)	1
Air gap (cm)	8
Take-up speed (m/min)	2.3
External coagulant	Tap water
Coagulant temperature (°C)	24
Dope temperature (°C)	27
Bore temperature (°C)	27
Ambient temperature (°C)	24.6
Humidity (%)	45
Spinneret dimensions (mm)	0.8 I.D./1.6 O.D.

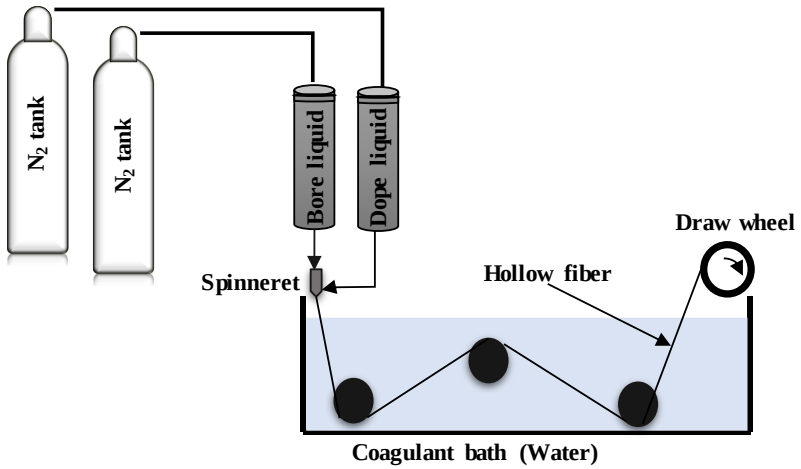

Figure 5.1. Schematic of the spinning apparatus for hollow fiber membrane fabrication

5.2.3. Peptoid Synthesis and Purification

Peptoids were synthesized via a submonomer protocol [20] on rink amide resin, as previously described [19]. Briefly, the resin was swelled with dimethylformamide (DMF) and the Fmoc protecting group was removed using 20% piperidine in DMF. The submonomer cycle begins with addition of 1.2 M bromoacetic acid in DMF in the presence of N, N'-diisopropylcarboniimide at a ratio of 4.3:1. Side chains were added by incubation with 0.5 M methoxyethylamine in NMP for 5 minutes. This cycle was repeated until the desired sequence was achieved. Peptoid was cleaved from the resin with a mixture of 95% trifluoroacetic acid (TFA), 2.5% water, and 2.5% triisoproylsilane for five minutes. The resin was filtered from the peptoid solution, TFA was removed using a Heidolph Laborota 4001 rotating evaporator (Elk Grove Village, IL), and the peptoid was diluted to a final concentration of ~3 mg/mL in a 25:75 solution of acetonitrile: water.

The peptoids were purified by preparative reversed-phase high performance liquid chromatography (HPLC; Waters Delta 600, Milford, MA) with a Duragel G C18 150 × 20 mm column (Peeke Scientific, Novato, CA) using a linear gradient of 0-65% solvent B in A (solvent A: water, 0.1% TFA; solvent B: acetonitrile, 5% water, 0.1% TFA) at room temperature over 60 minutes. Final peptoid purity was confirmed to be greater than 98% by analytical reversed-phase HPLC (Waters 2695 separations module) with a Duragel G C18 150 × 2.1 mm column (Peeke Scientific) and a linear gradient of 5 to 95% solvent D in C (solvent C: water, 0.1% TFA, solvent D: acetonitrile, 0.1% TFA) over 30 minutes at room temperature. The molecular weight of the peptoid was confirmed by matrix-assisted laser desorption/ionization mass spectrometry. Purified peptoid solutions were lyophilized (Labconco lyophilizer, Kansas City, MO) and stored at -20 °C prior to use.

5.2.4. Surface Modification of PSU Hollow Fiber Membrane

NMEG5 peptoid was attached to PSU fibers via polydopamine (PDA) as previously described [19]. PSU membranes were immersed in ethanol for 30 minutes and rinsed with ultrapure water. The membranes were immersed in fresh PDA solution at room temperature in the presence of oxygen for 3 hours. PSU-PDA membranes were washed with ultrapure water and incubated with 0.5 mg/ml peptoid in phosphate buffered saline (PBS, pH=7.4) at 60 ºC for 24 h. The peptoid modified membranes (PSU-PDA-NMEG5) were washed with ultrapure water to remove any unreacted peptoid and dried with nitrogen gas before storage.

5.2.5. Water Contact Angle

Surface hydrophobicity of hollow fibers was investigated by measuring water contact angle using the sessile drop method (OCA 15, DataPhysics Instruments GmbH, Filderstadt, Germany). Briefly, a drop of deionized water (1 μL) was formed at the tip of a needle and lowered to contact the HFM surface. DataPhysics SCA software was used to determine the contact angle. Contact angles were measured 10 times across the fiber surface. The measurements of water contact were performed at ambient conditions (25ºC and 50% relative humidity). All measurements were repeated three times.

5.2.6. Mechanical Properties

The mechanical properties of unmodified and modified PSU hollow fiber were measured membranes before and after exposure to bovine serum albumin using a uniaxial mechanical testing device (5994, Instron, Norwood, MA) at ambient temperature and humidity. Tensile stress at break, elongation at break and tensile modulus were measured to indicate the

mechanical strength of the fibers and the degree of deformation that could be expected under a given load.

The N_2-dried samples were deformed at a constant strain rate of 10% min^{-1} until failure using a 1N load cell while load and displacement values were recorded at 10 Hz prior to testing. Fiber diameters measured via calibrated microscopy software. All measurements were repeated three times.

Tensile strength at break was calculated using equation 5.1 (as the ratio of the breaking force divided to the cross-sectional area of the fiber):

$$Tensile\ strength\ at\ break = \left(\frac{F}{\pi(r_0^2 - r_1^2)}\right) \quad (5.1)$$

Young modulus was calculated as the ratio of the tensile strength at yield point divided to the strain (equation 5.2).

$$E = \frac{\sigma(yield\ point)}{Strain(in\ elastic\ deformation)} \quad (5.2)$$

The breaking tensile elongation was calculated as the ratio of the elongated length (ΔL) to the original length of the fiber (L_0) (Equation 5.3)

$$\delta = \left(\frac{\Delta L}{L_0}\right) \quad (5.3)$$

5.2.7. Measuring the amount of PDA and NMEG5 peptoid on PSU

The adsorption of PDA and NMEG5 peptoid to PSU membrane were performed using Quartz-Crystal Microbalance with Dissipation Monitoring (QCM-D; Q-Sense E4, Biolin Scientific). QCM-D is a nanogram sensitive instrument used for analyzing changes in the adsorbed mass and the viscoelasticity of the adsorbed layer happening at the surface in real-time [21]. Before coating the sensors with PSU, AT-cut piezoelectric quartz crystals with a 14 mm diameter and a fundamental resonant frequency of 4.95 MHz were treated with UV/ozone for 10 minutes. After that, sensors were dipped in a hydrogen peroxide and ammonium hydroxide solution (1:1:5 H2O v/v/v) at 348 K for 5 minutes. The sensors were then thoroughly washed with 18.2 MΩ deionized water and dried using N_2 gas. Finally, the sensors were treated again with UV/ozone for 10 minutes. The clean sensors were then spin coated with 1% (w/v) PSU dissolved in DMAc at 1000 rpm for 30 seconds. Spin coated layer was allowed to evaporate at room temperature for 1 hour.

The QCM-D used in this study is the Q-sense E4 (Biolin Scientific) system with 4 flow modules. Each module has approximately 140 µL internal volume. Before modification of PSU layers with PDA, sensors were equilibrated by establishing a baseline at 0 Hz using deionized water for 10 minutes at a flow rate of 100µL/min. Then, 10 mM Tris Buffer at pH = 8.5, titrated with 0.1 M HCl, was fed to the system for 10 minutes at the same speed. Later, 1 mg/ml of dopamine hydrochloride solution dissolved in 10 mM Tris buffer was flown across the sensors for 3 hours. After this step, the system was rinsed with 10 mM Tris buffer, deionized water and 10 mM PBS solution, for 10 minutes for each solution, respectively. After the rinsing step, NMEG5 peptoid was fed to the system. As soon as the peptoid started entering the flow chamber, the pump was

stopped and the change in frequency was recorded for 1 hour. Later, the flow chamber is washed with 10 mM PBS buffer for 10 minutes.

5.2.8. Measuring the Amount of BSA

The sensors that were coated with PDA and peptoid in the experiment described above are utilized to conduct fouling experiments using QCM-D. Bovine serum adsorption is chosen as the model foulant to study the fouling on these membranes. The baseline is re-established at 0 Hz for 2 minutes by flowing 10 mM PBS at 100 μL/min to not to exceed the sensitivity limit of the device during BSA adsorption. Then, 1 mg/ml of BSA dissolved in 10 mM PBS was fed to the system with a flow rate of 100 μL/min for 1 hour. Finally, the system was washed with 10 mM PBS solution at a flow rate of 100 μL/min for 10 minutes.

QCM-D output frequencies can be converted to adsorbed mass per unit area using the Sauerbrey equation 5.4 if the adsorbed mass is rigid and much smaller than the mass of the sensor itself. It relates the change in frequency (Δf) to change in mass (Δm), where n is the overtone number (n = 1, 3, 5, 7,.) and C is the mass sensitivity constant which has the value of -17.7 Hz ng/cm2 for a 5 MHz crystal [21].

$$\Delta m = -\frac{C}{n}\Delta f \tag{5.4}$$

In this study, the 7th overtone is used to calculate the adsorbed mass per unit area in ng/cm^2.

5.2.9. Oxygen Gas Transfer Rate Measurements

The experimental set-up for measuring oxygen gas transfer is shown in Figure 5.2. Each circuit contained a peristaltic pump, a membrane oxygenator chamber, connectors, and oxygen probe. Hollow fiber samples were fixed in polyvinyl chloride (PVC) tube test modules (n=5, 30 cm

length), in which both end of fibers were occluded with glue (mixture of Epikure 3030 and Epon 828). PVC tube were sealed using the glue at the both ends, causing the gas in the tube to permeate through the fibers. The lumen of the fibers at the outlet was connected to a gas outlet. The inlet of the PVC tube was fixed to an oxygen tank. All 5 fibers were places in parallel in the chamber for the test at ambient environment. The chamber was submerged in water bath at 37 °C. Bovine serum solution with concentration of 35 mg/ml in PBS were used for testing. Whole circulating loops were filled with bovine albumin solution and circulate at 88 ml/min using a peristaltic pump (Master Flex, Vernon Hills, IL). Nitrogen gas used to remove oxygen from bovine serum solution. Dissolved O_2 in the reservoir automatically recorded via an O_2 probe after different oxygenation times while bovine serum solution is circulating into the loops. To measure oxygen gas transfer equation 5.5 was used.

$$kLa = -\ln\frac{(C - C_0)}{C^* - C_0} \tag{5.5}$$

Where kLa is the volumetric oxygen transfer coefficient, C is the instantaneous dissolved O_2 concentration, C* is the liquid-vapor equilibrium O_2 concentration, and C_0 is the initial O_2 concentration.

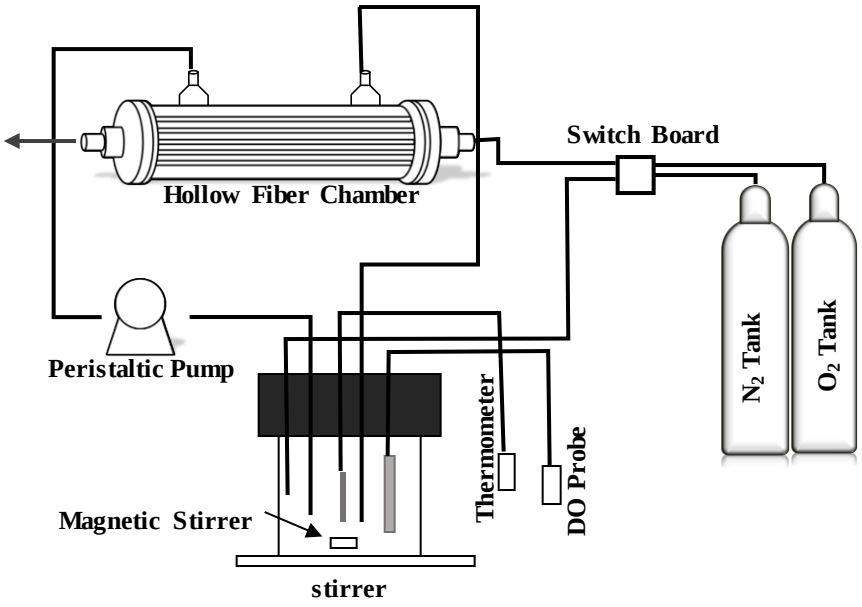

Figure 5.2. Experimental circulating loop for gas exchange test.

5.2.10. Statistical Analysis

All data were expressed as means ± SD. Data were evaluated by one-way ANOVA to assess any significant differences between groups. Moreover, student's t-test assuming equal sample variance with the least significant difference test was used to determine p-values and assess any statistically differences between two groups of samples. Differences were considered statistically significant when $p < 0.05$.

5.3. Results

NMEG5 peptoid can be named as effective antifouling polymer without any biodegradability problems. Peptoids are a class of biomimetic molecules that mimic peptide with the side chains attached to the amide nitrogen, rather than the α-carbon [22]. This change in side chain position

result in several backbone alteration that cause peptoids to resist protease degradation and increase biostability compared to peptides and make them promising candidate for biomedical applications [23]. NMEG5 peptoid has all the features previously found to resist biofouling. NMEG5 sequences are polar but uncharged, hydrophilic, contain hydrogen bond acceptors but not hydrogen bond donors. Moreover, NMEG5 has flexible backbone and high-water solubility to help reduce protein adsorption [24-26]. Studies have shown that a PEG-mimetic peptoid side chain, NMEG, has good antifouling properties [19, 26-28]. We previously successfully attached NMEG5 to PSU hollow fibers via PDA and significantly reduced fouling of bovine serum albumin, lysozyme, and fibrinogen [19].

5.3.1. Quantitative Analysis of NMEG5 Peptoid Surface Density

The amount of peptoid attached on the PDA coated surfaces was achieved using QCM-D method. XPS data could be used to calculate the grafting density of peptoid; however, since peptoid and PDA contain the same atoms, the grafting density of peptoid cannot be easily determined from XPS data [29]. To measure the amount of peptoid at first a monolayer adsorption of PDA onto PSU surface was accurately determined. PDA adsorbed on the PSU coated crystal for 3 hours. Figure 5.3A shows the frequency versus time graph; where each step of the process at the top and the two different adsorption regimes as dashed red and green lines were labeled. This graph suggests that the monolayer coverage is completed by 65 minutes of the experiment. Given that the initial rinsing steps takes 20 minutes, the monolayer coverage will be achieved by running 45 minutes of 1 mg/ml dopamine hydrochloride dissolved in 10 mM Tris HCl. Next, the adsorption of 0.5 mg/ml peptoid solution dissolved in 10 mM PBS on the PDA-PSU crystal covered was studied. The result shows that 665.58 ng/cm^2 NMEG5 peptoid is

adsorbed onto PSU-PDA after 1 hours. The adsorption rate of PDA and NMEG5 peptoid are shown in Figure 5.3B.

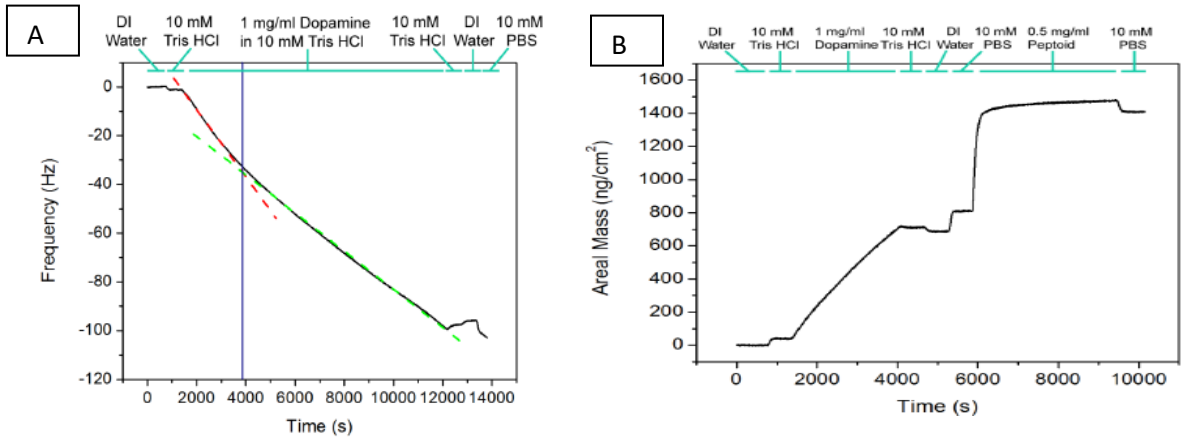

Figure 5.3 .A. Frequency change of QCM chips covered with PSU in dopamine hydrochloride/Tris Hcl buffer solution. Red and green dashed lines represent the two adsorption regimes monolayer and multilayer, respectively. Monolayer coverage is achieved by 65 min. B. The adsorption rate of PDA and peptoid.

5.3.2. Bovine Serum Albumin Adsorption

To evaluate protein adsorption onto solid surfaces a wide variety of techniques have been used such as optical methods including surface plasmon resonance [30], optical waveguide lightmode spectroscopy (OWLS) [31] and ellipsometry [32] provide high quality data. However, these methods are limited by the surface properties for example just highly transparent surfaces can be used in OWLS. Alternatively, a wide range of surfaces can be tested by QCM-D method which is an acoustic technique [33].

Here, the rate of bovine serum adsorption (1 mg/ml bovine serum dissolved in 10mM PBS, 1 hr) on PSU, PSU-PDA and PSU-PDA-NMEG5 peptoids layers were studied. As it can be seen at Figure 5.4 the highest rate of adsorption is observed on unmodified PSU layer compared to PDA and NMEG5 modified surfaces. Especially, the rate of adsorption of bovine serum on NMEG5

modified layers is the lowest suggesting that there is higher potential for antifouling behavior compared to PSU and PSU-PDA layers.

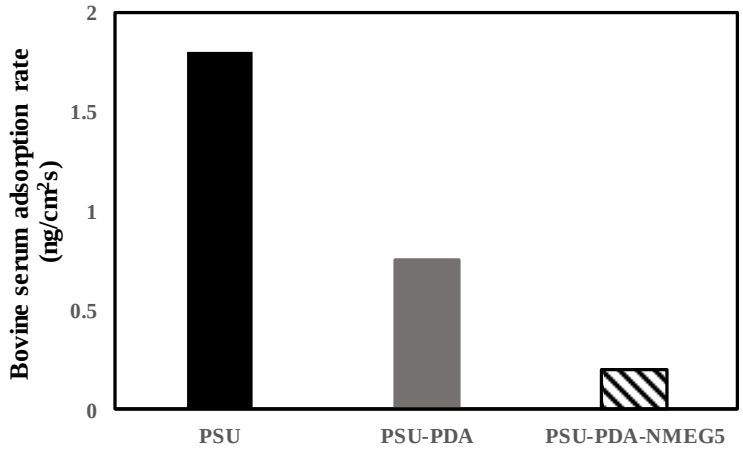

Figure 5.4. Bovine serum adsorption rate for PSU (black), PSU-PDA (gray) and PSU-PDA-NMEG5 peptoid (stripes) surfaces after 1 hr exposure.to bovine serum albumin solution.

To study the effect of bovine serum albumin on antifouling behavior of peptoid modified layers (0.5 mg/ml in PBS), three different bovine serum concentrations were selected (0.25 mg/ml, 0.5 mg/ml and 1mg/ml in PBS). Figure 5.5 shows that the amount of bovine serum adsorption is almost the same for 0.5 mg/ml and 0.25 mg/ml bovine serum albumin concentrations (~ 280 ng/cm^2). However, the adsorption of bovine serum with the concentration of 1 mg/ml onto PSU-PDA-NMEG5 peptoid shows a huge difference compare to 0.25 mg/ml and 0.5 mg/ml bovine serum concentrations, while the peptoid concentration is 0.5 mg/ml in PBS.

Moreover, the effect of different concentrations of NMEG5 on fouling behavior of membranes were investigated. The Figure 5.6 indicated that at 1 mg/ml bovine serum concentration there is

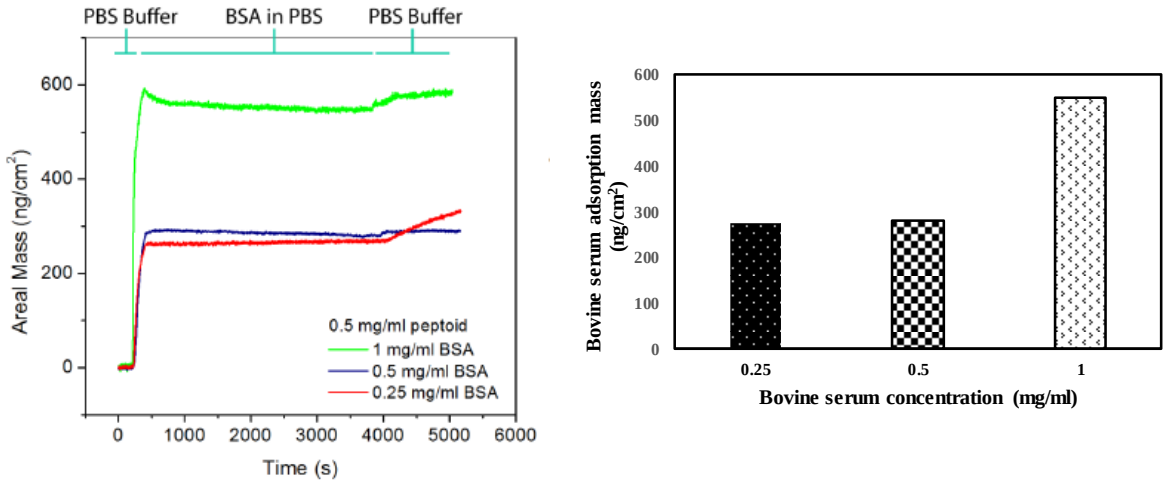

Figure 5 .5. Total mass adsorption of Bovine serum on peptoid-modified layer for .0.25 mg/mL, 0.50 mg/mL and 1 mg/mL of BSA solution concentration from QCM-D measurement.

no difference at different peptoid concentration while at 0.5 ml/ml bovine serum concentration

peptoid with 0.5 mg/ml concentration shows lower amount of bovine serum adsorption.

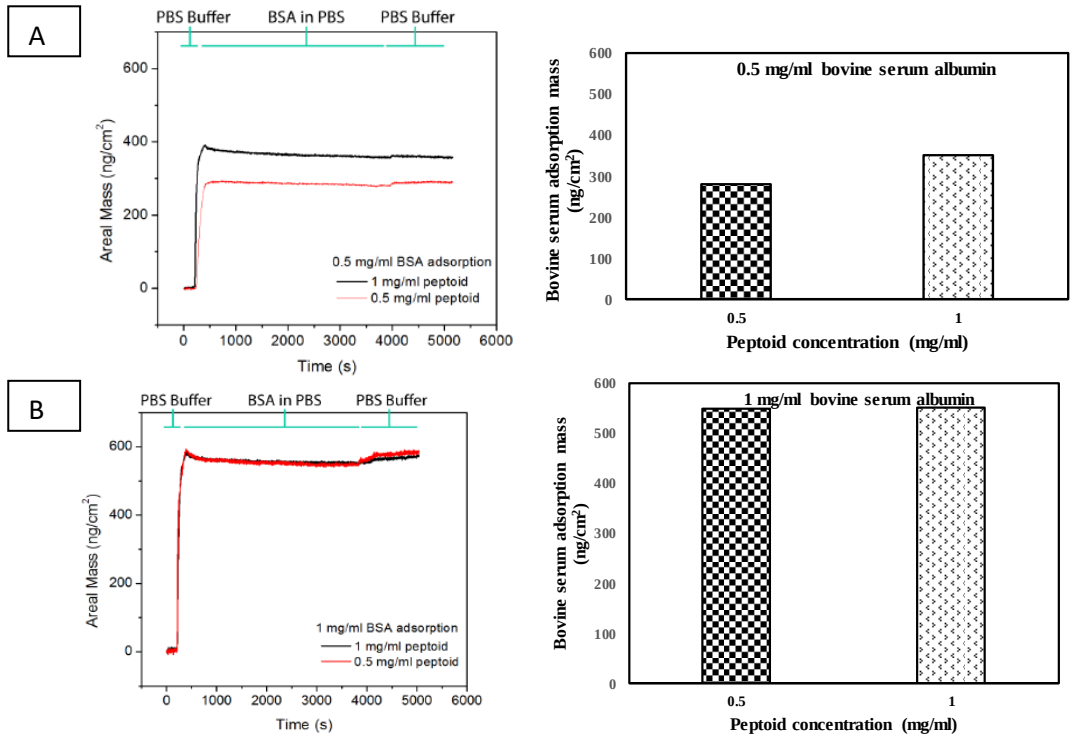

Figure 5.6. Total mass adsorption of Bovine serum on peptoid modified layer for 0.50 mg/mL and 1 mg/mL peptoid concentration at 0.5 ml/ml BSA concentration from QCM-D measurement. B. Total mass adsorption of Bovine serum on peptoid modified layer for 0.50 mg/mL and

5.3.3. Mechanical Property Measurements

To evaluate the mechanical propertied of the modified membranes, the tensile strength, Young`s modulus and elongation at break of unmodified and modified hollow fibers before and after exposure to bovine serum albumin solution (35 mg/ml concentration in PBS) were measured and the data are presented in Table 5.2. The tensile strength of the PSU-PDA-NMEG5 membranes is in the range of 7.8 MPa, which is approximately the same as the unmodified PSU membranes (8.7 MPA). A decrease in the Young`s modulus after modifying the surface with PDA and NMEG5 showed that the mechanical properties of the hollow fiber membranes were influenced. The Young`s modulus of PSU hollow fiber found a statistically significant difference from NMEG5 modified fibers, indicating an increase in flexibility of fibers after NMEG5 was added. Compared to unmodified PSU hollow fibers, the percent elongation was increased ~50.6% for NMEG5 modified membranes attributed to an increase in ductility of the fibers. Furthermore, it can be observed that after bovine serum adsorbed onto the surfaces of all types of hollow fibers there is no significant change in tensile strength, Young`s modules and elongation at break of native and fouled membranes.

Table 5.2. Mechanical properties of hollow fibers.

Membrane	**Tensile strength (MPa)**	**Young's modulus (MPa)**	**Elongation at break (%)**
PSU	8.9±1	231±8.4	33.9±7.6
PSU-PDA	8.5±1.8	207±16	36.3±10.6
PSU-PDA-NMEG5	7.8±1.9	184±15	50.6±2.86
PSU- fouled with BSA	7.8±1	220±11	26±5.3
PSU-PDA -fouled with BSA	9.6±1.2	214±2.1	26±2
PSU-PDA-NMEG5-fouled with BSA	8±2.2	175±15	46±10

5.3.4. Oxygen Gas Exchange Rate Measurements

The Figure 5.7 shows the O_2 gas transfer coefficient through PSU, PSU-PDA and PSU-PDA-NMEG5 hollow fibers by using bovine serum albumin solution. The concentration of bovine serum was selected 35 mg/ml which is similar to albumin concentration in blood [34]. NMEG5 modified hollow fibers showed slight increase in oxygen gas transfer rate compared with unmodified and PDA coated hollow fibers. The experiment times up to 6 h indicated that NMEG5 modified membranes retained the excellent oxygen gas transfer transmission properties while unmodified PSU hollow fibers showed a sharp decreased in oxygen transfer coefficient with negligible oxygen transfer after 3 hours exposure to a continuous flow loop of bovine serum solution. These finding demonstrated that the PSU-PDA-NMEG5 membranes could function as a suitable membrane oxygenator to pass oxygen through membranes in clinical applications.

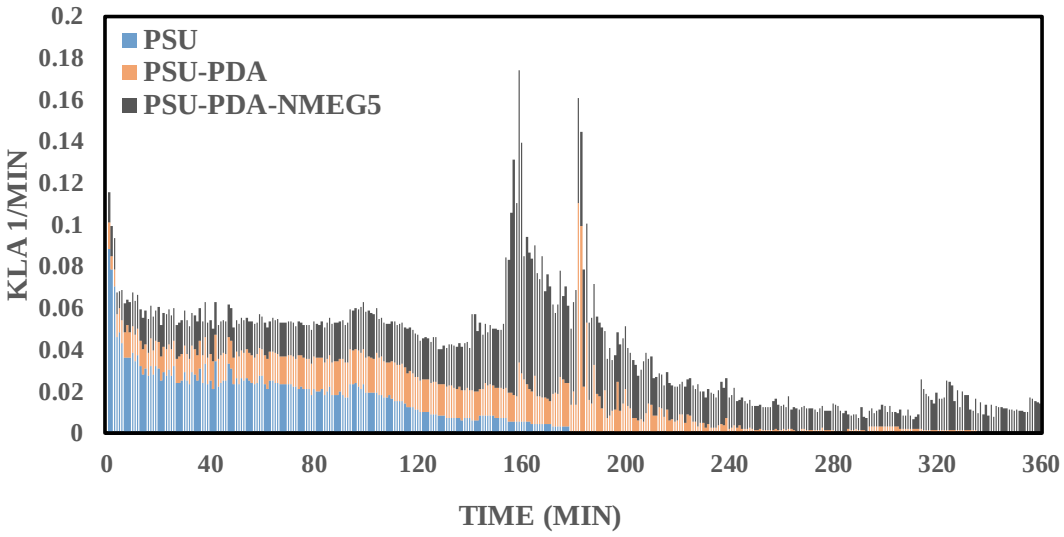

Figure 5.7. Oxygen transfer coefficient rate versus time

5.4. Conclusions

In conclusion, the impact of peptoid-modified hollow fibers on both biocompatibility and oxygen gas transfer from bovine solution were assessed. PSU hollow fibers were successfully modified with peptoid using polydopamine. Peptoid modified surfaces had significantly less bovine serum adsorption rate compared to unmodified and PDA-coated surfaces. Moreover, the mechanical properties data showed that peptoid modified hollow fibers have stability in physical properties. These finding suggest that the modified NMEG5 peptoid hollow fibers demonstrated a potential for biomedical application in membrane oxygenators. However, further studies are required to improve the biocompatibility of hollow fibers with higher gas exchange rate. If successful, the peptoid modified hollow fibers could be used in membrane oxygenators to improve respiratory support device ability to treat patients with severe respiratory failure.

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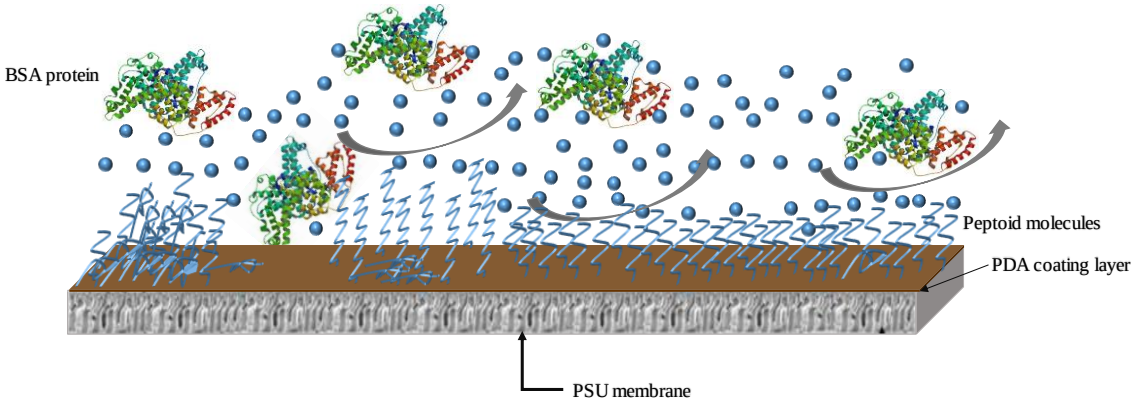
6. Chapter 6. Peptoid Functionalization of Polysulfone Ultrafiltration Membrane with Improved Antifouling Property and Blood Compatibility using Polydopamine

Abstract

Development of antifouling membranes to minimize nonspecific protein adsorption is important in various biomedical applications. In the present study, electrically neutral NMEG peptoids containing 2-methoxyethyl as side chains were attached onto polysulfone (PSU) membrane using polydopamine. A series of membranes containing NMEG peptoids with varying length (NMEG5, NMEG10, NMEG15 and NMEG20) were synthesized and attached onto PSU surface in order to improve surface antifouling performance. The effect of surface roughness, hydrophilicity, hydration capacity and electrical neutrality on antifouling behavior of membranes were determined. NMEG peptoids presented a high hydrophilicity and hydration capability compared to unmodified membranes. The antifouling performance of membranes was evaluated using bovine serum albumin filtration test. According to the cross-flow filtration results, NMEG modified membranes showed a significant improvement in antifouling ability. Furthermore, flux recovery ratios obtained from NMEG modified membranes were much higher than unmodified

membranes. These studies provide a convenient strategy to improve antifouling, hydrophilicity and hemocompatibility of PSU membranes for use in biomedical



6.1. Introduction

In recent years, polymeric materials are widely used to fabricate commercial microfiltration, ultra-filtration and nanofiltration membranes [1-3]. Chemical, mechanical stability and thermal resistance behavior of polymeric materials make them suitable candidate for different applications [4]. Polysulfone (PSU) is commonly used for the fabrication of microfiltration, ultra-filtration and nanofiltration membranes. PSU polymers have good advantages compared to other polymeric materials such as work in wide temperatures and pH limits, physiochemical stability, easy fabrication in a large variety of configuration and modules and wide range of pore sizes [5]. Despite its great promise, PSU has a key limitation of membrane fouling [6, 7]. Membrane fouling could decreases membrane flux either permanently or temporarily which can affect productivity, alter membrane selectivity, increased operating pressure, shorten membrane life, require intense chemical cleaning or frequent membrane replacement and significantly increase the operation cost by increasing osmotic pressure and circulating the feed solution [8,

9]. Membrane fouling generally can be classified into organic fouling, inorganic fouling and biofouling [10]. Among them, biofouling is considered as a serious fouling problem [11, 12]. To date, large number of methods have been employed to improve fouling behavior of membranes. They include hydrophilic surface modification by grafting, coating and blending methods [13-15]. Previous studies have shown that hydrophobic, highly charged and rough membranes exhibit a high intimacy in fouling while hydrophilic, electrically neutral and smooth membrane surfaces may foul less severely [16-21]. Therefore, several studies in membrane technology has been worked on the development of antifouling membranes [7, 13, 22-25]. In an effort to improve biocompatibility of membranes different factors should be considered such as, hydrophilicity, surface charge and surface roughness [26]. It is generally acknowledged that modification of surfaces with hydrophilic materials decreases protein adsorption since hydrophilic interface enables the minimizing of the interaction between membrane surface and proteins [12, 27]. Moreover, physical parameters such as roughness should be considered for example smoother dense surfaces usually result in lower protein adsorption [27, 28]. In this respect, many efforts have been made to enhance surface hydrophilicity of hydrophobic membranes. The common antifouling polymer to resist protein adsorption is poly (ethylene gycol) (PEG)-type materials [29, 30].

Studies showed that modified membranes could resist protein adsorption if the surface density and chain length of peptoid groups were controlled. Venault el al. were prepared modified poly (vinylidene fluride) membranes using zwitterionic diblock copolymers containing hydrophilic sulfobetaine methacrylate and hydrophobic propyleneoxide blocks via atom-transfer radical polymerization method. Membranes were modified using varying poly (sulfobetaine methacrylate) lengths. Protein adsorption tests evidenced that as the polymer amount absorbed

onto membrane surface increased, protein adsorptions (bovine serum albumin and lysozyme) decreased. zwitterionic diblock copolymers with shorter length (10 mer) of poly (sulfobetaine methacrylate) had the lowest protein adsorption, compared to 20 and 40 lengths of poly (sulfobetaine methacrylate) since uniform surface coverage can be reached using smaller copolymers. However, fibrinogen adsorption was the lowest in zwitterionic diblock copolymers with longest length of poly (sulfobetaine methacrylate) since steric hindrance is stronger and diffusion of higher molecular weight protein such as fibrinogen is not facilitated. Therefore, although zwitterionic with longer hydrophilic chains created cavities among themselves, fibrinogen could not diffuse into these cavities [27]. Song et al. proposed a novel zwitterionic organosilica monomer (zBPGH) and modified the membrane surface through sol-gel coating process. A uniform and smooth surface was achieved after modification of membrane the surfaces by organosilica xerogel coating with high hydrophilicity ability. Modified membranes exhibited stable antifouling and anti-bacterial behavior [12].

previously we showed that NMEG5 peptoid modified surfaces is regarded as an effective way to reduce protein adsorption of hydrophobic polymeric membrane [24]. To attach peptoid onto the membrane surface different methods can be used such as plasma treatment, UV-induced graft, radiation grafting technique, etc. Yet these methods are complex with a bad effect on the bulk properties of membrane materials [31]. Studies have shown that l-3,4 dihydroxyphenylalanine (DOPA) and its catecholic derivatives, for instance, 3,4- dihydroxyphenethylamine (dopamine) is can self-polymerize under mild conditions in presence of oxygen and adhere firmly to a variety of substrates such as metals, rocks, wood and polymers. Due to the strong adhesion behavior of polydopamine (PDA) a new and facile approach for surface modification of materials is put forward [32, 33]. In our recent work, it was found that peptoid could be

covalently attached on PSU hollow fiber membranes via PDA surface modification technique mentioned above [24].

In the present study, peptoids with varying length (5, 10, 15 and 20 mer) were synthesized and immobilized on PSU surface to modify flat sheet membranes. Then, the effect of different parameters including, hydrophilicity, hydration capacity, roughness surface charge on dynamic fouling were evaluated.

6.2. Materials and Methods

6.2.1. Materials

MBHA rink amide resin was purchased from NovaBiochem (Gibbstown, NJ). Piperdine, bovine serum albumin, and dihydroxyphenethylamine (dopamine) hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). PSU pellets (average MW ~35,000) were obtained from Nanostone (Oceanside, CA). All other reagents and materials were purchased from VWR and used without further modification unless otherwise noted. Ultrapure water was purified with a minimum resistivity of 18.2 MΩ cm, using a NANO pure DiamondTM Life Time system (Barnstead/Thermo scientific, Essex, United Kingdom). All reagents were of analytical grade and used without further purification.

6.2.2. Preparation and Purification of Peptoid

Peptoids were synthesized via a submonomer protocol [34] on rink amide resin, as previously described [24]. Briefly, the resin was swelled with dimethylformamide (DMF) and the Fmoc protecting group was removed using 20% piperidine in DMF. The submonomer cycle begins with addition of 1.2 M bromoacetic acid in DMF in the presence of N, N'-diisopropylcarboniimide at a ratio of 4.3:1. NMEG side chains were added by incubation with

0.5 M methoxyethylamine in N-methylpyrrilidone (NMP) for 5 minutes. This cycle was repeated until the desired sequence was achieved. Peptoid were cleaved from the resin using a mixture of 95% trifluoroacetic acid (TFA), 2.5% water and 2.5% triisoproylsilane for five minutes. The resin was filtered from the peptoid solution, TFA was removed using a Heidolph Laborota 4001 rotating evaporator (Elk Grove Village, IL), and the peptoid was diluted to a final concentration of ~3 mg/ml in a 25:75 solution of acetonitrile: water.

The peptoids were purified by preparative reversed-phase high performance liquid chromatography (HPLC; Waters Delta 600, Milford, MA) with a Duragel G C18 150 × 20 mm column (Peeke Scientific, Novato, CA) using a linear gradient of 0-65% solvent B in A (solvent A: water, 0.1% TFA; solvent B: acetonitrile, 5% water, 0.1% TFA) at room temperature over 60 minutes. Final peptoid purity was confirmed to be >98% by analytical reversed-phase HPLC (Waters 2695 separations module) with a Duragel G C18 150 × 2.1 mm column (Peeke Scientific) and a linear gradient of 5 to 95% solvent D in C (solvent C: water, 0.1% TFA, solvent D: acetonitrile, 0.1% TFA) over 30 minutes at room temperature. The molecular weight of the peptoids were confirmed by matrix-assisted laser desorption/ionization mass spectrometry. Purified peptoid solutions were lyophilized (Labconco lyophilizer, Kansas City, MO) and stored at -20 °C prior to use.

6.2.3. Preparation of PSU

The PSU membranes were prepared using a phase inversion technique as previously described [35]. 17.8 wt.% PSU were dissolved in NMP as a casting solution. Then mixture was stirred at 25 ºC for 24 h then allowed to rest for 8 h until the solution stopped bubbling. The degassed solution was cast onto a glass plate to form flat film. The casting films were immediately immersed into coagulation media in which there was a non-solvent pure water. In the

coagulation bath, solvent exchange every hour for the first four hours then changed every day for three days to remove solvents and form the polymer films to most of the solvent was removed.

6.2.4. Surface Modification of PSU Membranes

NMEG peptoids were attached to PSU fibers PDA as previously described [24]. Dopamine solution (0.5 mg/ml) was prepared by dissolving dopamine hydrochloride in Tris-HCl buffer solution (10 mM, pH 8.5). Circular pieces of PSU membranes (area of 13.8 cm^2) were immersed in ethanol for 30 minutes and rinsed with ultrapure water. Then membranes were immersed in fresh PDA solution and shaken at room temperature for 3 h. After that, PSU-PDA membranes washed with ultrapure water to remove most residual weakly bound PDA and incubated with 0.5 mg/ml peptoid concentrations in phosphate buffered saline (PBS, pH=7.4) at 60 ºC for 18 h (40-60 (chains/nm^2)). The peptoid modified membranes (PSU-PDA-NMEGs) were washed with ultrapure water to remove any unreacted peptoid and dried with nitrogen gas before storage.

6.2.5. Hydrophilicity of Membranes

To measure the surface hydrophilicity the static contact angle measurements of membranes were conducted at room temperature by contact angle goniometer (OCA 15, Data Physics Instruments GmbH, Filderstadt, Germany). For contact angle measurements the sessile drop technique was used and 1 µL of water was formed at the tip of a needle and lowered to membrane surface. Then using Data Physics surface contact angle (SCA) software the surface contact angle was calculated. Each reported value for contact angle represent an average value of ten separated drops on different positions of membrane and repeated three time on three different membranes. All water contact angle experiments were obtained at ambient laboratory condition.

6.2.6. Hydration Capacity of Membranes

Studies have shown that an effective way to reduce protein adsorption is to increase hydrophilicity of membranes, by reducing the hydrophobic interaction between membranes surface and proteins. The surface hydrophilicity can be measured using contact angle. However, when a porous membrane is considered rather than a dense media, surface hydrophilicity is not enough because proteins can still penetrate within the pores and interact with polymer inside the porous structure [36]. To evaluate the whole membrane hydrophilicity, hydration capacity can be used. Additionally, the capillary force causes the gradually penetration of water into the matrix so that it is not accurate to evaluate only the static water contact angle. We previously showed that grafting NMEG onto PSU surfaces result in a decrease of water contact angle [24]. As for hydration capacity, no evidence has been obtained so far related to the hydrophilicity of the whole PSU membranes thickness after modification by peptoids. Therefore, the hydration properties of the membranes can be assessed by evaluating their hydration capacity and their water contact angle. The hydration capacity was taken as the ratio of the difference in weight per unit surface area between the wet membranes and dry membranes after immersing in ultra-pure water for 24 h. Dry weighs of 4.2 cm diameter membranes were first recorded using a 10^{-5} g precision balance (Mettler, Toldedo). Subsequently, membranes were immersed in ultra-pure water for 24 h. Then, surface water was gently wiped out with kimwipe and placed in the closed container to weigh. For each membrane, five independent tests were performed, and the average value obtained taken as the final hydration capacity of the sample.

6.2.7. Surface Roughness

Atomic Force Microscopy (AFM) was used to measure the roughness of the membranes and the measurement was performed in the tapping mode in air atmosphere. A Bruker D3000 AFM with

Nanoscope III controller was used to obtain morphology of membranes. Probes with low stiffness (k = 8 N/m) were used in tapping mode to obtain images without modifying the soft surface.

6.2.8. Dynamic Antifouling Behavior

Permeation performance of pure water, PBS and protein solution were measured under a pressure of 15 psi at room temperature using a self-made cross flow flat apparatus, which consisted of a pump, reservoir (volume capacity of 500 ml), and a cross flow filtration cell with the effective filtration area of 13.8 cm^2 at room temperature. The membrane cell is made of stainless steel to resist high pressure (0-1000 psi) and flow rate was 100 ml/cm^2. Permeate was collected continuously and weighed automatically at the end of several periods. The balance was linked to a computer for automated data collection. In brief, membrane initial compacted with ultra-pure water for 15 min at 25 psi to obtain a stable permeation flux. Then, the operation pressure was lowered to 15 psi for 30 minutes to obtain the beginning pure water flux. After that PBS was running at 15 psi for 30 minutes and fallowed by bovine serum albumin (BSA) until BSA flux decreases to around 0 (mL/min cm^2), where the permeate flux is calculated by equation (6.1).

$$\text{Flux} = \frac{V}{A\Delta t} \tag{6.1}$$

Where V, A, and Δt are permeated volume (mL), membrane surface area (cm^2) and time (min).

6.2.9. Flux Recovery Rate (FRR)

Another way to evaluate biofouling property of membranes is using cyclic fouling. In present study, BSA was used as the representative of protein. The pressure was controlled. Typical three-

cycle filtration experiments [12] were conducted using a cross flow filtration system with BSA (5 mg/ml in PBS) as model foulants to investigate fouling performance of unmodified and modified membranes.

Prior to filtration, the membrane was pressurized with DI water at 25 psi for 30 min. This experiment mainly included three steps [37]: the pure water flux, Jw_0 (ml/ (cm^2 min)), was first measured at 15 psi pressure for 1 h. Then, the solution reservoir was emptied and refilled rapidly with BSA solution. Fouling was started by filtration with BSA solution for 60 minutes. After the measurement of BSA flux for 60 minutes, the reservoir solution (BSA solution) was emptied again and filled with water and the fouled membranes were washed for 30 minutes with ultrapure water. Afterward, water flux of cleaned membranes Jw_i (ml/ (cm^2 min)) was measured for 60 minutes at the end of one filtration cycle with aim to investigate the flux recovery rate. Flux recovery rate (FRR) in each filtration cycle was calculated in equation 6.2. Three sequential cycles of filtration tests were conducted to evaluate the fouling behavior of membranes. Higher FRR values of the membranes denoted a better antifouling property.

$$\text{FRR}\ (\%) = \frac{\text{Jw}_\text{i}}{\text{JW}_0} \times 100\% \tag{6.2}$$

Where J_{w0} denoted the initial pure water flux of membranes; J_{wi} denotes the stable flux at the last 10 minutes of water flux of fouled membrane after cleaning (i=1,2,3,).

6.2.10. Statistical Analysis

The data were presented as mean ± SD. Statistical analyses were performed using one-way analysis of variance (ANOVA) test and followed by a Tukey posthoc test. Single, double and

triple asterisk represent $p < 0.05$, $p < 0.01$, $p < 0.001$, respectively. A p value of <0.05 was considered statistically significant.

6.3. Results and Discussion

NMEG peptoid can be named as effective antifouling polymer without any biodegradability problems. Peptoids are a class of biomimetic molecules that mimic peptide with the side chains attached to the amide nitrogen, rather than the α-carbon [38]. This change in side chain position result in several backbone alteration that cause peptoids to resist protease degradation and increase biostability compared to peptides and make them promising candidate for biomedical applications [39]. NMEG peptoid has all the features previously found to resist biofouling. NMEG sequences are polar but uncharged, hydrophilic, contain hydrogen bond acceptors but not hydrogen bond donors. Moreover, NMEG5 has flexible backbone and high-water solubility to help reduce protein adsorption [40-42]. Studies have shown that a PEG-mimetic peptoid side chain, NMEG, has good antifouling properties [24, 42-44]. We previously successfully attached NMEG5 to PSU hollow fibers via PDA and significantly reduced fouling of bovine serum albumin, lysozyme, and fibrinogen [24].

6.3.1. Surface Roughness

AFM was used to evaluate the surface roughness of membranes. Mechanism of fouling is complicated and different mechanisms have been proposed including hydrogen bonding, hydrophobic interactions and π-π stacking [45]. Moreover, surface hydrophilicity, charge and roughness have an impact on protein adsorption. In the case of surface roughness, interaction between proteins increase with an increase in surface roughness; that is, proteins accumulate at the valleys of the rough membrane surfaces, after that valleys become blocked and fouling

becomes more severe for the rougher membrane surfaces and because of this reason, efforts are currently focused on the reduction of membrane roughness [21].

Three-dimensional images and the average roughness of PSU, PSU-PDA and PSU-PDA-NMEGs are shown in Figure 6.1. It was observed that the mean roughness (Ra) increased from 6.2 nm to 8 nm with PSU-PDA membranes due to the deposition of PDA nanoparticles on the membrane surface. Then Ra decreased from 8 nm to 3.6 nm after grafting NMEG5 onto the PSU-PDA surfaces, indicating more homogeneous surfaces. The roughness of all peptoids is quite the same, where is 3.6, 5.8, 4.6 and 4 nm for PSU-PDA-NMEG5, PSU-PDA-NMEG10, PSU-PDA-NMEG15 and PSU-PDA-NMEG20, respectively. The AFM images showed that the unmodified and PDA coated membranes had a surface with a larger area of ridge-valley structure, while NMEGs modified membranes seemed to be partially decreased in valley on the modified membranes; therefore, proteins could not adsorb in the “valleys”.

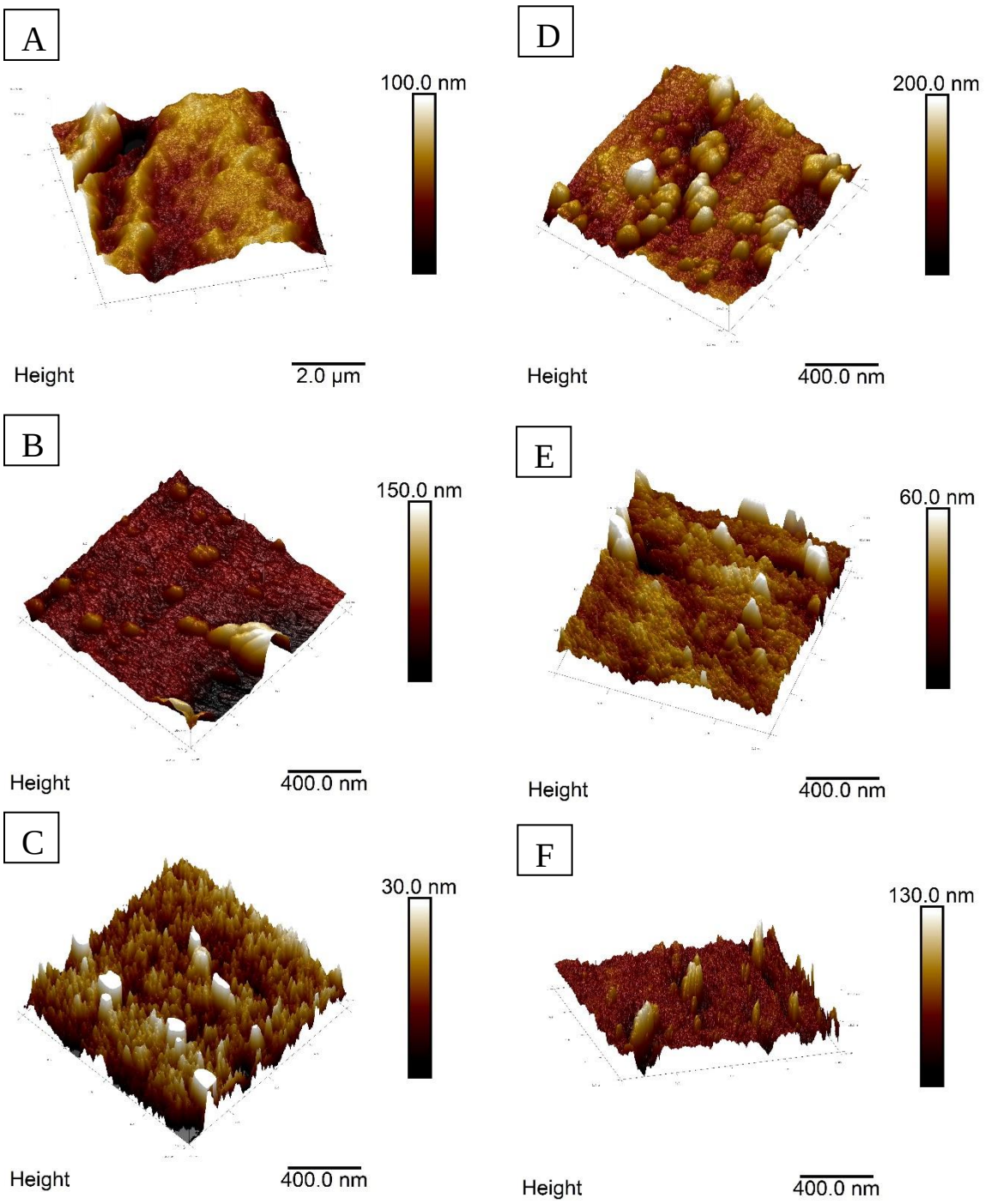


Figure 6. 1. AFM images of hollow fiber surfaces (A) PSU; (B) PSU-PDA; (C)PSU-PDA-NMEG5; (D) PSU-PDA-NMEG10; (E) PSU-PDA-NMEG15; (F) PSU-PDA-NMEG20

6.3.2. Hydrophilicity of Membranes

The membrane permeability and antifouling ability of membranes depends on the surface ability to be wetted [27]. The surface hydrophilicity of the PSU, PSU-PDA, PSU-PDA-NMEGs membranes were confirmed by static water contact angle measurements, as shown in Figure 6.2. According to the result, PSU membranes had a highest contact angle of 80 ± 5 corresponding the hydrophobic nature of PSU membranes, where this value decreased to 38.4 ±5 upon NMEG5 modified membranes. The contact angle value was found to increase to 45.3± 3 when NMEG side chain increase to 20 mer.

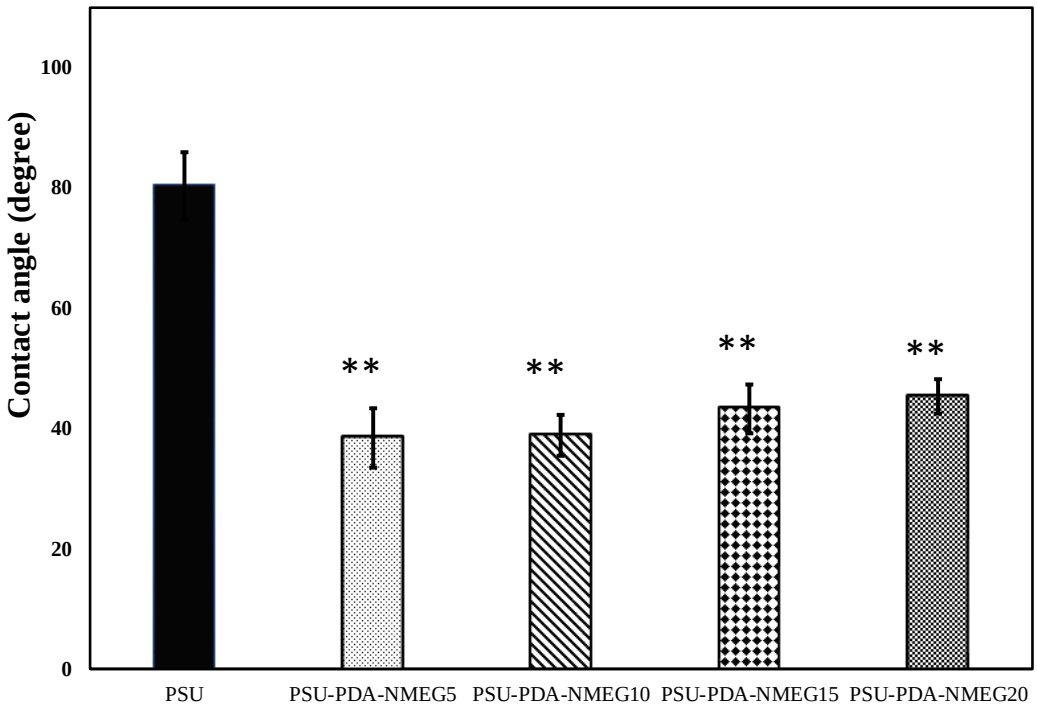


Figure 6.2. Contact angle measurements of PSU, PSU-PDA-NMEG5, PSU-PDA-NMEG10, PSU-PDA-NMEG15 and PSU-PDA-NMEG20 membranes Data are expressed as the mean ± standard deviation of three independent measurements. $p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$

6.3.3. Hydration Capacity of Membranes

The water contact angle depends on different factors such as surface roughness, porosity, pore size and distribution [12, 46] and also the contact angle measurement do not asses the hydrophilicity in the three dimensions so it is not enough to measure the hydrophilicity of the membranes [27]. Given this, hydration capacity was measured to evaluate the membranes hydrophilicity.

The peptoids length and flexibility can be associated with their capacity to capture water molecules. Figure 6.3 shows the hydration capacity of membranes (the difference in weight between the wet membranes and the dried one, divided by the total surface area of the membrane). It was clear that hydration capacity increased after modification the surface since unmodified PSU membranes almost has a limited hydration capacity due to its repulsion to water. Concerning modified membranes, an increase on hydration capacity from 1.2 ±0.4 for PSU to 3.6 ±0.9 was detected for PSU-PDA-NMEG5. Moreover, there is significant difference ($P<0.05$) between unmodified PSU and all peptoid side chain lengths, there is no significant difference between all peptoid although that hydration capacity decreased a little as side chain length increased. This increased in hydration capacity after modification surface with peptoids indicating that hydrophilic moieties of peptoids entrapped water molecules.

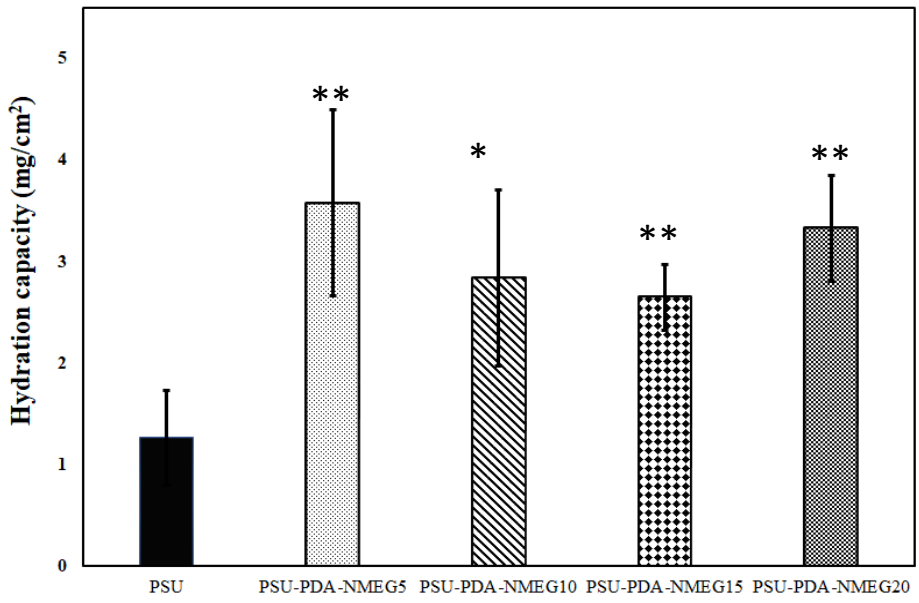


Figure 6.3. Hydration capacity of unmodified PSU and peptoid grafted PSU membranes.

6.3.4. Zeta Potential Measurements

The zeta potential of the prepared membranes measured from unmodified and modified PSU is shown in Table 6.1. The zeta potential, which determines the antifouling behavior of membranes, was increased by modification of membranes by NMEG peptoid. A membrane prepared by larger peptoid chain had a more positive zeta potential. value than pure membrane owing to the increase in the methyl groups. functional groups on the membrane surfaces. According to the results, the zeta potential values at pH=7 were -41.4±3.7 for PSU, -5.6±8.3 for NMEG5. As observed in the results, the PSU membranes showed a negative zeta potential than peptoid modified surfaces and peptoid surfaces the surface charge was almost neutral indicated no electrostatic interaction with blood proteins.

Table 6.1. Zeta potential data of PSU and peptoid grafted surfaces

Membrane	Zeta potential at =7
PSU	-41.4±3.7
NMEG5	-5.58333±8.3
NMEG10	5.153333±3
NMEG15	3.696667±0.6

6.3.5. Dynamic Antifouling Behavior

Protein adsorption on medical surfaces is counted as the first step of many undesired bio responses [27]. When membranes are in contact with proteins, the adsorption of proteins on the membrane surfaces could lead to sever membrane fouling and drastic flux decline [27]. BSA was selected as model protein to examine the antifouling properties of unmodified and modified membranes. The time-dependent normalized flux variations of PSU, PSU-PDA-NMEG5, PSU-PDA-NMEG10, PSU-PDA-NMEG15 and PSU-PDA-NMEG20 are presented in Figure 6.4.a. The BSA flux declined dramatically as a function of time for unmodified PSU membranes. The rate of flux decrease showed the higher fouling tendency happened by adsorption of BSA on the membrane pores and surface. The PSU-PDA-NMEGs modified membranes retained their fluxes well and showed the highest fluxes compare to the unmodified PSU membranes. This can be explained by the effort off peptoid, which improved membrane properties such as hydrophilicity, charge and morphology. Modifying the surface by peptoids promote the hydrophilicity of membranes and resistant to protein adsorption, and so decreasing biofouling and promoting BSA fluxes. In the first three-hour, severe membrane fouling caused by deposition and adsorption of BSA lead to drastic flux decline for unmodified membranes (by 82% flux reduction) while flux

decreased 38%, 60%, 57% and 49 % for PSU-PDA-NMEG5 and PSU-PDA-NMEG10, PSU-PDA-NMEG15 and PSU-PDA-NMEG20 respectively. Permeability measured for the membranes modified with NMEG5 is highest compared to that of other lengths since a decreasing of surface porosity probably occurs when the peptoid chain length become too long, moreover, the hydrophilicity of surface also decreased if side chain increased. When proteins adsorption and fouling happen, it can lead to pore narrowing and pore plugging and subsequently reduces the life span of membrane and declines the flux. The result showed that peptoid modified membranes exhibited more stable and higher resistance to BSA fouling than that unmodified membranes. This should be attributed to their different chemical and physical properties of immobilized surface.

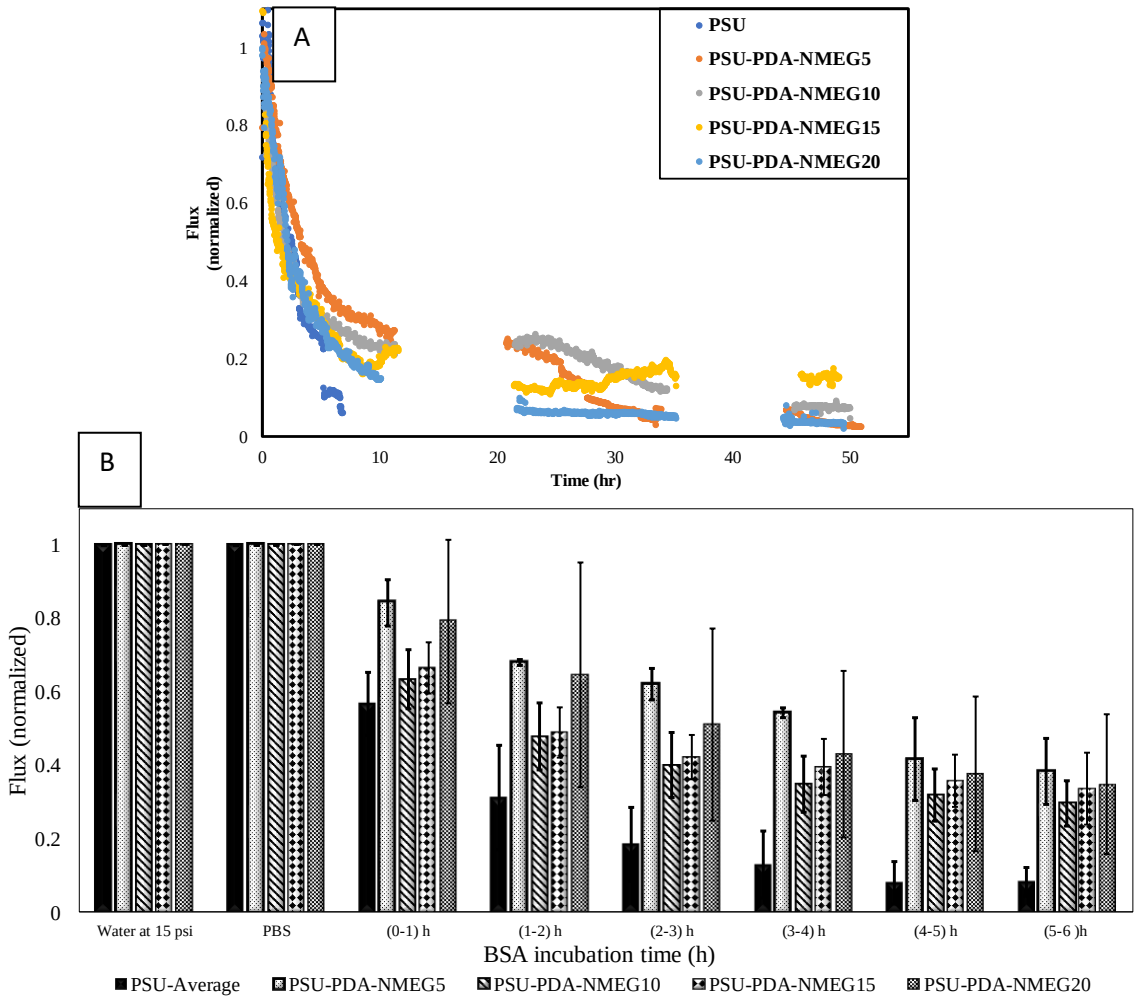


Figure 6. 4. Time-dependent normalized flux for unmodified and modified membranes (a). The normalized flux for the first 6 hours of BSA adsorption for PSU and peptoid modified membranes (b). The filtration process included three steps: pure water filtration, PBS

6.3.6. Flux Recovery Rate (FRR)

The FRR (%) was calculated to show the degree of irreversible flux to investigate the antifouling behavior of the evaluated membranes using typical three-cycle filtration test. BSA was used as model on unmodified and peptoid modified membranes (5 mg/ml in PBS). Figure 6.5 shows time dependent flux curves of unmodified and modified PSU membranes. Overall, comparing with initial pure flux, all membranes showed a rapid decrease on flux at the beginning of BSA solution filtration because of adsorption and deposition of BSA proteins. After one-hour

exposure of membranes to BSA solution, the fouled membranes were cleaning by water washing for 30 minutes; however, water flux were only recovered for irreversible fouling which caused by BSA adsorption on membrane surface and inside the pores. Finally, water flux was measured for one hour. Based on these flux data, the flux recovery rate was measured to calculate the fouling restorability. Here, antifouling protein ability was recognized as persistent of membrane to protein adsorption and temporal protein deposition, where the deposited proteins can be readily washed off by water washing was defined as reversible fouling and the adsorbed protein that cannot be removed by water washing was named the irreversible fouling [12].

After physical cleaning washing membranes with water, for the first cycle, varying FRR value rate (%) were obtained for all membranes where both peptoid modified membranes exhibited the highest FRR value of 76% and 66% for PSU-PDA-NMEG5 and PSU-PDA-NMEG20 membranes, respectively and the lowest value of 42% for unmodified membranes. These values indicated that the higher flux recovery was obtained by peptoid modified surface, higher FRR showed the higher reversibility of fouling and better antifouling ability. The fouling behavior of modified membranes was measured in subsequent cycle 2 and 3 to analyze their long-term antifouling behavior. As shown in Figure 6.5 FRR values of unmodified membranes were only 31% and 23% for cycle 2 and cycle 3, respectively, that was because of irreversible fouling induced by protein adsorption. The FRR values for all three cycle of unmodified PSU membrane indicated the severed irreversible fouling as well as the continuous fouling tendency.

For PSU-PDA-NMEG5 and PSU-PDA-NMEG20 only slight decline on FRR values observed cycle 2 (64% and 67%) and cycle 3 (59%) suggesting that no severe irreversible fouling further occurred throughout three filtration cycles. Therefore, FRR values indicated that peptoid

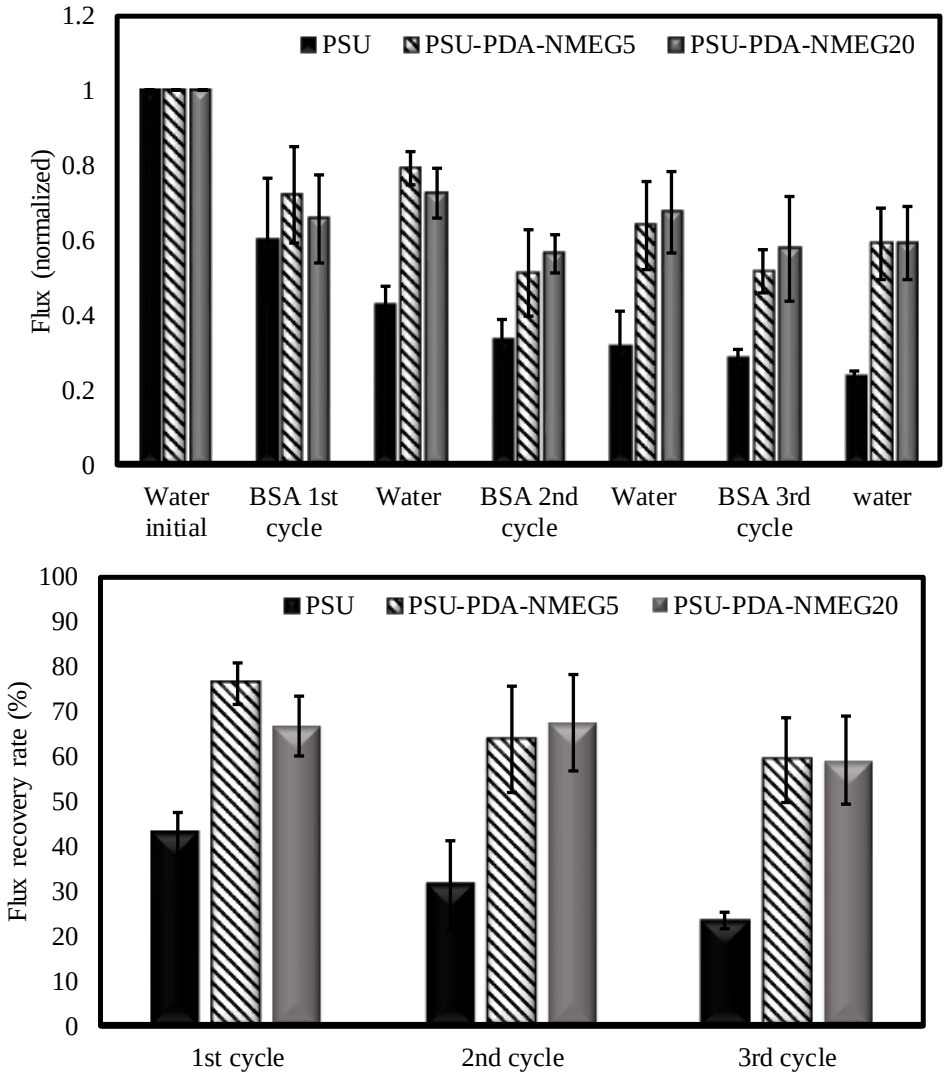


Figure 6.5. (a) Time-dependent normalized flux of unmodified and modified membranes for BSA solution (b) the flux recovery rates after physical cleaning in each cycle.

modified membranes possessed long-term antifouling ability to BSA solution. Moreover, there is significant improvement in fouling behavior of modified membranes compared to unmodified membranes ($P<0.05$).

This information was supported by the observation of BSA adsorption from long-term dynamic fouling, discussed above. However, all PSU membranes modified by NMEG5 and NMEG20

exhibited an obvious improvement of BSA flux recovery. It was clear that peptoids showed the decreasing of irreversible fouling by resisting BSA adsorption.

6.4. Conclusion

Studies show that hydrophobic, rough, and charged membrane surfaces show a tendency in protein adsorption, and it is hypothesized that hydrophilic, smooth and electrically neutral membranes may foul less [47]. The modification of surface improved hydrophilicity (decrease in contact angle), smoother surface (decrease in roughness of peptoid modified surfaces) and were almost electrically neutral (zeta potential around zero at pH=7). FRR values indicated that unmodified membranes had the lowest FRR value due to the hydrophobic interactions between membrane and hydrophobic moieties of proteins where tend to change the conformation of protein after contacting to the surface and lead to irreversible protein adsorption [48]. Moreover, irreversible protein fouling may increase the roughness of the membranes and induce more severe fouling in subsequent cycle 2 and 3 on membranes. However, after immobilization of surface by peptoids, NMEG peptoid could bind with water molecules via hydrogen interactions and form a hydration layer on surface, which could act as a barrier to reduce the contact between membrane surface and protein. Overall, this set results demonstrated very good biocompatibility of peptoid attached PSU membranes prepared by PDA coating. These results lend support to the assumption that biofouling of a hydrophobic membrane such as PSU membrane may decrease if surface modification process is carefully conducted.

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7. Conclusion and Future Directions

7.1. Conclusion

Peptoids with 2-methoxyethyl (NMEG) side chains were grafted on the polysulfone (PSU) membranes using polydopamine (PDA). The successful attachment of NMEG peptoid to the PSU surface was confirmed by X-ray photoelectron spectroscopy. Modified membranes by the NMEG peptoids showed higher hydrophilicity in compared to the unmodified PSU membranes. The NMEG-attached fibers decreased fouling compared to unmodified fibers by 40% for bovine serum albumin, 55% for lysozyme, and 66% for fibrinogen.

Protein adsorption experiments indicated that chain length and grafting density play important roles in membrane antifouling characteristics. In this work, to decrease the protein adsorption on the PSU membranes, the grafting density and chain length of the peptoids were changed to achieve a full surface coverage of NMEG on the PSU surface. We found that the key factor to have minimal protein adsorption is finding optimization of NMEG peptoids grafting density which depends on the length and grafting density of the peptoids. Peptoid-attached surfaces with low grafting density showed a poor antifouling behavior since proteins penetrated the gaps between peptoid chains and adsorbed on the PSU surface. At optimal surface coverage of peptoid, hydrated chains preventing proteins from reaching the membrane surface. However, by increasing the peptoid-grafted density, peptoid chains would lose their hydration and protein can adsorb on the PSU surface. The static fouling experimental results demonstrated that the minimum BSA adsorption after 12 h incubation time could be achieved when (4-8) μg/cm^2, (8-12) μg/cm^2, (8-16) μg/cm2 and (12-16) μg/cm^2 amount of NMEG5, NMEG10, NMEG15 and NMEG20, respectively immobilized onto the PDA coated surfaces. Additionally, platelet

adhesion test showed that hemocompatibility of peptoid modified surfaces were significantly improved.

To further evaluate the potential applications of NMEG-grafted membranes in biomedical fields, a gas exchange system was designed and built. Oxygen gas permeation experiments indicated that modified membranes retained an excellent performance compared with PSU and PDA-coated hollow fiber membranes after exposing to the bovine serum solution (35 mg/ml in PBS) for 6 h. Moreover, since surface properties such as hydrophilicity, surface charge, surface roughness and peptoid molecular weight have influence on fouling behavior of membranes the effect of surface properties on protein fouling was examined. The results show that bovine serum adsorption increases as the surface become rougher and more hydrophobic, while protein adsorption minimal when surface charge is neutral and increased with increasing charge. Here in this study, the result demonstrated that the hydration behavior is the determining factor to significantly improve fouling behavior of membranes while varying molecular weight of peptoid did not show any significant influence on protein adsorption behavior of membranes. Finally, flux recovery ratios obtained from NMEG-grafted membranes were much higher (59 %) than unmodified membranes (23 %). As a result, NMEG-grafted membranes have the potential to be used as antifouling membranes with broad applicability.

7.2. Future Work

Future studies can be continued in several directions. It can be the synthesis a peptoid with new side chain chemistry and compare the antifouling behavior of NMEG peptoid with new structure of peptoid. Moreover, nanomaterials such as TiO_2 and nano silver can be incorporated into the structure of modified membrane to improve surface properties and fouling behavior of modified membranes.

As in this work, the methods utilized to test the static antifouling effect of the peptoid modified membranes were based on the equipment available in our laboratory; it would be interesting if supplementary studies could be carried out to precisely measure the amount of foulants adhered to the surface. Further expansion to the measurement of adhesion of bacteria to the membrane surface would be highly desirable.

Peptoid modification was successful in reducing fouling in laboratory scale cross-flow filtration and gas exchange of bovine serum solution. It would then be a valuable contribution to evaluate its performance in real blood applications. More thoughts need to be given on the long-term (over 6-month) fouling stability of the modified membranes. To assess the long-term antifouling performance of membranes, cell-attachment studies can be performed by 3T3-Swiss albino fibroblasts and quantitative cell-attachment data can be obtained using a fluorescent microscope.