Structural Delineation of the Exeltra Plus 210 Hemodialyzer's Capillary Pores as Targets to Ameliorate its Filtration

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ABSTRACT

Aim: To examine potentials to improve solute flux of selected uremic toxins (1) across EXELTRA Plus 210 membrane.
Methodology: Scanning electron microscopy was used to measure the inner and outer fenestrations of the membrane. We selected endothelin, β2-microglobulin, and complement factor D as uremic toxins for this study. The effective diffusivities of these molecules, D_{eff}, were estimated using the equation $D_{eff} = K_{diff} D_o$, under different conditions of fenestration densities and capillary diameters, where the hindrance factor, K_{diff}, was calculated using the equation $K_{diff} = (\varepsilon / 2 - \varepsilon)^2$.
Results: The innermost and outermost fenestrations mean pore density of 2.71 % and 12.06 %, respectively. Provided that the fenestrations' density of the inner walls remained constant, doubling of the capillaries' diameter and reducing their numbers by half, reduced K_{diff} . Applying an electric potential across the outer surface of the capillaries increases the flux of the uremic toxins.
Conclusion: The potential to improve the diffusive transport of uremic toxins through the capillaries' walls could be enhanced by doubling the inner diameters of the capillaries, increasing the fenestrations density on the inner walls, and applying an electric potential.

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1. INTRODUCTION

The aim of hemodialysis is purifying the blood of uremic toxins. These toxins can either be small molecules like urea and creatinine with a molecular weight lower than 500 Da, or middle molecules that range in molecular weight from 300 to 12,000 Da [1]. Endothelin, β2-microglobulin, and complement factor D are examples of middle molecules uremic toxins, with molecular weights of 4,283 Da, 11,800 Da, and 23,750 Da, respectively [2]. Hemodialyzers broadly vary in pore sizes and pore densities [2], they have irregular pore forms and are characterized by inefficient filtration rates [3]. The molecular transport through hemodialysis membranes take place by three processes: (1) diffusion, which is driven by a concentration gradient across the membrane, (2) convection, which is driven by a hydrostatic pressure gradient between the two sides of the membrane, and (3) adsorption, which is governed by the adsorbed molecule and its interaction with the physiochemical characteristics of the membrane [4].

The limiting factors to middle molecules' clearance are the low pore densities in contact with the blood and the highly tortuous path from the inner surface of the capillary to its outer surface [5]. Recently we examined targets to improve hemodialysis of Polyflux 210H [6]. We aim in this study to compare similar analysis on the EXELTRA Plus 210. Exeltra Plus 210 is a single-use hemodialyzer with a 2.1 m^2 overall surface area. The capillaries are made of cellulose triacetate (CTA), and Baxter Healthcare Corporation, McGraw Park, IL, USA produce it. The dialyzer's capillaries have a 200 µms inner diameter, a 15 µm wall thickness, and are made of cellulose triacetate [7].

The objective of this research is to apply fluid mechanics theoretical principles to Exeltra Plus 210 dialysis membranes under different scenarios of percentage of open space, diameters of capillaries, number of capillaries, and combinations of these parameters. This can guide us to which combination of these parameters has potential to improve diffusivity of the membranes.

2. MATERIAL AND METHODS

2.1 Materials:

The hemodyalizer used in this research is a single-use EXELTRA Plus 210 distributed by Baxter Healthcare corporation, Deerfield, IL 60015 USA. We used a mixture of 2% paraformaldehyde and 2.5% glutardehyde in 0.1M phosphate buffer to fix the capillaries inside the EXELTRA Plus 210 dialyzer cartridge.

2.2 Apparatus:

A clamp and handsaw were used to open the EXELTRA Plus 210 hemodializer's casing. The capillaries were cut with scissors and dissected with a surgical scalpel. We used a Joel JSM-6010LV scanning electron microscope (SEM) and a Hitachi SU6600 field emission scanning electron microscope (FESEM) to characterize and measure the pores and cross-sections of the Exeltra Plus 210 hemodialyzer.

2.3. Procedures:

2.3.1 Fixing the EXELTRA Plus 210 capillaries: The capillaries of the EXELTRA Plus 210 were immersed in a 2% paraformaldehyde and 2.5% glutardehyde mixed in a 0.1 M phosphate buffer in a petri dish and fixed at room temperature overnight. The capillaries were then immersed three consecutive times in the phosphate buffer for the duration of ten minutes. They were then dehydrated using ethanol/water baths each lasting for 20 minutes in the volume ratio of 50/50,

70/30, 90/10, respectively. Finally, they were dehydrated twice in 100% ethanol, with each bath lasting 20 minutes.

2.3.2 Structural delineation of the EXELTRA Plus 210: The experimental procedure we followed to delineate the structure of the EXELTRA Plus 210 was identical to that detailed in our previous characterization of the Polyflux 210H capillaries [6]. We used the same uremic toxins molecules (endothelin, β2-microglobulin and compliment factor D) when characterizing the EXELTRA Plus 210 capillaries so that we can compare the results between the two hemodialyzers.

2.3.3 Estimation of the capillary wall tension: LaPlace Law was used to estimate the capillary wall tension in the EXELTRA Plus 210 dialyzer. Laplace Law states that the tension in the capillary wall is equal to the product of the applied pressure and the outside radius of the capillary, divided by the capillary's wall thickness. In order that we compare the tension exerted on the walls of the EXELTRA 210 capillaries with that exerted on the walls of the Polyflux 210H capillaries, we assumed that the proximal terminal of the hemodialyzer is subjected to an inlet pressure of 300 Hg. Given the dimensions of the capillaries and their overall surface area, we calculated the number of capillaries in an EXELTRA 210 dialyzer to be 16,438 capillaries.

2.3.4 Estimation of D_{eff} of selected molecules under different conditions: The diffusivity of molecules through pores cannot be characterized as free diffusivity, D_o. It is hindered by the interaction between the molecules and the walls surrounding the pores. K_{diff} denotes this hindrance [8]. Effective Diffusivity, D_{eff}, of a specific solute through the pores of a hemodialysis capillary is the product of K_{diff} and D_o. Also, $K_{diff} = [\varepsilon / (2 - \varepsilon)]^2$ where ε is the fraction of open space in a membrane [9], and $D_o = 1.76 \times 10^{-4} (MW)^{-0.552}$ [10].
We tabulated the data for the D_{eff} of urea, glucose, endothelin, β2-microglobulin, compliment factor D, and albumin through the EXELTRA 210 H under different conditions of the hindrance factor as we did in our previous research work with Polyflux 210H [11] in Table 3.

2.3.5 Evaluation of the effect of applying an electric potential across the walls of EXELTRA Plus 210 on selected molecules: Assuming an applied potential of 10 mV on the outer surfaces of the EXELTRA capillaries, the solute flux caused by electromigration, $J_{elelectromig}$, can be calculated using the following equation:

$$Jelectromigr = \left(\frac{D_{eff} A C_m z F}{RT}\right)\left(\frac{dV}{dx}\right) \qquad [12]$$

C_m is related to the concentration of the solute prior to filtration, C_{bulk} by the following formula:
$C_m = S \; C_{bulk}$ where S is the sieving coefficient of each solute [13].

The normal concentrations of endothelin, β2-microglobulin, and complement factor D and their sieving coefficients are given in Table 1.

Table 1. Normal blood concentrations of selected uremic toxins and their corresponding sieving coefficient

Uremic toxin	*Normal blood concentration **	*Sieving coefficient ***
Endothelin	28.8 +/- 3.8 ng/L [2]	0.70
β2-microglobulin	< 2.0 mg/L [2]	0.35
Complement factor D	1.9 +/- 0.5 mg/L [2]	0.07

* C_{bulk}
** Estimated from the sieving coefficient plot versus molecular weight [14].

3. RESULTS AND DISCUSSION

3.1 Structural delineation of the EXELTRA Plus 210: Both the SEM and FESEM yielded very interesting photomicrographs of the outer, inner, and cross-sectional surfaces of the EXELTRA Plus 210. Figure 1 shows the outer surface of the capillary. No macroscopic features are apparent from the image. Zooming in on the outer surface reveals the open surface with the dimensions as shown in Figure 2. Figure 3 shows the oval shaped fenestrations within the inner walls of a capillary. The mean of the openings' width and the percentage of open space for both the outer and inner surfaces of a capillary are summarized in Table 2.

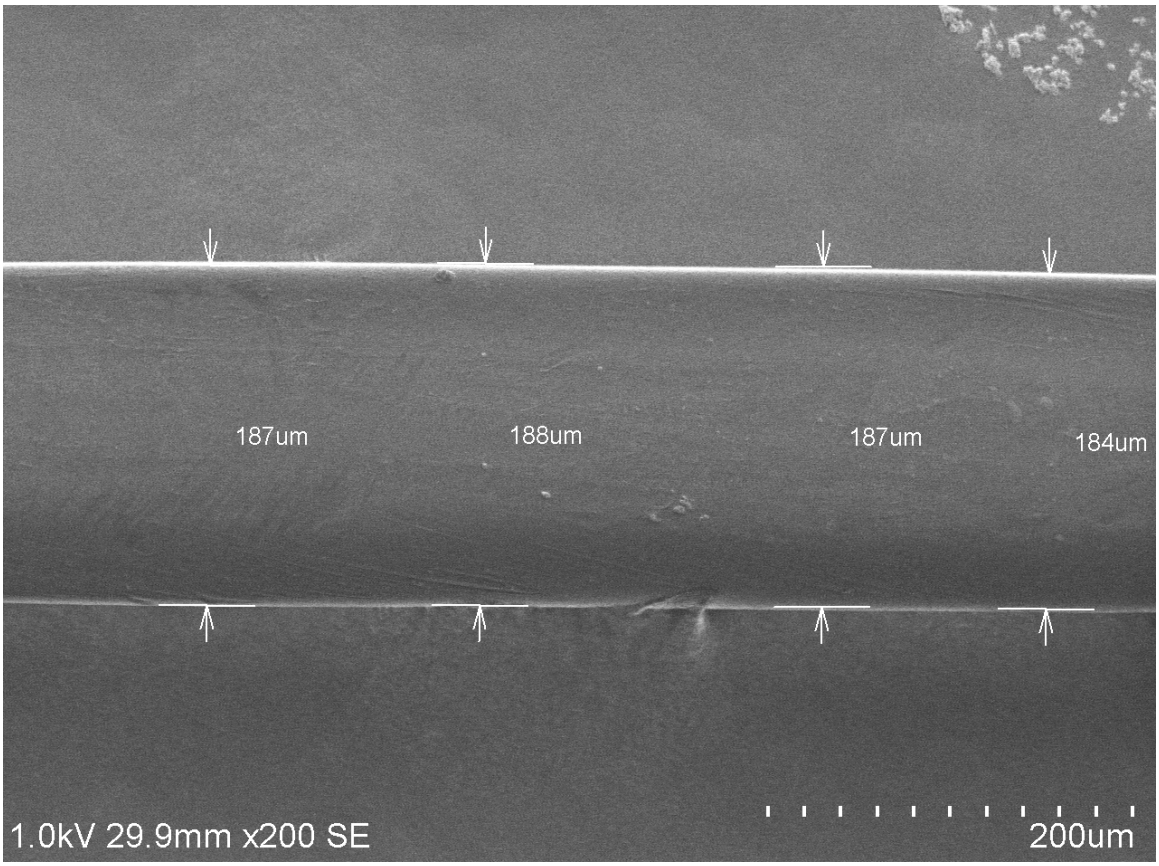

Fig. 1 Macro image of the outer surface of an EXELTRA Plus 210 capillary

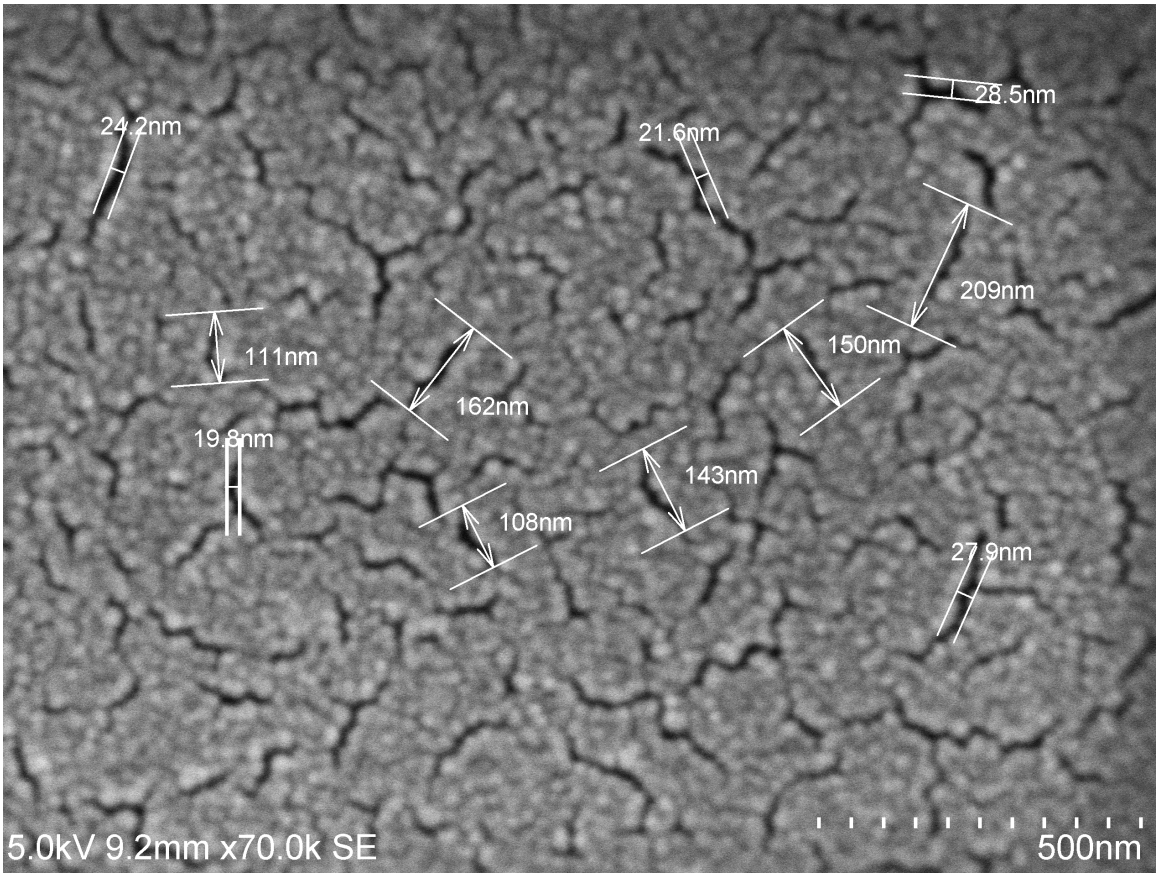

Fig. 2 Fenestrations in the outer surface of an EXELTRA Plus 210 capillary

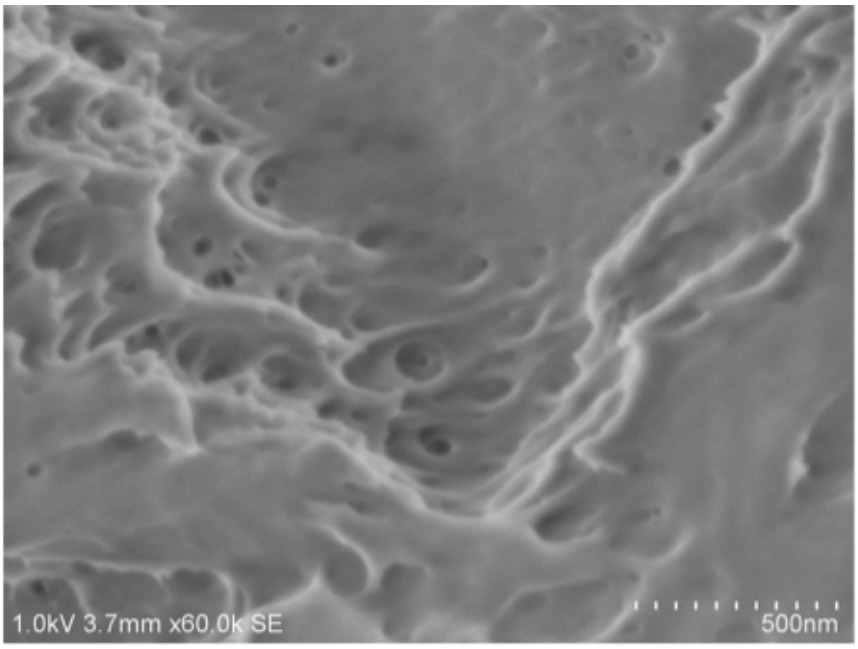

Fig. 3 Fenestrations in the inner wall surface of an EXELTRA Plus 210 capillary

Table 2. Summary of measurements of an EXELTRA Plus 210 capillary

Characteristic	***EXELTRA Plus 210 mean ± standard deviation***
Open space (inner surface - in contact with the blood)	2.71 ± 1.06 %
Open space (outer surface - in contact with the dialysate)	12.06 ± 3.07 %
Outer diameter	200 µm [7]
Wall thickness	15 µm [7]
Inner surface pore width	32.25 ± 6.40 nm
Outer surface pore width	24.40 ± 3.81 nm

The cross-section of the capillaries yielded a layered structure (Figure 4 (a)) that has circular holes emerging parallel to the cross-section. These holes varied in diameter from tens of nanometers (Figure 4 (b)) to about 200 nm (Figure 4 (c)). Compared to the polyflux 210H, the EXELTRA Plus 210H has relatively less pores, about 50 % less.

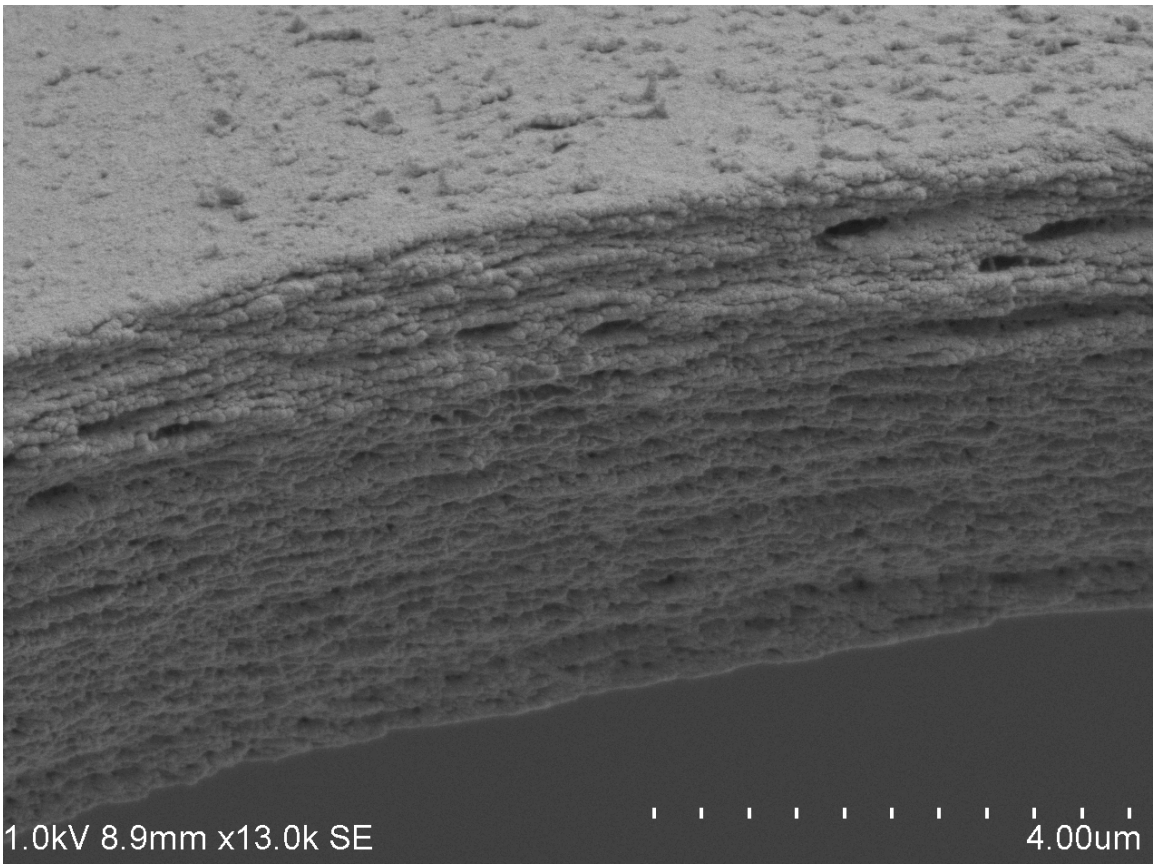

Fig. 4 (a) Cross-section of a capillary

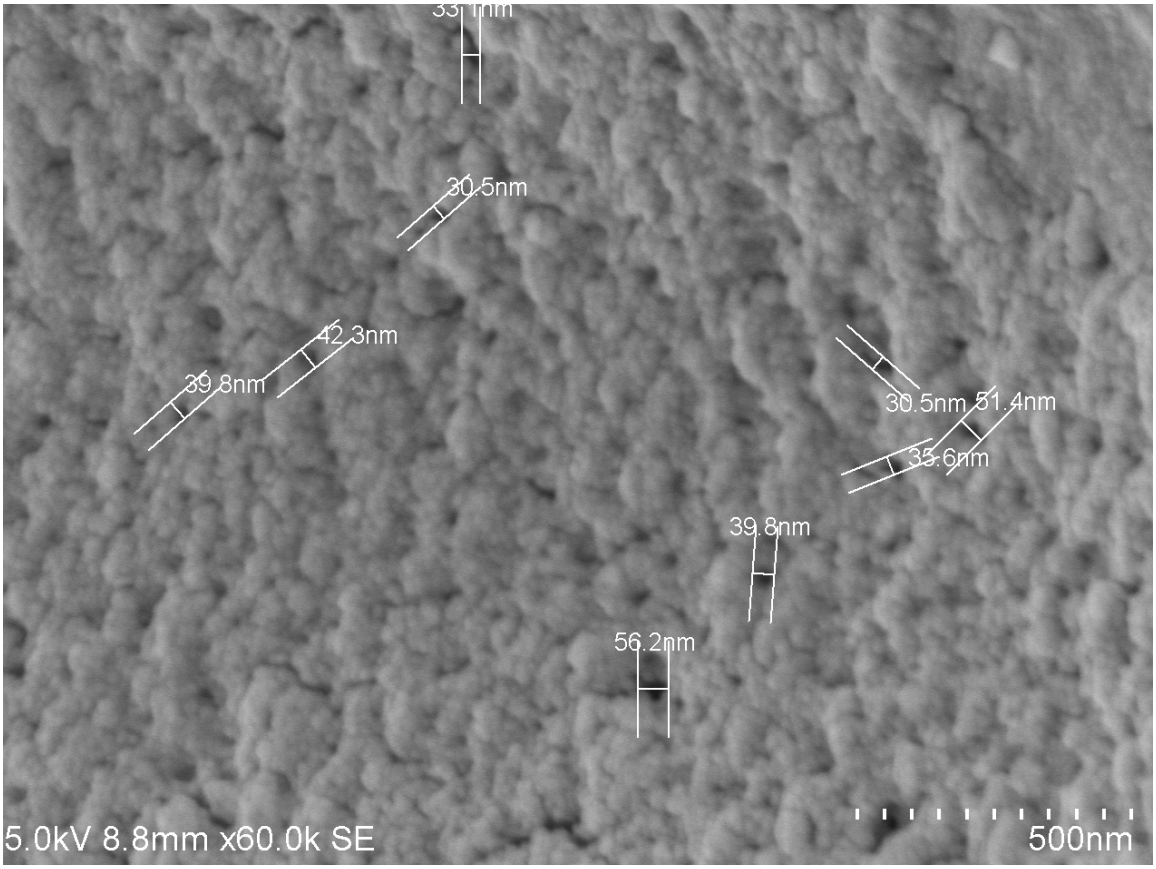

Fig. 4 (b) Relatively small size round holes in the cross-section of a capillary

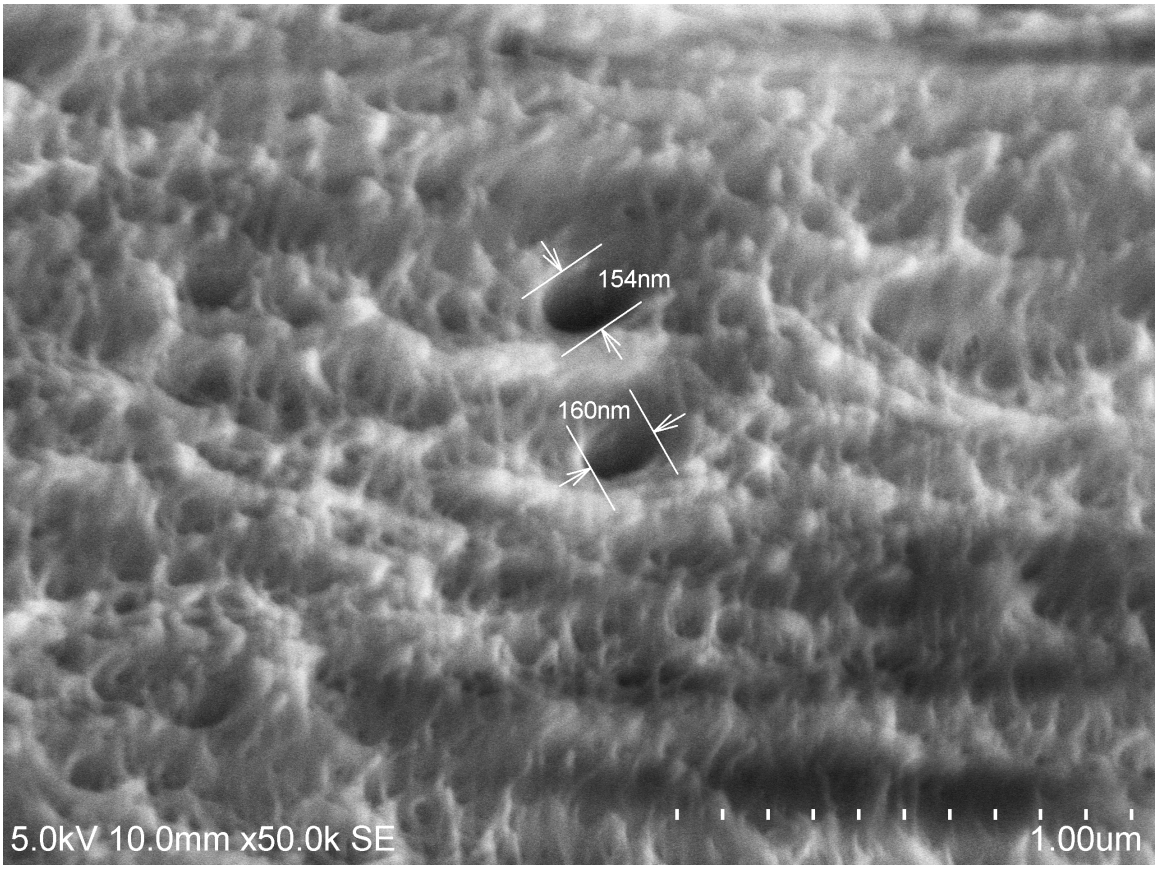

Fig. 4 (c) Relatively large round holes in the cross-section of a capillary

3.2 Estimation of the capillary wall tension: Using Laplace law, we calculated the tension exerted on the walls of each capillary to be 0.1399 mmHg or 2.705 x 10^{-3} psi / capillary. The wall tension in the EXELTRA Plus 210 is double that of the Polyflux 210H capillaries.

3.3 Estimation of D_{eff} of selected molecules under different conditions: Table 3. summarizes our findings. Note how diffusivity hindrance drops as we increase the pore density.

Table 3. Free and Effective Diffusivities of Albumin and Selected Uremic Toxins

		Conditions			
Molecule	***D_o x 10^{-8} (cm^2/s)*** ***[11]***	***(a) D_{eff} x 10^{-10} (cm^2/s) EXELTRA Plus 210 (ε = 0.0271) K_{diff}= 0.000188***	***(b) D_{eff} x 10^{-10} (cm^2/s) EXELTRA Plus 210 (ε =0.0542) K_{diff}=0.00078***	***(c) D_{eff} x 10^{-10} (cm^2/s) EXELTRA Plus 210 (ε=0.05826) K_{diff}=0.0009***	***(d) D_{eff} 10 x 10^{-10} (cm^2/s) EXELTRA Plus 210 (ε = 0.0291) K_{diff}= 0.0002***
Urea	1836.42	34.52	143.2	165.27	36.73
Glucose	1001.39	18.83	78.11	90.13	20.02
Endothelin	174.10	3.27	13.58	15.67	3.48
β_2-Microglobin	56.53	1.06	4.41	5.09	1.13
Complement Factor D	38.43	0.72	3.00	3.46	0.77
Albumin	21.33	0.40	1.66	1.92	0.42

(a) Calculations were based on an inner pore density of 2.71 ± 1.06 %, and a capillary's wall thickness of 15 µm.
(b) Calculations were based on doubling the pore density.
(c) Calculations were based on doubling the inner pore density, doubling the capillaries' inner diameter, and reducing the capillaries number by half.
(d) Calculations were based on the assumption of doubling the capillaries' diameter, and reducing the capillaries number by half.

3.4 Evaluation of the effect of applying an electric potential across the walls of EXELTRA Plus 210 on selected molecules: In a previous study [5], we proved that, theoretically, the application of an electric potential on the walls of membranes could enhance the flux transport across the membrane. Given the thickness of the membrane as 15 µm, and a K_{diff} of 188 x 10^{-6}, the flux increase, $J_{electromigr}$ for endothelin, β2-microglobulin, compliment factor D increased as follows when we applied an electric potential across the capillaries' surfaces:

For endothelin:

$$J_{electromigr} / z = 384.17 \times 10^{-10} \quad dV$$

For β2-microglobin:

$$J_{electromigr} / z = 15.69 \times 10^{-11} \quad dV$$

For Compliment Factor D:

$$J_{electromigr} / z = 14.37 \times 10^{-11} \quad dV$$

Where $J_{electromigr}$ is in mol/s, and V is in volts.

Several studies addressed the pore size characterization of CTA hollow-fiber dialysis membranes. In one study, the pores diameters were reported to range between 6.9 to 12.2 nm for the inner surface of the membrane, while those on the outer surface ranged from 3.2 to 3.6 nm. The open space (porosity) was estimated at 38% for both inner and outer surfaces of the membrane [15]. In another study, the pore radii ranged between 5.8 and 7.8 nm [16].

Our results indicate that the pore sizes in CTA are greater than those reported by Yamazaki et. al. [15] and Sunohara et. al. [16]. The difference may be attributed to the structural and dimensional changes occurring in the CTA membrane when dry. Our SEM characterization was pursued on a dry membrane, while that pursued by Yamazaki were pursued using the atomic force microscope (AFM) under water. Our results also yielded lower mean porosities 2.71 ± 1.06% and 12.06 ± 3.07% for the inner and outer surfaces of the hollow-fiber membrane, respectively, as compared to 38% for both surfaces as reported by Yamazaki et. al. [15]. The discrepancy can also be contributed to the characterization of the membrane under water in the study by Yamazaki et. al. [15] and our characterization, which was pursued under dry conditions. Using the segment of the Nernst-Planck equation relevant to solute flux attributed to diffusion that we used in our previous study [5]: $J_{diff} = - D_o A K_{diff} (dC/dx)$, where A is the overall area of the membrane, it is clear that a higher surface area of the membrane will yield higher solute fluxes.

It is interesting to notice that the mean width of the inner wall fenestrations of the EXELTRA Plus 210 is greater than that on the outer wall ones. It is also interesting to observe the layered structure of the capillary walls and the tangential round openings in them. We envision that the solutes follow two paths of transport. The first path is a tortuous path from the inner surface that is in contact with the blood to the outer surface in contact with the dialysate. The second path is the confined transport of the uremic toxins molecules into the cylindrical channels that are inside and parallel to the surface of the capillary walls. This is in contrast to the flow of toxins from the compact side adjacent to the blood through a tortuous path to the widely open structure in contact with the dialysate [6].

The mean density of the open pores on the inside surface of the capillaries in the EXELTRA Plus 210 (2.71 %) is about half of that of Polyflux 210H (5.45 %). However, at an inlet pressure of 300 Hg, the tension on the capillary walls of the EXELTRA Plus 210 (0.1399 mmHg or 2.705×10^{-3} psi / capillary) is double that exerted on the capillary walls of the Polyflux 210H capillaries (0.066 mmHg or 1.3×10^{-3} psi / capillary) [11]. This leads to conclude that the EXELTRA Plus 210 capillary is designed to compensate for the low pore density in contact with the dialysate by an increase in the tension applied to the capillary walls. This means that the convection transport mechanism plays a bigger role in the transport of uremic toxins than in the Polyflus 210H hemodialyzer. Also, we can deduce that the hindrance to the solute molecules due to convection, K_{conv}, in the EXELTRA Plus 210 dialyzer is less than that in the Polyflux 210H. Additional experimental work is needed to estimate K_{conv}.

Similar to our previous study that we pursued to characterize Polyflux 210H [6], K_{diff} for the urea, glucose, endothelin, β_2-Microglobin, and comlpliment factor D within the EXELTRA Plus 210 capillaries decreased with the increase in pore density, and doubling the capillaries' diameters quadrupled the D_{eff} of the same molecules through the membrane. The best theoretical scenario achieved in this study that yielded the best D_{eff} for the molecules is when the pore density was doubled, the diameters of the capillaries was doubled, and the number of capillaries was cut in half. The effective diffusivity calculation was based on the porosity of the inner surface in contact with the blood. It did not take into account the characteristics of the internal membrane layers and their porosity, nor the open space on the outer surface in contact with the dialysate. This may be the cause of the discrepancy between the value of the effective membrane diffusion coefficient for urea through the CTA membrane that we estimated to be 34.52×10^{-10} cm^2/s as compared to those reported in other studies [17]. In these studies, the effective membrane diffusion coefficient

for urea was estimated at 6.5 x 10^{-6} cm^2/s and 2.5 x 10^{-6} cm^2/s through polyamide high flux membranes and Cuprophan lowflux membranes, respectively.

The application of an electric potential theoretically indicated an increase electromigration flux for endothelin, β_2-Microglobin, and comlpliment factor D across EXELTRA Plus 210. Future research will be pursued to experimentally prove these results. Also, this study focused on the diffusion hindrance to molecular flux. The impact of convection hindrance on the molecular flux needs to be determined in the future.

This study has clinical relevance. First, it identifies some of the limitations of membrane under study. This membrane has an inner open area of less than 3% of the overall surface area which stresses a major potential for improvement as attested by our calculations. Secondly, the application of a slight electric potential will exponentially improve diffusion. Thirdly, future strategies may focus on elaborate pore designs and increasing open pore space.

3.5 Limitations:

1. The CTA membrane characterization was pursued under dry conditions.
2. Our model for estimating the effective diffusivities of solutes through the CTA dialysis membrane were based on the fraction of open space at the inner surface, and did not take into account the properties of the internal layers and the porosity of the outer surface into account.
3. The study is theoretical. The sieving coefficients of the middle molecules though CTA membranes need to be determined experimentally to yield accurate calculations of their electromigration flux.
4. Transmembrane pressure and hydraulic permeability govern convective transport and internal filtration of solutes through membrane dialyzers. This study focused only on the measure of convective transport. Hydraulic permeability was not taken into account.

4. CONCLUSIONS

1. EXELTRA Plus 210 has higher tension exerted on the capillary walls and a lower pore density adjacent with the blood when compared to Polyflux Plus 210.
2. We foresee that targeting pore density and / or doubling the diameter of the capillaries can achieve improved uremic toxins clearance. Both will increase D_{eff}.
3. The futuristic application of an electric potential across the walls of the capillaries will improve the flux of endothelin, β_2-Microglobin, and comlpliment factor D.

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COMPETING INTERESTS

The authors had no involvements that might raise the question of bias in the work reported or in the conclusions, implications, or opinions stated.

ABBREVIATIONS:

z is the valence
R is the gas constant

T is the temperature
F is Faraday's constant
D_{eff} is the effective diffusivity
C_m is the solute's concentration in the bulk of the membrane
A is the overall area of the membrane
x is the membrane's thickness
dV/dx is the electric potential difference across the membrane
dC/dx is the concentration gradient across the membrane

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