

Original Research Article

Structural delineation of the Exeltra plus 210 hemodialyzer's capillary pores as targets to ameliorate its filtration

Abstract

Background: Exeltra plus 210 is a high flux hemodialyzer made of cellulose triacetate (CTA). We applied theoretical principles of fluid mechanics to characterize the fenestrations on the outer and inner surfaces of the capillaries in order to examine potentials to improve its diffusivity.

Methods: The inner and outer fenestrations through the capillaries walls were delineated using scanning electron microscopy. We selected endothelin, β 2-microglobulin, and complement factor D as uremic toxins molecules. We calculated their effective diffusivity ($D_{\text{eff}} = K_{\text{diff}} D_o$), under different conditions of fenestration densities and capillary diameters, where the hindrance factor, $K_{\text{diff}} = (\epsilon / 2 - \epsilon)^2$. We also investigated the effect of electric potential on molecular flux.

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.

Conclusions: The transport of uremic toxins through the capillaries' walls could be enhanced through doubling the inner diameters of the capillaries, increasing the fenestrations density on the inner walls, and applying an electric potential.

Short title: Pore characterization in EXELTRA Plus 210

Key words: EXELTRA plus 210, hemodialysis, capillary, fenestrations, hindrance, effective diffusivity

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1. Introduction

Recently we examined targets to improve hemodialysis of Polyflux 210H [1]. We aim in this study to compare similar analysis on the EXELTRA Plus 210. 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 [2]. Endothelin, β 2-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 [3]. Hemodialyzers broadly vary in pore sizes and pore densities [3], they have irregular pore forms and are characterized by inefficient filtration rates [4]. 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 [5].

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. This hindrance is denoted by K_{diff} [6]. 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, $K_{diff} = [\epsilon / (2 - \epsilon)]^2$ where ϵ is the fraction of open space in a membrane [7], and $D_o = 1.76 \times 10^{-4} (MW)^{-0.552}$ [8].

In a recent study, we verified that reducing the membrane thickness and applying an electric potential on its surface are two different routes to enhance the transport of uremic toxins molecules through hemodialysis structures [1]. We also characterized the pores on the inner and outer surfaces of a Polyflux 210H hemodialyzer's capillaries. Our analysis demonstrated that the sizes of the pores from both the inside and the outside are wide enough to allow the uremic toxins molecules to pass through. 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 [9]. In this study, we delineate the structural of the Exeltra plus 210 hemodialyzer's capillary pores, and discuss targets to ameliorate its filtration. Exeltra plus 210 is a single-use hemodialyzer with a 2.1 m² overall surface area. The capillaries are made of cellulose triacetate (CTA), and it is produced by Baxter Healthcare Corporation, McGraw Park, IL, USA. The dialyzer's capillaries have a 200 μ m inner diameter, a 15 μ m wall thickness, and are made of cellulose triacetate [10].

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 [11]. In another study, the pore radii ranged between 5.8 to 7.8 nm [12].

2. Materials and Methods

2.1 Materials:

The hemodialyzer used in this research is a single-use EXELTRA plus 210 distributed by Baxter Healthcare corporation, Deerfield, IL 60015 USA. We fixed the hemodialyzer's capillaries using the same materials in our previous study on Polyflux 210H [1].

2.2 Apparatus:

A clamp and handsaw were used to open the EXELTRA plus 210 hemodialyzer'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 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 [1]. We used the same uremic toxins molecules (endothelin, β 2-microglobulin and compliment factor D) when characterizing the EXELTRA plus 210 capillaries so that we can compare the results between the two hemodialyzers.

2.3.2 Estimation of the capillary wall tension: LaPlace Law was used to estimate the capillary wall tension in the EXELTRA plus 210 dilyzer. 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.

2.3.3 Estimation of D_{eff} of selected molecules under different conditions: Similar to our analysis of D_{eff} for urea, glucose, endothelin, β 2-microglobulin, compliment factor D, and albumin through Polyflux 210H [9], we calculated the effective diffusivities for the same molecules under the following different conditions given in Table 3.

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

$$J_{electromigr} = \left(\frac{D_{eff} A C_m z F}{RT} \right) \left(\frac{dV}{dx} \right) \quad [13]$$

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 [14].

The normal concentrations of endothelin, β 2-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

<i>Uremic toxin</i>	<i>Normal blood concentration *</i>	<i>Sieving coefficient **</i>
Endothelin	28.8 +/- 3.8 ng/L [3]	0.70
β 2-microglobulin	< 2.0 mg/L [3]	0.35
Complement factor D	1.9 +/- 0.5 mg/L [3]	0.07

* C_{bulk}

** Estimated from the sieving coefficient plot versus molecular weight [15].

3. Results

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.

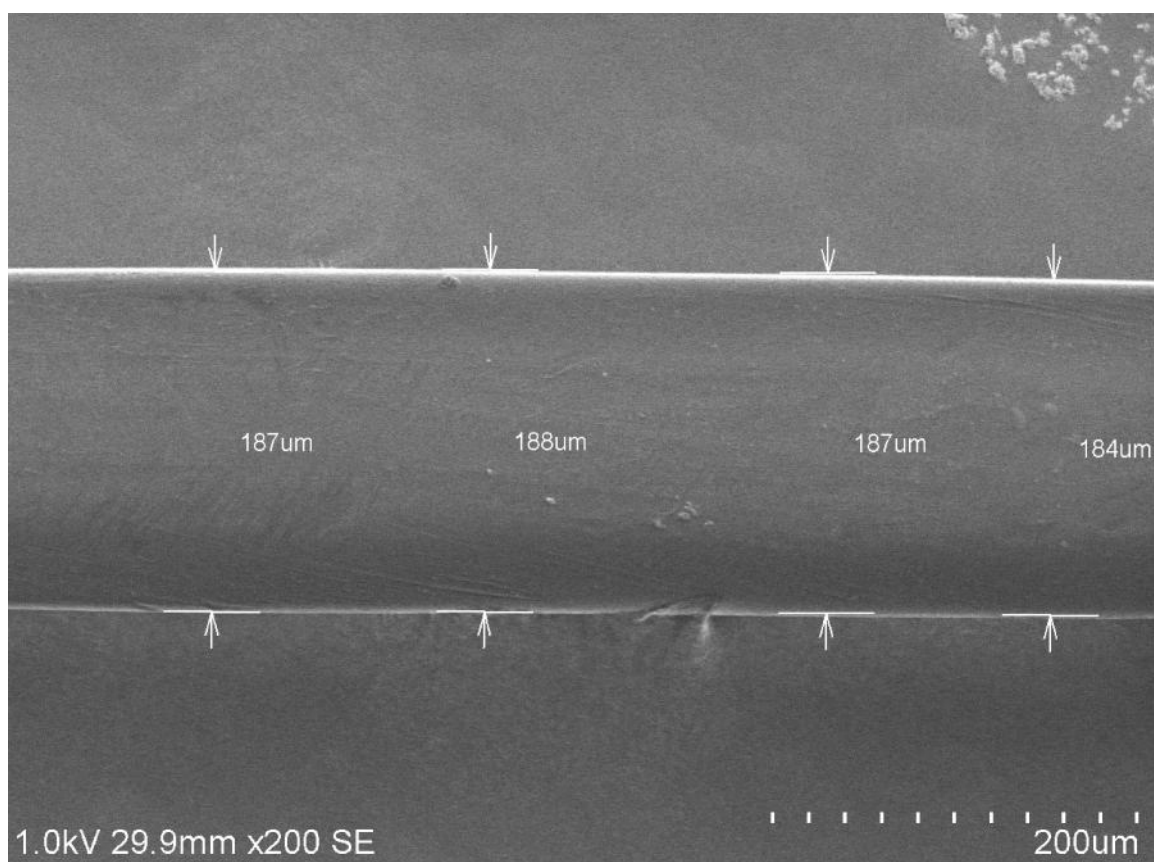


Fig. 1 Macro image of the outer surface of an EXELTRA capillary

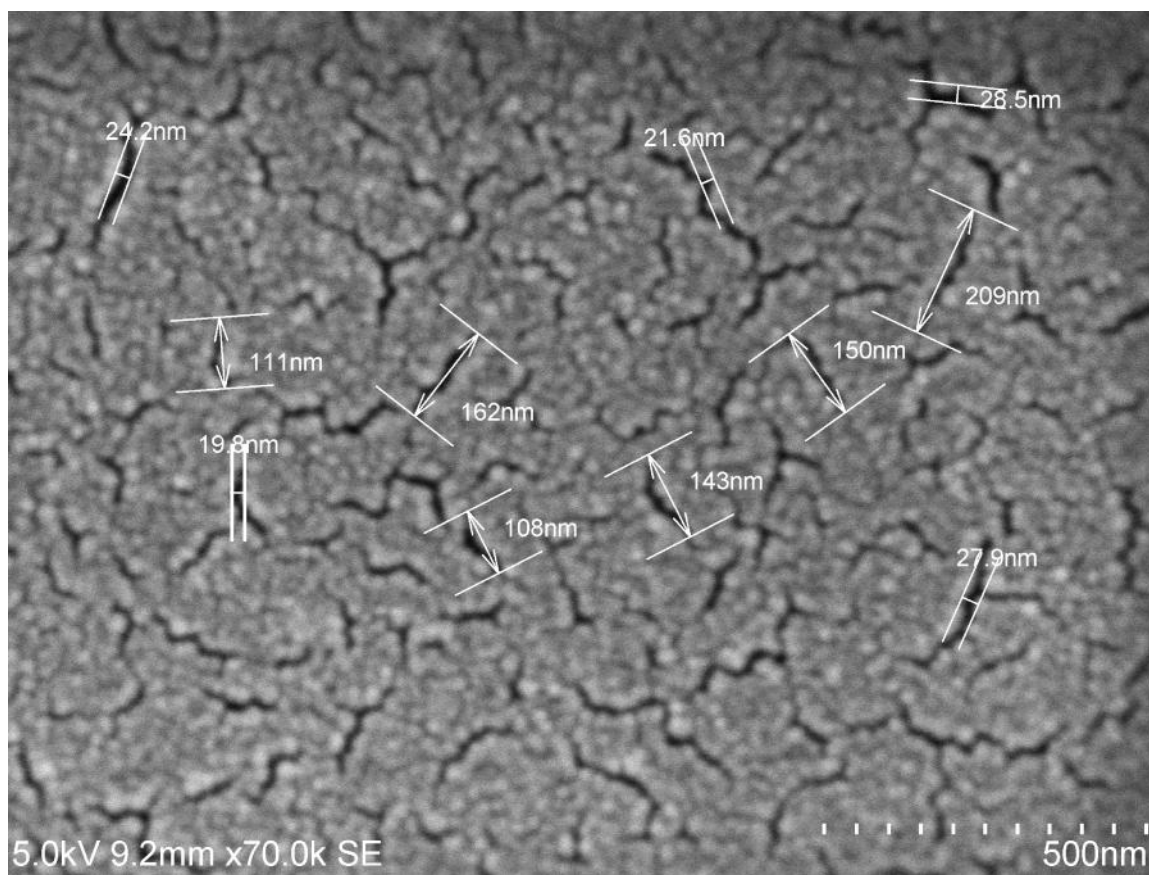


Fig. 2 Fenestrations in the outer surface of an EXELTRA plus capillary

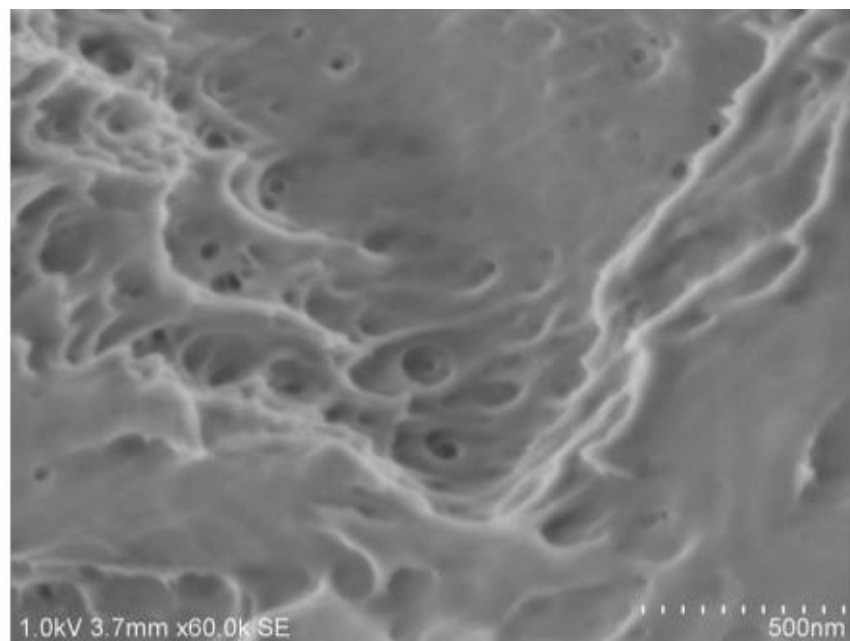


Fig. 3 Fenestrations in the inner wall surface of an EXELTRA plus capillary

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

<i>Characteristic</i>	<i>EXELTRA 210H mean $\pm$ standard deviation</i>
Open space (inner surface - in contact with the blood)	2.71 $\pm$ 1.06 %
Open space (outer surface - in contact with the dialysate)	12.06 $\pm$ 3.07 %
Outer diameter	200 μ m [10]
Wall thickness	15 μ m [10]
Inner surface pore width	32.25 $\pm$ 6.40 nm
Outer surface pore width	24.40 $\pm$ 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.

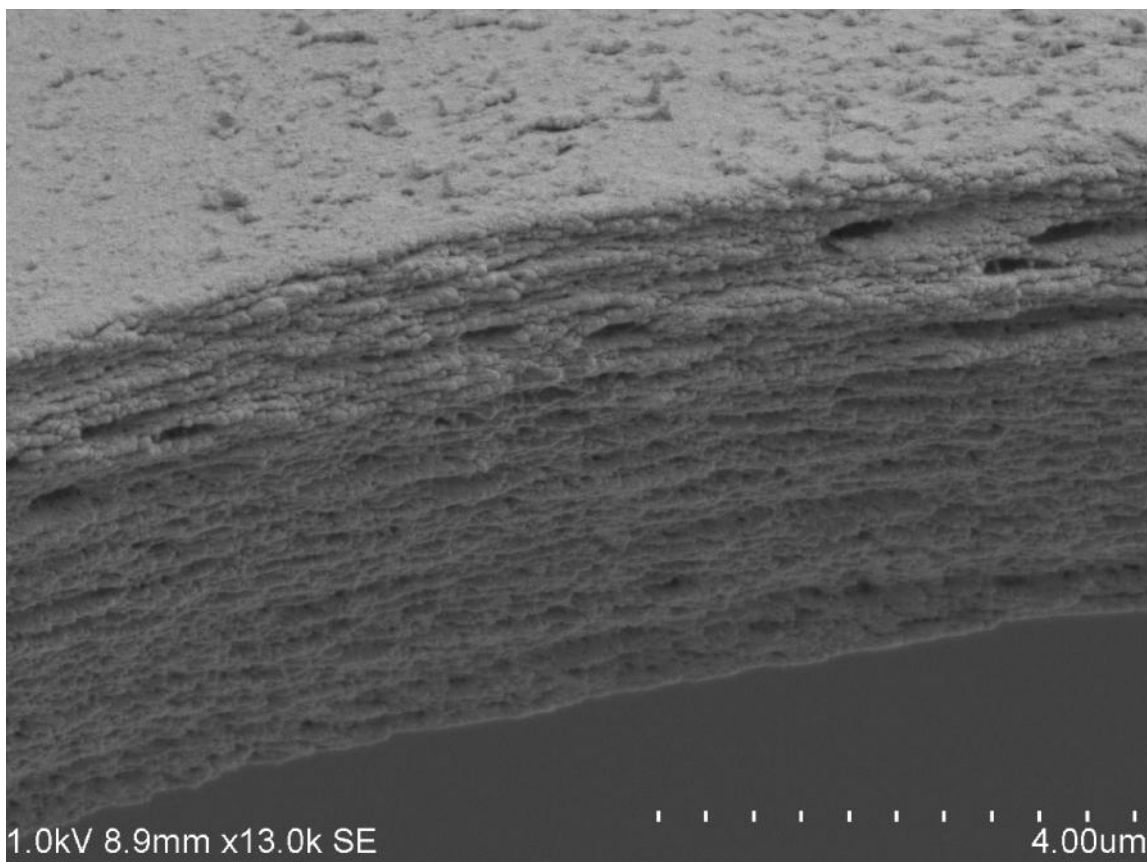


Fig. 4 (a) Cross-section of a capillary

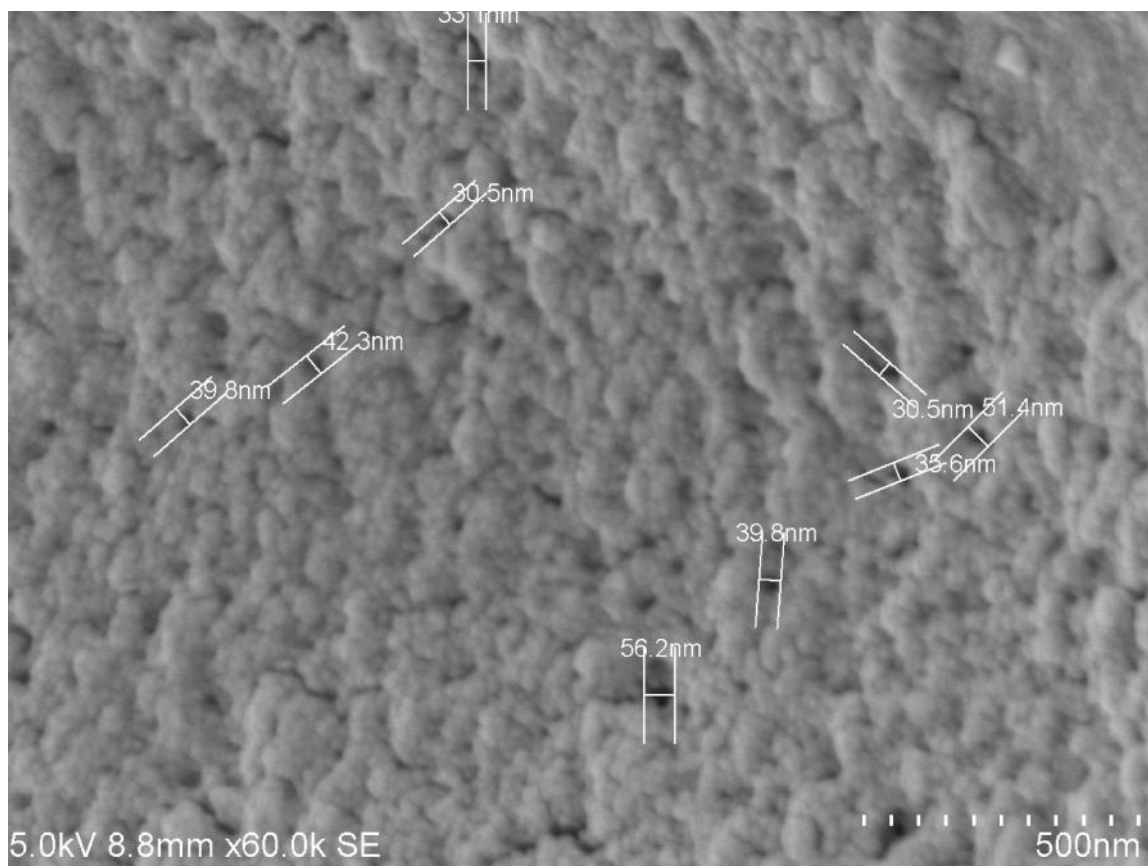


Fig. 4 (b) Relatively small size round holes in the cross-section of a capillary

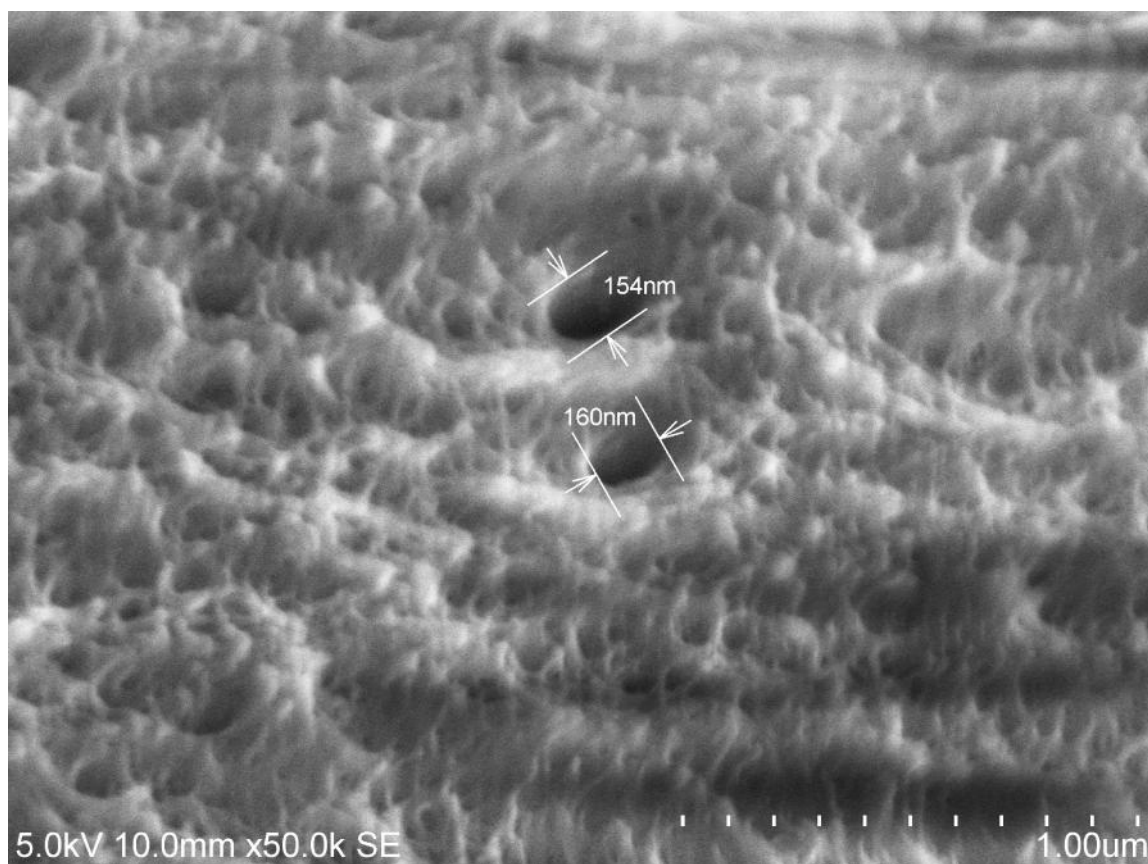


Fig. 4 (c) Relatively large round holes in the cross-section of a capillary

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

3.3 Estimation of D_{eff} of selected molecules under different conditions: We tabulated the data for the D_{eff} of urea, glucose, endothelin, β 2-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 [16]. 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

Molecule	$D_o \times 10^{-8}$ (cm^2/s) [16]	(a) $D_{eff} \times 10^{-10}$ (cm^2/s) EXELTRA 210H ($\varepsilon = 0.0271$) $K_{diff}=$ 0.000188	(b) $D_{eff} \times 10^{-10}$ (cm^2/s) EXELTRA 210H ($\varepsilon = 0.0542$) $K_{diff}=0.00078$	(c) $D_{eff} \times 10^{-10}$ (cm^2/s) EXELTRA 210H ($\varepsilon=0.05826$) $K_{diff}=0.0009$	(d) $D_{eff} 10 \times 10^{-10}$ (cm^2/s) Polyflux® 210H ($\varepsilon = 0.0291$) $K_{diff}=$ 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 - Microglobulin	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 based on inner pore density as 2.71 ± 1.06 %, and a capillary's wall thickness of 15 μm .
- (b) Calculations based on doubling the pore density.
- (c) Calculations based on doubling the pore density, doubling the capillaries' inner diameter, and reducing the capillaries number by half.
- (d) Calculations based on the assumption of doubling the capillaries' diameters, and reducing the capillaries number by half.

3.4 Evaluation of the effect of applying an electric potential across the walls of EXELTRA plus on selected molecules: In a previous study [9], we proved that, theoretically, the application of an electric potential on the walls of membranes can enhance the flux transport across the membrane. Given the thickness of the membrane as 15 μm , and a K_{diff} of 188×10^{-6} , the flux increase, Jelectromigr for endothelin, β_2 -microglobulin, compliment factor D increased as follows when we applied an electric potential across the capillaries' surfaces:

For endothelin:

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

For β 2-microglobulin:

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

For Compliment Factor D:

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

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

4. Discussion

Our results indicate that the pore sizes in CTA are greater than those reported by Yamazaki et. al. [11] and Sunohara et. al. [12]. 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 $2.71 \pm 1.06\%$ and $12.06 \pm 3.07\%$ 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. [11]. The discrepancy can also be contributed to the characterization of the membrane under water in the study by Yamazaki et. al [11] and our characterization, which was pursued under dry conditions.

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 [1].

The mean density of the open pores on the inside surface of the capillaries in the EXELTRA plus 210H (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

x 10^{-3} psi / capillary) [16]. This leads to conclude that the EXELTRA plus 210H 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 Polyflux 210H hemodialyzer. Also, we can deduce that the hindrance to the solute molecules due to convection, K_{conv} , in the XELTRA 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 [1], K_{diff} for the urea, glucose, endothelin, β_2 -Microglobulin, and compliment 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} cm²/s as compared to those reported in other studies [17]. In these studies, the effective membrane diffusion coefficient for urea was estimated at 6.5×10^{-6} cm²/s and 2.5×10^{-6} cm²/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 -Microglobulin, and compliment factor D across EXELTRA plus 210. Future research will be pursued to experimentally prove these results.

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.

Conclusions:

1. EXELTRA plus 210H has higher tension exerted on the capillary walls and a lower pore density adjacent with the blood when compared to Polyflux plus 210H.
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 -Microglobulin, and compliment factor D.

Conflict of interest statement

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

Abbreviation:

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

Bibliography

- [1] Hedayat A, Szpunar J, Kiran Kumar NAP, Peace R, Elmoselhi H, and Shoker A: Morphological characterization of the Polyflux 210H hemodialysis filter pores. International Journal of Nephrology. DOI: 10.1155/2012/304135 Available: <http://www.hindawi.com/journals/ijn/2012/304135/> Accessed 2012.
- [2] Vanholder R, De Smet R, Glorieux G, et. al. Review of uremic toxins: Classification, concentration, and interindividual variability. Kidney International 2003; 63:1934-1943.
- [3] Chelmcharla M, Leyboldt J, and Cheung A. Dialyzer Membranes as determinants of the adequacy of dialysis. Seminars in nephrology 2005;25(2):81-89.
- [4] Huang Z, and Van Geertruyden WH. A better membrane. Caramic Industry, May 1st 2009. (www.ceramicindustry.com).
- [5] Ford LL, Ward RA, and Cheung AK. Choice of the Hemodialysis Membrane. In Henrich WL. ed. Principles and Practice of Dialysis. 4th ed. Philadelphia, PA, USA: Lippincott Williams & Wilkins; 2009: 2.
- [6] Dechadilok P and Deen WH. Hindrence factors for diffusion and convection in pores. Ind. Eng. Chem. Res. 2006; 45 (21):6953–6959. DOI: 10.1021/ie051387n
- [7] Keurentjes JTF, Janssen AEM, Broek AP, Van Der Padt A, Wesselingh JA, and Van't Riet K. Multicomponent Diffusion in dialysis membranes. Chem Eng Sci 1992;47(8):1963-1971.
- [8] Peterson PA, Ervin PE, and Berggard I. Differentiation of glomerular, tubular, and normal proteinuria: Determinations of urinary excretion of beta2-microglobulin, albumin, and total protein. J Clin Invest 1969;48(7):1189-98.
- [9] Hedayat A, Elmoselhi H, Shoker A: Theoretical Application of Irreversible (Nonequilibrium) Thermodynamic principles to enhance solute fluxes across nanofabricated hemodialysis membranes. DOI: 10.1155/2012/718085. Available: <http://www.hindawi.com/journals/ijn/2012/718085/> Accessed 2012.
- [10] Baxter brochure. Retrieved from: http://www.baxter.com/downloads/healthcare_professionals/products/AL09086_cellulosic_brochure_final.pdf

[11] Yamazaki K, Matsuda M, Yamamoto K, Yakushiji T, and Sakai K. Internal and surface characterization of cellulose triacetate hollow-fiber dialysis membranes. *Journal of Membrane Science* 2011; 368: 34-40.

[12] Sunohara T and Masuda T: Cellulose Triacetate as a high-performance membrane. In: Saito A, Kawanishi H and Mineshima M, eds. *High-Performance Membrane Dialyzers*. Contrib Nephrol. Basel, Karger: 2011; vol 173: 156-163.

[13] Szymczyk A, Labbez C, Fievet P, et al. Contribution of convection, diffusion and migration to electrolyte transport through nanofiltration membranes. *Advances in Colloid and Interface Science* 2003; 103: 77-94.

[14] Rizvi SS. Membrane applications in biotechnology, food processing, life sciences, and energy conversion: introduction. In Pabby AK, Rizvi SSH, and Sastre AM, eds. *Handbook of Membrane Separations Chemical, Pharmaceutical, Food, and Biotechnological Applications*, Boca Raton, Fla, USA: CRC Press; 2009: 503.

[15] Gurland H, Samtleben W, Lysaght MJ, and Winchester JF. Extracorporeal blood purification techniques: plasmaspheres and hemoperfusion. In: Jacobs C, Kjellstrand CM, Koch KM, and Winchester JF, eds. *Replacement of Renal Function by Dialysis*. 4th edition, Dordrecht, The Netherlands: Kluwer Academic; 1996: 472-500.

[16] Hedayat A and Shoker A: Polyflux 210H hemodialysis membrane targets to improve filtration. Accepted for publication. *Saudi Journal of Kidney Disease and Transplantation*.

[17] Gohl H, Buck R, and Strathmann H. Basic features of polyamide membranes. In: Shaldon S and Koch KM, eds. *Polyamide: the evolution of a synthetic membrane for renal therapy*. Contrib Nephrol. Basel, Karger, 1992; vol 96: 1-25.