The Characterization of Hemodialysis Membranes in Miniaturized Dialyzers

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Medical Biophysics

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Abstract

Lack of an appropriate animal model prevents rapid evaluation of health outcomes associated with hemodialysis. We developed a miniaturized dialyzer for use in small rats. The aim of this study was to investigate whether the miniaturized dialyzers can differentiate performance characteristics representing hemodialysis membrane and fiber design. We evaluated internal filtration, water permeability, sieving coefficient (SC) and clearance of solutes, in the Medium Cut Off (MCO) and conventional High Flux (HF) miniaturized dialyzers. We found statistically significant differences between miniaturized MCO and conventional HF dialyzers with respect to ultrafiltration coefficient, clearance of Beta-2 Microglobulin (B2M), and SC of B2M. These outcomes suggest that the miniaturized dialyzers can effectively differentiate performance characteristics representing hemodialysis membrane and fiber design. This indicates that the miniaturized dialyzers are an appropriate component of a pre-clinical pipeline of dialysis technology impact.

Keywords

Medium Cut Off (MCO), High Flux (HF), Miniaturized Dialyzer, Hemodialysis, Beta-2 Microglobulin (B2M)
Summary for Lay Audience

Hemodialysis is the most common therapy provided to patients suffering from chronic kidney disease (CKD). Hemodialysis relies on the use of dialyzers (artificial kidneys), in which blood is filtered from waste products using clean dialysis fluid. However, the dialyzers used do not entirely fulfill the functionality of a healthy kidney. This is partly due to the membranes used in the dialyzers. The lack of an appropriate animal model prevents rapid evaluation of health issues associated with hemodialysis. Accordingly, we constructed a miniaturized dialyzer suitable for small rats using membranes harvested from existing clinical human dialyzers. The objective of this study was to investigate whether the dialyzers can effectively differentiate performance characteristics representing hemodialysis membrane and fiber design. We evaluated water permeability, internal filtration, and uremic toxin removal of the miniaturized dialyzers composed of harvested membranes from MCO and conventional HF human dialyzers. The results showed statistically significant differences between MCO and conventional HF miniaturized dialyzers with respect to the ultrafiltration coefficient, clearance of B2M, and the sieving coefficient of B2M. The main findings suggest that miniaturized dialyzers can effectively differentiate performance characteristics representing hemodialysis membrane and fiber design.
Co-Authorship Statement

The work contained in this thesis was conducted by Mehrshad Moradshahi, under direct supervision of Dr. Christopher McIntyre who aided in study design and reviewed this thesis. Dr. Barry Janssen aided in data interpretation and reviewing this thesis. Dr. Alireza Akbari was responsible for assembling the miniaturized dialyzers. The content of this thesis will be part of a larger animal study evaluating microvascular dysfunction in rodent models of hemodialysis.
Acknowledgments

Supervisory Committee

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List of Abbreviations

B2M – Beta-2 Microglobulin
CKD – Chronic Kidney Disease
ESRD – End Stage Renal Disease
EVAL – Ethylene Vinyl Alcohol Copolymer
FFP – Fresh Frozen Plasma
FGF-23 – Fibroblast Growth Factor-23
GDF-15 – Growth Differentiation Factor-15
GFR – Glomerular Filtration Rate
GLDH – Glutamate Dehydrogenase
HF – High Flux
Hct – Hematocrit
IL-6 – Interleukin-6
IS – Indoxyl Sulphate
IVM – Intravital Video Microscopy
LF – Low Flux
MCO – Medium Cut Off
MWCO – Molecular Weight Cut-Off
NAD^+(H) – Nicotinamide Adenine Dinucleotide
NaCl – Sodium Chloride
PAN – Polyacrylonitrile
PES – polyether sulfone
PEPA – polyester polymer alloy
PMMA – polymethyl methacrylate
PSF – Polysulfone
RBC – Red Blood Cell
SC – Sieving Coefficient
TNF-α – Tumor Necrosis Factor-alpha
Chapter 1

1 Background

This chapter provides relevant background information about the work described in this thesis. The chapter covers an introduction to functional and anatomical features of a healthy kidney, chronic kidney disease (CKD), and current replacement therapies with the focus on hemodialysis.

1.1 The Kidney

The kidneys lie bilaterally in the retroperitoneal space on each side of the vertebral column. Each kidney is about 12 cm long and weighs about 150 g. Longitudinally a kidney contains two different regions, including the Cortex (outer region) and the Medulla (inner region). The Medulla is composed of multiple conical areas called Renal Pyramid that extends towards the Renal Pelvis as shown in Figure 1-1. The nephrons are the functional units of the kidney which filter blood and produce urine to be excreted from the body. Each nephron is composed of a vascular part (glomerulus), Bowman’s capsule (encapsulating around glomerulus), a proximal tubule, a loop of Henle, and a distal tubule (Figure 1-2). Numerous distal tubules converge on a collecting duct, which joins the papillary duct of Bellini towards the renal pelvis [11]. The waste products in the renal pelvis are drained into the ureter through peristalsis towards the bladder as urine.
Figure 1–1: Labelled diagram of the human kidney, including cortex, medulla, calyx, renal pelvis, ureter, and renal capsule. Created with BioRender.com

Figure 1–2: Labelled diagram of the nephron, including the renal corpuscle (glomerulus and Bowman’s capsule), and the renal tubule (proximal convoluted tubule, loop of Henle, distal convoluted tubule, and collecting duct). Created with BioRender.com.
1.1.1 The Functions of Kidneys

The main function of the kidneys is to remove toxic by-products of metabolism and excess solutes smaller than albumin (MW: 68kDa) [12,13]. The kidneys regulate body fluid osmolarity that is imperative for the maintenance of normal cell volume. The kidneys also control volume of the body fluid by regulating the excretion of water and sodium chloride (NaCl) for normal function of cardiovascular system. The kidneys play a pivotal role in maintaining normal levels of electrolyte (e.g., sodium, chloride, bicarbonate, hydrogen, calcium, magnesium, phosphate) levels. To maintain appropriate balance, the excretion of the electrolytes in the kidneys must be balanced to the daily intake. The kidneys also maintain pH of the blood within normal range by excreting acids (e.g., sulfuric acid and phosphoric acid) and maintenance of fluid buffer store. The kidneys eliminate excess metabolic by-products such as urea (from amino acids), uric acid (from nucleic acids) and creatinine (from muscle creatine) and metabolites of hormones. The kidneys also remove drugs and ingested chemicals. In addition, the kidneys produce hormones, including renin, erythropoietin, and prostaglandins (vasodilator). Renin initiates renin-angiotensin-aldosterone system, which is involved in regulation of blood pressure, as well as sodium and potassium balance. The kidneys help in the conversion of vitamin D to 1,25-dihydroxvitamin D which regulate body calcium and phosphate levels. Kidneys also produce erythropoietin which stimulates production of erythrocytes in the bone marrow [12]. In addition, the kidneys maintain a normal level of glucose mainly through production of glucose from other precursor molecules (e.g., amino acids)

1.1.2 Renal Clearance

The removal of solutes in the kidneys reflects the coordinated action of glomerular and tubular segments of the nephrons. Specifically, glomerular filtration, reabsorption of fluid back into the blood, and secretion of substance from the blood into the tubular fluid determines the amount of solutes in the urine. As illustrated in Figure 1-3, the renal artery is the only route of solute entry, whereas the renal vein and ureter are the two output routes. Maintaining mass balance, the following relationship is derived [12]:
\[ P_x^a \times \text{RPF}^a = (P_x^v \times \text{RPF}^v) + (U_x \times \dot{V}) \]  \[1.1\]

where \( P_x^a \) and \( P_x^v \) represent the plasma concentrations of solute x in the renal artery and renal vein, respectively. \( \text{RPF}^a \) and \( \text{RPF}^v \) are the renal plasma flow rates in the artery and veins, respectively. \( U_x \) is the concentration of solute x in the urine; and \( \dot{V} \) is the urine flow rate. Renal clearance (\( C_x \)) represents only the excretory function of the kidney so that equation [1.1] could be modified to represent the rate at which solute x is removed from the plasma by the kidneys:

\[ C_x = \frac{U_x \times \dot{V}}{P_x} \]  \[1.2\]

**Figure 1–3: Solute transport in the kidneys. Renal Artery (solute entry route), and renal vein and ureter (output routes). Created with BioRender.com**

The function of the kidneys is commonly evaluated by the glomerular filtration rate (GFR) which is the volume of fluid filtered from the kidney glomerular capillaries into the Bowman’s capsule per unit of time. GFR for a single nephron is determined by hydrostatic pressure (\( P_h \)), oncotic pressure across the capillary membrane (\( \Delta \pi \)), and the capillary filtration coefficient in the glomerulus \( K_f \) [12]:

\[ \text{Single Nephron GFR} = K_f \times (P_h - \Delta \pi) \]  \[1.3\]
1.2 Chronic Kidney Disease (CKD)

According to Kidney Disease Improving Global Outcomes (KDIGO) guidelines, CKD is defined as the presence of structural and functional abnormalities of the kidneys for more than 3 months [14,15]. The diagnosis of CKD involves many factors, including evaluation of GFR (measure of function), presence of anatomical abnormalities, proteinuria (e.g., Albuminuria), and elevation of electrolytes [14]. The reduction of GFR and presence of kidney damage indicators assist clinicians in the determination of the severity of CKD. CKD is classified into 5 stages based upon evaluation of GFR (Table 1-1) [15,18]. GFR is normally estimated using clearance of creatinine or Cystatin C [16]. Albumin Creatinine Ratio (ACR) also helps clinicians to evaluate extent of glomerular injury and severity of CKD (Table 1-2) [15].

**Table 1-1: Description of severity of CKD based on GFR. Adapted from [15,18]**

<table>
<thead>
<tr>
<th>GFR categories (ml/min/1.73m²), descriptions, and ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
</tr>
<tr>
<td>≥90</td>
</tr>
<tr>
<td>Normal to high</td>
</tr>
</tbody>
</table>
Table 1-2: Description of severity of CKD based on ACR (A1-A3). Adapted from [15]

<table>
<thead>
<tr>
<th>Persistent albuminuria categories, descriptions, and ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
</tr>
<tr>
<td>Normal to mildly increased</td>
</tr>
<tr>
<td>&lt;30 mg/g</td>
</tr>
<tr>
<td>&lt;3 mg/mmol</td>
</tr>
</tbody>
</table>

CKD is associated with numerous complications because of the deterioration of kidney function. Patients suffering from CKD ultimately present with a uremic syndrome, which results from retention of compounds, causing disruption of biochemical and physiologic functions [19]. CKD is also associated with hyperkalemia due to impairment of kidneys in potassium excretion. High levels of potassium interfere with action potential activity of pacemaker cells in the heart triggering cardiac arrythmia [23,24]. CKD patients are often prone to acidosis that leads to interruption of many pH-dependent physiological reactions. Bone mineralization is also disrupted as malfunctional kidneys are unable to stimulate 1,25-dihydroxivitamin D production, retain calcium and secrete phosphate [25,26,27]. Furthermore, as the kidney function deteriorates, the ability of the kidneys to excrete water and sodium decreases. The reduction of GFR in later CKD results in the accumulation of sodium in the blood. This ultimately causes hypertension and volume overload [37,38,39]. Reduced kidney function independently is associated with an increased risk of death, and cardiovascular events [20,21,22,29]. CKD is also associated with acute and chronic inflammation and endothelial dysfunction which could aggravate cardiovascular disease in this population [28,30,31,32]. The endothelium serves multiple physiological functions, including regulation of vascular tone, leukocyte trafficking, hemostasis, angiogenesis, and innate and adaptive immunity [33]. Endothelial dysfunction happens in the early stages of CKD due to numerous factors, including inflammation, hypertension, and uremic environment [34]. Approximately, 13.4% of the population suffer from CKD globally [35]. In addition, the mortality rate associated with
CKD increased by 41.5% from 1990 to 2017 [36]. This suggests that CKD is a global health concern and requires further attention in research and global health policy.

### 1.3 Renal Replacement Therapies

There are two treatment modalities with regard to renal replacement therapies: dialysis and kidney transplantation. Dialysis is further sub-categorized into hemodialysis and peritoneal dialysis. Patients often wait for years to receive kidney transplant surgery, and only a minority of them make it onto the waiting list, so that dialysis is required in the preoperative period. The focus of this thesis is solely on hemodialysis which is the most common form of renal replacement therapy delivered to the patients.

### 1.4 Hemodialysis

Hemodialysis is a blood purifying therapy typically delivered three times a week 3-4 hours per session (intermittent hemodialysis). During a hemodialysis procedure, blood circulates through an apparatus called a dialyzer (Figure 1-4 B) wherein excess solutes and fluid are removed from the patient. Hemodialysis generally contains a vascular access, a blood circuit, a dialyzer, and a dialysis machine (Figure 1-4A).

A vascular access for a patient is needed for the blood to pass into the hemodialysis circuit. The vascular access techniques currently used in hemodialysis are arterial-venous fistulae (formed by connecting a patient’s artery to a vein), arterial-venous graft (created by joining patient’s artery to a vein using a hollow tube), and central venous catheter (a catheter inserted into the jugular vein, femoral vein, or subclavian vein).

A blood circuit contains an inflow bloodline which allows for blood entry from the patient to the dialyzer. This circuit also includes an outflow bloodline that returns the processed blood from the dialyzer back to the patient. The blood flow in the blood circuit is controlled by a roller pump on the inflow bloodline side. The blood flow rate prescription is based on body weight or surface area of the patients [42]. In adults, blood flow is typically set at 250-300ml/min, whereas in pediatric patients it ranges from 5ml/min/kg to 7ml/min/kg [2].
The dialysate circuit is where the dialysate fluid leaves its supply and is pumped through the dialyzer. Uremic toxins are exchanged from the blood into the dialysate fluid in a dialyzer. In a dialyzer, both blood and dialysate are pumped counter currently to enhance the concentration difference of the solutes and facilitate the removal of uremic toxins.

The dialysate fluid is prepared through a series of water detoxifications using activated carbon filter, reverse osmosis and, distillation [44]. The composition of solutes in the dialysate fluid is carefully regulated to ensure that physiological homeostasis is achieved during hemodialysis [43]. In adults, dialysate flow rate is normally set to 500 ml/min primarily to achieve adequate removal of small solutes (e.g., urea) [41,42].

Figure 1–4: A diagram of hemodialysis machine (A) and components of a dialyzer (B). Created with BioRender.com
1.5 Biophysics of Dialyzers

The dialyzers are designed to remove uremic toxins and excess water from the blood of patients as well as balancing the electrolyte content in the blood. Uremic toxins are classified based on their physicochemical characteristics [180] and molecular weight [1,45] as shown in Table 1-3 and Table 1-4.

Table 1-3: Classification of uremic toxins according to their physicochemical characteristics [180].

<table>
<thead>
<tr>
<th>Small water-soluble compounds</th>
<th>Protein bound solutes</th>
<th>Middle molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>e.g., urea, creatinine, uric acid</td>
<td>e.g., p-cresol sulfate</td>
<td>e.g., B2M and interleukin 6, interleukin 1β, Lambda-lg light chain</td>
</tr>
</tbody>
</table>

Table 1-4: Classification of uremic toxins according to their molecular weight [1,45]

<table>
<thead>
<tr>
<th>Class of solutes</th>
<th>Small molecules</th>
<th>Middle molecules</th>
<th>Large molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight range (Daltons)</td>
<td>&lt;500Da</td>
<td>500Da-25kDa</td>
<td>&gt;60kDa</td>
</tr>
<tr>
<td>e.g., urea (60Da), creatinine (125Da), phosphate (134Da)</td>
<td>e.g., vitamin B₁₂(1355Da), inulin(5200Da), B2M(12kDa)</td>
<td>e.g., albumin(68kDa), Transferrin (90kDa)</td>
<td></td>
</tr>
</tbody>
</table>
1.5.1 Basic Transport Mechanisms in the Dialyzers

There are three mechanisms that enable solute transport to occur in the hemodialyzers: diffusion, convection, and adsorption [71]. Many factors influence solute transport in a hollow fiber dialyzer. The characteristics of hemodialysis membranes are an important determinant of dialyzer performance.

1.5.1.1 Diffusion

The removal of solutes by diffusion is the result of concentration gradient across the semipermeable membrane in a dialyzer. The diffusion of solutes depends on many factors [45,46,47]. Hemodialysis membranes with more pores per square centimeter allow faster diffusion. Also, membranes with larger pores allow transport of larger solutes. The rate of diffusion is also enhanced with the use of membranes with better designed pore structures. Additionally, blood protein content affects diffusive transport of solutes across the membranes. The diffusible solutes (e.g., indoxyl sulfate (IS)) may bind to blood proteins such as albumin, forming dialysis membrane impermeable complex. Another impediment is the Gibbs-Donnan effect which refers to the protein-induced ion transport asymmetry. Negatively charged proteins that cannot cross the membranes tend to accumulate at the membrane surface during dialysis which results in retention of positively charged ions (e.g., sodium, calcium, and magnesium) in the blood. This hinders diffusive transport of such solutes by reducing the concentration gradient across the dialysis membrane.

Diffusion of uncharged solutes through a membrane follows Adolf Fick’s principle [48,49]:

\[ J = D_s \times A \times \frac{dC}{dX} = D_s \times T \times A \times \frac{\Delta C}{\Delta X} \]  \[1.4\]

where \( J \) is the net solute flow (mol/s), \( D_s \) is the solute diffusivity (m²/s) at a specific temperature (T), A is the available surface area of diffusion (m²) and \( \frac{dC}{dX} \) (\( \frac{\Delta C}{\Delta X} \)) refers to concentration difference \( \Delta C \) (mol/m³) over the membrane thickness \( \Delta X \) (m). Given membrane thickness \( \Delta X \) and solute diffusivity \( D_s \) is constant at a certain temperature, the
variables that solely determine the flux of a dialyzer are concentration gradient ($\Delta C$) and surface area ($A$). Therefore, Fick’s equation [1.5] can also be written as:

$$J = -K_0 \times A \times \Delta C$$  \[1.5\]

where $K_0$ is defined as the overall mass transfer coefficient in ml/min.

The diffusive dialysance $D$ is defined as the change in solute content in the blood inflow per unit of concentration driving force [118]:

$$D = \frac{Q_{Bi} \times (C_{Bi} - C_{Bo})}{c_{Bi} - c_{Di}} = \frac{Q_{Di} \times (C_{Do} - C_{Di})}{c_{Bi} - c_{Di}}$$  \[1.6\]

where $Q_{Bi}$ and $Q_{Di}$ are the blood flow at the blood-inlet and dialysate flow at the dialysate-inlet of the dialyzer, respectively. $C_{Bi}$ and $C_{Bo}$ are the concentration of the solute at the blood-inlet and blood-outlet of the dialyzer, respectively. $C_{Di}$ and $C_{Do}$ are the concentration of the solute at the dialysate-inlet and dialysate-outlet of the dialyzer, respectively. The concentration of most solutes is zero at the dialysate inlet. Hence, $C_{Di}$ parameter could be eliminated from the equation [1.6] to drive an expression for clearance $K_0$ (ml/min) [119]:

$$K_0 = \frac{Q_{Bi} \times (C_{Bi} - C_{Bo})}{c_{Bi} \times C_{Bi}} \approx \frac{Q_{Di} \times (C_{Do})}{c_{Bi} \times C_{Bi}}$$  \[1.7\]

Blood - Side  Dialysate - Side

Clearance could be measured using the blood-side or the dialysate-side. The presence of non-aqueous components in the blood decreases the accuracy of blood-side clearance measurements. That is why dialysate-side clearance is also considered for measurement of diffusive clearance [170].

The diffusive performance of a dialyzer could be evaluated by the mass transfer area coefficient ($K_0A$) for urea. In theory, $K_0A$ is the optimum value of small molecular clearance under conditions of infinite blood and dialysate flows [121]. In practice, $K_0A$ depends on blood flow rate ($Q_{B}$) and dialysate flow rate ($Q_{D}$) and it could be calculated using equation [1.8] with $K_d$ being the mean of blood and dialysate side urea clearance.
Increasing the dialysate flow rate enhances $K_{0A}$ due to a better flow distribution within the fiber bundle of dialyzers [122]. Likewise, this holds for blood flow rate up to a certain threshold, where $K_{0A}$ becomes independent of the blood flow rate due to less transient time for solute transfer to occur.

$$K_{0A} = \frac{1}{\frac{1}{Q_B} - \frac{1}{Q_D}} \times \ln \left( \frac{\frac{1}{Q_D} - \frac{1}{Q_B}}{\frac{1}{Q_B} - \frac{1}{Q_D}} \right)$$  \quad [1.8]

Urea clearance is commonly used as a measure of diffusive transport due to its small molecular weight [1]. The clearance of creatinine could also be used for evaluation of diffusive transport since its clearance is proportional to clearance of urea [1].

### 1.5.1.2 Convection

Convection refers to solute transport due to water flow through the membrane pores with the pressure gradient across the membrane (i.e., transmembrane pressure; TMP) as the driving force [71]. Convective transport of solutes depends on the relative size of the solute to the size of the membrane pores. Low molecular weight and non-protein-bound solutes are transported freely without any change in their concentration, whereas larger solutes tend to move at a relatively slower pace across the membrane. Convective transport depends on porosity of the membrane which could be quantitatively evaluated through the sieving coefficient (SC), reflecting both size and number of pores in the membranes (porosity). The SC ranges from 0 to 1.0 representing percentage of a particular solute able to pass through the membrane pores. The SC can be calculated using the following equation [8,9]:

$$SC = \frac{C_{Do}}{C_{Bi}}$$  \quad [1.9]

where $C_{Bi}$ and $C_{Do}$ are the concentration of solute of interest at the blood-inlet and dialysate-outlet, respectively.

The SC of B2M is normally used for evaluation of hemodialysis membrane characteristics due to its rather larger molecular weight [1]. Previously, the removal of vitamin B12 [1] used to be evaluated as a measure of the clinical assessment of dialyzer
performance. However, vitamin B12 is no longer used since it has a tendency to bind to plasma proteins that are impermeable to hemodialysis membranes [120].

Convective transport of solutes is vastly influenced by ultrafiltration rate that is required for removal of solutes (e.g., sodium) and water accumulated during the interdialytic (between dialysis) period. Ultrafiltration could be mathematically evaluated using Darcey’s law defined by the following equation:

\[
J_U = h_m \times A \times \Delta P
\]

[1.10]

where \( J_U \) is the volumetric flux (\( \text{m}^3/\text{s} \)), \( h_m \) is the hydraulic permeability (\( \text{m/s/Pa} \)), \( A \) is the area of ultrafiltration (\( \text{m}^2 \)), and \( \Delta P \) is the pressure difference (\( \text{Pa} \)). The ultrafiltration rate depends on factors such as hydrostatic pressure gradient across the membrane, protein coating formation onto the surface of the membrane, and the blood content. The effect of hydrostatic pressure gradient across the membrane on ultrafiltration rate could be mathematically evaluated using the ultrafiltration coefficient \( K_{UF} \). That is indicative of flux (permeability of dialyzer membrane to water) which depends on the characteristics of the membranes, including thickness and porosity. The ultrafiltration coefficient of a dialyzer is defined as the volume of fluid in milliliter, transferred across the membranes in an hour per mmHg pressure gradient applied across the membranes (equation [1.11][17]). The ultrafiltration coefficient could be quantified using evaluation of the slope of ultrafiltration rate \( Q_{UF} \) – transmembrane pressure TMP plot (Figure 1-5).

\[
K_{UF} = \frac{Q_{UF}}{\Delta P - \Delta \pi} = \frac{Q_{UF}}{\text{TMP}}
\]

[1.11]
Figure 1–5: Ultrafiltration rate $Q_{UF}$ – Transmembrane pressure TMP plot. Adapted from [156]

At low ultrafiltration rates, the slope of ultrafiltration rate $Q_{UF}$ – transmembrane pressure TMP plot in an isolated (with no dialysate flow) setting and closed (with dialysate flow) circuit is essentially the same in a dialyzer [156,157,165]. This means ultrafiltration coefficient $K_{UF}$ is the same at low ultrafiltration rates.

Proteins tend to stick to the inner surface of the membranes reducing the effective surface area available for ultrafiltration. High concentrations of red blood cells and molecules, such as proteins and lipids increase the viscosity and the oncotic pressure in the blood chamber of the dialyzer. Oncotic pressure is a form of osmotic pressure induced by the proteins (e.g., albumin) in the blood that causes an opposing pressure from the dialysate side into the blood side of the dialyzer. Consequently, the net pressure gradient across the membrane is diminished reducing the ultrafiltration rate.
1.5.1.2.1 Adsorption

Adsorptive solute removal occurs when solutes (mostly proteins) are removed from the blood by adhesion to the dialyzer membranes. Solute removal by adsorption highly depends on physicochemical characteristics of membranes and the solutes [169]. Adsorptive removal of proteins in HF dialyzers is significantly better, presumably due to a greater number of large pores compared to that of low flux (LF) dialyzers. Protein adsorption may reduce the effective membrane pore size of the membranes as dialysis session proceeds [168].

1.5.2 Hemodialysis Membrane Properties

The efficacy of the hemodialysis procedure depends greatly on the chemical composition of materials used in the membranes as well as the physical properties of the dialyzer fibers, cytocompatibility, hemocompatibility, and the ability to remove uremic toxins from the blood [71]. A list of assessment techniques for each factor is provided in Table 1-5. For the purpose of this thesis, the following sections provide detailed information on the evaluation of uremic toxin transport capacity and physical properties of hemodialysis membranes through the assessment of hydraulic (water) permeability and Molecular Weight Cut-Off (MWCO).
Table 1-5: List of assessment protocols for hemodialysis membrane characteristics [71]

<table>
<thead>
<tr>
<th>Physical characteristics of membranes</th>
<th>Cytocompatibility</th>
<th>Hemocompatibility</th>
<th>Uremic Toxin Transport capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permeability and MWCO</td>
<td>Cell metabolic activity</td>
<td>Hemolysis assay</td>
<td>Uremic toxin (e.g., urea and creatinine) transport rate across the membrane</td>
</tr>
<tr>
<td>Porosity of the membranes</td>
<td>Cell proliferation assay</td>
<td>Blood cell aggregation</td>
<td></td>
</tr>
<tr>
<td>Tensile strength</td>
<td>Oxidative stress analysis</td>
<td>Platelet adhesion assessment</td>
<td></td>
</tr>
<tr>
<td>Contact angle assessment</td>
<td>Total protein content assessment</td>
<td>Thrombus formation assessment</td>
<td></td>
</tr>
<tr>
<td>Membrane morphology</td>
<td>Cell attachment and morphology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface charge assessment</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.5.2.1 Hydraulic (Water) Permeability

Water permeability of a membrane is defined as the volume of pure water crossing the membrane pores per unit area, time, and pressure gradient applied across the membrane. More porous hemodialysis membranes have higher hydraulic permeability [71]. For the evaluation of hydraulic permeability of a membrane, the flux of water through the membrane can be calculated by the following equation:

\[ V_W = \frac{\Delta V}{A \times \Delta T} \] \[1.12\]

where \( V_W \) is pure water flux in \( \text{m}^3/\text{m}^2\text{s} \), \( \Delta V \) is the volume of permeating water in \( \text{m}^3 \), \( A \) is the effective filtration area in \( \text{m}^2 \), and \( \Delta T \) is the sampling time in seconds. The rate of water flux over transmembrane pressure is considered as hydraulic permeability of the membrane.
1.5.2.2 Molecular Weight Cut-Off (MWCO)

MWCO indicates the molecular weight above which solutes will be retained completely by the membrane and below which solutes will be allowed to cross the membrane [70,71].

For assessment of MWCO, the Percentage rejection (%R), i.e. fraction of solute that cannot pass the membrane, is evaluated for a wide range of solutes using the following equation:

\[ R = (1 - \frac{C_P}{C_F}) \times 100 \]  

where \( C_F \) and \( C_P \) are solute concentrations in before and after the filtration, respectively. Percent rejection (%R) for each considered solute is plotted against their molecular weights. The molecular weight corresponding to the point of 90% rejection is considered as MWCO of the membrane.

![Graph](image-url)  

*Figure 1–6: An example of MWCO of a membrane. Adapted from [71]*
1.5.2.3 Uremic Transport Capacity

The transport of uremic toxins is the most important function of hemodialysis membranes. To evaluate the performance of membranes in uremic toxins removal, uremic toxins (e.g., urea and creatinine) are pumped through the membrane. The concentration of uremic toxins is measured before, and after the filtration through the membranes. Lower concentrations after the filtration at a particular time indicates superior transport of a particular uremic toxin [71]. More porous membranes tend to have better uremic transport given that the solute of interest has a molecular range lower than the membrane MWCO.

1.5.3 The History of Dialyzers and Membranes

Thomas Graham is known as “the father of dialysis”. He demonstrated that solutes can be removed by diffusion from fluid containing colloids and crystalloids in a hoop dialyzer that he developed [51,52]. Subsequently, Adolf Eigen Fick described the process of diffusion quantitatively [53]. In 1913, the first artificial kidney was developed by John Abel and his colleagues using 8 parallel collodion tubes of 8 mm diameter with a length of about 40 cm [54]. However, the collodion tubes were fragile and anticoagulant was unavailable at the time. Shortly after, a non-toxic hirudin used as an anticoagulant was developed which enabled them to test their dialyzers on animals. Von Hess and McGuigan created a pulsatile blood flow and turbulent dialysate flow to prevent coagulation and formation of stagnant layers. In 1924, George Hass was the first person who performed hemodialysis on humans, though none of his patients survived [55]. Hass assembled a collodion tube (1.2m long) dialyzer with a surface area of 1.5-2.1m². Haas was the first person who used heparin as an anticoagulant and Ringer solution as dialysis fluid.

Willem Johan Kolff performed the first successful hemodialysis using a rotating drum dialyzer made from a wooden core [56]. This was a 30m cellophane tube with a diameter of 35mm wound around a cylinder. The drum was partially submerged into a stationary tank containing 70-100L dialysis fluid. The main problem with the rotating drum dialyzer was its large extracorporeal volume causing intradialytic hypotension and inadequate
ultrafiltration [56]. Kolff also developed a new generation of dialysis machine known as Kolff-Brigham Kidney which was able to remove solutes, but it did not allow for excess fluid removal [57].

The dialysis machine developed by Nils Alwall consisted of cellophane tubing around a stationary vertical metal drum, placed in a dialysate reservoir. Alwall constructed the first dialyzer with controllable ultrafiltration by applying negative pressures in the dialysate reservoir that allowed for both excess solutes and fluid removal [58,59].

In 1946, Gordon Murray, a Canadian surgeon built the first North American dialyzer which was constructed of a static coil wound in a steal frame [61]. Murray also, performed venovenous dialysis by placement of catheters into the inferior vena cava and femoral vein.

In 1947, Bondo Von Garrelts developed the first coiled dialyzer, made from a 13cm wide cellophane tube that was coiled and spread out between metal nettings [60]. The metal netting was meant to support the membrane and allow perfusion of dialysate through the dialyzer. The total area of the membrane was 1.5m² and with a capacity of 2L. The coil was placed on a porous disk in a container of dialysis solution stirred by the flow of air through the disk.

In 1947, Arthur MacNeil dialyzer constructed a parallel flow dialyzer made from cellophane tubes separated by a nylon mesh [60]. The year after, Skeggs and Leonards changed the design of the dialyzer by using two sheets of cellophane and two grooved rubber pads. In this setting, the blood flows between the cellophane sheets while dialysate flows in the grooves outside the sheets in an opposite direction to the blood flow.

In 1955, a twin coil dialyzer was developed by Willem Johan Kolff and Bruno Watschinger which was constructed using two parallel loops of cellophane tubing with spacers wrapped together around a metal core [60]. Twin coil dialyzers had several disadvantages, including the requirement for high priming volume, risk of bacterial contamination because of open tank design, and the possibility of membrane damage as a result of high pressure in the extracorporeal circuit.
In 1960, the Kiil dialyzer was developed by Frederic Kiil using double sheets of cuprophane membrane between grooved plastic boards [62]. The Kiil dialyzers had a relatively small blood compartment which didn’t require priming with donor blood.

In 1963, Zbylut Twardowski theoretically evaluated the characteristics of an ideal dialyzer and proposed the first design of a capillary dialyzer [62]. Zbylut Twardowski proposed that the ideal dialyzer is an apparatus consisting of 4 parallel dialyzing units in polyvinyl tubes, connected to the patient’s blood. The polyvinyl dialyzing units contained 100 cellophane capillary tubes, 50cm long, with an internal diameter of 1mm, and a wall thickness of 10 μm. In 1964, Dow Chemical Company initiated the first generation of hollow fiber dialyzers made from saponified cellulose triacetate. In 1968, the first hollow fiber dialyzers were used for treatment of a patient with CKD [63]. The hollow fiber dialyzers have been the only type of dialyzers in current use due to their superior advantages, such as relatively low capacity, low internal resistance, and high efficiency of dialysis and ultrafiltration.

Hemodialysis membranes have been constantly evolving with the advent of hemodialysis therapy. Cellulose acetate membranes were the first membranes used in the dialyzers. These membranes did not remove middle molecular weight uremic toxins due to their small pores [107]. The accumulation of middle molecules was associated with negative health outcomes such as neuropathy [69]. Additionally, the functional groups in the cellulose acetate membranes triggered complement activation responsible for overactive immune response towards antigens. Patients treated with cellulose acetate-based dialyzers were also at risk of hypoxia, and thrombocytopenia. Cellophane, Cuprophan, Bioflux, and Cuproammonium Rayon were other types of commercial cellulosic membranes that provided poor biocompatibility [72]. This issue was partially resolved by replacement of acetate groups with acetyl which also improved removal of middle molecules. The other issue with the cellulosic membrane was their thin walls (5-20μm), thus limiting convective removal of solutes [56]. These issues motivated commercial companies to make synthetic membranes with more biocompatibility, larger pores for better removal of middle molecular weight uremic toxins, and thicker walls for convective removal of solutes. The first generation of synthetic membranes provided limited diffusive removal
of solutes due to their thick walls. Polyacrylonitrile (PAN) membranes with pore size of 29 Å, showed superior advantage with regard to complement activation which was due to the adsorption of proteins responsible for complement activation [64,65]. The adsorption of B2M was also enhanced in PAN membranes [66]. Although PAN membranes were significantly better than cellulosic membranes, patients were very prone to anaphylactic reactions [64,65]. This prompted development of polysulfone (PSF) membranes that had relatively larger pores. PSF membranes showed both superior diffusive and convective removal of middle molecular weight uremic toxins [66,67]. Primary PSF membranes also were reported to be able to remove molecules with molecular weight between 11,400 to 40,000 Da [67]. Additionally, PSF membranes showed better removal of B2M and inflammatory cytokines, while retaining albumin (an essential solute) [68,73]. PSF-based membranes such as polyether sulfone (PES), polyester polymer alloy (PEPA) have been used in dialyzers for many years. Other types of commercial membrane materials such as ethylene vinyl alcohol copolymer (EVAL) and polymethyl methacrylate (PMMA) are currently available.
1.5.4 The Characteristics of Current Dialyzers

The type of hemodialyzers currently in use are the hollow fiber dialyzers which consist of many small semipermeable capillaries (Figure 1-7). Blood flows inside the capillaries, while dialysate flows counter currently around them. The dialyzer membrane acts as a sieve where solutes and fluid transport occur through the pores of the membranes. Dialyzers are classified as low, or high flux, which depends on water permeability of membranes. LF dialyzers (ultrafiltration coefficient lower than 15 ml/h/mmHg) provide adequate removal of small uremic toxins, but the removal of larger solutes is not efficient. In contrast, HF dialyzers (ultrafiltration coefficient higher than 15 ml/h/mmHg) provide relatively adequate removal of both small and larger uremic toxins [182]. This is due to relatively larger average membrane pore diameters in the HF dialyzers [50]. The ultrafiltration profile is volumetrically controlled by an ultrafiltration pump which creates a negative pressure gradient in the dialyzer [161].

Back-filtration in the distal portion of a dialyzer (Figure 1-8) can occur due to blood pressure drop inside the fibers (axial pressure drop). The axial pressure drop (\(\Delta P\)), depends on viscosity (\(\mu\)), the effective length of fibers (\(L\)), the blood flow rate (\(Q_b\)), and the hollow fiber inner radius (\(R\)) based on Hagen-Poiseuille equation [129,144]:

\[
\Delta P = \frac{8\mu L Q_b}{\pi R^4}
\]  

[1.14]

Herein, it is assumed that blood flow inside of the dialyzer is laminar. A reduction of internal diameter of individual membranes, and increasing the length of fibers results in the increase of blood pressure drop inside of membranes [144]. The back-filtration of dialysate fluid may lead to transport of pyrogens from bicarbonate-based dialysate fluid to the patient’s blood. The exposure of microbial compounds could trigger inflammation in hemodialysis patients [159,160].
Figure 1–7: Hollowfiber design. Hollowfiber membranes packed into the dialyzer housing. Adapted from [71]

Figure 1–8: Forward-filtration and back-filtration in hemodialyzers. Adapted from [184]
1.5.5 Novel MCO and Conventional HF Dialyzers

The goal in hemodialysis technology is to devise an artificial kidney model that mimics a functional kidney. The advances in membrane manufacturing industry have led to sufficient clearance of small water-soluble solutes. However, existing dialyzers still fail to remove adequate clearance of middle molecular weight solutes instigating cardiovascular events and higher mortality rates in maintenance hemodialysis patients. Expanded hemodialysis with a new generation of membranes, named Medium Cut-Off (MCO) membranes, have exhibited relatively better removal of middle molecular weight solutes, thereby improving health outcomes in maintenance hemodialysis patients. This is mainly due to the advanced physical characteristics of MCO membranes (Table 1-6), which is significantly affected by manufacturing processes. The membrane physical characteristics (mean pore size, pore size distribution, number of pores, and pore curviness) are affected by the type of additives that cause either swelling or shrinkage of the pores during the drying process of membrane synthesis. Furthermore, raw materials, spinning technology, precipitation methods, and regulation of temperature greatly influence the physical characteristics of membranes [123]. MCO membranes are made of a polyarylethersulfone and polyvinylpyrrolidone blend, with relatively higher numbers of large pores with a mean radius of 5 nm compared to conventional HF membranes [159,160]. This allows for the removal of an expanded range of uremic toxins removal up to 45kDa while avoiding the loss of essential larger solutes like albumin [124]. Also, the pore sizes are relatively more uniform compared to conventional HF membranes which enables high selectivity of MCO membranes [107,124]. Performance of hemodialysis membranes could be quantitatively evaluated through a sieving curve, obtained by plotting the SC of wide macromolecular polysaccharides with a broad molecular weight distribution, against their molecular weights. The evaluation of sieving curves for MCO membranes, conventional HF membranes, high cut-off membranes, and glomerular membrane, indicates that the permeability of MCO membranes is close to that of glomerulus. This implies that the performance of MCO membranes is closest to that of a healthy kidney [123,125, 126,127]. The unique sieving properties and enhanced internal filtration in MCO membranes allows for a wide range of uremic toxins (15kDa to 45kDa)
while limiting the loss of albumin. Structural characteristics of a MCO membrane is illustrated in Figure 1-9 [145].

Figure 1–9: Structural characteristics of the MCO membrane. Scanning electron microscopy images of the fiber (left), internal skin layer (middle) and fiber wall (right). Copyright © 2021 permitted by Thiago Reis [145]
Table 1-6: Technical specifications of clinical MCO (Theranova 400, Baxter, Canada) and conventional HF (FX 600 Helixone, Fresenius, Canada) dialyzers [146]

<table>
<thead>
<tr>
<th>Technical Specifications</th>
<th>MCO dialyzers</th>
<th>Conventional HF dialyzers (FX 600)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber diameter (Internal)</td>
<td>0.180mm</td>
<td>0.210mm</td>
</tr>
<tr>
<td><strong>Fiber material</strong></td>
<td>Medium Cut Off (MCO)</td>
<td>Polysulfone (PSF)</td>
</tr>
<tr>
<td></td>
<td>Polyarylethersulfone and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Polyvinylpyrrolidone blend BPA-free</td>
<td></td>
</tr>
<tr>
<td>Effective membrane total surface area</td>
<td>1.7m²</td>
<td>1.5m²</td>
</tr>
<tr>
<td>Ultrafiltration Coefficient (K$_{UF}$)</td>
<td>48 ml/h/mmHg (Theranova 400)</td>
<td>52 ml/h/mmHg</td>
</tr>
<tr>
<td></td>
<td>59 ml/h/mmHg (Theranova 500)</td>
<td></td>
</tr>
<tr>
<td>Number of microfibers</td>
<td>12,960</td>
<td>-</td>
</tr>
<tr>
<td>Fiber wall thickness</td>
<td>0.35 mm</td>
<td>0.35mm</td>
</tr>
<tr>
<td>Blood compartment volume</td>
<td>91ml (Theranova 400)</td>
<td>97ml</td>
</tr>
<tr>
<td></td>
<td>105ml (Theranova 500)</td>
<td></td>
</tr>
<tr>
<td>Housing Material</td>
<td>Polycarbonate</td>
<td>Polypropylene</td>
</tr>
<tr>
<td>Potting</td>
<td>Polyurethane</td>
<td>Polyurethane</td>
</tr>
<tr>
<td>Sterilization</td>
<td>In-line steam</td>
<td>In-line steam</td>
</tr>
</tbody>
</table>
1.5.5.1 Forward-Filtration and Back-Filtration in MCO Dialyzers

The internal diameter of MCO membranes is smaller than conventional HF membranes [128]. This results in a higher wall shear rate and blood flow velocity, preventing blockage of membrane pores by blood proteins which could hinder solute transport [129,130]. The reduction of internal diameter also increases axial pressure drop, and internal filtration which allows for the removal of middle molecular solutes by convective transport [131,144,166,167].

MCO membranes provide improved removal of middle molecular weight uremic toxins in a hemodialysis setting without the need for exogenous substitution fluid and complex setup as in the case of hemodiafiltration. This is due to an adequate amount of back-filtration in the distal end of MCO dialyzers [162,171]. In essence, MCO dialyzers provide internal hemodiafiltration to enhance convective transport [171]. Lorenzin et al., [132,162] quantitively evaluated internal filtration in the MCO dialyzers using nuclear imaging techniques in an in vitro setting. The finding showed that the amount of forward-filtration and back-filtration was more than 30 ml/min at zero net ultrafiltration rate and blood flow of 300 ml/min. The amount of forward-filtration and back-filtration could reach up to 50 ml/min at higher blood-flow rates.

The internal filtration from 30 ml/min to 50 ml/min and the sieving characteristics of MCO membranes allow for enhanced convective clearance of middle molecular weight uremic toxins. Prior to the advent of MCO membranes, hemodiafiltration provided improved convective removal of middle molecular weight uremic toxins. However, a complex hardware system is required for purification of the fluid replacement. In contrast, expanded hemodialysis using MCO membranes provides equal performance in terms of convective clearance of middle molecular weight uremic toxins due to its unique sieving properties and improved internal filtration.

As previously discussed, (section 1.5.4), back-filtration in the dialyzers may result in transport of pyrogens from dialysate side to patient’s blood which triggers inflammatory response. Previous research comparing MCO and conventional HF dialyzers showed that
MCO dialyzers effectively retain pyrogens at a similar level to other HF dialyzers even though MCO membranes have relatively larger pore sizes [159,160].

1.5.5.2 Clinical Studies Comparing MCO and Conventional HF Dialyzers

Numerous clinical studies have shown a significantly higher removal of B2M (a marker of middle molecular weight solute) in MCO membranes compared with HF membranes [132,133,134,135,136,164]. Previous studies presented mixed conclusions regarding removal of urea. Most clinical studies suggested that there is no difference in removal of urea between MCO membranes and conventional HF membranes [132,133,134,137,163,164]. However, one study showed enhanced removal of urea in patients treated with MCO membranes [138]. The health benefits of expanded hemodialysis with MCO membranes have been evaluated in many studies. A randomized crossover clinical trial indicated significant reduction in expression of Tumor Necrosis Factor-alpha (TNF-α) and interleukin-6 (IL-6) (proinflammatory cytokines) mRNA in patients who received expanded hemodialysis with MCO membranes compared to conventional HF dialyzers. However, this difference lost its significance when the study was expanded to 12 weeks [139]. Unfortunately, it is not entirely clear if expanded hemodialysis with MCO membranes results in significant reduction of all inflammatory agents compared to conventional HF membranes [132,134,133,137,139,140,141]. However, most studies indicated significant reduction in cardiovascular parameters (e.g., osteopontin, Growth Differentiation Factor-15 (GDF-15), and Fibroblast Growth Factor-23 (FGF-23)) in patients treated with expanded hemodialysis compared to patients who were treated with conventional HF dialyzers [142,143]. Despite the promising characteristics of MCO membranes further studies are required to evaluate its potential health benefits.
1.6 Challenges of Hemodialysis

Hemodialysis, though a lifesaving treatment for CKD, is associated with numerous health issues. Patients receiving hemodialysis have a poor quality of life due to many comorbidities associated with hemodialysis. The mortality rate is remarkably high in this population, with cardiovascular disease being the leading cause of death [40,74,75]. This is due to many factors related to inadequacy of currently available hemodialyzers. The accumulation of uremic toxins negatively affects many organ systems, notably the cardiovascular system [76,77]. The majority of uremic toxins include protein-bound, and middle molecular weight solutes that are inefficiently removed by conventional hemodialysis [77,78,79]. Many protein-bound molecules (e.g., Indoxyl Sulphate (IS)) bind to larger less permeable proteins, such as albumin which hinders their removal by hemodialysis. This triggers an inflammatory response, and development of cardiovascular disease. Accumulation of middle molecular weight solutes such as B2M increases the chance of atherosclerosis and myocardial disease [80]. Recent developments in hemodialysis membranes, including development of HF membranes with bigger pores, have not fully resolved the issues with ineffective removal of uremic toxins [78,80,158]. Additionally, accumulation of uremic toxins causes interdialytic volume overload which may trigger development of left ventricular hypertrophy and cardiac failure [81]. Intradialytic hypotension during hemodialysis can also, induce ischemic injuries in vital organs, such as the heart (causing myocardial stunning), the brain (causing cognitive impairment), and liver (causing endotoxemia) in hemodialysis patients [82,83,84,103,104].

Hemodialysis also aims to remove excess electrolytes like sodium that is not removed through any residual renal function. However, hemodialysis provides a limited regulation of sodium mainly due to complexity of its transport across the hemodialysis membrane. During intermittent hemodialysis, sodium is removed primarily through convective transport (~ 78 %), rather than diffusive transport (~ 22 %) [86,87]. The diffusion component depends on the concentration gradient between plasma sodium and dialysate sodium. The plasma concentration of sodium differs between individuals, and chiefly depends on the dietary intake and residual renal function [88]. Moreover, dialysis patients
have different unique set-points for plasma concentration, which is stable over long-term observations [89,90]. This is why the choice of dialysate sodium concentration remains among the most imperative and demanding challenges in the care of hemodialysis patients. Nephrology units have adopted high sodium bicarbonate dialysate to prevent incidences of symptomatic hypotension, disequilibrium, and reduce patients’ discomfort since 1980s [91]. However, a high concentration of dialysate sodium increases concentration of sodium in plasma. This results in volume overload, congestion, and hypertension [87]. Furthermore, higher sodium concentrations in plasma leads to tissue deposition of excess sodium in skin, and muscle causing irritation [113] and directly in the cardiac tissue further increasing the risk of mortality. Additionally, damage to the vascular epithelial glycocalyx can occur due to high concentrations of sodium exposure [114,115]. This further aggravates the ischemic vulnerability of tissue by inducing intradialytic endothelial dysfunction. In contrast, excessive sodium removal leads to disruption of plasma tonicity, hypotension, and circulatory collapse. Excessive sodium removal exacerbates hemodialysis-associated cardiovascular injury to the vital organs such as heart, brain, gut, and kidneys [84,103,116,117].

There have been many attempts to achieve a neutral sodium balance: reducing dialysate sodium concentration and alignment of concentration of sodium in plasma and dialysate. The first approach, reduction of dialysate sodium concentration, is associated with cramping and potential hypotension during dialysis. Yet, previous sodium MRI studies suggested more modest intradialytic weight gain [105], and reduced tissue deposition of sodium in patients who were treated with lower dialysate sodium concentration [106].

The second approach, alignment of concentration of sodium in plasma and dialysate, requires additional devices for direct on-site measurement of sodium during each hemodialysis session since dialysate conductivity monitoring is often inaccurate and prone to drift due to lack of appropriate calibration and maintenance [92].

The diffusive removal of sodium is limited for many reasons. Sodium interacts with many dissolved materials (bicarbonate, carbonate) due to its positive charge. In addition, sodium ions exchange for intracellular potassium and bind to skin proteoglycans reducing
the concentration of available sodium in plasma [108,109]. Consequently, only a small portion of sodium in plasma is available for diffusive removal. The diffusive removal of sodium also depends on environmental factors, including temperature and pH.

The removal of sodium by convection, also presents several problems. Convective removal of sodium requires increasing the ultrafiltration rate which can lead to hemodynamic instability and multiple organ dysfunction [93, 94, 95, 96, 97, 98, 99]. Furthermore, the removal of sodium could be hindered by the Gibbs-Donnan effect during dialysis that occurs when positively charged sodium ions bind to the negatively charged plasma proteins that are impermeable to the hemodialysis membrane in order to establish electroneutrality [100,110]. This also makes it possible for patients to experience hypernatremia even if they were treated with a low dialysate sodium concentration [111,112]. The characteristics of membranes used in the dialyzers, such as surface charge density [101] directly influence sodium removal. Furthermore, the porosity of membrane although speculative may influence sodium removal [102].

1.7 Rapid Throughput Research Platform Using Animal Models

The evaluation of issues with current hemodialysis therapy has been slow due to lack of an appropriate animal model and scarcity of high quality initial clinical studies. The use of animal models in hemodialysis provides a rapid throughput research platform to identify and evaluate benefits and issues with current and future renal replacement products. The use of pre-clinical animal models also provides robust biological plausibility and estimates of population and effect size, when designing clinical trials.

Previous studies mostly attempted using significantly large animals such as goats [172,173], sheep [151], rabbits [149] dogs, and cats [148,153]. However, producing stable severe CKD in larger animals is cumbersome.

There are several well-developed methods inducing severe CKD in small animals (e.g., rats). However, there has not been many hemodialysis studies in rats due to lack of an appropriate dialyzer suitable for their small body size. More importantly, the removal of
middle molecular weight uremic toxins has not been evaluated in previous hemodialysis studies in rats [150,152,154,155]. It is noteworthy that previous hemodialysis involved rather large rats (> 400 grams) [150,152,154,155]. Our team have successfully dialyzed healthy male Wistar Kyoto rats (weight approx. 250–300 g) using novel miniaturized conventional HF dialyzers in combination with intravital video microscopy to investigate hemodialysis-induced microvascular dysfunction [147]. It is imperative to investigate whether the novel miniaturized dialyzers can effectively differentiate performance characteristics demonstrating hemodialysis membrane and fiber design.

1.8 Study Objectives and Hypothesis

The aim of this study was to investigate whether the miniaturized dialyzers can effectively differentiate performance characteristics representing hemodialysis membrane and fiber design.

Hypothesis: We postulate that miniaturized self-produced dialyzers can effectively differentiate performance characteristics representing hemodialysis membrane and fiber design.
Chapter 2

2 The Characterization of Membranes in Miniaturized Dialyzers

2.1 Introduction

Hemodialysis is associated with numerous negative health outcomes primarily due to inefficacy of hemodialysis technology. Currently available dialyzers, as surrogate kidneys fail to meet the expectations of a functional kidney, most importantly inadequate removal of middle molecular weight uremic toxins and sodium. This in turn leads to numerous negative health outcomes, notably cardiovascular disease and increasing mortality rate in hemodialysis patients. Thus, there is an urgent need for an accelerated evaluation of negative health outcomes with hemodialysis therapy. Currently, lack of an appropriate pre-clinical animal model of hemodialysis further impedes rapid evaluation of the harms associated with hemodialysis. We introduced a functional miniaturized dialyzer for use in rodent hemodialysis models, which provides more flexibility for the in-vivo assessment of membranes (from any source) and the range of techniques that can be implemented within a rodent model. This particularly accelerates the investigation of dialyzer technology both within current hemodialysis pathophysiology paradigms (e.g., conventional concepts of clearance and biocompatibility), evolving paradigms (e.g., hemodialysis-induced multiorgan ischemic injury) and less considered ones (e.g., manipulation of ultrafiltration to maximize sodium removal). The versatility of this animal model allows investigation of biocompatibility of different fiber materials, and novel treatment protocols prior to clinical trials. In this study, we evaluated water permeability and a wide range of uremic toxin removal in the novel miniaturized rat dialyzers constructed from two different membranes in order to investigate whether the dialyzers can effectively differentiate performance characteristics representing hemodialysis membrane and fiber design. We also evaluated the internal filtration (forward-filtration and back-filtration) in miniaturized MCO dialyzers (previously used in vivo). Technical specifications of respective miniaturized dialyzers are provided in Table 2-1.
Table 2-1: Technical specifications of miniaturized conventional HF and MCO dialyzers.

<table>
<thead>
<tr>
<th>Technical Specifications</th>
<th>Conventional HF Mini-Dialyzers</th>
<th>MCO Mini-Dialyzers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber diameter (Internal)</td>
<td>0.210mm</td>
<td>0.180mm</td>
</tr>
<tr>
<td>Fiber material</td>
<td>Polysulfone (PSF)</td>
<td>Polyarethersulfone and Polyvinylpyrrolidone blend BPA-free</td>
</tr>
<tr>
<td>Effective membrane total surface area</td>
<td>0.0054m²</td>
<td>0.0047m²</td>
</tr>
<tr>
<td>Number of microfibers</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Effective length</td>
<td>110mm</td>
<td>110mm</td>
</tr>
<tr>
<td>Fiber wall thickness</td>
<td>35 μm</td>
<td>35 μm</td>
</tr>
<tr>
<td>Potting material</td>
<td>Marine Epoxy</td>
<td>Marine Epoxy</td>
</tr>
<tr>
<td>Disinfection Chemical</td>
<td>Sodium hypochlorite (bleach)</td>
<td>Sodium hypochlorite (bleach)</td>
</tr>
</tbody>
</table>
2.2 Experimental Overview

This is a study of novel miniaturized dialyzers performance with regard to characteristics of respective fibers used in the dialyzer housing. The miniaturized dialyzers used in the experiments were constructed using fibers harvested from existing clinical Medium Cut-Off (MCO) dialyzers (Theranova 400, Baxter, Canada) and conventional High Flux (HF) dialyzers (FX 600 Helixone, Fresenius, Canada). The dialyzers were evaluated using the whole blood (Hematocrit Hct 35%) obtained from LHSC core lab (London Health Sciences Center, London, Canada). The internal filtration was evaluated in 3 miniaturized dialyzers previously used in vivo. The water permeability and uremic toxin removal were evaluated in 12 miniaturized dialyzers (6 MCO and 6 conventional HF dialyzers) in a controlled ultrafiltration circuit. Moreover, sodium removal was separately evaluated in 10 miniaturized dialyzers (5 MCO and 5 conventional HF) using plasma.

2.3 Materials and Methods

This section provides the detailed information about the materials and techniques used in this study to evaluate internal filtration, water permeability and uremic toxin removal in the miniaturized dialyzers.

2.3.1 Miniaturized Dialyzers Production

The membranes were harvested from clinical human dialyzers and inserted into a clear polycarbonate tubing. Marine Epoxy (Henkel, Naperville, IL, USA) was subsequently used as a potting compound at the blood inlet and outlet of the miniaturized dialyzer tubing. After 24 minutes, the caps were glued to the blood inlet and outlet of the miniaturized dialyzers. Plastic luer-locks (ISM, Englewood, CO, USA) were screwed onto inlet and outlet dialysate sites. The gaps in the miniaturized dialyzer housing were sealed with Acrylic Cement (SCIGRIP, Durham NC, USA). The dialyzers were checked for potential leaks prior to the experiments.
2.3.2 Whole Blood and Dialysate Fluid Production

250 ml saline was added to the original packed RBC (Red Blood Cell) unit (75-150 ml) and centrifuged (42 rpm; 4 °C) for 7 min. A 600 ml dry pack was attached, and supernatant was removed. The content was weighed and an appropriate amount of FFP (Fresh Frozen Plasma) was added to the unit (RBC Volume × 1.6 = FFP). The whole blood was then stored in the fridge. Dialysate fluid was prepared in a 1L drain bag at the plasmapheresis unit (London Health Sciences Center, London, Canada) with electrolyte composition shown in the Table 2-2. Enoxaparin (2500IU (anti-factor Xa)/0.2 ml) was added to the whole blood and dialysate fluid prior to the experiments in order to prevent coagulation.

Table 2-2: Dialysate composition of electrolytes prepared for the experiments

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>K⁺</th>
<th>Na⁺</th>
<th>Ca²⁺</th>
<th>HCO₃⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrations</td>
<td>3.0 mmol/L</td>
<td>140 mmol/L</td>
<td>1.25 mmol/L</td>
<td>40 mmol/L</td>
</tr>
</tbody>
</table>

2.3.3 Experimental Setup

A miniaturized dialyzer was horizontally secured on a holder with all ports at the same level. The blood flow passing through the fibers was controlled by a peristaltic pump (P P720, Instech Lab., Plymouth Meeting, PA, USA) with an interconnected lumen tubing (FL-042S-LL, Instech Lab., Polymount Meeting, PA, USA). The blood flow rate was set at 0.59 ml/min. The dialysate flow was controlled using two separate, peristaltic infusion pumps (Sigma Spectrum V8 Infusion system, Baxter, Deerfield, III, USA) in a countercurrent flow to the direction of blood flow. The rate of infusion at dialysate-inlet was set at approximately 1 ml/min throughout the experiments. The infusion pump at the dialysate-outlet was used as an ultrafiltration control as suggested in previous studies [183]. The differential rates of the two peristaltic infusion pumps, was used to set the desired ultrafiltration rates (equation [2.1]) (Table 2-3).

\[ Q_{UF} = Q_{Do} - Q_{Din} \]  \[2.1\]
where $Q_{Do}$ and $Q_{Din}$ are the dialysate flow rates at the outlet and inlet of the miniaturized dialyzer, respectively.

In adults, hemodialysis prescription is set at standardized blood flow rates ($\geq 300$ ml/min) and dialysate flow rates ($\geq 500$ ml/min) [2]. Considering that these miniaturized dialyzers are adapted to be used in in-vivo experiments with small rats (250-300 g) [147] both blood flow and dialysate flow rates were reduced to 0.59 ml/min and 1ml/min, respectively.

Pressure measurements at each port of the dialyzer was recorded every minute using a pressure meter (90XL Technician Meter, MesaLabs, CO, USA). We used 3-way stopcocks to connect the pressure extension lines pointing at the same direction. We calculated time-averaged TMP at corresponding ultrafiltration rates shown in Table 2-3. A schematic of our in-vitro experimental set-up is provided in Figure 2-1. Prior to commencement of dialysis, the dialyzers were primed for 15 minutes using dialysate fluid mixed with Enoxaparin (2500 IU (anti-factor Xa)/0.2ml) to ensure patency of fibers and optimize dialyzer performance [3]. The miniaturized dialyzers were disinfected using sodium hypochlorite (bleach) at the end of experiments [181].

**Table 2-3: Ultrafiltration rates set by differential infusion rates of dialysate pumps**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0-15</th>
<th>15-30</th>
<th>30-45</th>
<th>45-60</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dialysate Outlet</strong>&lt;br&gt;Pump Rate&lt;br&gt;(ml/min)</td>
<td>1</td>
<td>1.10</td>
<td>1.17</td>
<td>1.20</td>
</tr>
<tr>
<td><strong>Dialysate Inlet</strong>&lt;br&gt;Pump Rate&lt;br&gt;(ml/min)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>$Q_{UF}$ (ml/min)</td>
<td>0</td>
<td>0.10</td>
<td>0.17</td>
<td>0.20</td>
</tr>
</tbody>
</table>
2.3.4 Measurements and Calculations

The required measurements were done within intervals of 15 minutes at each desired ultrafiltration rates (Table 2-3). This duration (i.e., 15 minutes) was chosen to ensure that the whole ultrafiltration cycle was completed without disruption of the infusion pumps due to accumulation of air bubbles in the dialysate lines at higher ultrafiltration rates.

2.3.4.1 Evaluation of Internal Filtration (forward- and back-filtration)

The evaluation of internal filtration (forward-filtration and back-filtration) was performed in miniaturized MCO dialyzers that were previously used in-vivo. To evaluate forward- and back-filtration, pressure measurements at all ports of the dialyzer were concurrently recorded under zero net ultrafiltration setting. For this experiment, dialysate fluid with the same composition (shown in Table 2-3) pumped through the fibers in 3 used miniaturized MCO dialyzers. The same composition of dialysate was used to minimize the osmotic effect in measurement of ultrafiltration rate.
Transmembrane pressure in the proximal portion and distal portion (relative to blood-inlet) of dialyzer was calculated using the following equations:

\[
\text{TMP}_{\text{proximal}} = P_{\text{blood-inlet}} - P_{\text{dialysate-outlet}} \tag{2.2}
\]

\[
\text{TMP}_{\text{distal}} = P_{\text{blood-outlet}} - P_{\text{dialysate-inlet}} \tag{2.3}
\]

where positive and negative TMP indicate forward-filtration and back-filtration, respectively.

The net TMP was also calculated using the following equation [2.4] in order to evaluate direction of internal filtration.

\[
\text{TMP}_{\text{net}} = \text{TMP}_{\text{proximal}} + \text{TMP}_{\text{distal}} \tag{2.4}
\]

Ultrafiltration rate was also calculated using the following equation [4]:

\[
Q_{\text{UF}} = \frac{(V_{\text{Bo}})_D}{\Delta T} - Q_{\text{Bi}} \tag{2.5}
\]

where \((V_{\text{Bo}})_D\) is the volume of dialysate fluid collected at the blood-outlet of the dialyzer, \(Q_{\text{Bi}}\) is the blood flow set by the peristaltic pump at the blood-inlet of the dialyzer, and \(\Delta T\) represents the duration of dialysis.

### 2.3.4.2 Evaluation of Water Permeability

The total volume of whole blood at blood outlet of the miniaturized dialyzers was measured gravimetrically using a high precision lab scale with accuracy of 0.01 grams (CGOLDENWALL, California, USA) at each corresponding ultrafiltration rate settings (Table 2-3). Assuming the density of blood is approximately 1g/ml, average ultrafiltration rate was calculated via the following equation [4]:

\[
Q_{\text{UF}} = \frac{(V_{\text{Bo}})_B}{\Delta T} - Q_{\text{Bi}} \tag{2.6}
\]

where \(Q_{\text{Bi}}\) is the blood flow rate, \(\Delta T\) is the duration of dialysis at corresponding ultrafiltration rates (Table 2-3), and \((V_{\text{Bo}})_B\) is the total volume of blood measured at blood-outlet of the miniaturized dialyzers.
The mean TMP was derived from pressure readings at blood-and dialysate-outlets at corresponding ultrafiltration rate settings. The pressure line connected to the blood-inlet was clamped to prevent blood entry into the pressure diaphragms. Blood entry into the pressure lines results in coagulation and inaccurate pressure measurements. Therefore, the pressure line connected to the dialysate-inlet was also closed-off to use a 2-point approach for calculation of TMP [5].

\[ \text{TMP} = (P_{\text{Bo}} - P_{\text{Do}}) - \Delta \pi \]  

[2.7]

where \( P_{\text{Bo}} \) and \( P_{\text{Do}} \) are the pressure recordings at the blood outlet and the dialysate outlet respectively, and \( \Delta \pi \) is the oncotic pressure created by the proteins in the blood.

For the evaluation of \( K_{\text{UF}} \) (ml/h/mmHg), ultrafiltration flow (ml/min) was plotted as a function of transmembrane pressure TMP (mmHg). At low ultrafiltration rates, the slope of ultrafiltration rate \( Q_{\text{UF}} \) – transmembrane pressure TMP plot in an isolated (with no dialysate flow) setting and closed (with dialysate flow) circuit is essentially the same in a dialyzer [156,157,165]. Therefore, we determined the ultrafiltration rate range where the slope of ultrafiltration rate \( Q_{\text{UF}} \) – transmembrane pressure TMP plot is linear. To compare ultrafiltration coefficient \( K_{\text{UF}} \) of miniaturized MCO and conventional HF, the rate of ultrafiltration rate \( Q_{\text{UF}} \) over transmembrane pressure TMP was separately calculated within the linear range [4]:

\[ K_{\text{UF}} = \frac{Q_{\text{UF}}}{\text{TMP}} \]  

[2.8]

### 2.3.4.3 Evaluation of Uremic Toxins Removal

The removal of various uremic toxins with a wide range of molecular weights was evaluated. The clearance of small (urea and creatinine), and middle (B2M and vitamin B\(_{12}\)) molecular weight uremic toxins was evaluated with increasing ultrafiltration rates (Table 2-3). The purpose of this evaluation was to verify the effect of the ultrafiltration rate on the removal of small and middle molecular weight uremic toxins in the miniaturized dialyzers based on previous knowledge of solute removal in dialyzers [45,132]. The Sieving Coefficient (SC) of middle (B2M and vitamin B\(_{12}\)) and large (albumin) solutes were also evaluated in the miniaturized dialyzers.
2.3.4.3.1 Evaluation of Clearance of Small Molecular Weight Uremic Toxins

Blood-side clearance of small molecular weight solutes, including urea and creatinine was investigated at each ultrafiltration rate as shown in Table 2-3. Immediately after each ultrafiltration session, whole blood samples were collected from the inlet and outlet of blood lines in designated vacutainer tubes.

The concentration of urea was analyzed by enzymatic photometric assay using Roche Cobas 702 (Roche Diagnostics, Basal, Switzerland) in the LHSC core lab. In this setting, urea is hydrolyzed by urease to form ammonium and carbonate. The accumulated ammonium then reacts with the coenzyme Nicotinamide Adenine Dinucleotide (NADH) and 2-oxoglutarate facilitated by glutamate dehydrogenase (GLDH) producing L-glutamate, NAD$^+$ and H$_2$O. In this reaction for each mole of urea, two moles of NADH are oxidized to NAD$^+$. To estimate the concentration of urea, the concentration of NADH is measured photometrically with main wavelength of 340 nm (Sub:700nm) (Roche Diagnostics, Basal, Switzerland).

The concentration of creatinine was analyzed by enzymatic assay (standardized against isotope dilution mass spectroscopy) using Roche Cobas 702 (Roche Diagnostics, Basal, Switzerland) in the LHSC core lab as well. In this setting, creatinine is converted to glycine, formaldehyde, and hydrogen peroxide in the presence of creatininase, creatinase, and sarcosine oxidase. The accumulated hydrogen peroxide in this reaction then reacts with 4-aminophenazone and HTIB (2,4,6-triido-3-hydroxybenzoic acid) to form a quinone imine chromogen with the aim of peroxidase. The color intensity of the quinone imine was evaluated for estimation of creatinine concentration with the main wavelength of 546 nm (Sub: 700 nm) (Roche Diagnostics, Basal, Switzerland).

The clearance $K_0$ (ml/min), is a function of the blood inlet $C_{Bi}$, blood outlet $C_{Bo}$ concentrations and blood flow rate $Q_B$ (ml/min). For this evaluation, only the blood side clearance was used due to the low concentration of solutes (out of range) in the dialysate fluid.
\[ K_0 = \frac{c_{Bi} - c_{Bo}}{c_{Bi}} \times Q_B \]  

[2.9]

\[ K_0 A \] for urea also was calculated at corresponding ultrafiltration rate settings using the following standard equation [6,7]:

\[ K_0 A = \frac{1}{1 - \frac{1}{Q_{Bi}} - \frac{1}{Q_{Di}}} \times \ln \left( \frac{1 - K_0}{1 - \frac{K_0}{Q_{Di}} / Q_{Bi}} \right) \]  

[2.10]

where \( Q_{Bi} \) and \( Q_{Di} \) represent blood and dialysate flow rates at the inlet sides respectively. \( K_0 \) is the clearance (blood-side) of urea defined by equation [2.9].

### 2.3.4.3.2 Evaluation of Middle Molecular Weight Clearance

Blood-side clearance of middle molecular weight uremic toxins, including vitamin B\(_{12}\) and B2M, was evaluated at the desired ultrafiltration rates (Table 2-3) using standard equation [2.9] in order to examine the effect of ultrafiltration rate on clearance of middle molecular weight uremic toxins.

The concentration of vitamin B\(_{12}\) was quantified using electrochemiluminescence immunoassay analysis by Roche Cobas e801 (Roche Diagnostics, Basal, Switzerland) in the LHSC core lab. In this setting, vitamin B\(_{12}\) is incubated with biotin which enables measurement of vitamin B\(_{12}\) concentration by a photomultiplier.

The concentration of B2M was analyzed using immunoturbidimetric assay by Roche Cobas 502 (Roche Diagnostics, Basal, Switzerland). Immunoturbidimetric assay makes use of the antigen/antibody agglutination technique.

### 2.3.4.4 Evaluation of Sieving Coefficient (SC)

To investigate the permeability of miniaturized dialyzers to middle and large molecular weight uremic toxins, vitamin B\(_{12}\), B2M, and albumin were selected as surrogate markers in the whole blood. The SC of B2M was further evaluated using plasma passing through the fibers of the miniaturized dialyzers under the same experimental conditions.

Immediately after each ultrafiltration session, whole blood samples were collected from the inlet of the blood chamber in the designated vacutainer tubes. Dialysate samples were
taken from the outlet lines in sterile containers at the end of each ultrafiltration session. The concentrations of vitamin B\textsubscript{12} and B2M was measured in each sample as explained in section 2.3.4.3.2.

The concentration of albumin was evaluated using the colorimetric endpoint technique by Roche Cobas 702 (Roche Diagnostics, Basal, Switzerland). At pH value of 4.1, albumin exhibits a sufficiently cationic characteristics to be able to bind to a blue-green anionic dye called bromocresol green. This enables estimation of the concentration of albumin which is measured photometrically with a main wavelength of 570 nm (Sub 505 nm) (Roche Diagnostics, Basal, Switzerland).

The SC of vitamin B\textsubscript{12}, B2M and albumin were calculated using the following equation [8,9]:

\[
\text{SC} = \frac{C_{Do}}{C_{Bin}}
\]

[2.11]

where \( C_{Do} \) and \( C_{Bin} \) are the concentrations of representative solute in the dialysate outlet and blood inlet, respectively.

2.3.4.5 Evaluation of Sodium Permeability

To assess sodium permeability in the miniaturized dialyzers, plasma was pumped through the dialysis fibers. Using plasma instead of whole blood during hemodialysis excludes the effect of potential red blood cell hemolysis on plasma sodium levels [10]. Prior to the experiment, a sample of prepared dialysate was collected in a sterile container for measurement of pre-dialysis sodium concentration. After each successive ultrafiltration rates, samples of dialysate at the outflow line were collected for measurement of post-dialysis sodium concentrations. The concentration of sodium was analyzed by indirect ion selective using Roche Cobas ISE (Roche Diagnostics, Basal, Switzerland) electrodes in LHSC core lab (LHSC, London Health Sciences Center, Laboratory Test Information Guide, London, Canada).
To evaluate sodium permeability, sodium mass removal was evaluated in the miniaturized dialyzers using the following equations [85]:

\[ M_{D - \text{instantaneous}} = Q_{Di} \times C_{Di_{\text{Na}^+}} - Q_{Do} \times C_{Do_{\text{Na}^+}} \]  \[2.12\]

\[ M_D = M_{D - \text{instantaneous}} \times t_d \]  \[2.13\]

where \( Q_{Di} \) and \( Q_{Do} \) represent the dialysate flow at the inlet and outlet lines, respectively. \( C_{Di_{\text{Na}^+}} \) and \( C_{Do_{\text{Na}^+}} \) are the sodium concentrations at the inlet and outlet lines of the dialyzer, respectively. \( M_{D - \text{instantaneous}} \) represents sodium instantaneous flux difference between the dialysate ports. \( t_d \) represents the duration of dialysis. \( M_D \) is the total sodium mass removal at corresponding ultrafiltration rate settings. It is important to note that sodium mass removal could have been evaluated using Sodium Ionic Flux approach which relies on both diffusive and convective forces [85]. However, for our experiment an extra 90XL prob was required.

2.3.5 Data Analysis

Statistical analysis was performed using SPSS Statistics, Version 28.0.1.1 (14) (IBM, SPSS Inc, Chicago). The generation of graphs was performed using the Microsoft Office Excel, Version 16.54 (Microsoft, Washington). Regression analysis was used to evaluate the relationship between parameters. The Shapiro-Wilk test was performed to examine if the data distribution was normal. Leven’s test was also performed to test for equality of variance. Non-parametric tests were used where assumptions of equivalent parametric tests (which includes normality and equality of variance) were violated. To evaluate comparison between parameters, \( p \)-value<0.05 was considered statistically significant.
2.4 Results

This section provides key findings for evaluation of internal filtration, including the effect of forward-filtration and back-filtration profile on the ultrafiltration rate in miniaturized MCO dialyzers. The findings indicated the presence of forward-filtration in the proximal end and back-filtration in the distal end of dialyzers which directly reflects the net ultrafiltration rate measured gravimetrically. The evaluation of water permeability shows that ultrafiltration rate increases linearly with transmembrane pressure and low ultrafiltration rates in miniaturized dialyzers. We also found that the ultrafiltration coefficient of miniaturized MCO dialyzers is significantly different from that of miniaturized conventional HF dialyzers. The evaluation of the clearance of small molecular weight uremic toxins revealed that there are no significant differences between miniaturized dialyzers with respect to the clearance of urea and creatinine (except for ultrafiltration rate of 0.10 ml/min). We also found that the clearance of urea and creatinine is not significantly affected by ultrafiltration rate. The evaluation of the clearance of vitamin B$_{12}$ showed no significant differences between miniaturized MCO and conventional HF dialyzers. Also, the clearance of vitamin B$_{12}$ became negative with increasing ultrafiltration rate. The findings showed that there are statically significant differences between miniaturized MCO and conventional HF dialyzers with respect to the clearance of B2M at lower ultrafiltration rates (0 ml/min and 0.10 ml/min). We also found that the SC of B2M (using plasma) is enhanced with an increasing ultrafiltration rate in miniaturized MCO dialyzers while the SC of B2M is zero in all miniaturized HF dialyzers. The evaluation of SC using whole blood revealed that the SC of B2M is enhanced with increasing ultrafiltration rate in miniaturized MCO dialyzers, but the SC of B2M is zero at higher ultrafiltration rate of 0.20 ml/min. We found that the SC of albumin and vitamin B$_{12}$ are zero in both miniaturized MCO and conventional HF dialyzers. The findings demonstrate that there are no significant differences between miniaturized MCO and conventional HF dialyzers with respect to the sodium mass removal with increasing ultrafiltration rate.
2.4.1 Evaluation of Internal Filtration

Table 2-4 shows time-averaged transmembrane pressures and gravimetric ultrafiltration rate findings in the miniaturized MCO dialyzers. The positive transmembrane pressure in the proximal end (relative to blood-inlet) implies that the higher pressure in the blood chamber creates a pressure gradient that causes forward-filtration from blood side to the dialysate chamber of the dialyzers. Likewise, the negative transmembrane pressure in the distal end implies that higher pressure in the dialysate chamber creates a pressure gradient that causes back-filtration from the dialysate chamber to the blood side of the dialyzer. The data suggests that net transmembrane pressure directly affects the ultrafiltration rate measured in the miniaturized dialyzers. The positive net transmembrane pressure in dialyzer 2 (Figure 2-3) indicates that there is a net forward filtration that increases the ultrafiltration rate. In contrast, the negative net transmembrane pressure in dialyzer 1 (Figure 2-2) and dialyzer 3 (Figure 2-4) implies there is a net back-filtration, decreasing the ultrafiltration rate.

**Table 2-4: Mean transmembrane pressure TMP (mmHg) and ultrafiltration rate Q_{UF} (ml/min) measurements in miniaturized MCO dialyzers.**

<table>
<thead>
<tr>
<th>Dialyzer</th>
<th>TMP_{Proximal} (mmHg)</th>
<th>TMP_{Distal} (mmHg)</th>
<th>TMP_{Net} (mmHg)</th>
<th>Q_{UF} (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.5±2.4</td>
<td>−17.3±2.4</td>
<td>−9.8±4.7</td>
<td>−0.4</td>
</tr>
<tr>
<td>2</td>
<td>14.9±1.1</td>
<td>−6.9±0.8</td>
<td>8.0±1.4</td>
<td>0.29</td>
</tr>
<tr>
<td>3</td>
<td>16.2±1.6</td>
<td>−18.0±1.3</td>
<td>−1.8±2.6</td>
<td>−0.29</td>
</tr>
</tbody>
</table>
Figure 2–2: Forward-filtration in the proximal section and back-filtration in the distal section of the miniaturized MCO dialyzer (1)

Figure 2–3: Forward-filtration in the proximal section and back-filtration in the distal section of the miniaturized MCO dialyzer (2)

Figure 2–4: Forward-filtration in the proximal section and back-filtration in the distal section of the miniaturized MCO dialyzer (3)
2.4.2 Evaluation of Water Permeability

Figure 2-5 and Figure 2-6 illustrate the ultrafiltration rate (ml/min) as a function of transmembrane pressure (mmHg) in miniaturized MCO and conventional HF dialyzers respectively. The plots suggest that the ultrafiltration rate $Q_{UF}$ increases linearly with transmembrane pressure $TMP$ at low ultrafiltration rates. The plots indicate that ultrafiltration coefficient $K_{UF}$ (slope of $Q_{UF} - TMP$ plot) is constant at low ultrafiltration rates between 0 ml/min and 0.1 ml/min. Accordingly, ultrafiltration coefficient $K_{UF}$ of miniaturized MCO and conventional HF dialyzers was compared between ultrafiltration rates of 0 ml/min and 0.1 ml/min. The Mann Whitney U test revealed that the ultrafiltration coefficient $K_{UF}$ of miniaturized MCO dialyzers (Median 0.44; IQR 0.09; N 6) is significantly higher compared to miniaturized conventional HF dialyzers (Median 0.20; IQR 0.05; N 6) as shown in Figure 2-7 ($U = 0; p<0.05$).

Figure 2–5: Ultrafiltration rate $Q_{UF}$ – Transmembrane Pressure TMP plot for the miniaturized MCO dialyzers. Each data point represents the mean of ultrafiltration rates (measured gravimetrically) and transmembrane pressures measured in miniaturized MCO dialyzers at corresponding ultrafiltration rate settings. Error bars represent the standard deviation values for each data point.
Figure 2–6: Ultrafiltration rate $Q_{UF}$ – Transmembrane Pressure (TMP) plot for miniaturized conventional HF dialyzers. Each data point represents the mean of ultrafiltration rates (measured gravimetrically) and transmembrane pressures measured in miniaturized conventional HF dialyzers at corresponding ultrafiltration rate settings. Error bars represent the standard deviation values for each data point.
Figure 2–7: Ultrafiltration coefficient $K_{UF}$ at ultrafiltration rate range of 0 ml/min and 0.1 ml/min in miniaturized MCO (Mean 0.41; Median 0.44; IQR 0.09; N 6) and conventional HF (Mean 0.18; Median 0.20; IQR 0.05; N 6) dialyzers. $\times$ denotes the mean of ultrafiltration coefficient $K_{UF}$. * denotes $p<0.05$ for comparison between $K_{UF}$ of miniaturized MCO and conventional HF.
2.4.3 Clearance of Small Molecular Weight Uremic Toxins

The analysis of data from Kruskal Wallis test showed no significant differences between miniaturized MCO and conventional HF dialyzers with respect to the clearance of urea. Also, the data suggests that urea clearance is not significantly affected by the ultrafiltration rate as shown in Figure 2-8.

![Figure 2-8: Clearance of urea at ultrafiltration rates of 0 ml/min (MCO:Mean 0.33, Median 0.30,IQR 0.12,N 6;Conventional HF: Mean 0.31,Median 0.33,IQR 0.04,N 6), 0.10 ml/min (MCO: Mean 0.28,Median 0.28,IQR 0.22,N 6;Conventional HF: Mean 0.32,Median 0.33,IQR 0.04,N 6), 0.17 ml/min (MCO: Mean 0.17,Median 0.08,IQR 0.24, N 6;Conventional HF: Mean 0.30,Median 0.29,IQR 0.03,N 6) and 0.20 ml/min (MCO: Mean 0.28,Median 0.28,IQR 0.13,N 6;Conventional HF: Mean 0.32,Median 0.33,IQR 0.02,N 6) in miniaturized dialyzers. × denotes the mean of urea clearance at corresponding ultrafiltration rates]
The analysis of data from Kruskal Wallis showed no statistically significant differences between Mass Transfer Area Coefficient $K_0A$ of miniaturized MCO and conventional HF dialyzers. The evaluations suggest that Mass Transfer Area Coefficient $K_0A$ for urea is not significantly affected by ultrafiltration rate (Figure 2-9).

**Figure 2–9**: Evaluation of Mass Transfer Area Coefficient $K_0A$ for urea at ultrafiltration rates of 0 ml/min (MCO: Mean 0.67, Median 0.52, IQR 0.45, N 6; Conventional HF: Mean 0.56, Median 0.61, IQR 0.12, N 6), 0.10 ml/min (MCO: Mean 0.58, Median 0.52, IQR 0.59, N 6; Conventional HF: Mean 0.58, Median 0.62, IQR 0.13, N 6), 0.17 ml/min (MCO: Mean 0.31, Median 0.09, IQR 0.46, N 6; Conventional HF: Mean 0.52, Median 0.47, IQR 0.08, N 6) and 0.20 ml/min (MCO: Mean 0.57, Median 0.57, IQR 0.37, N 6; Conventional HF: Mean 0.61, Median 0.61, IQR 0.07, N 6) in miniaturized dialyzers. × denotes the mean of $K_0A$ for urea clearance at corresponding ultrafiltration rates.
The analysis of data from Kruskal Wallis test revealed significant differences between miniaturized MCO and conventional HF dialyzers ($\chi^2 = 5.041; p$-value <0.05). For further exploration, the analysis of data from the Mann Whitney U output showed significant differences between the clearance of creatinine in miniaturized MCO (Median 0.20; IQR 0.12; n 6) and conventional HF (Median 0.35; IQR 0.03; n 6) dialyzers at ultrafiltration rate of 0.10 ml/min as shown in Figure 2-10 (U 4.0; $p$-value <0.05). Also, the data suggests that clearance of creatinine is not significantly affected by the ultrafiltration rate.

![Creatinine](image)

**Figure 2–10:** Clearance of creatinine at ultrafiltration rates of 0 ml/min (MCO: Mean 0.30, Median 0.32,IQR 0.10,N 6;Conventional HF: Mean 0.33,Median 0.32,IQR 0.06,N 6) 0.10 ml/min (MCO: Mean 0.27,Median 0.27,IQR 0.06,N 6;Conventional HF: Mean 0.34,Median 0.34,IQR 0.02,N 6), 0.17 ml/min (MCO: Mean 0.29,Median 0.29,IQR 0.12,N 6;Conventional HF: Mean 0.34,Median 0.35,IQR 0.02,N 6)and 0.20 ml/min (MCO: Mean 0.27,Median 0.27,IQR 0.12,N 6;Conventional HF: Mean 0.35,Median 0.35,IQR 0.02,N 6) in miniaturized dialyzers. * denotes $p$<0.05 for comparison between clearance of creatinine in MCO and conventional HF dialyzers at ultrafiltration rate of 0.10 ml/min. × denotes the mean of creatinine clearance at corresponding ultrafiltration rates.

* denotes $p$<0.05 for comparison between clearance of creatinine in MCO and conventional HF dialyzers at ultrafiltration rate of 0.10 ml/min.
2.4.4 Clearance of Middle Molecular Weight Uremic Toxins

The analysis of data from the Kruskal Wallis test showed no significant difference between miniaturized MCO and conventional HF dialyzers with regard to vitamin B\textsubscript{12} clearance. Additionally, the clearance of vitamin B\textsubscript{12} is negative with increasing ultrafiltration rate as shown in Figure 2-11.

Figure 2–11: Clearance of vitamin B\textsubscript{12} in miniaturized MCO and conventional HF dialyzers at ultrafiltration rates at ultrafiltration rates of 0 ml/min (MCO: Mean 0.08, Median 0.08, IQR 0.03, N 6; Conventional HF: Mean 0.06, Median 0.05, IQR 0.02, N 6), 0.10 ml/min (MCO: Mean -0.05, Median -0.06, IQR 0.04, N 6; Conventional HF: Mean -0.02, Median -0.02, IQR 0.06, N 6), 0.17 ml/min (MCO: Mean -0.17, Median -0.15, IQR 0.08, N 6; Conventional HF: Mean -0.14, Median -0.15, IQR 0.12, N 6) and 0.20 ml/min (MCO: Mean -0.19, Median -0.19, IQR 0.03, N 6; Conventional HF: Mean -0.14, Median -0.16, IQR 0.14, N 6) in miniaturized dialyzers. × denotes the mean of vitamin B\textsubscript{12} clearance at corresponding ultrafiltration rates.
The analysis of data from Kruskal Wallis test revealed significant differences between miniaturized MCO and conventional HF dialyzers ($\chi^2=5.041; p$-value <0.05). For further analysis, data from the Mann Whitney U test showed significant differences between clearance of B2M in miniaturized MCO (Median 0.37; IQR 0.36; N 6), and conventional HF (Median 0.13; IQR 0.03; N 6) at ultrafiltration rate of 0 ml/min. The data also suggests significant differences between clearance of B2M in miniaturized MCO (Median 0.30; IQR 0.35; N 6) and conventional HF (Median 0.08; IQR 0.03, N 6) at ultrafiltration rate of 0.10 ml/min. The data suggests that clearance of B2M is not significantly affected by ultrafiltration rate.

![Figure 2–12: Clearance of B2M in miniaturized MCO and conventional HF dialyzers at ultrafiltration rates at ultrafiltration rates of 0 ml/min (MCO: Mean 0.51, Median 0.37, IQR 0.36, N 6; Conventional HF: Mean 0.14, Median 0.13, IQR 0.03, N 6), 0.10 ml/min (MCO: Mean 0.46, Median 0.30, IQR 0.35, N 6; Conventional HF: Mean 0.08, Median 0.08, IQR 0.03, N 6), 0.17 ml/min (MCO: Mean 0.45, Median 0.27, IQR 0.45, N 6; Conventional HF: Mean 0.03, Median 0.02, IQR 0.04, N 6) and 0.20 ml/min (MCO: Mean 0.42, Median 0.42, IQR 0.27, N 6; Conventional HF: Mean 0.03, Median 0.02, IQR 0.02, N 6) in miniaturized dialyzers. * denotes $p$<0.05 for comparison between clearance of B2M in MCO and conventional HF dialyzers at ultrafiltration rates of 0 ml/min and 0.10 ml/min. × denotes the mean of B2M clearance at corresponding ultrafiltration rates.]
2.4.5 Evaluation of Sieving Coefficient (SC)

The evaluation of the SC using plasma suggests that the SC of B2M is enhanced with an increasing ultrafiltration rate in miniaturized MCO dialyzers (Figure 2-13). However, the analysis using whole blood, indicates that the SC of B2M is zero at ultrafiltration rate of 0.20 ml/min (Figure 2-14). The SC of B2M is zero in miniaturized HF dialyzers using whole blood and plasma. Also, the SC of vitamin B₁₂ and albumin are zero in both miniaturized MCO and conventional HF dialyzers.

![Figure 2–13: Evaluation of B2M Sieving Coefficient (SC) using plasma in miniaturized MCO dialyzers. Each data point represents the mean of Sieving Coefficient (SC) evaluated in miniaturized MCO dialyzers at corresponding ultrafiltration rates. Error bars represent the standard deviation values for each data point](image-url)
Figure 2–14: Evaluation of B2M Sieving Coefficient (SC) using whole blood in miniaturized MCO dialyzers. Each data point represents the mean of Sieving Coefficient (SC) evaluated in miniaturized MCO dialyzers at corresponding ultrafiltration rates. Error bars represent the standard deviation values for each data point.
2.4.6 Evaluation of Sodium Permeability

The analysis of the Kruskal-Wallis test showed no significant differences between the miniaturized MCO and conventional HF dialyzers with respect to absolute sodium mass removal across all ultrafiltration rates as shown in Figure 2-15.

**Figure 2–15:** Absolute sodium mass removal in miniaturized MCO and conventional HF dialyzers at ultrafiltration rates of 0 ml/min (MCO: Mean 27.0, Median 30.0,IQR 30.0,N 5;Conventional HF: Mean 45.0,Median 45.0,IQR 0.0,N 5), 0.10 ml/min (MCO: Mean 312.0,Median 339.0,IQR 48.0,N 5;Conventional HF: Mean 266.4,Median 276.0,IQR 48.0,N 5), 0.17 ml/min (MCO: Mean 489.9,Median 515.0,IQR 80.1,N 5;Conventional HF: Mean 401.5,Median 393.4,IQR 42.0,N 5) and 0.20 ml/min (MCO: Mean 557.0,Median 582.0,IQR 49.5,N 5;Conventional HF: Mean 486.0 ,Median 492.0,IQR 45.0,N 5) in miniaturized dialyzers. × denotes the mean of absolute sodium mass removal at corresponding ultrafiltration rates.
2.5 Discussion

The aim of present study was to investigate whether the novel miniaturized dialyzers can differentiate performance characteristics representing hemodialysis membranes and fiber design. To assess this aim, we evaluated internal filtration, water permeability and solute transport in the novel miniaturized dialyzers with MCO and conventional HF membranes.

In this study, the evaluation of internal filtration findings showed that there is a forward-filtration in the proximal portion, and back-filtration in the distal portion (relative to blood-inlet) of miniaturized MCO dialyzers which directly reflects the net ultrafiltration rate. This demonstrates that miniaturized MCO dialyzers mimic the behavior of a typical dialyzer in terms of internal hemodynamic characteristics [50,161,162].

We also found that the ultrafiltration rate increases linearly with increasing transmembrane pressure at low ultrafiltration rates. This suggests that the ultrafiltration coefficient $K_{UF}$ of miniaturized dialyzers is constant at low ultrafiltration rates. This finding is consistent with preexisting literature on the evaluation of water permeability in clinical dialyzers [156,157,165]. We also found that there was a statistically significant difference between the ultrafiltration coefficient $K_{UF}$ of miniaturized MCO and conventional HF dialyzers. This finding reflects the differences between physical characteristics of MCO and conventional HF membranes. MCO membranes have relatively higher number and larger pores compared to conventional HF membranes [159,160].

We found no significant differences between miniaturized MCO and conventional HF dialyzers with regard to the clearance of urea. The findings in our study also suggest that the clearance of urea is not significantly affected by the ultrafiltration rate. Given that the membrane thickness and the surface area of miniaturized dialyzers are the same, the clearance of urea is not expected to be significantly different in miniaturized MCO and conventional HF dialyzers. This is in line with previous knowledge about clearance of the small molecular weight uremic toxins. Urea is primarily removed by diffusion which mainly depends on the membrane thickness, surface area of the dialyzer and concentration gradient across the hemodialysis membranes [132]. The findings of
previous clinical studies confirm that MCO and conventional HF dialyzers have equal performance in terms of clearance of urea [132,133, 134, 137]. As part of an observational prospective study, the reduction ratio of urea was evaluated in patients who were dialyzed with a PSF-based conventional HF (Rexeed-21A) and MCO (Theranova 400) dialyzers. The findings of this study showed no significant differences between the two groups [163]. Similarly, the study of Cho et al. [135] found no significant difference between clearance of urea in MCO (Theranova 400) and conventional HF dialyzers (FX Cor Diax 80).

The findings of our study revealed significant differences between miniaturized MCO and conventional HF dialyzers with relation to the clearance of creatinine only at an ultrafiltration rate of 0.10 ml/min. Previous studies have shown that there are no significant differences between the clearance of creatinine in conventional HF dialyzers and MCO dialyzers [164]. However, the study of Kirsch et al. indicated significant removal of creatinine in MCO dialyzers compared to HF dialyzers (FX CorDiax 80) [136]. The findings of our study showed that the clearance of creatinine is not significantly affected by the ultrafiltration rate in miniaturized dialyzers. Similar to urea, creatinine is classified as a small solute which is primarily removed by diffusive transport [45,132].

The results of our study showed no significant results between the clearance of vitamin B_{12} in miniaturized MCO and conventional HF dialyzers. Notably, the data demonstrated that the clearance of vitamin B_{12} is negative at higher ultrafiltration rates. This is indicative of hemoconcentration of vitamin B_{12} at higher ultrafiltration rates due to the impermeability of vitamin B_{12} in the miniaturized dialyzers. The evaluations also showed that SC of vitamin B_{12} was zero in both miniaturized MCO and conventional HF dialyzers. This is also indicative of the impermeability of vitamin B_{12} in both miniaturized MCO and conventional HF dialyzers. Previous studies evaluating clinical dialyzers showed that there are no significant differences between clearance of vitamin B_{12} MCO and conventional HF (CorDiax 80) dialyzers [135]. In the past, the removal of vitamin B_{12} used to be evaluated as a measure of dialyzer performance. However, vitamin B_{12} tends to bind to plasma proteins that might be impermeable to hemodialysis
membranes [120]. Consequently, evaluation of vitamin B\textsubscript{12} removal is not an ideal measure of the performance of dialyzers in removal of middle molecular weight uremic toxins.

The findings of our study demonstrate that there are statistically significant differences in clearance of B2M between miniaturized MCO and conventional HF dialyzers at low ultrafiltration rates of 0 ml/min and 0.10 ml/min, whereas the results were not statistically significant at higher ultrafiltration rates of 0.17ml/min and 0.20ml/min. The outcomes also showed that clearance of B2M is not significantly affected by ultrafiltration rate.

The assessment of the SC of B2M in plasma suggests that increasing ultrafiltration rate enhances the removal of B2M in miniaturized MCO dialyzers. However, the evaluation of the SC of B2M in whole blood indicates that miniaturized MCO dialyzers are impermeable to B2M at the higher ultrafiltration rate of 0.20ml/min. This might be due to an increase in the concentration of red blood cells and impermeable molecules at higher ultrafiltration rates. High concentrations of red blood cells and impermeable molecules increase the viscosity and oncotic pressure in the dialyzer [71]. Consequently, the internal filtration necessary for removal of B2M is hindered. Also, the SC of B2M in miniaturized conventional HF dialyzers was zero even when the ultrafiltration rate was increased. These findings are consistent with pre-existing knowledge about differences between MCO and conventional HF membranes. The higher SC of B2M in miniaturized MCO dialyzers reflects the superiority of MCO membranes in the removal of middle molecular weight uremic toxins. MCO membranes have a relatively higher number, and larger pores compared to conventional HF fibers, which improves removal of middle molecular weight uremic toxins [124]. In addition, the smaller internal diameter of MCO fibers compared to that of conventional HF dialyzers results in reduction of the axial pressure gradient. This in turn enhances internal forward filtration, and convective transport of middle molecular weight uremic toxins such as B2M [166,167]. Previous studies in clinical dialyzers showed significantly higher removal of B2M compared to conventional HF dialyzers [132,133,134,135,164]. We found the SC of albumin is zero in both miniaturized MCO and conventional HF dialyzers indicating impermeability of miniaturized dialyzers to albumin.
The results of sodium mass removal in our study suggests that there are no significant differences between miniaturized MCO and conventional HF dialyzers with increasing ultrafiltration rate. To our knowledge, this is the first study comparing sodium permeability of MCO membranes and conventional HF membranes in-vitro. The characteristics of membranes used in the dialyzer, such as surface charge density may influence sodium removal [101]. There are several other factors that should be taken into consideration when evaluating diffusive and convective removal of sodium. Sodium tends to bind to negatively charged plasma proteins that might be impermeable to the hemodialysis membranes (Gibbs–Donnan effect) [100,111]. Further robust experiments are required to conclusively evaluate the effect of membrane characteristics such as porosity and membrane diameter on sodium removal in the dialyzers.

We found that miniaturized MCO dialyzers have significantly different water permeability and a higher SC of B2M compared to miniaturized conventional HF dialyzers. This reflects the differences between characteristics of MCO (i.e., higher porosity, reduced membrane diameter) and conventional HF membranes. We also found no significant differences between miniaturized MCO and conventional HF dialyzers with regard to small molecular weight uremic toxins, with the exception of clearance of creatinine at ultrafiltration rate of 0.10 ml/min. The main findings strongly suggest that the performance of miniaturized dialyzers can be directly related to hemodialysis membrane and fiber design. This indicates that the miniaturized dialyzers are an appropriate component of a pre-clinical pipeline of hemodialysis impact. This is remarkable since we can expedite the investigation of health outcomes associated with hemodialysis using a miniaturized version of clinical hemodialyzers in a small rodent model of hemodialysis.
2.5.1 Limitations

This study had several limitations. For the evaluation of internal filtration, the measurement of pressure at the blood inlet of the miniaturized dialyzer was not feasible using whole blood due to blood entry into the pressure meter diaphragms. That is why we were limited to use dialysate fluid to be able to measure pressure at all ports of the miniaturized dialyzer.

It is noteworthy that the ultrafiltration coefficient $K_{UF}$ is typically evaluated in an isolated (with no dialysate flow) circuit [157]. However, this setting could not be implemented for the evaluation of the ultrafiltration coefficient $K_{UF}$ in the miniaturized dialyzers presumably due to the packing density of membranes and the design of the dialyzer housing.

The miniaturized dialyzers provide limited surface area for mass transport across the membranes. Consequently, the concentration of solutes in the dialysate is relatively miniscule. In evaluation of the clearance of small and middle molecular weight uremic toxins, we only accounted for the clearance of solutes in the blood side. However, the true clearance is the average of both blood-side and dialysate-side clearances [170]. Also, in evaluation of the SC, we were unable to detect any vitamin $B_{12}$ and albumin in the dialysate. Perhaps more advanced measurement techniques (e.g., nuclear imaging techniques) are required to enable measurement of low concentration of larger and relatively impermeable solutes in the dialysate fluid. The evaluation of sodium removal revealed no significant differences between MCO and conventional HF dialyzers. However, further research is required to account for other factors involved such as membrane charge density affecting sodium permeability of the dialyzers [101]. Furthermore, more experiments are required to enhance the validity of our results.
Chapter 3

3 Conclusions and Future Work

3.1 Summary

This thesis presents the evaluation of MCO and conventional HF hemodialysis membranes performance in miniaturized rat dialyzers. We found that the water permeability of miniaturized MCO and conventional dialyzers are significantly different, reflecting a reduced membrane diameter and higher porosity of MCO membranes. We also found that the SC of B2M in miniaturized MCO dialyzers is higher than miniaturized HF dialyzers, suggesting MCO membranes have better permeability to middle molecular weight uremic toxins, which also reflects relatively higher porosity of MCO membranes. The main findings also showed no significant differences between the clearance of small molecular weight uremic toxins and sodium mass removal in miniaturized MCO and conventional HF dialyzers. The main findings in this study strongly suggest that the miniaturized dialyzers can effectively differentiate performance characteristics representing hemodialysis membrane and fiber design.

3.1.1 Qualification of Internal Filtration in Hollow Fiber Miniaturized Dialyzers using Albumin Macro-aggregated Labeled with 99Tc Metastable

Given the importance of internal filtration in miniaturized dialyzers, it is essential to empirically quantify the forward-filtration and back-filtration in the novel miniaturized dialyzers. Nuclear imaging techniques could be used to evaluate local cross filtration at different points along the length of miniaturized dialyzers in an in-vitro setting [162]. A non-diffusible marker molecule such as albumin macroaggregates labeled with 99Tc metastable is injected in the blood compartment and a gamma camera is used to record nuclear emission. To calculate local cross filtration, relative variations in the concentration of the marker molecule along the length of the filter is used. The increase in concentration of the marker molecule describes forward-filtration, whereas the decrease in concentration of the marker molecule describes back-filtration. The forward-
filtration (FF) and backward-filtration (BF) could be calculated using following equations:

\[ \text{FF} = Q_B \times (1 - \text{Hct}) \times \left(1 - \frac{C_{in}}{C_{max}}\right) \]  

\[ \text{BF} = Q_B \times (1 - \text{Hct}) \times \left(1 - \frac{C_{out}}{C_{max}}\right) \]

where \( Q_B \) is the blood flow rate, Hct is the hematocrit, \( C_{in} \) is the count of the marker molecule at the inlet of the dialyzer, \( C_{out} \) is the count of the marker molecule at the outlet of the dialyzer and \( C_{max} \) is the peak count of the marker molecule along the length of the dialyzer.

### 3.1.2 Intravital Video Microscopy (IVM) Observation of Microvasculature During Hemodialysis in Chronic Kidney Disease (CKD) Rats

Microvascular dysfunction is frequently found in hemodialysis patients resulting in inadequate blood flow to vital organs, ultimately leading to irreversible tissue damage and multiple organ failure. This warrants an investigation of microvascular dysfunction in relation to hemodialysis. Our team is planning to use a combination of IVM with hemodialysis in CKD rats. To proceed, rats may undergo nephrectomy procedure [39,150,154]. Alternatively, an addition of an adenine-based diet reliably could reliably induce kidney dysfunction in rats [152,174,175]. An assessment of GFR could be used to evaluate the extent of residual kidney function. Subsequently, CKD rats could undergo hemodialysis therapy while IVM is used for the evaluation of microvascular perfusion. This would allow us to directly investigate the effect of different membrane materials in the development of microvascular dysfunction.

### 3.1.3 IVM Observation of Microvasculature During Hemodialysis in Septic Rats

End-Stage Renal Disease (ESRD) patients are very prone to sepsis and bacterial infections increasing the mortality rate in this population [176,177,178]. Sepsis is associated with release of interleukins [179], microvascular dysfunction and eventual
organ failure. The effectiveness of dialyzer membranes in removal of interleukins could be evaluated in miniaturized dialyzers. Concurrently, IVM could be utilized for evaluation of microcirculation during hemodialysis in septic rat models. Also, the effectiveness of numerous anticoagulants could be evaluated.

3.1.4 Evaluation of Uremic Toxin Removal in Miniaturized MCO and Polynephron Dialyzers

Polynephron is relatively new generation of hemodialysis membranes with the inner diameter of 0.200 mm and membrane thickness of 40 μm (Polynephron, Nipro Medical Co, USA). The inner diameter of polynephron membranes is greater than that of MCO membranes (0.180 mm). Hence, the internal filtration is expected to be relatively lower in polynephone dialyzers. However, the thickness of polynephon membranes is greater in polynephrons compared to MCO membranes (35 μm). Consequently, the diffusive removal of uremic toxins is expected to be rather greater in MCO membranes. Our team is planning to compare uremic toxin removal in miniaturized polynephron and MCO dialyzers in the same experimental setup developed in this study.
3.2 References


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