Biomimetic Membranes for Kidney Dialysis

Effective way for dialysate regeneration for peritoneal dialysis

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Abstract— Aquaporin-Z (AQPZ) membranes has high applicability for use in kidney dialysate regeneration due to its ability to selectively transport water molecules through while blocking out all other solutes. In this study, water transport through an AQPZ membrane was investigated through the Forward Osmosis process. To study the effectiveness of the AQPZ membrane for kidney dialysate regeneration, the rejection of the membrane against a waste material, creatinine and the water flux through the membrane was investigated. The results indicated a desirable creatinine rejection for the AQPZ membrane of over 90% creatinine rejection, which is significantly higher creatinine rejection of the control setup of about 70% rejection. In addition, the AQPZ membranes indicated a good water flux of over 1.5 g m^{-2} sec^{-1} water flux as compared to the over 1.2 g m^{-2} sec^{-1} water flux for the control setup.

Keywords- Biomimetic membranes, kidney dialysis, AquaporinZ, artificial kidney

I. INTRODUCTION

Development of filtration devices for continuous and dependable renal replacement treatment make them a promising and feasible alternative to current treatments methods such as kidney transplant. Peritoneal dialysis (PD) allows the 24-hour filtration of the kidneys, enabling patients the freedom to carry out everyday activities. Attempts to create a functional WAK (wearable artificial kidney) has largely been met with failure (Gura et al., 2009).This mainly arises from the inability of the currently used sorbent systems to properly remove waste molecules such as urea and creatinine (Ronco, 2007). Another concern is the bulkiness of the machine, inconveniencing patients traveling for prolonged periods of time.

The suitability of biomimetic membranes in regenerating spent dialysate is tested. Akin to cell membranes, this simulates low energy transport of waste materials and water, reducing the need for the pump and its power source, making the device lighter. In addition, it may possibly increase convenience for the patient as the membrane need not be replaced as often as the sorbent cartridge (Davenport, 2012).

The amount of waste materials removed by the biomimetic membranes in comparison to the body's kidneys must be investigated to determine its effectiveness. Creatinine was selected as the waste material to be filtered as it is a major component filtered by kidneys and other PD systems. Although urea was also initially chosen, along with creatinine, as a waste material, it was unable to be tested due to the inability to accurately measure the concentration of urea as well as additional modifications required to protect the AQPZ membranes against the denaturing properties of urea. Given that the aquaporin incorporated biomimetic membranes is a very recent development (NUS, 2015), testing its effectiveness in kidney dialysis applications promises to be a novel experience in this relatively unexplored field.

II. METHODOLOGY

a) Substrate preparation

Cellulose Acetate substrate of both 15% and 18% by mass was prepared by dissolving cellulose acetate powder in N-methyl-2-pyrrolidone. The mixture was then cast onto a glass plate with thickness of 250 μm. The cast substrate was then immersed into distilled water for 48 hours, allowing phase inversion to occur.

b) Spacer chain attachment to the substrate surface

The substrate surface was modified by incubating it in 7.0% (by mass) sodium periodate solution in darkness for 6 hours, allowing aldehyde groups to be attached to the substrate surface.

NTA-PEG-NH_2 powder was dissolved in pH 10 sodium hydrogen carbonate buffer to form a 1mM solution and topped up with 50mM sodium borohydride solution. The membrane was then submerged in the resulting solution and incubated overnight at room temperature.

c) Cheleate formation and APQZ proteins anchoring

The membrane was then incubated in 50mM nickel(II) chloride solution for 2 hours. This allows for the Ni(II) ions to form chelates with the NTA (nitrilotriacetic acid) on the other end of the chain. Milli-Q purified water was then used to flush the membrane to remove excess Nickel(II) chloride solution.

AQPZ proteins were prepared at concentration 0.0025g/litre in DDM/PBS buffer solution containing 0.2% DDM solution by mass. The membrane was incubated in the AQPZ solution overnight. It was then washed with 0.1% by weight of TWEEN 20 in PBS buffer solution to stop the reaction.

d) Vesicle Preparation

Approximately 10mg of PMOXA-PDMS-PMOXA (ABA triblock copolymer) was dissolved in 15ml of chloroform in a 25ml round-bottom flask. The flask was then attached to a rotary evaporator and left overnight with pressure set at 100mbar. The film was rehydrated the next day by adding 0.05% DDM solution in PBS buffer solution such that the resulting mixture contained 5mg/ml of ABA polymer. The mixture was left to stir overnight again in the rotary evaporator. The flask was topped up the next day by an equivalent volume of PBS buffer to the total mixture, before being left to stir on a magnetic stirrer until all the polymer had dissolved. Biobeads were added at a concentration 3 times the CMC (critical micelle concentration) of 0.2mg/ml for 4 hours to remove the DDM. The biobeads were then removed to obtain the vesicles.

e) Polymer cross linking

2mg/ml of the vesicle solution was added to the membrane and the set-up incubated overnight. Thereafter, the set-up was cross-linked in a UV cross-linker for 15 minutes.

III. CHARACTERIZATION

Forward osmosis was primarily employed to characterise the membrane in its properties to remove creatinine due to it functioning purely on osmotic pressure with the absence of other external pressures.

35ml of 0.6M sucrose solution was used as the draw solution and 35ml of 2.8mg/dl creatinine solution as the feed solution. The circular effective membrane surface used was of radius 3mm. The set-up was then left to stand at room temperature for 2 hours and the feed and draw solutions were retrieved after.

Two parameters were observed using forward osmosis: water flux and percentage rejection of creatinine. The modification and testing was done on 2 different types of substrate: 15% (by mass) Cellulose Acetate substrates and 18% (by mass) Cellulose Acetate substrates. For each substrate type, forward osmosis was carried out with the unmodified substrates, substrates modified with and without the AQPZ proteins. Triplicates were done and both the average creatinine rejection and the average water flux were compared.

a) Creatinine Rejection

To calculate the percentage creatinine rejection, concentrations of creatinine in the feed solution were found. 200 µl of the solution was pipetted into a microplate and placed into a TECAN Infinite M200 Pro UV microplate reader set at wavelength 250nm, and the absorbance reading was recorded. A calibration of concentration against absorbance units was plotted as shown in Figure 1 below. The amount of creatinine rejected by the membrane, and hence the percentage creatinine rejection, was found.

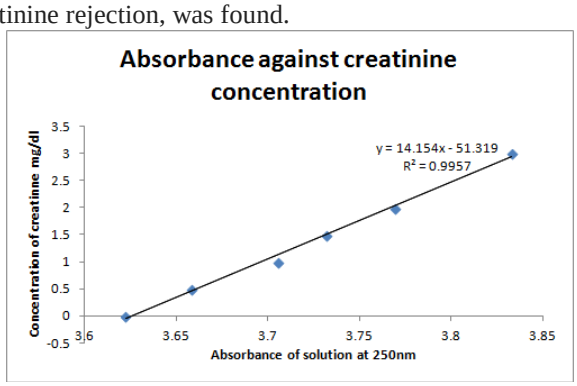


Figure 1: Calibration curve of creatinine concentration against absorbance of solution at 250nm

b) Water flux

$$\varphi = \Delta W / S_m(\Delta t) \quad (1)$$

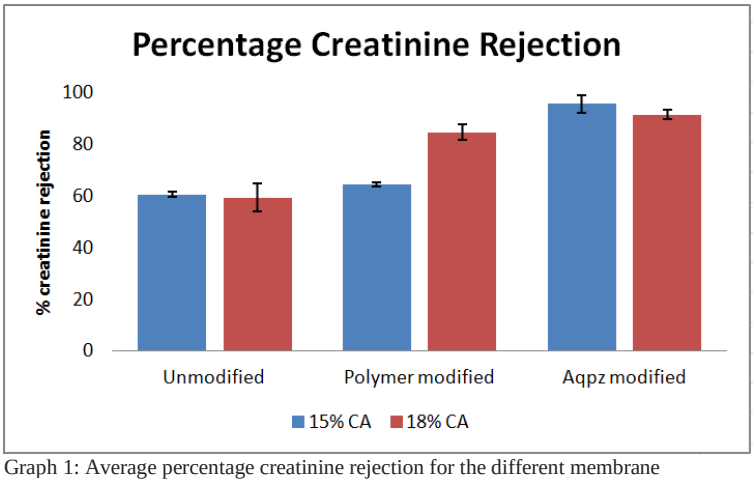
where:

φ is the water flux in g m^{-2} sec^{-1}
ΔW is the change in mass of the draw solution in g
S_m is the effective membrane surface area in m^{-2}
Δt is the time taken for osmosis to occur

c) Scanning electron microscopy

Images taken of the controls and modified membranes will be used to further substantiate our results in the discussion section below.

IV. RESULTS AND DISCUSSION



Graph 1: Average percentage creatinine rejection for the different membrane types

a) Creatinine rejection between differently modified substrates

As shown in Graph 1, there is a significant increase in the creatinine rejection from the unmodified substrate to the non-AQPZ modified substrate to the AQPZ modified substrate. This is likely due to the presence of the polymer bilayer formed from ruptured vesicles.

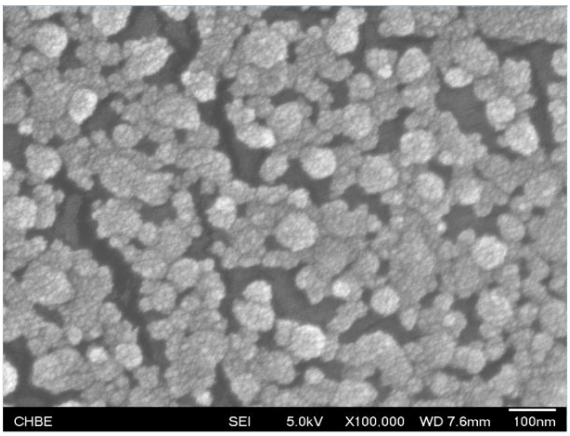


Figure 2: Presence of unruptured vesicles as seen by the SEM image at 100,000X magnification

However, it is interesting to note that the percentage rejection of creatinine is lower for the control than the AQPZ incorporated membranes. A possible reason for this is that the absence of the AQPZ proteins results in reduced interaction between the lipid bilayer and the hydrophobic exterior of the AQPZ proteins. Hence, the vesicles did not rupture completely, as shown in Figure 2, resulting in reduced creatinine rejection.

b) Creatinine rejection between different percentage Cellulose acetate(CA) substrates

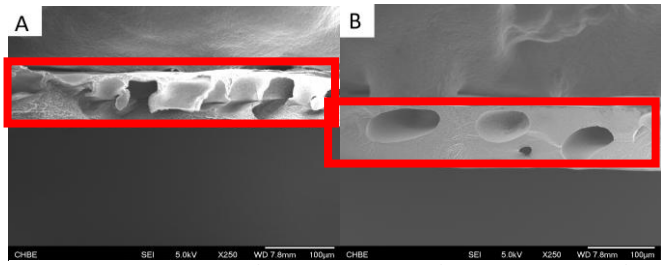


Figure 3: Cross section area for the membrane surface (outlined in red) for the (A): 15% substrate and the (B): 18% CA substrate

Comparing the 15% and 18% CA membranes, in general, the 15% CA membrane has a better creatinine rejection. This is supported by the pictures from the SEM that indicates that the 15% substrates have a more uneven surface as compared to the 18% substrates, as shown in Figures 3(A) and 3(B).

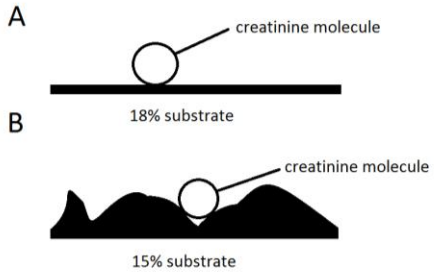
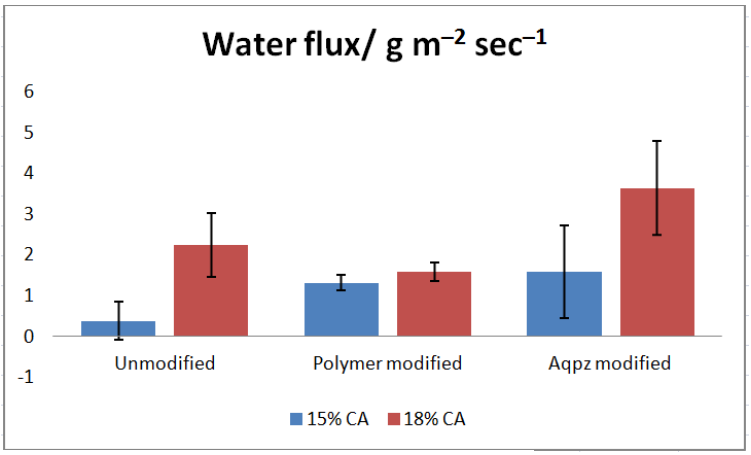


Figure 4: Effect of surface unevenness on creatinine obstruction for the (A) even 18% substrate and the (B) more uneven 15% substrate

As such, it is more likely for the large creatinine molecules to obstruct a greater surface area on the substrate for the 15% substrates as compared to the 18% substrates as shown in Figure 4. Hence, less creatinine molecules will be able to permeate the substrate for the 15% substrate as compared to the 18% substrate.

Another plausible reason for the 15% membranes having higher percentage creatinine rejection and lower water flux is that the weaker support for the 15% membrane as shown in Figure 3(A) and 3(B) collapses under osmotic pressure. This causes the internal resistance of the 15% CA membranes to increase as the molecules will have to pass through more obstacles in their path, leading to higher percentage creatinine rejection and lower water flux.



Graph 2: Average water flux for the different membrane types

c) Water flux between differently modified substrates

For water flux, it is noted from Graph 2 that it increases in the order of non-AQPZ modified substrates, unmodified substrates and AQPZ modified substrates.

While the relatively low flux of the non-AQPZ modified membranes is expected, it is interesting to note that the AQPZ modified membranes have higher flux than the unmodified ones. A possible reason for this is that the polymer bilayer for the AQPZ membrane blocks out the large creatinine molecules, preventing them from clogging up the pores in the

substrate surface as with the unmodified membranes, where all the creatinine molecules are in direct contact with the substrate surface. This will increase the water flux of the AQPZ modified ones.

d) Anormalous results

The non-AQPZ modified substrate also displayed anomalous results for both water flux and percentage creatinine rejection, with the results being too high and too low respectively. A possible explanation could be that the osmotic pressure caused the membrane structure to collapse such that the membrane had large defects. This resulted in the overall observation of anomalous results as shown in Graphs 1 and 2.

V. CONCLUSION AND FUTURE WORK

In conclusion, AQPZ-incorporated biomimetic membranes have high potential in being utilized for dialysate regeneration purposes due to the high creatinine rejection of the membrane and a good water flux.

Moreover, the recent developments in creating self-sustaining synthetic membranes capable of continuous growth to increase the lifespan of biomimetic membranes render its application in dialysate regeneration technologies to be highly promising.

However, the inevitable problem large molecules such as creatinine clogging membrane pores may be a hurdle in its applicability in the peritoneal dialysis systems. This could be alleviated by having an automated system which is able to rotate the use of membranes in the peritoneal dialysis system as this will reduce the effect of having large molecules obstructing the membrane.

Improvements can also made to how the aquaporins are incorporated into the membrane so as to improve protection of the AQPZ proteins from the osmotic pressure; this can be done by intrusion of AQPZ proteins into polymer vesicles, which will be further reinforced by polymerization.

Future works can include testing the membrane against other waste materials such as urea and Beta-2 microglobulin, as well as other considerations such as means by which the biomimetic membranes can be possibly replaced.

ACKNOWLEDGMENT

We would like to express our gratitude towards Associate Professor Tong Yen Wah and his biomimetic membrane department for so graciously aiding us in our project, as well as the National University of Singapore and Victoria Junior College for providing us this opportunity in the Science Research Programme.

REFERENCES

[1] **Davenport, A. (2012).** Portable or Wearable Peritoneal Devices— The Next Step Forward for Peritoneal Dialysis? *Advances in Peritoneal Dialysis, 28*, 97-101.

[2] **Faubel, S. (2009).** Acute kidney injury and multiple organ dysfunction syndrome. *Minerva Urol Nefrol*. Retrieved July 7, 2015, from http://www.ncbi.nlm.nih.gov/pubmed/19773721

[3] **Gura, V., Macy, A., Beizai, M., Ezon, C., & Golper, T. (2009).** Technical Breakthroughs in the Wearable Artificial Kidney (WAK). *Clinical Journal of the American Society of Nephrology, 4*, 1441-1448. doi:10.2215/CJN.02790409

[4] **Fissell, W., Roy, S., & Davenport, A. (2013).** Achieving more frequent and longer dialysis for the majority: Wearable dialysis and implantable artificial kidney devices. *Kidney Int Kidney International*, 256-264. doi:10.1038/ki.2012.466

[5] **Harrison, P. (2015, November 7).** Wearable Artificial Kidney Potential Alternative to Dialysis. *Medscape*. Retrieved August 3, 2015, from http://www.medscape.com/viewarticle/854106

[6] **Hub, J., & De Groot, B. (2007).** Mechanism of selectivity in aquaporins and aquaglyceroporins. *Proceedings of the National Academy of Sciences, 105*(4).

[7] **Humes, H., Fissell, W., & Tiranathanagul, K. (2006).** The future of hemodialysis membranes. *Kidney International*. doi:10.1038/sj.ki.5000204

[8] **Mcdonald, K. (2015, June 22).** Scientists create synthetic membranes that grow like living cells. *Eurekalert!* Retrieved October 12, 2015, from http://www.eurekalert.org/pub_releases/2015-06/uoc--scs061715.php

[9] **National Kidney Foundation. (n.d.).** Leading Causes of Kidney Failure. Retrieved August 3, 2015, from http://www.nkfs.org/kidney-disease/leading-causes-of-kidney-failure/causes-symptoms-and-treatment/

[10] **NUS. (2015, December 9).** NUS researchers develop highly efficient nature-inspired membrane that can potentially lower cost of water purification by 30 per cent. *NUS News*. Retrieved December 24, 2015, from http://news.nus.edu.sg/press-releases/9790-biomimetic-membrane-for-water-purification

[11] **Nolph, K. (1985).** The Peritoneal Dialysis System. In *Peritoneal dialysis*(Second, revised and enlarged ed.). Dordrecht: Springer Netherlands.

[12] **Ronco, C. (2009).** The Wearable Artificial Kidney: Is Peritoneal Dialysis the Solution? *Peritoneal Dialysis - From Basic Concepts to Clinical Excellence Contributions to Nephrology, 163*, 300-305. doi:10.1159/000223814

[13] **Ronco, C., Davenport, A., Gura, V. (2008).** A wearable artificial kidney: Dream or reality? *Nature Clinical Practice Neurology, 4*(11). doi:10.1038/ncpneph0929

[14] **Ronco, C., Fecondini, L. (2007).** The Vicenza Wearable Artificial Kidney for Peritoneal Dialysis (ViWAK PD). *Blood Purification*. Retrieved September 7, 2015, from http://www.karger.com/Article/Pdf/107775

[15] **Shaw, R., Kotowich, S., Mantsch, H., & Leroux, M. (1996).** Quantitation of Protein, Creatinine, and Urea in Urine by Near-Infrared Spectroscopy.*Clinical Biochemistry, 29*(1). doi:0009-9120(95)02011-A

[16] **Storrs, C. (2015, November 11).** Could wearable 'artificial kidney' free patients of big dialysis machines? *Cable News Network*. Retrieved June 29, 2015, from http://edition.cnn.com/2015/11/11/health/wearable-artificial-kidney-dialysis-study/

[17] **Stumpe, M., & GrubMuller, H. (2007).** Aqueous Urea Solutions: Structure, Energetics, and Urea Aggregation. *The Journal of Physical Chemistry B*. doi:10.1021/jp066474n

[18] **Wang, H., Chung, T., Tong, Y., Jeyaseelan, K., Armugam, A., Chen, Z., Meier, W. (2012).** Highly Permeable and Selective Pore-Spanning Biomimetic Membrane Embedded with Aquaporin Z. *Small*. doi:10.1002/smll.201102120

[19] **Wang, H., Chung, T., Tong, Y., Jeyaseelan, K., Armugam, A., Duong, H., Hong, M. (2013).** Mechanically robust and highly permeable AquaporinZ biomimetic membranes. *Journal of Membrane Science*.

[20] **Wang, H., Chung, T., & Tong, Y. (2013).** Study on water transport through a mechanically robust Aquaporin Z biomimetic membrane. *Journal of Membrane Science*.

[21] **Xie, W., He, F., Wang, B., Chung, T., Jeyaseelan, K., Armugam, A., Tong, Y. (2013).** An aquaporin-based vesicle-embedded polymeric membrane for low energy water filtration. *Journal of Materials Chemistry A*. doi:10.1039/c3ta10731k

[22] **Zhao, Y., Qiu, C., Li, X., Vararattanavech, A., Shen, W., Torres, J., Tang, C. (2012).** Synthesis of robust and high-performance aquaporin-based biomimetic membranes by interfacial polymerization-membrane preparation and RO performance characterization. *Journal of Membrane Science*.