Nanoporous Membrane Robustness / Stability In Small Form Factor Microfluidic Filtration System

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***Abstract*— The development of wearable hemodialysis (HD) devices that replace center-based HD holds the promise to improve both outcomes and quality-of-life for patients with end-stage-renal disease (ERD). A prerequisite for these devices is the development of highly efficient membranes that can achieve high toxin clearance in small footprints. The ultrathin nanoporous membrane material developed by our group is orders of magnitude more permeable than conventional HD membranes. We report on our progress making a prototype wearable dialysis unit. First, we present data from benchtop studies confirming that clinical levels of urea clearance can be obtained in a small animal model with low blood flow rates. Second, we report on efforts to improve the mechanical robustness of high membrane area dialysis devices.**

I. Introduction

ERD affects 2 million people worldwide [1]. The silicon-based ultrathin nanoporous membrane materials developed by our group are orders of magnitude more permeable than conventional HD membranes and were originally described for use in size separations [2], and the uses for ultrathin nanoporous membranes has expanded to include co-cultures [4], electro-osmotic pumping [5], and dialysis devices [6].

By controlling pore sizes during manufacturing, the membranes can be engineered to pass middle-molecular-weight protein toxins while retaining albumin, mimicking the healthy kidney. A microfluidic dialysis device developed with these membranes achieved urea clearance rates that confirm that the membrane offers effectively no resistance to urea passage [3]. Two factors of interest are the clearance rates for uremic toxins of the membrane as well as the stability of the membranes in biologic fluids.

II. Long Term Benchtop Clearance Test

A. Experimental Set up

The setup used for the long term clearance testing [6] is shown in Figure 1. The fraction collector can be set to advance to the next collection tube at intervals from 1 min to 99 min. Samples were collected every hour for 12 hours. The collected samples were assayed for urea concentrations by absorbance using a urea assay kit as described by the manufacturer (ABCAM, Cambridge, MA).

B. Method

The dialysis tests were performed in two parts to avoid interference and masking by similar emission wavelengths. First, a 9.4 mM concentration of urea in 100% serum was used, then a 1 mg/mL cytochrome c and 1 mg/mL fluorescent BSA in 100% serum. Cytochrome c absorbs at 405 nm. The OxiRed probe in the urea assay kit emits at 570 nm, and FITC tagged BSA, (excitation 492 nm, emission 520 nm) was used. Isopropanol was pumped through the single membrane chip followed by degassed PBS pumped for 12 hours to eliminate bubble formation. Serum was pumped at 5.6 µL/min for 12 hours with collections taken at one-hour intervals.

A TECAN M200 multimode plate reader (Tecan Trading AG, Switzerland) was used to measure the serum retentate. The expectation was the albumin would maintain its initial concentration and the cytochrome c and urea were expected to decrease in concentration due to diffusion with cytochrome c having a smaller decrease over urea due to its larger size (13 kD and 60 kD respectively.)

III. Flow Pressure Burst Tests

In order to assess the robustness requirements of the membranes, the pressures within the tubing and the small dialysis device were explored. The goal was to design a system tolerant of the normal pressures experienced during a four-hour animal dialysis experiment.

Multichannel devices were constructed by incorporating fluidic components formed in polydimethylsiloxane (PDMS) with two 22 mm x 24 mm membrane chips with 11 parallel microfluidic channels [6]. The geometry of the membranes, which can be fabricated with thicknesses from 15 nm to 75 nm, brings with it concerns of pressure stability. In co-cultures and size separations, where the typical membrane area is 2.7 mm^2 to 15 mm^2, pressure has not been an issue, but for applications requiring an increased surface area to be feasible, such as hemodialysis,

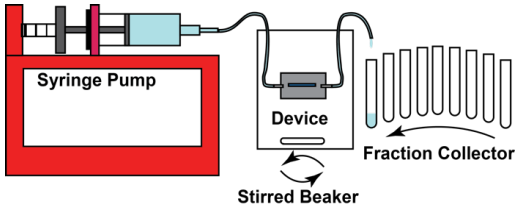

Figure 1: Long-term dialysis clearance set up. Syringe pump: 5 mL of 4.1 mM urea (or 1mg/mL Cytochrome-C and 1mg/mL Albumin), 5.6 µL/min. Single channel dialyzer, 10 mm long, 0.5 mm wide. Fraction Collector programed to rotate every hour. Setup in refrigerator at 40 ºC.

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trans-membrane pressure is an important consideration.

An eleven-membrane chip device was placed into a pumping system to mimic a small animal dialysis device to be used in four-hour clearance experiments. Pressures were measured at the inlet and outlet of both the blood and dialysate sides of the chip. Figure 2 shows a two-chip dialysis device within the fixture used for the single chip flow tests.

In HD, blood is pumped via a peristaltic pump along one side of a filtration membrane and dialysate (water, glucose, salts, etc.) is pumped in a counter flow along the other side of the membrane. This µHD system uses a silicon chip based filter with 11 channels (10 mm long, 0.5 mm wide) with a nanoporous filter material (50 nm or 75 nm thick) separating the two fluids. A fluidic circuit directs blood into the channels (300 µm deep, determined by wafer thickness) along the filter and collects it exiting the chip. Dialysate is moved across the flat side of the chip in a single channel covering the entire membrane surface. The flow device is housed in two acrylic plates with blunt needles used for fluidic access, and is connected to the blood flow from a mini peristaltic pump (VWR International, Radnor, PA, USA) and dialysate from an IV bag via gravity as shown in Figure 3. Pressure sensors (LabSmith) are located at both inlets and both outlets of the system. The lab smith software captured relative pressures to determine transmembrane pressure.

We investigated normal operating pressures of the µHD system. The expected pressure drop across the various fluidic resistances was calculated using either the formula for circular pipes,

$$\Delta P = \frac{8Q\mu l}{\pi L^4} \quad (1)$$

for the tubing between the sensor and the first silicon gasket flow channel, or for low profile rectangular channels,

$$\Delta P = \frac{a\mu Q l}{\pi W} \quad (2)$$

where,

$$\mathrm{a} = 12\left[1 - \frac{192\mathrm{H}}{\pi^5 \mathrm{W}} \tanh\left(\frac{\pi \mathrm{W}}{2\mathrm{H}}\right)\right]^{-1} \quad (3)$$

which includes the gasket channels and the membrane channels themselves. The majority of the pressure drop is across the membrane channel due to its lower height of 300 µm. The dialysate side of the membrane does not experience as much of a pressure drop as the 'plenum' on the flat side is both wider and deeper.

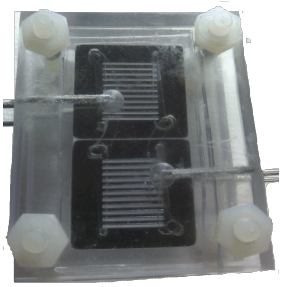

Figure 2: Two-chip dialyzer. 'Blood-side' shown, inlet from left divides fluid to both chips then evenly into the channels in the surface of the chips. Fluid was then collected from the channels and exits the single outlet on the right. Similar fluids route the dialysate across the smooth underside of the chip.

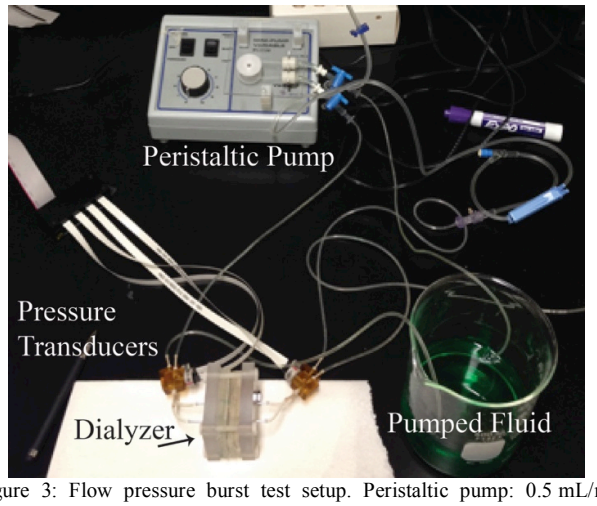

Figure 3: Flow pressure burst test setup. Peristaltic pump: 0.5 mL/min pumps fluid to and from beaker (at atmosphere) through 'blood-side' of the single chip dialyzer. 'Dialysate-side' is supplied via gravity feed.

A. Experimental Setups

The chips are clamped in between two plastic plates for fixation. There are two PDMS gaskets on each side of the chips. The PDMS gaskets in direct contact with the plates have punched holes that allow fluid to flow through the inlet and outlet channels, and the PDMS gaskets that contact the chip has channels that distribute fluid into two chips with equal pathway length. The top part of this device circulates 'blood', which will be different fluids in our experiment in which water was used as a control. The 'blood' circulates to the chip and returns to a beaker, while the bottom part delivers dialysate. The fluid channels on the chip directly contact the top PDMS and the membrane side contacts the bottom PDMS with the gasket in between.

The four sensors are positioned as close to the dialyzer as possible as shown in Figure 3. The Tees are connected to either the peristaltic pump on the blood side, or the gravity fed IV bag on the dialysate side. Water was used in these baseline experiments. We measure pressure on the 'blood' side and the 'dialysate' side at both the inlet and outlets of the dialyzer.

B. Method

In the first part of the experiment, a non-porous membrane chip was used to determine the pressure difference between the side of the entering 'blood' and that of entering dialysate.

In the second part of the experiment, a chip with a nanoporous membrane was used. Deionized water was used in both the 'blood' circulation side and the dialysate side. Burst pressure required to break the membrane was found by pinching off the return line of the 'blood'. The 'blood' fluid in the beaker was colored using green food coloring, and breakage of the membrane can be visualized when

green fluid is seen in the bottom plate side. Breakage was also detected from the maximum pressure recorded with the LabSmith software. This was repeated to find the burst pressure after running water for 4 hours.

IV. Membrane Strength and Stability

The purpose of the static burst pressure test is twofold. First, the strength of the membranes as manufactured is important for evaluating their ability to survive in an environment with fluid flow and transmembrane pressure. Secondly, it is imperative to evaluate the potential chemical effects of the salt in physiological fluid on the strength of the membranes.

A. Experimental set up

An aluminum pressure cell, as shown in Figure 4, was used to determine the burst pressure of membranes on 5.4 mm square chips. The membrane was placed, trench side down, between a rubber O-ring and a top plate with a viewing window. The membrane was exposed to compressed gas from below, controlled with a gas cylinder regulator and measured with an electronic manometer, until it bursts. The maximum pressure before bursting was recorded on the manometer.

B. Method

Burst pressure of rectangular windows was used to find the influence of membrane geometry on burst pressure and therefore membrane strength. Membranes soaked in PBS were used to determine the effects of fluids on burst pressure. Membranes of two different thicknesses were used to determine the effects membrane thickness on burst pressure.

1) Window Geometry

SiMPore produced wafers of 50 nm and 75 nm NPN with a matrix of four widths by five lengths (one membrane per chip). Widths of 100 µm, 300 µm, 500 µm, and 700 µm, and lengths of 1 mm, 1.5 mm, 2 mm, 2.5 mm, and 3 mm were tested in the burst pressure set-up (n=4).

2) Stability in Salt

This experiment investigated if soaking in PBS would significantly weaken the membrane and lead to low burst pressure. All chips in this experiment had the same membrane dimensions. Three groups of chips were tested. Fresh chips that were not soaked in any solution were tested as the control group (n=5). Chips soaked in 1x PBS for 4 hours, 24 hours, 48 hours and those soaked with 100% fetal bovine serum (FBS) for 1 hour, 2 hours and 4 hours were also tested (n=5).

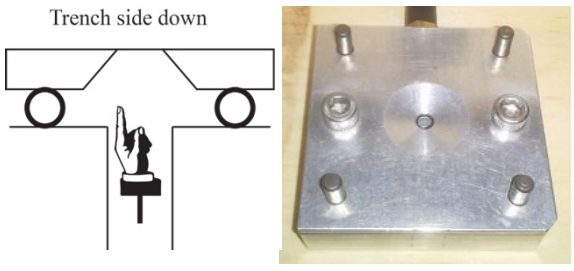

Figure 1: Static burst pressure setup. 5.4 mm chips were placed on the rubber o-ring over the compressed gas inlet port. The chip is held in place with an aluminum plate with an access hole that allows visual inspection of the membrane and acts as an outlet for the compressed gas after the membrane has burst. An electronic manometer recorded the burst pressure.

The chips were rinsed with DI water, dried then soaked in PBS or FBS, then re-rinsed with DI water and air dried before testing in the pressure cell.

3) Membrane Thickness

Chips with square membranes of varying size (side lengths from 0.3 mm to 1.8 mm) were fabricated with one of two membrane thicknesses, 50 nm or 75 nm. Burst pressure tests, as described above were performed. The expectation was that the 75 nm membranes would have higher burst pressures over the 50 nm membranes at the same membrane areas geometries, and that burst pressures would increase for smaller membrane areas.

V. Results

A. Clearance Rates

Benchtop dialysis experiments were conducted in a counter flow system in which 100% serum was spiked with either urea, cytochrome C (a 13kD colored surrogate for ß2 microglobulin - B2M), or fluorescently labeled albumin and dialyzed against PBS buffer for 12 hours. Samples taken at the output of the dialyzer show steady clearance of urea for 12 hours at the reduction predicted by the COMSOL model for the flow rates used. Figure 5 shows the dialyzer exit concentrations for urea, cytochrome c and f-BSA normalized to initial concentrations of 4.1 mM, 1 mg/mL, and 1 mg/mL respectively. Note that the 13kD clearance is also steady over 12 hours while albumin is fully retained. Consistent clearance over 12 hours indicates a lack of membrane fouling despite very high protein content in serum.

B. Analysis of pumping burst pressures of HD membranes

The normal operating transmembrane pressure was between 0.5 psi and 1.5 psi, (initial and after DI or PBS flow for four hours). Extrapolating from burst pressure tests for the 10 mm long HD chips with 50 nm membranes, a burst pressure of between 1 psi and 2 psi was expected. This indicates that the 50 nm thick membranes may burst with the expected operational pressures of the pump and dialysis system.

C. Static Burst Pressure

1) Window Geometry

Figure 6 groups the chips by side length. While there was some increase in burst pressure with the shorter lengths, the variation is much higher for decreasing the width. The width increases up to sevenfold while the length only increases threefold. Even increasing the length from 100 µm to 300 µm (threefold increase) the burst pressure decreases more than the same threefold increase in width.

2) Stability in Salt

When compared to the burst pressure of chips that were not soaked, the chips soaked in PBS showed no change. This indicates that the membranes will not become

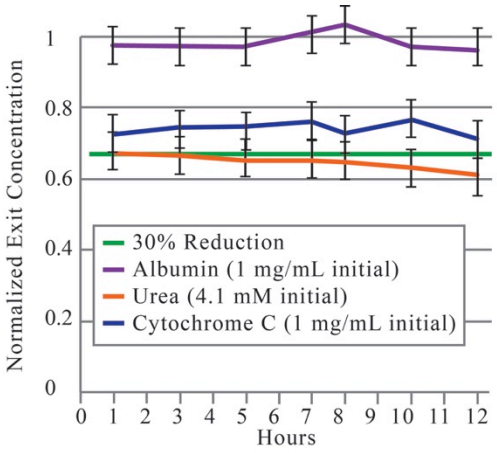

Figure 5: 12-hour clearance study. Exit concentrations normalized to inlet concentrations. Urea (4.1 mM), Cytochrome-C (1 mg/mL), and Albumin (1 mg/mL) in 100% serum were pumped through a single pass counter flow device. While the filter retained albumin, urea met its expected 30% reduction. Cytochrome-C (13 kDa) was also reduced.

weakened in a biological environment. The membranes also showed no decrease in burst pressure from soaking in FBS.

3) Membrane Thickness

Both 50 nm and 75 nm thick membranes were tested for static burst strength, and flow burst strength. Figure 7 shows the burst pressure results for square membranes of 50 nm and 75 nm thicknesses. There was a marked increase in burst pressure for the thicker membranes that should put the burst pressure for the HD devices above the operating pressures of the system.

The 50 nm and 75 nm thick hemodialysis chips were tested in the dialysis flow system. The burst pressures were found to be 1.5 psi (n=3) and 3.62 psi (n=4) respectively. Increasing the thickness 1.5 fold lead to an increase in burst pressure of more than double.

Importantly, the increase in burst pressure will allow the membranes to survive the transmembrane pressure experienced in the hemodialysis system (1 to 1.5 psi).

VI. Conclusion

The ultimate goal was to develop design rules and

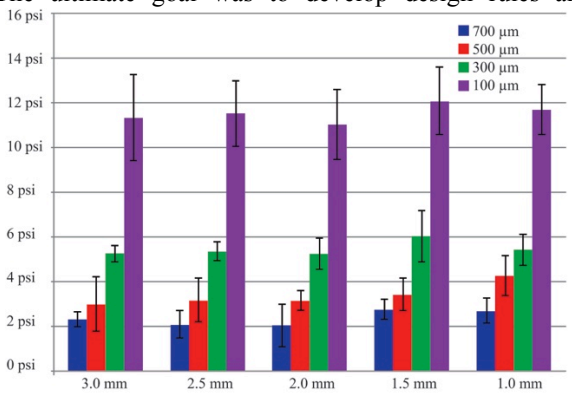

Figure 6: Burst pressures of rectangular membranes, lengths of 1.0 mm to 3 mm, and widths of 0.1 mm to 0.7 mm. Clearly, membrane width plays a more important role in determining the burst pressure than does lengths, for these dimension ranges.

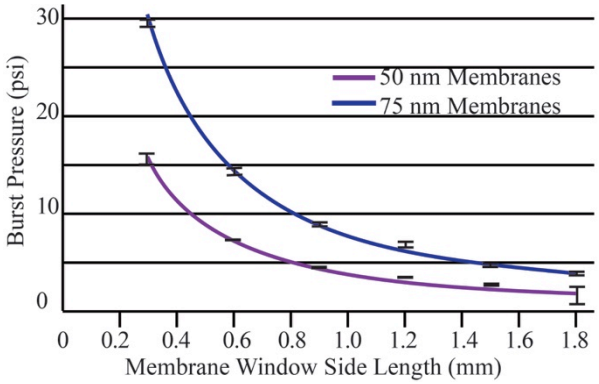

Figure 7: Static burst pressure results of 75 nm membranes vs. 50 nm membranes (square membranes). Burst pressures are doubled for a 1.5 fold increase in membrane thickness.

fabrication methods for implementing a small footprint dialyzer for a four-hour dialysis experiment with small animal models. The dialyzer must be able to tolerate normal pressures expected to exist in the test devices.

75 nm thick membranes (0.5 mm wide by 10 mm long) can easily withstand the pressures expected in the µHD system. Additional robustness can be gained by decreasing the membrane width to 300 µm. Such a membrane can be expected to have a burst pressure of greater than 6.9 psi. Other methods of boosting the burst pressure in the µHD system include inclusion of fluidic capacitors before and after the dialyzer such that it is kept away from the pulsatile pressures of the peristaltic pump and any pressure spikes that may enter the system from the biological system.

References

1] C. R. Blagg, "The 50th anniversary of long-term hemodialysis: University of Washington Hospital, March 9th, 1960," in *J Nephrol.* vol. 24 Suppl 17, ed Italy, 2011, pp. S84-8.

[2] C. C. Striemer, T. R. Gaborski, J. L. McGrath, and P. M. Fauchet, "Charge- and size-based separation of macromolecules using ultrathin silicon membranes," *Nature,* vol. 445, pp. 749-53, Feb 2007.

[3] J. L. Snyder, A. Clark, D. Z. Fang, T. R. Gaborski, C. C. Striemer, P. M. Fauchet*, et al.*, "An experimental and theoretical analysis of molecular separations by diffusion through ultrathin nanoporous membranes," *Journal of Membrane Science,* vol. 369, pp. 119-129, 2011.

[4] A. A. Agrawal, B. J. Nehilla, K. V. Reisig, T. R. Gaborski, D. Z. Fang, C. C. Striemer*, et al.*, "Porous nanocrystalline silicon membranes as highly permeable and molecularly thin substrates for cell culture," *Biomaterials,* vol. 31, pp. 5408-17, Jul 2010.

[5] J. Getpreecharsawas, J. L. McGrath, and D. A. Borkholder, "The electric field strength in orifice-like nanopores of ultrathin membranes," *Nanotechnology,* vol. 26, p. 045704, Jan 2015.

[6] D. G. Johnson, T. S. Khire, Y. L. Lyubarskaya, K. J. P. Smith, J.-P. S. DesOrmeaux, J. G. Taylor*, et al.*, "Ultrathin Silicon Membranes for Wearable Dialysis," *Advances in chronic kidney disease,* vol. 20, pp. 508-515, 2013.

[7] D. Yorimitsu, M. Satoh, M. Koremoto, Y. Haruna, H. Nagasu, A. Kuwabara*, et al.*, "Establishment of a blood purification system for renal failure rats using small-size dialyzer membranes," *Ther Apher Dial,* vol. 16, pp. 566-72, Dec 2012.

[8] H. Y. Yokozawa T Fau - Chung, T. W. Chung Hy Fau - Lee, H. Lee Tw Fau - Oura, and H. Oura, "Renal function and urinary prostaglandins in rats given an adenine diet," 19891117 DCOM-19891117 1989