Combinatorial synthesis with high throughput discovery of protein-resistant membrane surfaces

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\begin{abstract}
Using combinatorial methods, we synthesized a series of new vinyl amide monomers and graft-polymerized them to light-sensitive poly(ether sulfone) (PES) porous films for protein resistance. To increase the discovery rate and statistical confidence, we developed high throughput surface modification methods (HTP) that allow synthesis, screening and selection of desirable monomers from a large library in a relatively short time (days). A series of amide monomers were synthesized by amidation of methacryloyl chloride with amines and grafted onto commercial poly(ether sulfone) (PES) membranes using irradiation from atmospheric pressure plasma (APP). The modified PES membrane surfaces were then tested and screened for static protein adhesion using HTP. Hydroxyl amide monomers N-(3-hydroxypropyl)methacrylamide (A3), N-(4-hydroxybutyl)methacrylamide (A4), and N-(4-hydroxybutyl)methacrylamide (A6), ethylene glycol (EG) monomer N-(3-methoxypropyl)methacrylamide (A7), and N-(2-(dimethylamino)ethyl)-N-methylmethacrylamide (A8), and N-(2-(diethylamino)ethyl)-N-methylmethacrylamide (A9) all terminated with tertiary amines and were shown to have protein resistance. The PES membranes modified with these monomers exhibited both low protein adhesion (i.e. membrane plugging or fouling) and high flux. Their performance is comparable with previously identified best performing PEG and zwitterionic monomers, i.e. the so-called gold-standard for protein resistance. Combining a Hansen solubility parameter (HSP) analysis of the amide monomers and the HTP filtration results, we conclude that monomer solubility in water correlates with protein-resistant surfaces, presumably through its effects on surface–water interactions.

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1. Introduction

Nonspecific protein adsorption on surfaces has a significant negative effect on the performance of materials as drug delivery [1], implantable medical device and biosensors [2] and synthetic membrane filtration application (cite) [3,4]. Whitesides have developed criteria for protein resistance with four molecular characteristics: surfaces should (i) be hydrophilic (high wettability), (ii) incorporate hydrogen bond acceptors, (iii) not include hydrogen bond donors, and (iv) be net electrically neutral [5,6]. Examples include self-assembled monolayers (SAMs) of oligo(ethylene glycol) (OEG), tertiary amines, zwitterionics and combination of positively charged and negatively charged moieties [7–9]. The exception to Whitesides criteria is sugar-based SAMs with multiple hydroxyl functional groups that possess hydrogen bond donors [5,10].

To date few non-fouling materials have been discovered over the past 30 years. Polyethylene glycol (PEG) meets the above criteria and has been widely used for biomedical applications [11,12]. However, PEG-based materials have limitations since they decompose in the presence of oxygen and transition metal ions [13]. To overcome this limitation, zwitterionic polymers or polyampholytes constructed with charge balance of positively and negatively charged monomers have been developed [5]. They exhibit comparable non-fouling characteristics comparable to PEG but also more stable [7,8,14]. Non-fouling materials also have been...
developed by tailoring existing material surfaces with functional polymer chains. This approach does not substantially alter the bulk material properties, but enhance the wetting, charge, dyeing, adhesion or non-fouling properties of these materials [15–19].

Fouling is a significant challenge for membrane filtration since it diminishes the separation capabilities and increases the cost for membrane cleaning and replacement. Fouling is caused by accumulation, deposition and adsorption of particles, colloids or biological molecules on the membrane surface (external fouling) or within membrane pores (internal fouling) [3]. Researchers have focused on the surface modification of synthetic membranes using graft polymerization to reduce fouling [20–23]. However, only a few low fouling membranes have been discovered over the past 30 years. Previously, we described a high throughput platform (HTP) combined with a photo-grafting method that facilitated quick synthesis and screening of protein-resistant membrane surfaces from a library of commercial vinyl monomers [24–27]. Membrane surfaces grafted with hydroxyl, PEG, amine and zwitterionic monomers exhibited protein resistance. Moreover, based on these HTP results, the mechanism of non-fouling has also been investigated with the help of structure—property relationships [26]. As the number of the commercially available vinyl monomers is limited, there are opportunities to expand this library approach, increase the variety of protein-resistant surfaces, and explore protein adhesion or fouling mechanisms.

Here we develop combinatorial methods for acquiring protein-resistant surfaces [23–28]. Related approaches have been utilized to rapidly synthesize a library of molecules and materials, such as catalysts [28], enzymes inhibitors [29], protein receptors [30], peptide ligands [31], and polymers [32]. To this end, we synthesized ten amide monomers by amination of methacryloyl chloride with amine compounds (Fig. 1) and confirmed their target masses with mass spectrometry (Figs. S1–S10). The amide monomers were functionalized with alkane (A1), bis-amide (A2), hydroxyl (A3–A6), ethylene glycol amine (A7), tertiary amine (A8, A9) and morpholino amine (A10) groups. Combined with the HTP method, these monomers were rapidly graft polymerized onto poly(ethersulfone) (PES) ultrafiltration membrane surfaces using atmospheric

![Fig. 1. (A) Synthesis of amide monomers by reactions of methacryloyl chloride with amines, and (B) list of names and chemical structures of the synthesized amide monomer, A1–A10. A1 is an internal control as it has not hydroxyl group at the terminus; A3–A5 increased in length by one carbon group; A6 is a branched version of A4; A7 is a methyl-terminated PEG; A8–A10 are terminated with tertiary amines. A10 is slightly positive.](image-url)
pressure plasma (APP) [26]. The anti-fouling performance of these modified membranes was evaluated with a static protein adsorption assay and the results were correlated with Hansen solubility parameters (HSPs).

2. Experimental

2.1. Materials

Amlyamine (99%), 1,4-diaminobutane (≥99.0%, GC grade), 3-amino-1-propanol (≥99.0%), 4-amino-1-butanol (98%), 6-amino-1-hexanol (97%), 2-amino-1-butanol (97%), 3-methoxypropylamine (99%), N,N,N′-trimethyl-ethylenediamine (97%), N,N-diethyl-N’-methylethylenediamine (97%), 4-(2-aminoethyl)morpholine (99%), dichloromethane (DCM, ≥99.5%, HPLC grade), N,N-disopropylethylamine (DIEPA, 99.5%, biotech grade), hexane (≥95%, HPLC grade), ethyl acetate (ETAC, ≥99.5%, HPLC grade), ethanol (≥99.5%), and sodium chloride (≥95%) were purchased from Sigma Aldrich (St. Louis, MO), which were used without further purification. 96-well filter plates (CMR #1746-3, Seahorse Labware, Chicopee, MA) were used for the HTP grafting and filtration. Silica Gel (230–400 Mesh, Grade 60) was purchased from Fisher Scientific (Pittsburg, PA). Magnesium sulfate powder (99.5%, anhydrous) was purchased from Alfa Aesar (Ward Hill, MA). PES membranes (100 kDa) having an effective area of 19.95 mm² were mounted and sealed by the manufacturer (Seahorse Bioscience, North Billerica, MA) on the bottom of each well of a 96-well plate (volume of 200 µL). The membranes were washed and soaked in DI water overnight before use to remove any excess surface silica gel. High purity helium gas was used as a plasma source (Noble Gas Solutions, Albany, NY). Solution for the static protein adsorption assay was prepared by dissolving 1 mg/mL bovine serum albumin (BSA, molecular weight (MW) 67 kDa, pI 4.7) in 200 µL phosphate buffered saline (PBS) solution. BSA and PBS tablets were purchased from Sigma Aldrich (St. Louis, MO). When dissolved in 200 mL of water, a PBS tablet yields 10 mmol L⁻¹ phosphate buffer, 2.7 mmol L⁻¹ potassium chloride and 117 mmol L⁻¹ sodium chloride with pH 7.4 at 25 °C.

2.2. Methods

2.2.1. Monomer synthesis

Amine (1 mmol), and DIEPA (3 mmol) were first added to DCM (4 mL) and then an abundant amount of methacryloyl chloride (1.5 mmol) was added. The mixture was stirred for 0.5 h at 0 °C for 2 h at room temperature and under a nitrogen blanket. The crude products were then washed by extraction with brine twice, and the organic phase was collected which was then concentrated by evaporation at room temperature under vacuum (VWR 1410, VWR, Radnor, PA). The resultant crude products were purified by a silica gel column (50% vol/50% vol) concentrated by evaporation at room temperature under vacuum oven, and dried with magnesium sulfate under vacuum desiccator (VWR Desi-Vac™, WR, Radnor, PA) overnight.

2.2.2. HTP-APP

Membranes located at the base of each well in the 96 filter plate were, first exposed to an atmospheric pressure (AP) plasma source (Model ATOMFLO, Surfx Technologies LLC, Culver City, CA) at a helium exposure to an atmospheric pressure (AP) plasma source (Model ATOMFLO, Surfx Technologies LLC, Winooski, VT) placed under the 96-well receiver plate (Corning Inc., Corning, NY). Permeation of permeate in each receiver well was calculated by measuring the absorbance at 977 nm of permeate solution in the receiver plate wells using a Microplate Spectrophotometer (PowerWave XS, BioTek Instruments Inc., Winooski, VT). The protein adhesion and subsequent pore blocking (i.e. anti-fouling performance) was measured in terms of a fouling index, R (Eq. S12):
chain length (six units of repeated alkane groups) and the highest \( R \) value, suggesting that there may be a maximum chain length for hydroxyl amide methacrylates, above which \( R > 1 \). Tertiary amide monomers A8 and A9 showed comparable protein adhesion (anti-fouling) to hydroxyl amide monomers A3, A4 and A6 and did not exhibit monomer concentration dependency.

### 3.2. Surface characterization with Hansen solubility parameters

Empirical and modeling studies have determined that protein resistance is mainly due to surface–water interactions that prevent a protein’s approach to a surface [11,36–38]. Hower et al. [39] quantified protein resistance of a surface by evaluating the intrinsic hydration capacity of different chemical groups (ethylene glycols, sugar alcohols, and glycline analogs). Here, the Hansen solubility parameters (HSPs) are used to quantify the grafted surface–water interactions by assuming that the properties of the monomers sufficiently represent the interactive properties of the grafted polymers. Non-polar (\( \delta_p \)), polar (\( \delta_a \)), and hydrogen-bonding (\( \delta_h \)) components of the HSPs for monomers A1–A10 were estimated using a group contribution method [35]. \( \delta_d \) versus \( (\delta_p^2 + \delta_h^2)^{1/2} \) for the interaction of each monomer with water is plotted in Fig. 3. The distance from the water point is considered as a measure of water affinity (dashed circles are to help guide the eye). Monomers A1 and A2 were further away from the water coordinate than the other amide monomers. This indicates weaker interactions between A1 and A2 modified surfaces with water, which is consistent with their higher \( R \) values (Fig. 2). As the length of the alkane chain increases from A3 to A5, the distance from the water coordinate also increases. Therefore, the lower \( R \) values for membranes modified with monomers A3 and A4 compared to those modified with A5 could be attributed to stronger interactions between the water and the modified surface. Oligo(ethylene glycol) amide monomer A7 showed weak interactions with the water, but membranes modified with this monomer still had \( R < 1 \). This is consistent with our previous findings in which PEG monomers (\#s 6–12 [26]) exhibited relatively weak interactions with water but PEG-modified membranes still gave \( R < 1 \) [26]. It suggests that protein resistance for PEG or OEG-grafted surfaces is not only due to strong interactions with water, but is also determined by the flexible helical conformation of PEG or OEG which facilitates formation of a tight hydration layer [11,36,37,40]. In Fig. 4, the fouling index, \( R \), of the modified surfaces at 0.3 M monomer concentration (Fig. 2) is plotted against the distance, \( d_{M-H_2O} \), between a monomer point and the water point in Fig. 3. Their correlation suggests that a monomer highly soluble in water (smaller value of \( d_{M-H_2O} \)), will result in lower protein fouling. Hence water–interface interactions are important.

### 3.3. Development of protein-resistant membranes

Protein-resistant membranes require both low fouling (\( R \), or protein adhesion) and high permeation (or low resistance to flow through the membrane). Here, the ratio of the resistance to flow of the modified membrane to that of the control, or the ratio of the inverse PBS fluxes, is plotted against fouling index, \( R \) (Fig. 5). Desirable performance is in the direction of the arrow or toward the origin. Data for three additional membranes are included for reference: an unmodified membrane (control), as well as PEG (#8) and zwitterionic (#40) modified membranes from earlier work [22]. PES membrane modified with monomers A8 and A9 displayed lower \( R \) values than the control membrane (as in Fig. 2), but also had higher resistance. In contrast, A3, A4 and A7 modified PES membranes gave both lower \( R \) and higher resistance.

![Fig. 2](image-url) Fouling index or \( R \) values of PES membranes grafted with amide monomer A1–A10 by HTP-APP after 1 mg/mL BSA adsorption for 44 h at \( T = 22 \pm 1 \degree C \) and \( pH \) 7.4. Ethanol/water (20/80 wt%wt%) was used as the solvent for A1 and A5, and ethanol was used as the solvent for A2. \( R \) values for A1–A4, A6, A8 and A9 were obtained with 0.1 M, 0.2 M and 0.3 M monomer solution (from left to right for each monomer). \( R \) values of 0.3 M A5 and 0.3 M A7, and 0.2 M and 0.3 M A10 were not shown due to the very low permeation after adsorption (difficult to measure). Each data point was obtained in quadruplicate.

![Fig. 3](image-url) Hansen solubility parameters (HSPs) of \( \delta_d \) versus \( (\delta_p^2 + \delta_h^2)^{1/2} \) of amide monomers and water.
values and higher flux than the control membrane. Surfaces modified with monomer A7 had slightly lower fluxes but similar R values to those modified with monomer A4 as expected (Fig. 3). Monomers A4 and A7 yielded surfaces having comparable filtration performance with those modified with previously discovered best monomers (PEG #8 and zwitterionic #40).

3.4. Membrane permeability versus selectivity (dynamic fouling)

In order to compare the performance for different gas separation polymer membranes, Robeson [41,42] developed a log–log plot of separation factor versus permeability similar to the classic plot of capacity versus selectivity for most separation processes. The data obtained from binary gas mixtures revealed an “upper bound”, above which nearly no data exists. Here, selectivity is measured as the ratio of the permeability of the small molecules (water and ions) to that of the less permeable BSA and is plotted as the function of the permeability of the modified and the control membrane (Fig. 6) [43]. A trade-off between selectivity and permeability is observed, as modified membranes with high selectivity displayed low permeability and vice versa. Membranes modified with monomers A3–A5 and A7 exhibited higher average permeability than the control membrane. Notably, modification with monomers A3 (3.05 LMH/kPa) and A7 (3.11 LMH/kPa) yielded membranes having higher average permeability than the previous PEG #8 (2.66 LMH/kPa) and zwitterionic #40 (2.58 LMH/kPa) modified membranes. The highest selectivity ($\gamma = 4.49 \pm 0.39$) and the lowest average permeability (0.61 LMH/kPa) were obtained from grafting with monomer A10. This is possibly due to the high electrostatic attraction between the A10 modified membrane surface with its positive polyelectrolyte and the negatively charged BSA at pH 7.4.

4. Conclusions

Combinatorial methods offer opportunities to expand the chemical space of monomers for protein-resistant (or anti-fouling) membrane surfaces. A series of amide monomers were synthesized from vinyl chloride and amine compounds with different functional groups. These newly synthesized amide monomers were used to modify commercial PES membranes by high throughput atmospheric pressure plasma (HTP-APP). After challenging these membranes with BSA (static fouling), the PES membrane surfaces grafted with N-(3-hydroxypropyl)methacrylamide (A3), N-(4-hydroxybutyl)methacrylamide (A4), N-(4-hydroxybutyl)methacrylamide (A6), N-(3-methoxypropyl)methacrylamide (A7), N-(2-(dimethylamino)ethyl)-N-methylmethacrylamide (A8) and N-(2-(diethylamino)ethyl)-N-methylmethacrylamide (A9) were analyzed for protein resistance. A4 and A7 modified PES membranes exhibited both lower fouling and higher permeation flux when compared with the control membrane, and their
performance was comparable with previously identified best monomers, PE GB and zwitterionic #40. After challenging membranes with a BSA filtration assay (dynamic fouling), A3 and A7 modified PES membranes exhibited the highest average permeability, and these membranes could be useful for BSA purification from blood (large industrial separation challenge). The A10 modified PES membrane showed the highest selectivity and could be useful for removing BSA from other permeable blood components. Structure–property analysis from the Hansen solubility parameters (HSPs) was predictive for experimental measurements and thus revealed the importance of surface–water interactions for reducing protein fouling.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2013.04.051.

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