High flux mixed matrix membrane with low albumin leakage for blood plasma detoxification

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CURRENT HEMODIALYSIS (HD) THERAPY REMOVES WELL SMALL SIZED TOXINS BUT REMOVES LESS EFFECTIVELY MIDDLE MOLECULES AND PROTEIN-BOUND UREMIC TOXINS (PBUTs). THIS LIMITED REMOVAL HAS BEEN ASSOCIATED TO HIGH MORTALITY OF PATIENTS DUE TO INCREASED CARDIOVASCULAR EVENTS. THE HEMODIAFILTRATION TREATMENT, WHICH COMBINES DIFFUSIVE AND CONVECTIVE TRANSPORT USING HIGH FLUX MEMBRANES, CAN ACHIEVE HIGHER REMOVAL OF MIDDLE MOLECULES. HOWEVER, STILL THE REMOVAL OF PBUT IS LIMITED. OUR EARLIER STUDIES SHOWED THAT COMBINATION OF FILTRATION AND ADSORPTION ON ONE HOLLOW FIBER MEMBRANE, SO CALLED MIXED MATRIX MEMBRANES (MMMs) CAN ACHIEVE REMOVAL OF RANGE OF TOXINS, INCLUDING PBUTs, HOWEVER, THESE MMMs EITHER HAD LOW FLUX AND THEREFORE WERE NOT SUITABLE FOR CONVECTIVE THERAPIES OR HAD HIGH FLUX BUT ALSO ALBUMIN LEAKAGE WHICH IS UNDESIRABLE FOR THE HD THERAPIES. IN THIS WORK, WE PRESENT FOR THE FIRST TIME A NEW GENERATION OF MMM WHICH COMBINES HIGH WATER FLUX WITH HIGH ALBUMIN RETENTION AND VERY LOW PROTEIN ADSORPTION AND PROTEIN LEAKAGE. THE MEMBRANE FABRICATION CONDITIONS ARE OPTIMIZED FOR ACHIEVING FIBERS WITH SMALL DIAMETER AND OPTIMAL DISTRIBUTION OF THE SORBENT PARTICLES LEADING TO SUPERIOR REMOVAL OF PBUT FROM HUMAN PLASMA, IN COMPARISON TO THE PREVIOUS MMMs, AS WELL AS, TO SERIES OF COMMERCIAL MEMBRANES USED CURRENTLY IN THE CLINIC.

1. Introduction

Since the first successful renal replacement therapy, developed by Kolff in 1940s [1–3], hemodialysis (HD) is now considered as the most successful therapy for prolonging the life of patients with end-stage renal disease (ESRD). A key element of the therapy is an artificial kidney, a membrane module containing thousands of hollow fibers for filtration and removal of uremic toxins. The European Uremic Toxin Work Group (EUTox) of the European Society for Artificial Organs (ESAO) suggests that the artificial kidney should remove a range of sizes of uremic toxins [4]: small sized and water soluble [(Mw < 500 g mol⁻¹), such as urea (60 g mol⁻¹), creatinine (113 g mol⁻¹)], middle sized [(Mw > 500 g mol⁻¹, such as β₂-microglobulin (11 800 g mol⁻¹), parathyroid hormone (9225 g mol⁻¹)]; and protein-bound uremic toxins [PBUTs, such as indoxyl sulfate (IS, 251 g mol⁻¹), p-cresol sulfate (pCS, 188 g mol⁻¹) hippuric acid (HA, 179 g mol⁻¹) etc. For the PBUTs, it is also important to consider the percentage of their binding to albumin, for example: IS is bound to protein >93% whereas pCS and HA are bound to protein >95% and >39%, respectively [4–7]. Most HD membranes have almost similar or even better performance on the clearance of small-sized toxins compared to the healthy kidney. However, they have poor performance on removing the middle sized uremic toxins and the PBUTs [8]. The latter are associated to increased risk of cardiovascular events and mortality [9–11] and therefore should be removed. Besides, as healthy kidney processes the blood for 24 h, more frequent and prolonged blood purification treatment can achieve better removal of the middle-sized and PBUTs, too. Therefore, new membranes and systems for dialysis at home (for example nocturnal hemodialysis [12]) and for portable/wearable artificial kidney [13] are required. There, the membrane modules size should be minimized and the toxin removal should be significantly improved.

Earlier studies of our group showed that mixed matrix membranes (MMMs) can effectively remove PBUTs [14–16]. The MMM is a dual layer hollow fiber membrane consisting of an inner selective layer composed of polyethersulfone (PES) and polyvinylpyrrolidone (PVP) blend and an outer layer composed of activated carbon (AC) microparticles embedded in PES/PVP matrix [14,15,17]. Based on modelling studies, the adsorption of the toxins to the sorbent particles lowers the toxin concentration in the MMM and therefore leads to higher concentration gradient across the membrane and to higher toxin removal [18].

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The efficiency of the toxin adsorption removal can be tuned by applying different types of sorbents.

The first generation MMM developed in our lab (marked as MMM1 in the present study) was a high flux membrane (Ultrafiltration coefficient, $K_{UF}$) but had albumin leakage, which is undesirable in the clinic [14]. The second generation MMM (marked as MMM2 in this present study) [15] was a low flux membrane ($K_{UF}$ = 3.4 ml h⁻¹ m⁻² mmHg⁻¹) and had better PBUT removal and no albumin leakage. As many evidence in regard to the higher toxin removal by high flux HD membranes are available [19–21], the low flux MMM may reduce the effect of the convective solute transport during HD and eventually limit the PBUT toxin removal. Therefore, in this study, we focus on developing a new high flux MMM with no/or very low albumin leakage. Moreover, we optimize the fiber fabrication protocols (such as spinneret dimensions, flow rate of dope and bore solutions, air-gap, and take-up speed) for minimizing the fiber dimensions and for improving the removal of PBUTs. The morphology of new membranes is characterized and their transport properties are investigated using pure water, model solutions of PBUTs and human plasma spiked with PBUTs, and human plasma (Sanquin, Deventer, The Netherlands) were applied for the toxin removal studies. For the transport studies a dialysate solution was used, see composition in the supplement, Table 1S. Three commercial membranes, Fresenius, F8HPS dialyzer, indicated as COM1; Fresenius, FX100 dialyzer indicated as COM2; and Baxter, Polylux 2H dialyzer indicated as COM3, were used as controls (all kindly supplied by a research group of Dr. K.G.F. Gerritsen in UMC Utrecht).

### 2.2. Polymer dope solution characterization

The rheology of polymer dope solutions was investigated by using a rheometer (Physica MCR 301, Anton Paar) at shear rate of 1 s⁻¹ at room temperature.

### 2.3. Preparation of dope solutions

For the preparation of the sorbent free dope solution, 15 wt% PES and 7 wt% PVP were dissolved in NMP at room temperature for a day. To obtain the sorbent-based dope solution, AC was sieved with a sieve (0.45 mm) first. Then 60 wt% AC (Norit A suprima) of the total polymer amount was added in the homogeneous dope solution of 14 wt% PES/1.4 wt% PVP/84.6 wt% NMP and dispersed homogenously for 3 days at room temperature. Before the membrane fabrication, all dope solutions were degassed for a day.

### 2.4. Preparation of hollow fiber membranes

To fabricate dual layer hollow fiber MMMs, the polymer dope solutions (sorbent free and with sorbent particles) where placed in stainless steel syringes (diameter: 29 mm and length: 15 cm) and were pumped with certain flow rates (as described in Table 1) through the spinnerets using two syringe pumps. The bore fluid (water) was pumped via an HPLC pump. All solutions were extruded at the same time and the hollow fiber membranes were formed by phase separation into a coagulant bath that contained pure water as a non-solvent. The air gap was also tailored to achieve optimal membrane properties, see Table 1.

For tuning the diameter of hollow fiber MMMs, the following parameters were investigated: the flow rate of outer and inner dope solutions and of the bore fluid; the airgap; the take-up speed and the application of two different spinnerets (one large, indicated here as “L” and one small, indicated here as “S”, see the supporting information, Fig. 1S).

The spinning conditions were selected based on the viscosity of dope solutions combined with the dimensions of the spinnerets and the linear force of pumps (see Table 2S in supporting information). The representative spinning conditions are summarized in Table 1.

### 2.5. Module preparation

All modules were composed of 10 hollow fiber membranes and the initial module length was 8.5–12 cm. Two ends were potted with an epoxy adhesive (Griffon Combi) composed of 2 components, the epoxy resin and hardener. The effective surface area of all membranes was calculated by subtracting the length of two glued ends (approximately 2 cm per a glued end) from the initial module length.

### 2.6. Membrane morphology study

The inner surface membrane morphology was studied by Field
Fig. 1. (a) Effect of the various spinning parameters to the OD and ID of the produced fibers. The details of the spinning protocols are summarized in Table 2. (b) SEM images of the membranes produced under the conditions described in Table 2.
Emission Scanning Electron Microscopy (FE-SEM, JEOL, JSM-7610F) and the membrane cross-section was analyzed using SEM (JEOL, JSM-IT100). For cross-sectional images, the membrane samples were fractured in liquid nitrogen, and then coated with gold by Cressington IT100. For inner surface images, with kind support by European Membrane Institute (EMI) in the University of Twente, we studied the membrane surface roughness and pores using FE-SEM after coating the membrane samples with chromium.

### 2.7. Membrane characterization

The water transport through the membrane at different transmembrane pressures (TMP) up to 5 bars was measured for 1 h using a dead-end filtration set-up. The membrane water permeance \( L m \ m^{-2} h^{-1} \) bar \(^{-1} \) was estimated by the following equation:

\[
\text{Water permeance} = \frac{Q}{A \times \Delta P^{-1}}
\]

where \( Q \) is the water flow through the membrane (L h \(^{-1} \)), \( A \) is the effective membrane area (m\(^2\)), and \( \Delta P \) is the applied transmembrane pressure (bar). Ultrafiltration coefficient \( K_{uf} \) (mL m\(^{-2} \) h\(^{-1} \) mmHg\(^{-1} \)) was converted from results of the water permeance.

For the estimation of membrane pore size, a range of PEGs and PEOs was used with small (Mw 200 g mol\(^{-1} \)), middle (Mw 1500, 6000, and 10 000 g mol\(^{-1} \)), and large (Mw 35 000 and 100 000 g mol\(^{-1} \)) sized molecules. The rejection (R\%) and sieving coefficient (SC) for PEGs (SC\( \text{PEG} \)) and albumin (SC\( \text{albumin} \)) were calculated by the following equations:

\[
R(\%) = \left[ 1 - \frac{C_{\text{perm}}}{C_{\text{feed}}} \right] \times 100(\%)
\]

\[
SC = \frac{C_{\text{perm}}}{C_{\text{feed}}} = \frac{\left[ 100 - R(\%) \right]}{100}
\]

where \( C_{\text{perm}} \) and \( C_{\text{feed}} \) are the concentrations of the permeate and feed solutions, respectively, which were analyzed by UV Spectroscopy (Nanodrop) for albumin and by gel permeation chromatography (GPC) for PEGs with PSS SUPREMA analytical pre-column and columns in water with kind support by EMI–Twente. The membranes’ pore size, pore distribution, and MWCO were estimated based on the results of SC\( \text{albumin} \) and SC\( \text{PEG} \) and the solute Stokes radius. The calculation of the Stokes radius for PEGs and PEO [22,23] are described in Table 3 in supporting information.

### 2.8. Toxin removal

For the study of the PBUTs, indoxyl sulfate (IS, 40 mg L\(^{-1} \)) and hippuric acid (HA, 110 mg L\(^{-1} \)) were spiked in human plasma purchased from Sanquin (Deventer, the Netherlands). The human plasma was then incubated for 4 h at 37 °C under gentle shaking. Before the toxin removal test, the pure water flux test was carried out to ensure the membrane pores are open and wet completely. The toxin removal test was carried out for 4 h by a lab scale dialysis system (Convergence inspector, Enschede, The Netherlands) with the human plasma including the protein bound uremic toxins (IS and HA) and the lab-made dialysate (the composition is listed in Table 1). The initial volumes of the human plasma and of the lab-made dialysate were 50 mL, each. The human plasma and dialysate were recirculated with flow of 1 and 10 mL min\(^{-1} \), respectively. All fluids were freshly prepared for every experiment. The toxin removal set-up was monitored to ensure no transmembrane pressure (TMP), 0 mmHg.

\[
\text{TMP} = \frac{(P2 - P1)}{2} - \frac{(P3 - P4)}{2}
\]

where the pressures of inlet (P1) and outlet (P2) in hollow fiber lumen (human plasma side) and inlet (P3) and outlet (P4) in hollow fiber outer side (dialysate side) were recorded every second by the pressure monitoring system in the HD test set-up.

To observe the changes of the toxin removal, samples of 1 mL (of human plasma and of dialysate) were collected every hour. The collected samples were 4 times diluted with deionized water and deproteinized by heat denaturation at 95 °C for 30 min. Then the samples were cooled down immediately (in ice for 15 min) and were placed on filters (Millipore, Amicon Ultra 0.5 mL centrifugal filters MWCO 10 kg mol\(^{-1} \)) for centrifugation at 14 000 RCF for 15 min. The concentration of IS and HA was measured by high performance liquid chromatography (HPLC, Jasco) equipped with UV (at the wavelength of 254 nm) and fluorescence (at the wavelength of 280 and 340 nm) detectors and with XBridge BEH C8 2.5 μm 4.6 × 150 mm XP Column.

For the quantification of the total protein loss and leakage in human plasma and dialysate, the protein concentration of samples collected from human plasma and dialysate every hour was measured by UV-spectroscopy (Nanodrop, wavelength of 280 nm).

### 3. Results and discussion

#### 3.1. Dope solution viscosity

For the fiber fabrication, we used a lab scale spinning set up and a stable extrusion of the dope solution through the spinneret was observed. The viscosity of the sorbent free dope solution was approximately 2 times more viscous comparing to the viscosity of the sorbent free dope solution (13.8 ± 1 Pa s at shear rate 1 s\(^{-1} \) and 7.25 ± 0.48 Pa s, respectively). Table 2 (see the supporting information for the Table 2: Information of spinning conditions used in this study. Seven spinning protocols were applied with the large spinneret (indicated as L1 - L7) and two protocols with the small spinneret (indicated as S1 - S2).
Fig. 2. Typical SEM images of the cross sections of the fibers studied here.
Fig. 3. Typical (FE)SEM images of the inner and outer surfaces of the studied membranes. Magnifications in each case are 50 000 x (inner surface) and 1000 x (outer surface).
3.2. Membrane morphology

Fig. 1a and Table 2 present the influence of different spinning conditions on the inner diameter (ID) and outer diameter (OD) of the produced fibers. As expected, the application of the smaller spinneret is the most effective parameter for reducing the diameter of the produced fiber. The ID and OD of S1 spun by the small spinneret were 33% smaller comparing to L1 which was spun under the same spinning conditions but with the large spinneret. Another important spinning parameter for effectively reducing the size of the MMM fibers is the flow rate of the outer dope solution; decrease of the flow rate of the outer dope solution from 1.6 to 0.4 ml min⁻¹, results in decrease of the fiber OD from 1.2 to approximately 0.9 mm (see L1 and L2). Concerning other parameters, the fiber’s OD and ID can be reduced by decreasing the flow rate of the outer (sorbent based) and inner (sorbent free) dope solutions and of the bore fluids (Finner, Fonter, and Ffree) and by increasing the air-gap and fiber take-up speed. Fig. 1b presents typical SEM images of some of the produced fiber membranes, whereas, the SEM images of all produced membranes are shown in Fig. 2S of the supporting information. The MMM prepared with the protocol S2 has the smaller OD and ID. In the rest of this study, it will be indicated as MMM3 and will be further studied together with three commercial membranes COM1, COM2, and COM3 as controls.

Fig. 2 compares, in more detail, the morphology of optimal MMM3 to the commercial control membranes. The MMM3 has ID close to that of the control membranes (MMM3: 206 μm, COM1: 185 μm, COM2: 185 μm, and COM3: 214 μm), the thickness of particle free selective layer is approximately 50 μm, similar to the thickness of all control membranes (COM1, COM2, and COM3 have about 40 μm). The layer with the activated carbon particles has thickness of about 100 μm. The sorbent free layer is composed of the sponge-like structure of 1/3 and finger-like structure of 2/3. The pores in the selective layer are open and interconnected. The sorbent-based layer is also quite porous and AC particles, with the size of approximately 5–20 μm, are well distributed there. COM1 and COM2 have the sponge-like structure whereas the COM3 has a more finger-like structure.

Table 3 presents the transport properties of the studied membranes. During all transport experiments we did not observe any leakage of the sorbent AC. The MMM3 has the highest water permeance and the highest albumin retention of all membranes. It is important to note that the KUF of the control membranes measured here is much higher to those reported by the manufacturers and the results are consistent to the results reported earlier by Eloot et al. [28]. The difference is resulted from different test set up and modules used. In our study, we used small modules with low packing density and the lab-scale dialysis setup with dead end filtration mode. Perhaps, the test of the manufacturers was done in the test setup which is closer to the actual clinic system with the highly packed-full size module.

Fig. 4 presents the clean water flux of the MMM3 at TMPs up to 3750 mmHg (5 bar). The linear plot indicates good membrane mechanical stability and no membrane compaction at this pressure range. In the clinic, the applied TMP for convective therapies is mostly up to 750 mmHg (1 bar), so the MMM3 is very suitable for those. It is also worth noting that the maximum TMP recommended by the manufacturers for applying to the COM1-3 commercial membranes is up to 600 mmHg (0.8 bar) which might indicate limitations in their stability at higher TMPs.

### Table 3

Transport properties of the studied membranes.

| Membrane   | Inner diameter (ID, μm) | Water permeance (Lm⁻²h⁻¹bar⁻¹) (n ≥ 3) | Ultrafiltration coeff. (KUF) (ml m⁻²h⁻¹mmHg⁻¹) | BSA rejection (%) |
|------------|-------------------------|----------------------------------------|-----------------------------------------------|------------------|
| MMM1 [14] | 699 ± 9                 | 58.4 ± 9.3                             | 78                                            | –                |
| MMM2 [15] | 450                     | 2.5                                    | 3.4                                           | –                |
| MMM3      | 208 ± 5                 | 132 ± 15                               | 174                                           | –                |
| COM1 (FHPS) | 184 ± 4               | 13.3 ± 1.7                             | 18                                            | 100              |
| COM2 (FX1000) | 185 ± 9               | 102 ± 19                               | 136                                           | 34               |
| COM3 (Polyflux2H) | 215 ± 2          | 120 ± 1                                | 160                                           | 75               |

| Current study | Company information | Difference(%) |
|---------------|---------------------|---------------|
|               | 78                  | –             |
|               | 3.4                 | –             |
|               | –                   | 99            |
|               | –                   | –             |
|               | 99 ± 1              | –             |
|               | 97 ± 3              | –             |
|               | 95 ± 1              | –             |
|               | 91 ± 2              | –             |

* Values were estimated based on the ultrafiltration coefficient, in ml h⁻¹mmHg⁻¹ provided by the manufacturers normalized with the membrane surface area. The surface area was 1.8, 2.2 and 0.2 m² for the COM, COM2 and COM3, respectively.

* Difference (%) between the ultrafiltration coefficient values measured here and estimated from the data of the manufacturers.
Fig. 5 presents the sieving coefficient (SC) curves and pore size distribution of the studied membranes. Based on the PEGs and albumin rejection data (see Table 3S and equation (4) in the supporting information), the MWCO (SC = 0.1) of the MMM3 was estimated to be about 47 kDa whereas the MWCO of the COM 1, 2, and 3 membranes is estimated to be 53, 68, and 73 kDa, respectively (see insert of Fig. 5a and Table 3S (supporting information). Based on these estimations, the protein leakage by the MMM3 and COM1 is expected to be lower than the COM2 and COM3. The protein leakage during the toxin removal test for 4 h is investigated in section 3.4. As shown in Fig. 5b, the mean pores of MMM3 and COM1 are around 4.5 nm and are smaller than those of COM2 and COM3 (around 6 nm). Furthermore, the pore size distribution of MMM3 is narrower than that of COM1. These results are consistent to the images of the inner surface obtained by FE-SEM (see Fig. 3) where it is shown that the inner surface pore size of the MMM3 is smaller than that of the commercial membranes. The relatively high clean water permeance of the MMM3, in comparison to the commercial membranes (especially in comparison to COM1), is probably due to the higher surface porosity and pore connectivity of the MMM3.

3.4. PBUT removal by the membranes

Prior to performing transport tests through the membranes, we checked the amount of toxins bound to protein in the model albumin solution and in human plasma. Similar to other studies [7, 29], 78–97% of IS and 30–43% of HA were bound to protein after 4 h (see Fig. 3S in the supporting information). We then carried out the toxin removal test from a BSA solution (40 g L⁻¹ BSA in PBS, 40 mg L⁻¹ IS and 110 mg L⁻¹ HA spiked) to study the PBUT removal of membranes (MMM3 and COM1) with restricted components. The total IS and HA removal of MMM3 were approximately 3-fold higher than those of COM1 (Fig. 4S and Table 4S in the supporting information). It is important to note that we did not observe any leaching of AC from the MMM3 during the 4 h of toxin removal experiments and no AC was found in dialysate.

Fig. 6 and Table 4 present the results of toxins removal from human plasma by the studied membranes. The adsorption and diffusion were determined by the mass balance calculation (Total toxins removed from the plasma = diffused toxins to dialysate + adsorbed toxins to the membranes). The IS removal after 4 h by the MMM3 is 500 mg m⁻², which is approximately 2 times higher than COM1 (272 mg m⁻²) and approximately 1.4 times higher than COM2 and COM3. High flux membranes (COM2 and 3) have higher IS removal compared to the low flux membrane (COM1). In principle, for the standard HD membranes the toxin removal is mainly due to diffusion caused by the toxin concentration gradient between blood plasma and dialysate. However, for the MMMs, as explained earlier, the adsorptive layer increases the concentration gradient across the membranes leading to higher toxin removal [18]. In fact, the removal of both toxins, IS and HA, by the MMM3 is mainly due to adsorption, which is unlikely to other commercial membranes. Additionally, although the toxin removal rate decreases in time, still the toxin removal by adsorption does not seem to reach saturation during 4 h of the HD experiment (Fig. 6a).

### Table 4

|                  | MMM1 [14] | MMM2 [15] | MMM3 Current study | COM1 | COM2 | COM3 |
|------------------|-----------|-----------|--------------------|------|------|------|
| Total IS removal | 255 ± 102 | 367 ± 176 | 500 ± 43 (n = 3)   | 272 ± 43 (n = 4) | 377 ± 91 (n = 5) | 370 ± 24 (n = 5) |
| Total HA removal | 784 ± 142 | –         | 2478 ± 361 (n = 3) | 2611 ± 564 (n = 3) | 2674 ± 574 (n = 3) | 2599 ± 574 (n = 3) |
| after 4 h (mg m⁻²) |          |          |                    |      |      |      |
Fig. 6. The removal results of the protein bound indoxyl sulfate (IS) and hippuric acid (HA) with the human plasma: (a) MMM3, (b) COM1, (c) COM2, and (d) COM3 at zero TMP after 4 h.
Fig. 7. PBUT removal in the current study and references [14,15,29,31–34]: (a) IS and (b) HA. MMM3 has outstanding IS toxin removal comparing to all membranes.
COM1, the IS removal is mainly due to diffusion, see Fig. 6b. However, for the COM2 and COM3 membranes significant IS removal appears to be due to toxin adsorption (see Fig. 6c and d). This may be due to interaction of the toxin with the membranes and/or due to toxin bound protein layer on the membrane surface, consistent to an earlier report by Elloot et al. [28].

In the case of HA, although the main removal mechanism is different, mainly diffusion for commercial membranes and adsorption for MMM3, the total removed amount is approximately 2500 mg m$^{-2}$ for all membranes. The high diffuse removal of HA is due to its high free fraction (binding to albumin >30%, see Fig. 3S in the supporting information) in comparison to IS (which is bound to albumin >80% in our system, Fig. 3S in the supporting information). Since the molecular size of HA is much smaller than the pores of the selective layer of all membranes, the transport of HA across all membranes is similar and high for all membranes.

During the filtration experiments with PBUT in human plasma, we have monitored the changes in total protein concentration in the plasma and dialysate and we estimated the protein leakage to the dialysate, as well as, the total protein adsorption on the membrane surface and/or within the membrane structure. The total protein adsorption was the lowest on the MMM3 reaching 2.0 g/g membrane compared to COM1, 2, and 3 which adsorbed 25, 30, and 12 g/g of membrane, respectively during 4 h of HD. We hypothesize that this is due to low surface roughness (see Fig. 3), and low protein adhesion properties of the inner layer of the MMM3. For the MMM3, we also measured very low protein leakage to the dialysate of 0.1 g (calculated based on the protein concentration in dialysate ($C_{d}$), dialysate flow rate ($Q_d = 10 ml min^{-1}$), and time (250 min) [20,30]) compared to 1.8–2 g measured for the other 3 commercial membranes. We hypothesize that this is due to the lower MWCO and rather narrow pore distribution of the MMM3 (see Fig. 5).

Fig. 6 compares the removal of uremic toxins by the MMM3 to the MM1 and MM2 as well as to other membranes reported in literature. However, it should be noted that the IS and HA removal results in references were obtained from HD with whole blood using commercial membranes. As the whole blood includes many components which can cause different blood viscosity and clotting problems, it may result in reducing the PBUT removal comparing to human plasma. In any case, MMM3 has approximately 4 times higher $K_{d}$ and higher toxin removal compared to the references and earlier MMMs (see also Table 5S of the supporting information).

This high toxin removal combined to very low albumin leakage makes the MMM3 very attractive not only for conventional HD therapy but also for portable and/or wearable artificial devices. In fact, due to high toxin removal, a small MMM3 module with the surface area 0.22 m$^2$ can be utilized there [assumptions: similar toxin removal in vivo, uremic toxin concentrations in patient blood with kidney failure: 37.07 ± 26.5 for IS and 109.43 ± 64.66 mg l$^{-1}$ for HA (data from EUTox [4]), and 2–3 L of patient blood [35] is purified during 4 h hemodialysis session in the clinic] in comparison to the surface area 2.2 m$^2$ (COM2 sourced by the manufactured companies) used in the current therapy.

4. Conclusions and outlook

We successfully developed the dual layered MMMs (MMM3) with small ID and selective inner layer similar to commercial membranes as well as a sorbent-based layer with optimal particle accessibility leading to superior removal of PBUTs. Importantly, the new MMM3 has high $K_{d}$ low protein adhesion and low protein leakage in comparison to membranes used currently in the clinic. In the future, we plan to systematically investigate these membranes with full blood and in vivo with small animals.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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