ADVANCED HEALTHCARE MATERIALS

# Supporting Information

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## Second Generation Nanoporous Silicon Nitride Membranes for High Toxin Clearance and Small Format Hemodialysis

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## 1. Computational Modeling

A geometrically accurate fluidic and mass transport model of the single-pass clearance experiments was developed using the COMSOL Multiphysics 5.3 software package (Burlington, MA). The model was designed using COMSOL Multiphysics and the Chemical Reaction Engineering Module. Specifically, it used Laminar Flow, Transport of Diluted Species, and Transport of Diluted Species in Porous Media (porosity = 0.15). Both diffusion (diffusion coefficient was set to $1.38 \times 10^{-9}$ $m^2 s^{-1}$) and convection are accounted for in the model, with the convection through the membrane set to zero to match the ideal of no ultrafiltration. The geometry consists of the central portion of the fluidic device directly surrounding the membrane area and is the same dimensions (**Figure S1a**). It also includes the 300-µm tall trench with the slanted sides, which result from the membrane release process. The membrane itself is drawn to have the same surface dimensions (2 mm by 0.7 mm) but is drawn two orders of magnitude thicker to simplify the mesh and keep calculation times short (<4 hours). The change in thickness is accounted for by changing the diffusion coefficient. We did not model individual pores as this would be computationally intensive (each membrane has > 50,000,000 pores). Instead we used pointwise constraints to model the selective nature of the porous membrane surface. The constraint is applied to both the top and bottom surface of the membrane and control the passage of the dilute species (**Figure S1b**). The constraint and constraint force expression used were c2-K*c1 and test(c2)-test(c1) respectively, where c1 is the concentration in the fluidic channels and c2 is the concentration in the membrane pores. K is the dimensionless partition coefficient and represents the concentration ratio at the membrane boundaries. For standard dialysis membranes K ~ 0.7 and this is the value used in the simulations.

The study reported flow velocity and species concentration. The maximum velocity of the fluid, at the membrane surface, is ~10 µm $s^{-1}$. (**Figure S1c**). We set the initial concentration of the analyte, 'blood', as c1=1 to represent the normalized initial concentration. The continuous concentration at the output of the analyte flow was $C_{out}$ = 0.73 which represents a 27% reduction of the analyte. (**Figure S1d**).

**2.0 Cytochrome c as a surrogate for β-2 Microglobulin**

**2.1 Results**

Cytochrome c (13 kDa) has been used as a surrogate for β-2 Microglobulin (13 kDa) because of its similar size and the molecule's strong visible absorbance and reliable detection at 405 nm. An experiment was designed to ascertain whether cytochrome c is a good surrogate in diffusion experiments. Cytochrome c was purchased from Sigma-Aldrich (St. Louis, MO, USA) while β-2 Microglobulin was purchased from Lee Biosolutions (Maryland Heights, MO, USA).

After performing the assay for three sets of standards of cytochrome c protein, a standard curve was obtained. The $R^2$ value of the curve was 0.9982 with a y = 0.4777x equation relating the absorbance values (y) to the corresponding concentration (x).

Three devices were assembled to carry out three double-membrane, single-pass clearance experiments, the averaged data is shown in **Figure S2**. The slope of the curve was 0.0023.

In a similar manner, the following β-2 Microglobulin standard curve was obtained after carrying out the ELISA kit's procedure. The $R^2$ value of the curve was 0.9924 and the $y = -0.0179x^2 + 0.3201x + 0.0869$ equation related the absorbances to the corresponding concentrations.

The first and last data points of each protein filtration is plotted in **Figure S2**, showing that the device's performance is steady and that cytochrome c is a good substitute for β-2 Microglobulin in clearance experiments.

**2.2. Experimental**

Devices, as used in the single-membrane devices (**Figure 2a**), were constructed with a double-membrane chip, three 300-µm layers of silicone gasket material, two 120-µm double-sided tape layers, and an upper layer of PDMS to hold the inlet and outlet dialysis tubing. The design of these devices was based on the previously made single-membrane devices and was created using a Cameo cutter and studio software (Silhouette America®, Inc. Lindon, Utah). The geometry of each layer allowed for the counter flow of the analyte and dialysate. All the layers were bonded, along with the chip, on top of a glass slide via an UV-ozone bonding process.

The starting concentration for cytochrome c and β-2 Microglobulin was 1 mg $mL^{-1}$ in phosphate-buffered saline (PBS). A 720 µL analyte volume of each protein was filtered through the double NPN-O membranes, while samples were collected every hour using an automated fraction collection system for a period of 6 hours at 3°C (to prevent any evaporation). The analyte and dialysate fluid flow rates were 2 µL $min^{-1}$ and 4 µL $min^{-1}$, respectively, for all experiments. This 1:2 ratio was shown to be the optimal

ratio in clearing the middle weight proteins from earlier experiments. A triplicate of each experiment was done, using new devices, to confirm the results.

An appropriate 1.44 mL dialysate (1X PBS) volume was set based on its flow rate and analyte volume and flow rate. The analyte and dialysate flow rates were maintained using a programmable syringe pump.

The cytochrome c protein was assayed using a simple protocol that measured its light absorbance at 405 nm in a clear 96-well plate. On the other hand, an appropriate β-2 Microglobulin ELISA-kit (#1814648) was purchased from IBL America (Minneapolis, MN, USA). The kit's reaction was based on the indirect enzyme immunoassay (ELISA) method. The β-2 Microglobulin binds to the antibody coated plate wells forming an antigen-antibody complex. Then, washing of the microwells removed any other unbound molecules. During incubation with anti- β-2- microglobulin enzyme-conjugate immunologically a conjugate / antibody / antigen complex is formed. Again, the wells were washed to remove unbound conjugate. Consequently, an enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. Then, the addition of an acid stops the reaction forming a yellow end-product. Finally, the intensity of this yellow color is measured photometrically at 405 nm. The amount of color is directly proportional to the concentration of antibodies present in the original sample. All protein absorbances were measured using a Tecan Infinite M200 Microplate Reader (Tecan Trading AG, Switzerland).

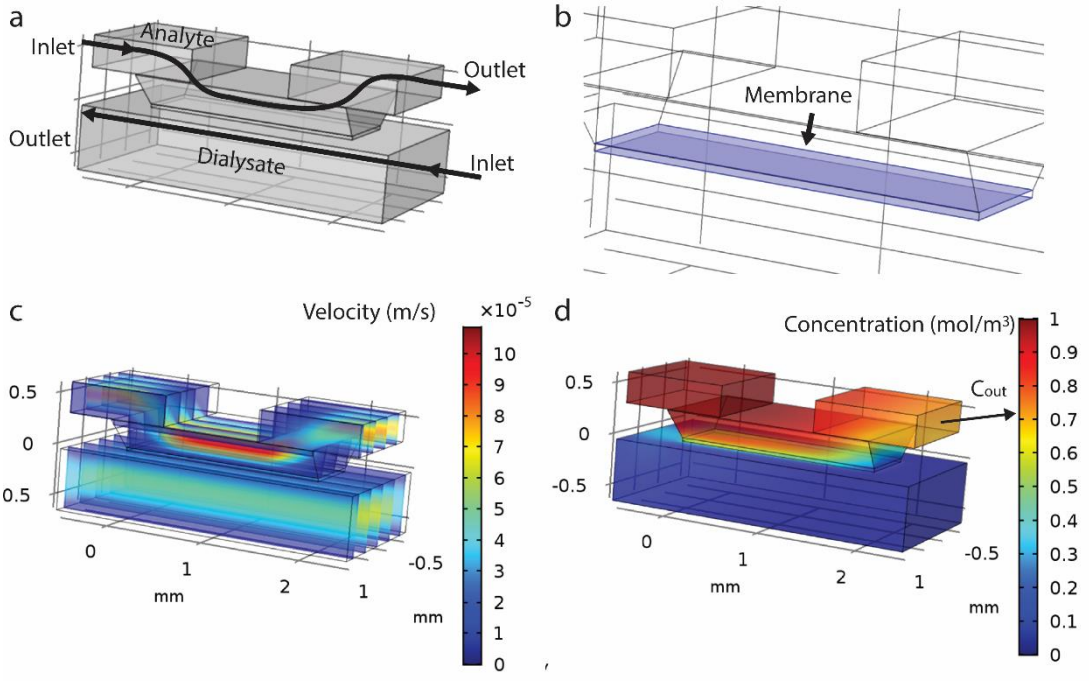

**Figure S1: Computational Modeling of Single-Membrane Hemodialyzer. a: Counter flow pattern of analyte and dialysate shown. b: Modeling the porous membrane surfaces used a point-wise constraint. c: Fluid flow velocity is shown. d: Concentration at analyte outlet (upper right) Cout = 0.73 mol $m^{-3}$.**

**Figure S2: Clearance Comparison of β-2 Microglobulin and cytochrome c. Single-pass experiments, with β-2 M and cytochrome c. There is no significant difference in the clearance over time, as shown by the p-values between the first and last values. There is a significant, but small, difference between β-2 M and cytochrome c clearance indicating that cytochrome c is a good surrogate for β-2 M in clearance studies. In fact, the clearance for and cytochrome c is lower than β-2 M and most likely underestimates its clearance.**