

Preparation of high performance nanofiltration (NF) membranes incorporated with aquaporin Z

Xuesong Li^{1,2}, Rong Wang*^{1,2}, Filicia Wicaksana³, Chuyang Tang^{1,2}, Jaime Torres^{1,4},
Anthony Gordon Fane^{1,2}

*1. Singapore Membrane Technology Centre, Nanyang Environment and Water
Research Institute, Nanyang Technological University, 1 Cleantech Loop, Singapore
637141*

*2. School of Civil and Environmental Engineering, Nanyang Technological University,
Singapore 639798*

*3. Department of Chemical and Materials Engineering, the University of Auckland, Auckland,
New Zealand 1142*

*4. Structural and Computational Biology, School of Biological Sciences, Nanyang
Technological University, Singapore 637551*

*Corresponding author at: School of Civil and Environmental Engineering,

Nanyang Technological University, 639798 Singapore,

Singapore. Tel.: +65 6790 5327; fax: +65 6791 0676.

E-mail address: rwang@ntu.edu.sg (R. Wang).

Abstract

Aquaporin (AQP) based biomimetic membrane has attracted increasing attention in recent years because of its potential application for water purification and seawater desalination, attributed to the exceptionally high permeability and selectivity of AQPs. Despite its superior performance, AQP based membrane faces several challenges associated with the design and preparation of such membranes for practical application. In this current study, a novel and simple method was introduced to prepare an aquaporin Z (AqpZ) based biomimetic nanofiltration (NF) membrane with a relatively large membrane area of 28.26 cm². The proteoliposome, incorporated with AqpZ, was fully encapsulated into the selective layer through crosslinking of a polyelectrolyte with the membrane substrate made by poly(amide-imide) (PAI). The water flux of the AqpZ based membrane was around 50% higher than the mutant one. At optimal preparation conditions, the AqpZ based membrane could offer a water flux of 36.6 L·m⁻²·h⁻¹ with a MgCl₂ rejection of 95% at 1 bar. Results from this study showed that AqpZ could maintain its activity even under harsh environmental conditions of thermal treatment at 70°C for 2 hours. These results and findings may provide useful insights on developing next generation of biomimetic membranes.

Keywords: aquaporin Z; biomimetic nanofiltration (NF) membrane; polyelectrolyte crosslinking; PAI membrane substrate.

1. Introduction

Currently nanofiltration (NF) and reverse osmosis (RO) are the leading membrane technologies for water purification and seawater desalination. However, their applications are constrained by the high energy input to supply power for the pump operation. Numerous efforts have been made to improve the performance of membranes, thereby reducing the energy cost [1]. One main protocol is to design or tailor new membrane materials to increase the separation performance of the membranes. The discovery of the water channel protein, aquaporin (AQP), has provided a new opportunity for innovation of membrane materials. Different from conventional polymeric membrane materials where water molecules diffuse faster than salts, it is reported that certain groups of AQPs could only allow passage of water molecules at an extremely high rate (as many as ~3 billion water molecules per second per subunit) while achieving nearly complete rejection of all other molecules [2-3]. Therefore, theoretically an AQP based membrane could exhibit an extraordinary desalination performance compared to conventional commercial NF or RO membranes [1].

Being part of integral membrane proteins, AQPs need to be integrated into specific amphiphilic carriers such as lipids [2] or block copolymers [4], to maintain their unique function in an aqueous environment. The integration of these carriers with a specific substrate is the first critical step to successfully design a biomimetic membrane for practical application. One commonly employed protocol is the fusion of the vesicles incorporated with AQPs on a porous substrate [5-8]. After the incorporation of AQPs into the liposomes or polymersomes, these vesicles, also called proteoliposomes or proteopolymersomes, would tend to fuse to a thin planar dense layer upon interaction with specific substrates[8], or upon reaction with the substrate [7]. It was expected that this thin layer formed by vesicle fusion

would function as a selective layer, while the embedded AQPs would play a role in facilitating the efficient transport of water molecules. However, the vesicle fusion was susceptible to the substrates and many other factors [5]. More critically, it is quite challenging to obtain a membrane that is sufficiently large for practical needs by this protocol [6-8]. Instead of fusing the proteoliposomes or proteopolymersomes on a porous substrate, Sun *et al* [9] reported one different method: intact proteoliposomes were immobilized on membrane surface first and then these immobilized vesicles were sealed by a polydopamine layer, which could function as a salt-rejection layer. This layer could be constructed by some other chemicals such as polyelectrolytes via layer-by-layer method, as reported by Sun *et al* [10]. Wang *et al* [11] immobilized the crosslinked proteopolymersomes on a membrane substrate and used a layer-by-layer polydopamine-histidine coating process to seal these vesicles. It was also reported an *in situ* surface imprinting polymerization method had been applied to coat the immobilized proteopolymersomes and thereby obtain a dense composite layer [12]. The amount of AQPs in the membrane would determine the performance of prepared membrane as more AQPs could produce a higher water flux. Sun *et al* [13] managed to encapsulate magnetic particles into proteoliposomes and more proteoliposomes tended to move towards the substrate surface under a magnetic field. All the membranes prepared by this protocol were nanofiltration (NF)-type membranes. In addition, for some membranes prepared by this protocol, the thickness of the selective layer constructed to link or coat the immobilized vesicles was far less than the mean size of those vesicles. This made most of incorporated AQPs being exposed to the external environment [9, 11-12]. This brought one big concern about the stability of membrane as the toxic chemicals in the feed may affect the activity of AQPs, and the membrane fouling or cleaning occurred during membrane operation process may bring more uncertainty to the AQPs exposed. It would be also very difficult to perform any surface medication for tailoring surface properties of those membranes.

Moreover, although some membranes prepared by this method presented some interesting results, all these reported membranes had quite small membrane areas ($< 0.8 \text{ cm}^2$), which might imply that this protocol might encounter a big challenge for scaling up, even in a bench-scale at current stage. To prepare a sizeable biomimetic membrane suitable for practical applications, some basic criteria have to be met: a scalable substrate is essential and the membrane should maintain defect-free and present a reproducible result after scaling up. Recently our research team developed a unique protocol that had successfully overcome this shortcoming [14]: we managed to fully incorporate the intact proteoliposomes into the polyamide layer of a RO membrane, which was prepared by interfacial polymerization. This membrane presented a promising desalination performance with a scalable size. However, the improvement was modest compared to the theoretically high permeability of AQPs, probably because the chemicals used for interfacial polymerization had negative effects on AQP activity, ~~or~~ and the loading amount of proteoliposome in the selective layer was also relatively low because of gas sweeping used in the interfacial polymerization process.

In present study, a high performance NF membrane was prepared based on AQPs by polymer crosslinking. During this preparation process, the chemicals used had been minimized in order to minimize the effects of chemicals on the activity of AQPs. The proteoliposomes were initially immobilized on a poly(amide-imide) (PAI) membrane substrate and subsequently encapsulated in a selective layer formed by polyelectrolyte crosslinking with the substrate. Before incorporating the proteoliposomes into this selective layer, the proteoliposomes were decorated with polydopamine (PDA) to enhance its affinity to the membrane substrate. PDA is a novel bio-compatible polymer that can adhere to many surfaces, including liposome [15] via covalent or non-covalent bonds [16]. It was reported that the polyelectrolyte with primary amine groups, such as poly(ethyleneimine) (PEI), could

crosslink with the PAI membrane very efficiently at elevated temperatures, a dense and defect-free selective layer could be formed to offer a fairly good rejection to divalent ions or small organic molecules, as well as reasonable water permeability [17-19]. This efficient reaction also guarantees that the method is applicable to fabricate a sizable defect-free membrane. The crosslinking reaction could be easily controlled by adjusting the temperature or selecting reactive polymers, thereby being able to tailor the performance of membrane [19-20]. The thickness of selective layer could reach several hundred nanometers [18, 20-22], which allows to encapsulate the proteoliposomes completely as the mean size of proteoliposomes is usually smaller than 200 nm in present conditions [23]. This layer could offer a fairly good rejection to divalent ions, as well as a reasonable water permeability [17-18]. If the introduced proteoliposomes were completely encapsulated by this layer, this polyelectrolyte layer with embedded proteoliposomes is expected to be able to function as a highly permeable selective layer. The whole procedures for the formation of the selective layer are schematically illustrated in Figure 1. This technique is relatively simple for preparation and scaling up as compared to other methods for AQP based membrane formation, thus it provides a potential way to design and fabricate an AQP based biomimetic membrane for practical application. To date, there was no report to use similar approach to incorporate AQPs into the selective NF membrane layer.

2. Experimental

2.1. Chemicals and materials

The main chemicals used in this study were 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) in chloroform (*Avanti Polar Lipids, Alabaster, USA*), *n*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt

(NBD-PE) (*Invitrogen, Singapore*), detergent 1-n-octyl- β -D-glucopyranoside (OG) and biobeads (*Calbiochem®*, *Singapore*), poly(ethyleneimine) solution (average Mw ~750,000 by LS, 50 wt. % in H₂O) and dopamine hydrochloride (*Sigma-Aldrich, Singapore*). Other chemicals or materials were purchased from Merck chemicals (*Singapore*) unless otherwise stated. All chemicals were used without further purification. Milli-Q water (*Millipore, integral 10*) with a resistivity of 18.2 M Ω ·cm was used. Tris buffer solution was prepared by dissolving 10 mM tris(hydroxymethyl)aminomethane in Milli-Q water, the pH was then adjusted to 8 by HCl addition. Aquaporin Z (AqpZ) and mutant were expressed and purified in term of the previously reported procedures [5, 14]. Torlon® 4000T (copolymer of amide and imide) (PAI) was supplied by Solvay Advanced Polymers (*Alpharetta GA*). NF-270 and NTR-7450 flat sheet membranes were supplied by Dow Chemical Company (*USA*) and Nitto Denko Corporation (*Japan*), respectively.

2.2. Preparation of Liposome and Proteoliposome Solutions

DOPC chloroform solution was dried by slowly passing a pure N₂ stream and then vacuumed for at least 12 hours. The dried lipid film was then hydrated in Tris buffer. After agitating for 10 min and three freeze-thawing cycles, the solution was extruded many times through a polycarbonate membrane with a mean pore size of 200 nm by an extruder (*Avanti, US*). The final concentration of the DOPC vesicle solution was 8 mM. A certain amount of the AqpZ solution in terms of nominal lipid-to-protein ratio (LPR) was added into 8 mM DOPC liposomes Tris buffer containing 1% OG at various lipid/AqpZ ratios. Stepwise addition of 0.2 g biobeads into 1 ml solution was performed. The mixture was rotated for 2.5 hours to remove the OG. The proteoliposome solution was then extruded through a polycarbonate

membrane (200 nm mean pore size) for many times for further use. The size and zeta potential of the liposome were measured by a zetasizer Nano ZS (*Malvern, UK*) at 22 °C.

2.3. Preparation of Substrates

Torlon® 4000T was dried in a 50 °C vacuum oven for at least one day to remove the moisture prior to the dope preparation. 14% (wt%) PAI and 3% (wt%) LiCl were dissolved in NMP using a mechanical stirrer at 70 °C for 3 days. PAI membrane was casted by a casting knife on a flat-sheet casting machine. The thickness was set at 200 µm. The casted PAI membranes were then soaked in Milli-Q water for at least one day.

2.4. Polydopamine (PDA) coating of proteoliposomes

A 2 ml 4 mM extruded proteoliposome solution was injected into a 2 ml 0.1 mg/ml dopamine in Tris buffer. The mixed solution was continuously stirred for 12 hours and then extruded through a polycarbonate membrane with a mean pore size of 200 nm.

2.5. Preparation of nanofiltration membrane encapsulated with proteoliposomes

The extruded PDA coated proteoliposomes were deposited on the PAI membrane substrate for 1.5 hours. Upon removal of the proteoliposome solution, a 15 ml 0.5% (wt%) PEI in Tris buffer solution was deposited on the membrane surface for crosslinking in water bath at 70 °C for 2 hours. At a temperature of 70 °C, branched PEI molecules could react with PAI membrane surface to form a several hundred nanometers thick rejection layer [20].

2.6. Membrane characterization and water flux & rejection measurements

Fluorescence images were obtained with LSM 710 confocal microscopy system (*Carl Zeiss, Germany*) using a 40× objective lens (excitation line 458 nm). For fluorescence characterizations, the lipid was mixed with 1 mol% NBD-PE in all experiments. Samples for fluorescence microscopic characterization were prepared differently from other samples; these samples were heated at 70°C for only 20 minutes in order to control the thickness of PEI layer and to prevent the proteoliposomes from being fully covered by the PEI layer. Field emission scanning electronic microscopy (FESEM) images were obtained at room temperature. Prior to the FESEM characterization, samples were dried in desiccators for at least 24 hours. The permeabilities of liposomes and proteoliposomes were analyzed by a stopped flow apparatus (*SX20, Applied Photophysics*) at 22°C. The vesicles were rapidly mixed with a hyperosmolar solution containing 600 mM sucrose. The osmolarity difference between intravesicular and extravesicular could cause the shrinkage of the vesicles. The vesicle with a higher permeability would exhibit a more rapid shrinkage rate. The shrinkage rates of vesicles were monitored by light scattering. One curve was obtained by averaging five light scattering traces got in each stopped flow experiment. The initial rising rate (k) was calculated by fitting the averaged curve. The water permeability of liposomes or proteoliposomes, P_f ($\mu\text{m/s}$), was calculated using the following equation[2, 5]:

$$P_f = \frac{k}{S/V \times V_w \times \Delta_{osm}} \quad (1)$$

where S/V is the ratio of the initial surface area to the volume of vesicles, V_w is the partial molar volume of water ($18 \text{ cm}^3 \cdot \text{mol}^{-1}$) and Δ_{osm} (osmol/L) is the osmolarity difference between the intravesicular and extravesicular solutions.

The water flux and salt rejection were measured using a 100 ppm MgCl₂ solution as feed under 1 bar pressure in a stirred dead-end cell. The water flux, J_w (L·m⁻²·h⁻¹) was calculated according to equation 2:

$$J_w = \frac{\Delta w}{S \cdot \Delta t} \quad (2)$$

where Δw (kg) is the increase of permeate weight over a certain period of time, Δt (hour) is the time interval; S (m²) is the effective membrane area; The salt rejection, R (%) was calculated according to equation 3:

$$R = \frac{C_f - C_p}{C_f} \times 100\% \quad (3)$$

where C_f and C_p are the salt concentration in the feed and permeate, respectively. The effective area of the membrane was 28.26 cm². All flux and rejection measurements were carried out at 22°C.

3. Results and Discussion

3.1. Properties of proteoliposomes with AqpZ or mutants

It has been proposed that water permeability of AqpZ channel was mediated by the 189th amino acid, arginine [24-25]. The **mutants AqpZ molecules** used for the experimentation protocol were mutated in the arginine. The mutation of highly conserved arginine into a different amino acid like alanine resulted in inactivation of channel without change in its tetrameric state. The main advantage of using these mutants is maintaining the same structure as AqpZ but with a loss in functionality. Figure 2 shows typical normalized stopped flow curves of liposome, proteoliposomes with mutants and proteoliposomes with AqpZ. In comparison with the liposomes and proteoliposomes with mutants, the proteoliposomes with

AqpZ gave a notably higher shrinkage rate. Their calculated permeability values are displayed in Table 1. Correspondingly, the AqpZ incorporated proteoliposomes provided the highest water permeability. In our previous report, the AqpZ was incorporated into DOPC in a phosphate buffer with a pH value 7.8 and gave a high water permeability [5]. In this study the reconstitution of AqpZ into DOPC was performed in Tris buffer (pH 8.0, 10mM) because this buffer and pH were required for PDA formation [16] and its calculated permeability value per subunit was comparable to our previously reported value [5], which confirmed that AqpZ could maintain its activity in present conditions. Meanwhile, the mutant one gave a significantly lower water permeability due to the change in its channel structure [14]. Nevertheless, its permeability was still higher than that of liposome, indicating that the mutant still could facilitate water transport to some degree.

The incorporation of AqpZ/mutants into liposomes had altered the size of proteoliposomes to become considerably smaller but more uniform (as shown in Table 1). Moreover, proteoliposome carried more negative charges than liposomes because the incorporated proteins were negatively charged in the buffer pH condition. It was also reported that the size and surface charge of the vesicle had substantial effects on the interaction between the vesicle and the solid surface [26-27]. In addition, the proteoliposome possessed a greater mechanical strength than liposomes and therefore the proteoliposome could nearly maintain its vesicle shape under pressure, while the liposome would fuse into bilayer under the same condition [5, 23, 28]. In comparison to liposome, the proteoliposome with mutants presented more similar properties as the proteoliposome with AqpZ, only except for the water permeability. Therefore, in present study, the proteoliposome with mutants was selected as the control group instead of the liposome without AQPs.

3.2. PDA coating and thermal treatment effects on AqpZ

In present protocol, PDA was used to modify the surface of proteoliposomes, while PEI was used to facilitate the encapsulation of proteoliposomes by crosslinking. The effects of PDA and thermal treatment on the activity of AqpZ and mutant were investigated by stopped flow tests. The results are shown in Figure 3. Upon coating with PDA, there was a little increase in the permeability of proteoliposome with mutants, while no significant change in the permeability was observed even after being heated at 70°C for 2 hours. Slightly different from its mutant counterpart, the permeability of the proteoliposome with AqpZ did not significantly change after being coated with PDA, however, an approximately 10% decrease in the permeability occurred after the thermal treatment. Although the thermal treatment caused different effects on the permeability of different proteoliposomes, the permeability values of the proteoliposomes with AqpZ were nearly twice the permeabilities of those with mutants after the same treatment. This result also demonstrated that the AqpZ tetramers were fairly stable, even in a harsh in-vitro environment.

3.3. Performance and structure of the membranes with proteoliposomes

During the encapsulation of proteoliposomes, PDA could serve as a bridge between the proteoliposomes and the membrane surface due to its excellent adhesion to a wide range of materials [16]. Moreover, the internal gap between proteoliposome and PEI layer could also be eliminated by PDA via amine-catechol reactions [16]. In order to minimize experimental errors, PAI membranes with a water permeability of around $120 \pm 20 \text{ L} \cdot \text{m}^{-2} \cdot \text{h}^{-1} \cdot \text{bar}^{-1}$ were selected as the substrate and this substrate had no rejection to salt. Figure 4 shows the results

of water flux and salt rejection of membranes after PEI crosslinking with the PAI membrane. The flux of the PAI-PEI membrane without immobilized proteoliposomes in its selective layer was around $32.7 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ while the rejection could only reach to 93.8%. The membranes crosslinked with proteoliposome with mutants (LPR 400) had a lower flux of around $19.9 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ but a higher salt rejection ($\sim 95.6\%$). The AqpZ-incorporated membrane (LPR 400) exhibited an equivalent salt rejection but with a much higher water flux ($30.5 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$), which is $\sim 50\%$ higher than that of the mutant one. The AqpZ based membrane exhibited a superior separation performance to the control group, which should be attributed to the high permeability and selectivity of active AqpZ molecules in the selective layer.

To understand the above results, it would be necessary to analyze the structures of the selective layers of different membranes. After the proteoliposomes were introduced into the PEI layer, the integration of proteoliposomes with the crosslinked PEI layer might be presented in two different ways: proteoliposomes partially embedded into the layer or entirely embedded. These would be determined by the size of proteoliposomes and the thickness of the crosslinked PEI layer. The FESEM images in Figure 5 (A, B) clearly showed that the proteoliposomes were completely embedded into the crosslinked PEI layer and no gap was observed between the proteoliposome and the PEI layer. Additionally, these embedded proteoliposomes could maintain their spherical shapes even after PEI crosslinking and flux measurements. The confocal fluorescence images (Figure 5a, 5b) also showed a reasonable coverage of the proteoliposomes in the membranes.

The relationship between the water flux and membrane resistance as well as the relationship between the rejection and trans-membrane pressure can be described as follows;

$$J_v = (\Delta P - \Delta \pi) / \mu R \quad (4)$$

$$r = \left\{ 1 + \frac{B}{A(\Delta P - \Delta \pi)} \right\}^{-1} \quad (5)$$

where J_v represents the water flux, ΔP is the driving force, $\Delta \pi$ is the osmotic pressure, μ is the dynamic viscosity of water and R is the resistance of membrane. A and B are the intrinsic water permeability and salt permeability, respectively. In this study, a low concentration of salt solution and high stirring speed were used to minimize the effect of osmotic pressure. By assuming that all introduced proteoliposomes were fully encapsulated into the selective layer, a simplified model was given in Figure 6 to help understand the experimental results. J_1 , J_2 , J_3 and r_1 , r_2 , r_3 represent the flux and rejection of the crosslinked PEI layer, the proteoliposome with mutant and the proteoliposome with AqpZ, respectively. Based on the experimental results, one could obtain that $J_1 > J_3 > J_2$ while $r_3 = r_2 > r_1$. As the mutants could not transport water molecules efficiently, one proteoliposome with mutants could be considered as an impermeable (or less permeable) vesicle (Figure 6B). The introduction of proteoliposomes with mutants increased the resistance of the selective layer, resulting in a decrease of water permeance. In contrast, the AqpZ incorporated proteoliposome could be considered as a highly water-permeable vesicle which could facilitate transport of water molecules (Figure 6C) and dramatically decreased the resistance of the selective layer, thus leading to a much higher flux than the mutant one. Since both the lipid bilayer and AqpZ had high rejections to salt molecules[3], a lower B value would be obtained by integrating the proteoliposomes with the PEI rejection layer. Although AqpZ could facilitate the transport of water molecules, the flux of AqpZ based membrane was still slightly lower than the PEI layer ($J_1 > J_3$). This indicated that the proteoliposome with this LPR (400) still had a higher water flux resistance than crosslinked PEI layer.

3.4. Water flux and salt rejection of membranes with proteoliposomes with higher AqpZ density

As it had been demonstrated that the incorporated AqpZ could enhance the water permeability of the proteoliposome, the performance of AqpZ based biomimetic membrane may benefit from further increase of AqpZ density incorporated in the proteoliposome. Proteoliposomes with LPR 200 and LPR 100 were used to prepare the biomimetic membranes by the same procedures. The stopped flow results had shown that these two groups of proteoliposomes owned much higher permeability even after PDA coating and thermal treatment, as shown in Figure 7A. The membrane with LPR 200 produced the highest flux, $\sim 36.6 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ with similarly high salt rejection ($\sim 95\%$) to the one with LPR 400 (Figure 7B). This demonstrated that a greater amount of AqpZ molecules would provide more paths for the passage of water molecules. It was interesting to observe that when the AqpZ density increased to LPR 100, the water flux of membranes did not increase; instead, the flux decreased to around $27.3 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ though the salt rejection still remained a high level. However, the stopped flow results showed that the proteoliposome with LPR 100 could still provide higher permeability even after PDA coating and thermal treatment (Figure 7A). Based on the previous analysis, the decrease of water flux was thought to be caused by the increase of the resistance in the rejection layer. Thus it was suspected that the proteoliposomes with a higher AqpZ density (LPR 100) became less permeable than those with LPR 200 after being embedded into the crosslinked PEI layer. When the density of AqpZ was further increased to LPR 50, the permeability of proteoliposome didn't increase but decreased as shown in the stopped flow test. A similar phenomenon was also reported by other researchers [2, 7]. Nevertheless, the permeability of proteoliposome with LPR 50 was still higher than those of proteoliposomes with LPR 400 and LPR 200. However, the

membrane embedded with proteoliposome with LPR 50 gave the lowest water flux but a similarly high salt rejection. These results suggested that proteoliposome with the highest density of AqpZ became significantly less permeable but still kept a high rejection to salt, after being encapsulated into the PEI layer. In present study, the LPR 200 might be close to the optimal ratio for water flux and salt rejection. Although the same proteoliposome was used in the stopped flow test and for the preparation of the biomimetic membranes, two different optimal LPRs were found in two different systems. One probable reason was the crosslinking process between the PEI molecules and the PAI substrate may interfere with the interaction between the tetramers at a high density, leading to that the proteoliposome with a high protein ratio lost its function. However, the exact mechanism in these two systems needs to be further investigated in the future study.

As the introduced proteoliposome was completely embedded in a crosslinked PEI layer, the composite layer could withstand a relatively higher pressure than the isolated or semi-embedded proteoliposome. In addition, this layer could also provide a shield to protect the proteoliposome from the impurities or toxic chemicals in the feed, which could help maintain the activity of AQP embedded. Different from some other ways of constructing the selective layer, this layer was anchored with the substrate by covalent bonds instead of electrostatic force or hydrophobicity interaction [20]. This could ensure a strong integration of this selective layer with the substrate, which is able to withstand a high tangential flow flushing in membrane process. Besides, the modification or post-treatment of membrane surface would probably have little effects on the completely embedded proteoliposomes.

Table 2 summarizes the permeation and separation performances of different AQP based biomimetic NF membranes reported by some recent studies. It showed that the AQP based

biomimetic membrane obtained in current work offered a superior performance to other biomimetic NF membranes, in terms of the effective membrane area, water flux and salt rejection under comparable testing conditions. The results showed that the proteoliposomes embedded contributed to a flux higher than 36 LMH/bar, higher than those of AQP-based biomimetic NF membranes ($\sim 3\text{-}34 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}\cdot\text{bar}^{-1}$) [6, 8-10, 12] or RO membrane ($\sim 4 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}\cdot\text{bar}^{-1}$) [14], indicating that AQPs embedded maintained higher activity than others or the loading amount of proteoliposomes in the selective layer was superior to theirs, or it was due to both of the two mechanisms. The biomimetic membrane designed in current work still exhibited a promising performance as compared to selected commercial NF membranes under the same testing conditions. As discussed above, the performance of membrane could be further enhanced by improving the loading amount of proteoliposomes in the crosslinked PEI layer.

4. Conclusions

A NF-type biomimetic membrane based on AqpZ has been designed and developed in the present study. PDA coated proteoliposomes were first deposited on the substrate surface and PEI was then crosslinked with the PAI substrate to encapsulate these deposited proteoliposomes. The FESEM images showed that the proteoliposome could be entirely embedded into the PEI selective layer. Compared to the mutant group, the AqpZ based membrane provided a much higher water flux. At LPR of 200, the water flux and the rejection to divalent salt could reach $36.6 \text{ L}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ and 95%, respectively. However, further increase of AqpZ density in the proteoliposomes could not improve the water flux any more though the proteoliposome had a higher water permeability as shown in the stopped flow measurements. Our experiment results revealed that the AqpZ molecules could maintain a

relatively high activity even under harsh environmental conditions of thermal treatment at 70°C for 2 hours. More importantly, our findings provided useful protocols for the preparation of next generation of biomimetic membranes in a simple and scalable approach.

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