Electrifying solutions for a miniature dialysis device

M. Wester

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Electrifying solutions for a miniature dialysis device

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(met een samenvatting in het Nederlands)

PROEFSCHRIFT

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Promotor:	Prof.dr. M.C. Verhaar
Copromotoren:	Dr. J.A. Joles
	Dr. K.G.F. Gerritsen

Voor Marloes, Abel, Boaz, Milou
en mijn ouders

Beoordelingscommissie:	Prof. dr. C.A.J.M. Gaillard (voorzitter)
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	Prof. R. Masereeuw
	Prof. R. Vanholder
Paranimfen:	De heer A. Wester
	De heer T. van den Bosch

“Ik kan het niet alleen”
(De Dijk)

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General introduction, aims and outline of the thesis

Adapted from:

From portable dialysis to a bioengineered kidney

Maaike K. van Gelder, Silvia M. Mihaila, Jitske Jansen, Maarten Wester, Marianne C. Verhaar, Jaap A. Joles, Dimitrios Stamatialis, Roos Masereeuw and Karin G. F. Gerritsen

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GENERAL INTRODUCTION

Worldwide, approximately 3 million patients receive dialysis treatment [1]. The best solution for patients with end-stage kidney disease (ESKD) is kidney transplantation. However, the supply of viable organs cannot cope with current demand [2]. This shortage is expected to worsen with the expanding growing elderly, diabetic, and metabolic syndrome patient populations [3].

Besides kidney transplantation, long-term chronic dialysis, either hemodialysis (HD) or peritoneal dialysis (PD), has become standard renal replacement therapy (RRT). However, although life sustaining, existing dialysis techniques are a poor substitute of normal kidney function. They partially compensate for renal glomerular filtration and correct fluid and electrolyte imbalances but fail to replace the complex renal tubular function: the endocrine, metabolic and secretory activities. While small (<500 Da) water-soluble waste solutes (i.e. uremic toxins) are relatively effectively removed, middle molecules and protein-bound uremic toxins (PBUT) are retained due to the limited pore size of low- and high-flux dialyzers. Only the free fraction of PBUT is available for diffusion across the dialyzer. Clearance of fluid and uremic toxins with conventional HD (CHD) is also low because of the discontinuous character of treatment (typically three sessions of 4 h per week) compared with the continuous mode of action of healthy kidneys (168 h per week). As a result, fluid and uremic toxins accumulate during the interdialytic interval, followed by rapid shifts in fluid balance and uremic toxin and electrolyte concentrations during dialysis. This is in sharp contrast with homeostasis maintained by healthy kidneys.

More continuous dialysis is provided by PD, that is based on diffusion of toxins and osmosis of excess water from the blood across the peritoneal membrane into dialysate in the abdominal cavity, which is exchanged 4–6 times per day via an abdominal catheter (either manually during the day or automatically by a machine at night [automated PD (APD)]. However, blood clearance is relatively low because the toxin concentration gradient between blood and dialysate rapidly decreases during the dwell which limits solute transport. Other disadvantages of PD are time-consuming exchange procedures during the day or preparation of the APD equipment in the evening and limited technique survival (median 3.7 years [4]). The latter is due to high incidence of recurrent peritonitis and deterioration of the peritoneal membrane because of exposure to very high glucose concentrations in the dialysate used to induce osmotic water removal [5].
The inadequacy of conventional dialysis treatment results in poor clinical outcomes, high mortality and low quality of life. To improve the situation of dialysis patients, novel RRTs are under development (Figure 1).

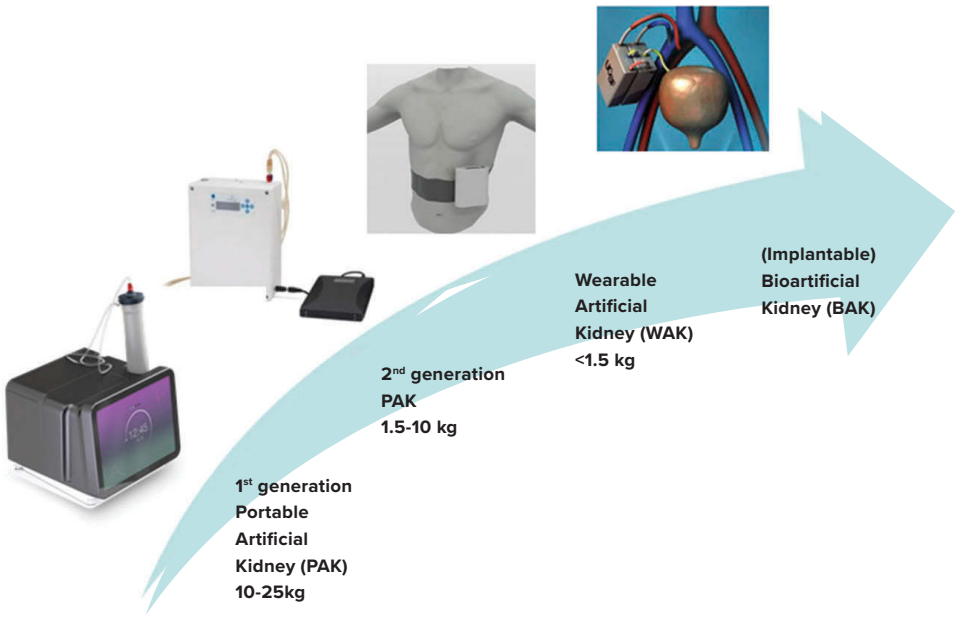

FIGURE 1. Roadmap toward development of novel renal replacement therapies. 1st generation PAK image courtesy of Neokidney. Implantable BAK image courtesy of dr. Shuvo Roy, UCSF.

The utilization of emerging disciplines, such as microfluidics and nanotechnology, has led to the development of sophisticated new devices and equipment that challenge the current dialysis treatment paradigms, as devices become smaller, lighter, and are intended for use outside the clinic. The improvement closest to clinical introduction could be offered by a miniaturized dialysis machine. The first generation of dialysis devices will be portable, or rather transportable (weight: ~10–25 kg), intended to be used during sedentary activities or as a bedside device. Further miniaturization may allow for the creation of a wearable device (weight: <1.5 kg), that can be worn on the body during the day. Such portable artificial kidney (PAK) or wearable artificial kidney (WAK) devices can be used at home and during travel, enhancing patient's freedom and autonomy and facilitating participation in economic and social activities, while reducing healthcare costs. PAK and WAK devices can facilitate longer and more frequent RRT, providing patients with less fluctuations in fluid status and internal environment, and possibly improved blood clearance. This may improve clinical outcomes, including survival and quality of life, as observed in observational studies with more frequent and/or prolonged HD [6–8]. However, PAK and WAK devices, like conventional RRT, fail to replace tubular functions, and accordingly, are only partial substitutive therapies. To address this, strategies are being explored to build an on-demand functional kidney [9–14]. The development of such a bioartificial kidney (BAK), combining

synthetic materials with specialized renal epithelial cells, partially replacing tubular function, to be used in combination with standard HD, incorporated in a PAK or WAK or as an implantable device, is making progress. A final goal would be to fully replace kidney function by organ repair or (re)generation.

Efficient water utilization is an important challenge for achieving a portable or wearable dialysis device. Current therapies are characterized by high water consumption. With HD, approximately 280–500 L of water is used to generate 120 L of dialysate per 4-h treatment (dialysate flow of 500 mL/min), depending on efficiency of reverse osmosis [15]. In addition, water treatment systems for HD are stationary and demand high-maintenance and home adaptations to meet specific plumbing and electricity requirements. PD patients use 8–12 L of bagged dialysis fluid per day which also hampers their mobility.

Electrolyte balance and urea removal

Portable and wearable dialysis devices are based on continuous regeneration of a small volume of spent dialysate by a purification unit in a closed-loop system, independent of a fixed water-source. Current purification units make use of cation and anion exchangers for the removal of potassium and phosphate, respectively, and activated carbon for the removal of organic waste solutes (e.g. creatinine and middle molecules). Urea removal from spent dialysate is difficult because of its hydrophilic character and low reactivity, but this is essential for dialysate regeneration. Although urea is generally considered biologically inert, chronically elevated urea concentrations >20 mM (as commonly observed in dialysis patients) are associated with toxicity caused by the direct effects of urea or by urea-derived (iso)cyanate and ammonia (e.g. urea-/isocyanate-induced carbamylation of proteins and peptides) [16].

Several urea removal methods are available: (1) urease, an enzyme that catalyzes hydrolysis of urea into ammonium and carbonate, was applied in the Recirculating DialYsis (REDY) sorbent system. The REDY system was widely used for dialysate regeneration in HD in the 1970s and 1980s [17,18] but was withdrawn from clinical practice due to inferior treatment adequacy because of limited dialysate flow rates (max. 250 mL/min), reports of aluminum toxicity (i.e. osteomalacia and dementia), spillover acidosis, concerns about the release of zirconium, and low cost competitiveness [19–24]. Redesign of the REDY system has eliminated the risk of aluminum toxicity by replacement with a non-aluminum urease immobilization substrate. Remaining disadvantages are ammonium generation requiring binding by a cation exchanger, simultaneous binding of calcium, magnesium and (too much) potassium by the cation exchanger and release of sodium and hydrogen in exchange for bound cations. This necessitates replenishment of calcium, magnesium and potassium, and a

system to prevent sodium release to the patient (the released hydrogen is neutralized by the urease-generated bicarbonate). (2) A relatively large quantity of activated carbon (~2–5 kg) can be used to remove the daily urea production (230– 470 mmol/day depending on protein intake) [25–27]), as urea has a low-binding affinity for activated carbon (~0.1 mmol/g) [28].

Other urea removal strategies, all not yet available for clinical use, include (3) electrochemical degradation of urea to carbon dioxide and nitrogen gas [29-40], and (4) removal by adsorbents (e.g. zeolites, resins, silica, and chitosan) [41,42].

Removal of protein bound uremic toxin*s*

Another challenge is the removal of PBUT, since in conventional dialysis this is hampered by the fact that only part of the free fraction of these compounds is filtered. Several methods to increase PBUT removal have been explored: (1) Extending dialysis sessions and increasing the dialyzer mass transfer area coefficient and dialysate flow rate improve removal of PBUT only to a limited extent [7,43,44]. (2) Pre-dialyzer infusion of PBUT-binding competitors (tryptophan, ibuprofen and furosemide) increase PBUT removal in vitro (1.4- and 2.9-fold increase of indoxyl sulfate (IS) removal and 1.3- and 2.1-fold increase of indoleactic acid removal by infusion of tryptophan or a combination of ibuprofen and furosemide, respectively) [45]. However, to achieve removal of a broad spectrum of PBUT, several PBUT binding competitors should be infused, considering clinically acceptable plasma concentrations. Studies are ongoing to find the ideal-binding competitors that are both efficacious and safe for long term routine clinical use. 3) Hypertonic predilution hemodiafiltration or haemodiafiltration at increased plasma ionic strength (HDF-IPIS) is a technique that infuses hypertonic saline into pre-filter replacement fluid to enhance plasma ionic strength, stimulating release of toxins from proteins, thus making them available for clearance through filtration [46,47]. HDF-IPIS was shown to increase clearance of the free-fraction of IS by 1.4-fold compared with HD in humans [46]. 4) Hemodiafiltration with endogenous reinfusion (HFR) combining convection, adsorption and diffusion using a double-chamber system improved p-cresol removal. The ultrafiltrate, containing small amounts of albumin generated in the first chamber via a high-flux filter (sieving coefficient for albumin of 0.02), passes through a resin cartridge for PBUT removal, and is reinfused into the blood that subsequently enters a second chamber for diffusive removal via a low-flux filter [48]. 5) Combined fractionated plasma separation and adsorption was shown to increase PBUT removal (1.3-fold increase for phenylacetic acid and p-cresol removal and 1.9-fold for IS). With this technique, plasma is separated and passed over two hydrophobic and cationic adsorbers for PBUT removal, followed by plasma reinfusion into the blood circuit and passage through a high-flux dialyzer [49].

So-called hollow-fiber mixed-matrix membrane (MMM), that combine diffusion and adsorption into one membrane, could offer an alternative for the above-mentioned strategies to remove PBUT. MMM consist of two layers: an outer layer with adsorptive particles (e.g. activated carbon) incorporated in a porous polymer and a particle-free hemocompatible inner membrane. The diffused free fraction is continuously adsorbed, thus maintaining a high concentration gradient across the dialyzer that drives diffusion. These MMM could be incorporated in a miniature dialysis device or added to conventional dialysis to increase PBUT removal. However, PBUT removal by MMM is limited by the fact that only the free faction can pass the filter and the predialysis protein-bound/free fraction equilibrium is not completely restored during the treatment (the percentage protein binding increases) [50].

Besides water efficiency, other technical requirements for a WAK include: light weight (ideally <1.5 kg), a miniaturized battery-operated pump, ergonomic design and safety mechanisms (e.g. automatic discontinuation of the blood pump in case of fluid leakage or air bubble detection). Remote monitoring systems in home dialysis devices enable close surveillance of patients and treatment parameters outside of the hospital and facilitate remote assistance by the medical team [51]. Like with conventional therapies, biocompatible materials, including non-clotting membranes, should be used.

Prelude - At the start of Nephron+, an EU consortium (Nephron+ grant FP7- 430-ICT-2009-4; 2010-2014) that aimed to design a wearable device, we had a number of expectations that were published in 2013 [52]. In our discussion, we will look back at these expectations and put them into perspective.

AIMS AND OUTLINE OF THE THESIS

The studies described in this thesis were performed to evaluate efficacy and safety of strategies to remove excess electrolytes and urea in a miniature dialysis device. We used sorbents to remove excess potassium and phosphate and re-explored whether electrochemical degradation of urea could be an efficacious and safe alternative for urease. Additionally, removal of creatinine and PBUT by MMM was evaluated, as a prospective asset to conventional HD or for incorporation in a miniature dialysis device.

Part I – Electrolyte balance

In part I of this thesis we explored efficacy and safety of potassium and phosphate binding sorbents to enable re-use of spent dialysate. These sorbents adsorb potassium and phosphate in exchange for sodium, hydrogen and hydroxyl ions. To evaluate potential excessive release of these ions, sodium levels and pH were monitored. Not only monovalent but also bivalent ions are bound by the ion exchangers, so we also explored whether preloading of the sorbents with calcium and magnesium could counteract net removal of these ions. **Chapter 2** describes *in vitro* testing of the binding capacity of these sorbents. Regenerability of the sorbents was explored to select the sorbents that could most easily be prepared for re-use. **Chapter 3** describes the *in vivo* validation in a large animal experimental model of our *in vitro* results. In addition, the effect on vital parameters is documented to evaluate potential toxic side effects.

Part II - Urea removal by electro-oxidation

Part II covers the testing and optimization of electro-oxidation of urea and neutralization of toxic chloride oxidation by-products. In **chapter 4** we compared electrode materials, studied the effect of urea concentration and current on urea degradation efficiency and chlorine generation *in vitro* and investigated whether activated carbon can be used to reduce release of chlorine species. In **chapter 5** we tested selected graphite electrodes *in vivo* in a large animal model. We explored whether increasing total electrode surface area and varying dialysate flow increased cumulative urea removal. Potential side effects of electro-oxidation were evaluated by investigating influence on electrolyte and acid-base balance, ammonium release, release of toxic chlorine species and by monitoring parameters of hemolysis.

Part III – Uremic toxin removal by mixed matrix membranes

In part III new dialysis membranes, MMM, were developed and tested. These dual-layer mixed matrix membranes contain activated carbon particles in the outer layer to increase removal of both creatinine and PBUT extracorporeally. **Chapter 6** shows the development

and proof of concept of these membranes. In **chapter 7** removal of PBUT was quantified.

Epilogue - In **chapter 8** The implications of our findings are discussed and integration of the investigated components in a miniature dialysis device is proposed.

REFERENCES

1. Fresenius Medical Care. Annual Report 2016. [Cited 2017 Oct 30]. Available from: https://www.freseniusmedicalcare.com/fileadmin/ data/com/pdf/investors/News_Publications/Annual_Reports/2016/FMC_AnnualReport_2016_en.pdf
2. Rosen L, Vining AR, Weimer DL. Addressing the shortage of kidneys for transplantation: purchase and allocation through chain auctions. J Health Polit Policy Law. 2011;36:717–755.
3. Chapman JR. What are the key challenges we face in kidney transplantation today? Transplant Res. 2013;2:S1.
4. Mujais S, Story K. Peritoneal dialysis in the US: evaluation of outcomes in contemporary cohorts. Kidney Int Suppl. 2006;103:S21–6.
5. Wu HY, Hung KY, Huang TM, et al. Safety issues of long-term glucose load in patients on peritoneal dialysis–a 7-year cohort study. PLoS One. 2012;7:e30337.
6. Beby AT, Cornelis T, Zinck R, et al. Cost-effectiveness of high dose hemodialysis in comparison to conventional in-center hemodialysis in the Netherlands. Adv Ther. 2016;33:2032–2048.
7. Lacson E Jr, Xu J, Suri RS, et al. Survival with three-times weekly incenter nocturnal versus conventional hemodialysis. J Am Soc Nephrol. 2012;23:687–695.
8. Ok E, Duman S, Asci G, et al. Comparison of 4- and 8-h dialysis sessions in thrice-weekly in-centre haemodialysis: a prospective, case-controlled study. Nephrol Dial Transplant. 2011;26:1287–1296.
9. Fleming GM. Renal replacement therapy review: past, present and future. Organogenesis. 2011;7:2–12.
10. Sanavi S, Afshar R, Mirsepasi Z. Renal Replacement Therapy: Challenge on the Best Therapeutic Modality. Internal Medicine. 2011;1.
11. Humes HD. Stem cells: the next therapeutic frontier. Trans Am Clin Climatol Assoc. 2005;116:167–183.
12. Chung HC, Ko IK, Atala A, et al. Cell-based therapy for kidney disease. Korean J Urol. 2015;56:412–421.
13. Peired AJ, Sisti A, Romagnani P. Mesenchymal stem cell-based therapy for kidney disease: a review of clinical evidence. Stem Cells Int. 2016;2016:4798639.
14. Hickson LJ, Eirin A, Lerman LO. Challenges and opportunities for stem cell therapy in patients with chronic kidney disease. Kidney Int. 2016;89:767–778.
15. Agar JW. Reusing and recycling dialysis reverse osmosis system reject water. Kidney Int. 2015;88:653–657.
16. Vanholder R, Gryp T, Glorieux G. Urea and chronic kidney disease: the comeback of the century? (in uraemia research). Nephrol Dial Transplant. 2018;33:4–12.
17. Gordon A, Better OS, Greenbaum MA, et al. Clinical maintenance hemodialysis with a sorbent-based, low-volume dialysate regeneration system. Trans Am Soc Artif Intern Organs. 1971;17:253–258.
18. Blumenkrantz MJ, Gordon A, Roberts M, et al. Applications of the Redy sorbent system to hemodialysis and peritoneal dialysis. Artif Organs. 1979;3:230–236.
19. Lameire NH, De Vriese AS. Adsorption techniques and the use of sorbents. In: Berlyne GM, Ronco C, editors. Dialysis, dialyzers and sorbents. Where are we going? Basel: Karger Medical and Scientific Publishers; 2001. p. 140–153.

20. Mion C, Branger B, Issautier R, et al. Dialysis fracturing osteomalacia without hyperparathyroidism in patients treated with HCO3 rinsed Redy cartridge. Trans Am Soc Artif Intern Organs. 1981;27:634–638.
21. Pierides AM, Frohnert PP. Aluminum related dialysis osteomalacia and dementia after prolonged use of the Redy cartridge. Trans Am Soc Artif Intern Organs. 1981;27:629–633.
22. Drury PJ, Harston GA, Ineson PR, et al. Aluminium release from the Sorbsystem D-3160 and D-3260 cartridges. Life Support Syst. 1986;4:211–219.
23. Pierides AM, Myli MP. Iron and aluminum osteomalacia in hemodialysis patients. N Engl J Med. 1984;310:23.
24. Agar JW. Review: understanding sorbent dialysis systems. Nephrology (Carlton). 2010;15:406–411.
25. Shinaberger CS, Kilpatrick RD, Regidor DL, et al. Longitudinal associations between dietary protein intake and survival in hemodialysis patients. Am J Kidney Dis. 2006;48:37–49.
26. Ikizler TA, Greene JH, Yenicesu M, et al. Nitrogen balance in hospitalized chronic hemodialysis patients. Kidney Int Suppl. 1996;57: S53–6.
27. Weiner ID, Mitch WE, Sands JM. Urea and ammonia metabolism and the control of renal nitrogen excretion. Clin J Am Soc Nephrol. 2015;10:1444–1458.
28. Stephens RL, Jacobsen SC, Atkin-Thor E, et al. Portable/wearable artificial kidney (WAK)––initial evaluation. Proc Eur Dial Transplant Assoc. 1976;12:511–518.
29. Koster K, Wendt H, Gallus J, Krisam G, Lehmann HD. Regeneration of hemofiltrate by anodic oxidation of urea. Artif Organs 1983; 7: 163-168.
30. Grinval'd V, Leshchinskii GM, Rodin VV, Strelkov SI, Yakovleva AA. Development and Testing of a Unit for Electrochemical Oxidation of Products of Hemodialysis. Biomedical Engineering 2003; 37: 67-72.
31. Fels M. Recycle of dialysate from the artificial kidney by electrochemical degradation of waste metabolites: small-scale laboratory investigations. Med Biol Eng Comput 1978; 16: 25-30
32. Fels M. Recycle of dialysate from the artificial kidney by electrochemical degradation of waste metabolites: continuous reactor investigations. Med Biol Eng Comput 1982; 20: 257-263
33. Hintzen K, Stiller S, Brunner H, Rautenbach R, Mann H. Electrodialysis and reverse osmosis as a regeneration system for hemofiltrate. Artif Organs 1983; 7: 169-175
34. Keller Jr R, Yao S, Brown J, Wolfson S, Zeller M. Electrochemical Removal of Urea from Physiological Buffer as the Basis for a Regenerative Dialysis System. Bioelectrochemistry and Bioenergetics 1980; 7: 469-485
35. Schuenemann B, Quellhorst E, Kaiser H, Richter G, Mundt K, Weidlich E et al. Regeneration of filtrate and dialysis fluid by electro-oxidation and absorption. Trans Am Soc Artif Intern Organs 1982; 28: 49-53
36. Wright J, Michaels AS, Appleby AJ. Electrooxidation of Urea at the Ruthenium Titanium Oxide Electrode. AIChE Journal 1986; 32: 1450-1458.
37. Yao SJ, Wolfson SK, Jr., Ahn BK, Liu CC. Anodic oxidation of urea and an electrochemical approach to de-ureation. Nature 1973; 241: 471-472
38. Yao S, Wolfson Jr S, Tokarsky J, Ahn B. De-ureation by Electrochemical Oxidation. Bioelectrochemistry and Bioenergetics 1974; 1: 180-186
39. Yao S, Wolfson Jr S, Krupper M, Wu K. Controlled-potential controlled-current electrolysis: In vitro and in vivo electrolysis of urea. Bioelectrochemistry and Bioenergetics 1984; 13: 15-24
40. Simka W, Piotrowski J, Robak, A, Nawrat G.

Electrochemical treatment of aqueous solutions containing urea. J Appl Electrochem 2009; 39: 1137-1143.

41. Urbańczyk E, Sowa M, Simka W. Urea removal from aqueous solutions—a review. J Appl Electrochemistry. 2016;46:1011–1029.
42. Smakman R, van Doorn AW. Urea removal by means of direct binding. Clin Nephrol. 1986;26(Suppl 1):S58–S62.
43. Cornelis T, Eloot S, Vanholder R, et al. Protein-bound uraemic toxins, dicarbonyl stress and advanced glycation end products in conventional and extended haemodialysis and haemodiafiltration. Nephrol Dial Transplant. 2015;30:1395–1402.
44. Meyer TW, Leeper EC, Bartlett DW, et al. Increasing dialysate flow and dialyzer mass transfer area coefficient to increase the clearance of protein-bound solutes. J Am Soc Nephrol. 2004;15:1927–1935.
45. Tao X, Thijssen S, Kotanko P, et al. Improved dialytic removal of protein-bound uraemic toxins with use of albumin binding competitors: an in vitro human whole blood study. Sci Rep. 2016;6:23389.
46. Krieter DH, Devine E, Korner T, et al. Haemodiafiltration at increased plasma ionic strength for improved protein-bound toxin removal. Acta Physiol (Oxf). 2017;219:510–520.
47. Bohringer F, Jankowski V, Gajjala PR, et al. Release of uremic retention solutes from protein binding by hypertonic predilution hemodiafiltration. Asaio J. 2015;61:55–60.
48. Esquivias-Motta E, Martin-Malo A, Buendia P, et al. Hemodiafiltration with endogenous reinfusion improved microinflammation and endothelial damage compared with online-hemodiafiltration: a hypothesis generating study. Artif Organs. 2017;41:88–98.
49. Brettschneider F, Tölle M, von der Giet M, et al. Removal of proteinbound, hydrophobic uremic toxins by a combined fractionated plasma separation and adsorption technique. Artif Organs. 2013;37:409–416.
50. Deltombe O, van Biesen W, Glorieux G, et al. Exploring protein binding of uremic toxins in patients with different stages of chronic kidney disease and during hemodialysis. Toxins (Basel). 2015;7:3933–3946.
51. Rosner MH, Lew SQ, Conway P, et al. Perspectives from the kidney health initiative on advancing technologies to facilitate remote monitoring of patient self-care in RRT. Clin J Am Soc Nephrol. 2017;12:1900–1909.
52. Wester M, Gerritsen KG, Boer WH, Joles JA, Kooman JP. [The wearable artificial kidney: a promise for the future?]. Ned Tijdschr Geneeskd. 2013;157(52):A6965. Dutch.

PART I

Electrolyte balance

2

A regenerable potassium and phosphate sorbent system to enhance dialysis efficacy and device portability: an in vitro study

Maarten Wester, Frank Simonis, Karin G. Gerritsen, Walther H. Boer, Will K. Wodzig, Jeroen P. Kooman and Jaap A. Joles

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ABSTRACT

Background

Continuous dialysis could provide benefit by constant removal of potassium and phosphate. This study investigates the suitability of specific potassium and phosphate sorbents for incorporation in an extracorporeal device by capacity and regenerability testing.

Methods

Capacity testing was performed in uremic plasma. Regenerability was tested for potassium sorbents, with adsorption based on cationic exchange for sodium, with 0.1 M and 1.0 M NaCl. To regenerate phosphate sorbents, with adsorption based on anionic exchange for hydroxide, 0.1 M and 1.0 M $NaHCO_3$ and NaOH were used. Subsequently, sodium polystyrene divinylbenzene sulphonate (RES-A) and iron oxide hydroxide (FeOOH) beads were incorporated in a cartridge for testing in bovine blood using a recirculating blood circuit and a dialysis circuit separated by a high flux dialyzer (dynamic set-up). Preloading was tested to assess whether this could limit calcium and magnesium adsorption.

Results

In the batch binding assays zirconium phosphate most potently adsorbed potassium (0.44±0.05 mmol/g) and RES-A was the best regenerable potassium sorbent (92.9±5.7% with 0.1M NaCl). Zirconium oxide hydroxide most potently adsorbed phosphate (0.44±0.05 mmol/g) and the polymeric amine sevelamer carbonate was the best regenerable sorbent (85.7±5.2% with 0.1M $NaHCO_3$). In the dynamic set-up a potassium adsorption of 10.72±2.06 mmol in 3h was achieved using 111 g of RES-A and a phosphate adsorption of 4.73±0.53 mmol in 3h using 55 g of FeOOH. Calcium and magnesium preloading was shown to reduce the net adsorption in three hours from 3.57±0.91 to -0.29±1.85 and 1.02±0.05 to -0.31±0.18 mmol respectively.

Conclusions

RES-A and FeOOH are suitable, regenerable sorbents for potassium and phosphate removal in dialysate regeneration. Use of zirconium carbonate and zirconium oxide hydroxide may further increase phosphate adsorption but may compromise sorbent regenerability. Use of polymeric amines for phosphate adsorption may enhance sorbent regenerability. Calcium and magnesium preloading considerably reduced net adsorption of these ions.

INTRODUCTION

Conventional haemodialysis and peritoneal dialysis have major shortcomings. The quality of life on dialysis is low due to inadequate removal of uremic waste molecules and excess fluid [1-3], and morbidity and mortality in dialysis patients are high [3,4]. Increasing the dialysis dose would be a major improvement in renal replacement therapy [3,5-9]. This could be achieved with a wearable artificial kidney [10]. In addition, a miniaturized design that is independent of a fixed water supply because of dialysate regeneration will offer more freedom and autonomy to the patient.

In designing such a portable device, effective removal strategies have to be developed for all uremic waste products and excess sodium and water. Amongst the substances that have to be removed by a wearable device are potassium and phosphate. In this paper, we explored ion exchange as removal strategy for these ions. Ideally, ion exchangers should have a high binding capacity and selectivity for the ions to be removed and should not release unwanted substances into the fluid to be treated. Furthermore, regenerability of ion exchangers is an important topic. Regenerating instead of replacing exchangers several times daily, as applied in other sorbent-based artificial kidneys [10-17], will substantially limit the costs of the wearable system. We therefore investigated the binding capacity, selectivity and regenerability of several ion exchangers for potassium and phosphate in short batch binding assays. Furthermore, we incorporated a selected cation and anion exchanger in a prototype of a miniature artificial kidney device and used fresh bovine blood spiked with potassium and phosphate to simulate the *in vivo* situation.

MATERIALS AND METHODS

Materials

For the batch binding assays zeolite (ZEO) ($Na_{12}[(AlO_2)_{12}(SiO_2)_{12}]\cdot xH_2O$, Sigma Zwijndrecht, The Netherlands), a cationic sodium polystyrene/divinylbenzene sulphonate exchanger (RES-A) (Resonium A, Sanofi-Aventis Gouda, The Netherlands) and zirconium phosphate (ZIR-phos) ($Zr(HPO_4)_2$, Sigma) were tested for potassium adsorption. For phosphate adsorption zirconium oxide hydroxide (ZIR-hydr) ($ZrO(OH)_2$, Sigma), zirconium carbonate (ZIR-car) ($Zr(CO_3)_2$, Sigma), iron oxide hydroxide (FeOOH, Sigma) and sevelamer carbonate (SEV-car) (Renvela, Sanofi-Aventis) were tested. To allow comparison of the sorbents, all were tested in powder form.

Pilot studies have shown that powdered sorbents will cause obstruction of the dialysate circuit, so dynamic tests were performed using sorbent beads to allow incorporation in the prototype miniature artificial kidney. For potassium adsorption RES-A beads (diameter 0.6-0.8 mm) were available and for phosphate adsorption polystyrene/divinylbenzene (PS/DVB) beads loaded with FeOOH (FeOOH beads, diameter 0.3-0.4 mm). The RES-A beads wholly consist of potassium sorbent, whereas the PS/DVB beads served as a non-adsorptive holder for phosphate adsorbing FeOOH. The bead diameter of 0.3 to 0.8 mm, as used, was a compromise between flow resistance and performance. Beads were provided by Nanodialysis, The Netherlands.

Batch binding assays

Batch binding assays were performed at room temperature with plasma obtained by plasmapheresis from three patients with Goodpasture syndrome.
Capacity testing was performed by the following steps:

1. Four millilitre fresh plasma was added to 100 mg sorbent.
2. The resulting mixture of plasma and sorbent was placed on a shaker and centrifuged after 1 hour.
3. The supernatant was stored for analyses.
4. Steps 1-3 were repeated twice with the used sorbents.

The amount of adsorbed potassium (mmol/g sorbent), phosphate and calcium was calculated as follows:

$$\text{Amount adsorbed (mmol/g sorbent)} = (((Cb\text{-}Cs_1) + (Cb\text{-}Cs_2) + (Cb\text{-}Cs_3)) \times V)/W$$

where Cb is the concentration (mmol/L) in the blank plasma, Cs_n is the concentration

(mmol/L) in the plasma after contact with the sorbents with n = 1, 2 and 3 for the consecutive plasma's, V is the volume (L) of plasma and W is the mass (g) of the added sorbent. Because ion-exchangers may also release substances, similar calculations were used to measure the release of sodium by RES-A and iron by the FeOOH beads into the supernatant. Magnesium concentrations were not measured in the batch binding assays, as the divalent cations magnesium and calcium are known to show similar binding characteristics [18].

Subsequently, regenerability of the sorbents was tested by adding regeneration solutions to the potassium- and phosphate-loaded sorbents. Adsorption by the selected potassium sorbents is based on cationic exchange for sodium, so 0.1 M and 1.0 M NaCl were used to regenerate the potassium sorbents. To regenerate the phosphate sorbents, with adsorption based on anionic exchange for OH^- or CO_3^{2-}, 0.1 M and 1.0 M $NaHCO_3$ and 0.1 M and 1.0 M NaOH were used. Regenerability testing was performed by the following steps:

1. Four millilitre regeneration solution was added to the potassium- and phosphate-loaded sorbents, used in capacity testing.
2. The resulting mixture of regeneration solution and sorbent was placed on a shaker and centrifuged after 20 min.
3. The supernatant was stored for analyses.
4. Steps 1-3 were repeated twice with the used sorbents.

The amount of desorbed potassium and phosphate was calculated as follows:

$$\text{Amount desorbed (mmol/g sorbent)} = (Cr_1+Cr_2+Cr_3) \times V$$

Cr_n is the concentration (mmol/L) in the regeneration solution after contact with the sorbents with n = 1, 2 and 3 for the consecutive solutions and V is the volume (L) of regeneration solution.

A desorption/adsorption ratio was calculated by dividing the total amount that was desorbed by the total amount that was adsorbed:

$$\text{Desorption/Adsorption ratio} = (Cr_1+Cr_2+Cr_3) / ((Cb\text{-}Cs_1) + (Cb\text{-}Cs_2) + (Cb\text{-}Cs_3))$$

A desorption/adsorption ratio of 1.0 would mean complete regenerability of the sorbent.

Dynamic tests with RES-A beads and FeOOH beads

An adsorption unit was provided by Nanodialysis BV (Oirschot, The Netherlands). It consists of a polyoxymethylene housing (Nanodialysis BV, Oirschot, The Netherlands), with a mixture of 111 and 55 grams of the selected potassium and phosphate sorbents, respec-

tively. These amounts were derived from pilot experiments.

Commercially available high flux dialyzers (Gambro 2H 0.2m^2, cut-off 50 kD; polymer blend of polyamide, polyarylether-sulfone and polyvinylpyrrolidone; Gambro Dialysatoren GmbH Hechingen, Germany) were used to separate blood and dialysate (figure 1) to prevent proteins from entering the adsorption unit. Commercially available Thomas pumps (Gardner Denver Thomas, Sheboygan, WI, USA) were used.

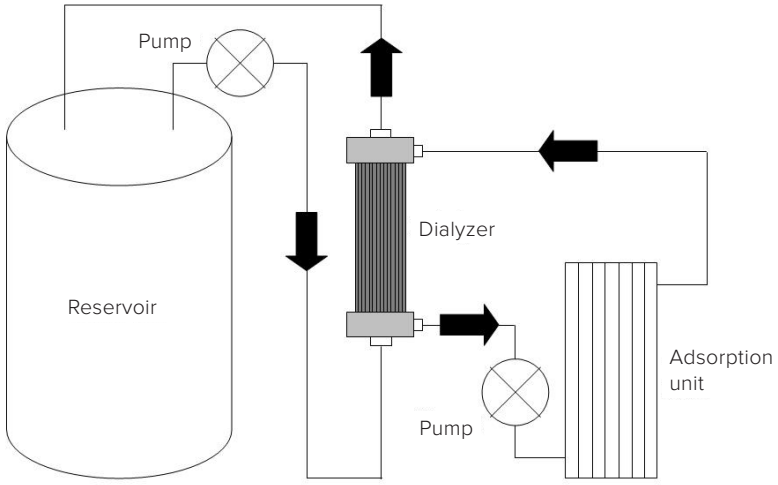

FIGURE 1. Dialysis circuit consisting of a 3L blood reservoir, 2 Thomas pumps (speed of pump 1 and 2: 120 and 22 ml/min respectively), a 0.2 m^2 Gambro 2H high flux dialysis membrane (a polyarylethersulfone polymer) and an adsorption unit (a polyoxymethylene housing) containing a mixture of 111 grams of RES-A beads and 55 grams of FeOOH beads.

Three batches of fresh bovine blood (2.0-3.0 L, collected at an abattoir) were re-circulated over the dialyzer with an average flow rate of 120 mL/min for 3 hours after rinsing the sorbents with 500 mL of an isotonic solution containing 154 mM NaCl. Because the selected sorbents also bind calcium and magnesium to some extent, $CaCl_2$ (2.25 mM) and $MgCl_2$ (0.84 mM) were added to this isotonic solution to preload the sorbents with calcium and magnesium and prevent net calcium and magnesium adsorption during dialysis. The reservoir was stirred continuously with a magnetic stirrer. The isotonic solution was pumped through the adsorption unit and the dialyzer in counter current direction with an average flow rate of 22 ml/min. Samples were withdrawn directly from the reservoir. At 0, 60 and

120 min potassium phosphate ($K_2PO_4.3H_2O$) was added to the reservoir to maintain the potassium and phosphate concentrations, mimicking inter-compartmental transport of potassium and phosphate from tissue compartments to the plasma. The total amounts of potassium, phosphate, calcium and magnesium adsorbed per hour were calculated as follows:

Amount adsorbed (mmol) = (Cb-Cs) x V

Cb and Cs are the concentrations (mmol/L) in the reservoir at the start and the end, respectively, of each hour during the experiment and V is the batch volume (L). Similar calculations were used to measure the release of sodium.

Analyses

Potassium and sodium concentrations were measured with a Corning 480 Flame Photometer. Phosphate concentrations were determined with DiaSys Phosphate FS (1 5211 99 10 021) and a Starrcol standard SC-60-S photometer. Calcium concentrations were determined with DiaSys Calcium CPC FS (1 1121 99 10 021) and a Bio-Rad Microplate reader Benchmark 16-channel photometer. pH was measured with a Radiometer Copenhagen PHM lab pH meter. Magnesium concentrations were measured by photometry with a Cobas 6000 automated chemistry analyzer (Roche). Iron concentrations were measured by colorimetry with an AU5811 chemistry analyzer (Beckman Coulter).

Statistical analysis

All experiments were performed in triplicate, unless specified otherwise. Data are shown as mean ± standard deviation. Statistical significance was determined using Sigmastat 11.0 one-way ANOVA for grouped analyses and (paired) t-tests for subgroup analyses.

RESULTS

Batch binding assays with potassium sorbents

All sorbents showed significant adsorption of potassium (figure 2.A). ZIR-phos appeared to be the most potent sorbent of potassium (0.44±0.05 mmol/g). Interestingly, the least effective potassium sorbents, ZEO and Resonium A, showed considerable calcium adsorption (figure 2.B, 0.16±0.04 mmol/g and 0.15±0.03 mmol/g respectively), whereas calcium adsorption by ZIR-phos was limited (0.04±0.02 mmol/g). Figure 2.C underlines that the ratio of calcium versus potassium adsorption is most favourable for ZIR-phos. This implies that this sorbent would necessitate the lowest levels of calcium infusion in a dynamic setup to compensate for undesired calcium adsorption.

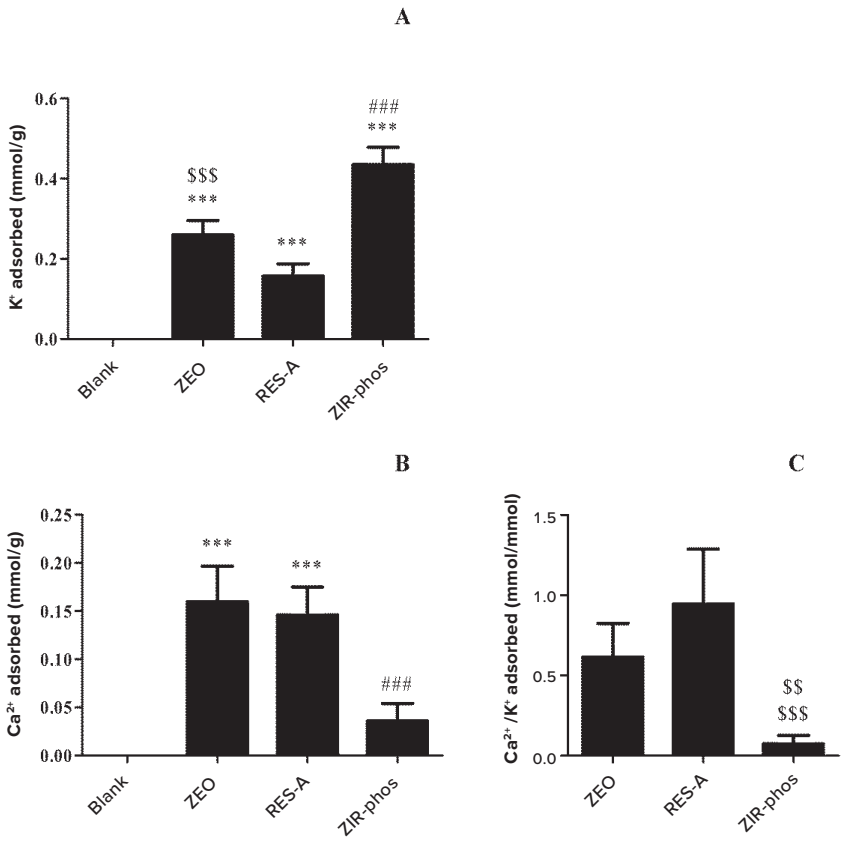

FIGURE 2. Batch binding assays with potassium adsorbents. A: Potassium adsorption (mmol/g sorbent, n=6). B: Calcium adsorption (mmol/g sorbent, n=6). C: Calcium/potassium adsorption (mmol/ mmol, n=6). *** p<0.001 versus blank; ### p<0.001 versus ZEO and RES-A; $$$ p<0.001 versus RES-A; $$ p<0.01 versus ZEO.

ZEO and RES-A released sodium (0.94±0.19 and 1.51±0.25 mmol/g respectively, p<0.001 versus blank) while ZIR-phos adsorbed sodium (2.01±0.19 mmol/g, p<0.001 vs. blank). There was a marked acidifying effect of ZIR-phos (pH 4.80±0.23 versus 8.13±0.07 in the blank plasma, p<0.001), and a mild alkalizing effect of ZEO (pH 8.68±0.10, p≤0.005), with only RES-A not affecting pH (pH 8.20±0.05).

Regenerability tests showed full regenerability of RES-A and ZEO with 1.0 M NaCl, whereas ZIR-phos reached less than half regenerability (desorption/adsorption ratio 0.46±0.17) with the concentrated NaCl solution (figure 3). Only RES-A could be regenerated to a large extent (desorption/adsorption ratio 0.93±0.06) under mild conditions using 0.1 M NaCl (figure 3).

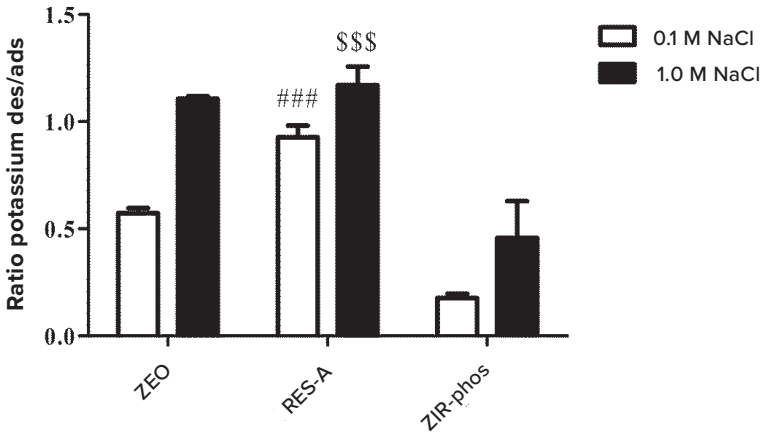

FIGURE 3. Regenerability of potassium adsorbents: potassium desorption/adsorption ratio (mmol/mmol, n=3). *###* p<0.001 versus ZEO and ZIR-phos 0.1M NaCl; $$$ p<0.001 versus ZIR-phos 1.0M NaCl.

Batch binding assays with phosphate sorbents

All sorbents showed significant adsorption of phosphate (figure 4.A). ZIR-car and ZIR-hydr appeared to be the most powerful sorbents (0.22±0.04 and 0.23±0.05 mmol/g respectively). The most powerful phosphate sorbents also showed more calcium adsorption (figure 4.B). However, there was no difference in the ratio adsorbed calcium/phosphate between the phosphate sorbents (figure 4.C). ZIR-car had an acidifying effect and SEV-car a marked alkalizing effect (pH 7.59±0.09 and 10.11±0.04 respectively versus 8.13±0.07 in blank plasma, p<0.001). ZIR-hydr (pH 8.19±0.10) and FeOOH (pH 8.25±0.08) had little effect on pH.

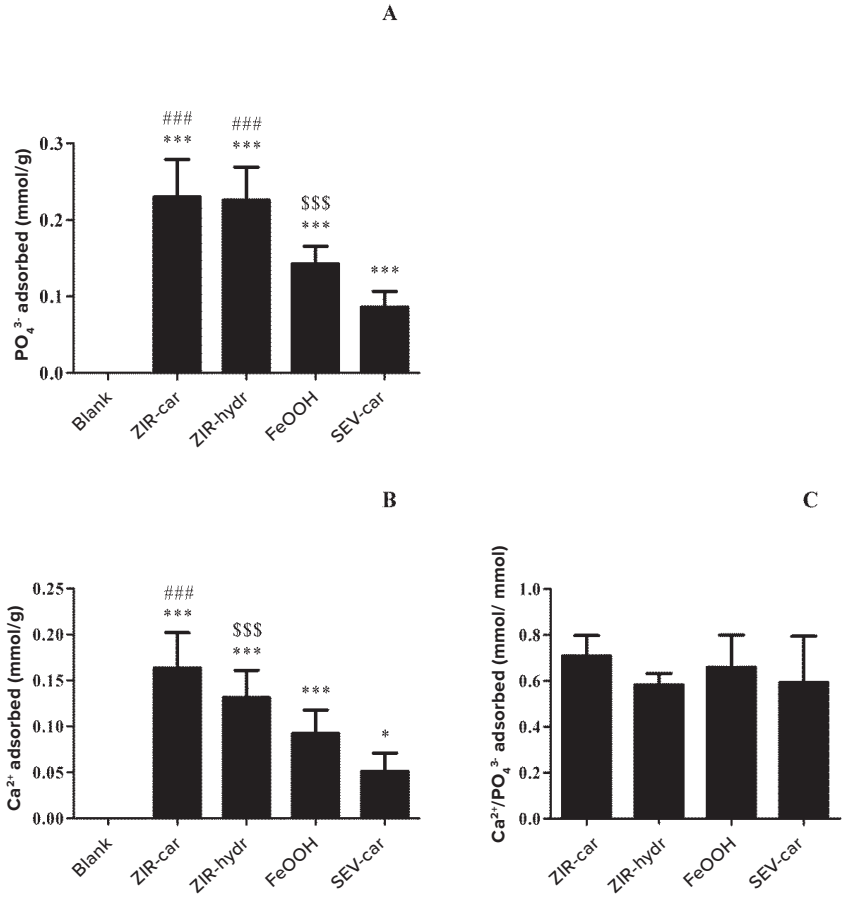

FIGURE 4. Batch binding assays with phosphate adsorbents. A: Phosphate adsorption (mmol/g sorbent, n=12). B: Calcium adsorption (mmol/g sorbent, n=6). C: Calcium/phosphate adsorption (mmol/ mmol, n=6). *** p<0.001 and * p<0.05 versus blank; ### p<0.001 versus FeOOH and SEV-car; $$$ p<0.001 versus SEV-car.

Regenerability tests (figure 5) showed that all phosphate sorbents could be more or less completely regenerated using 1.0M NaOH. Only SEV-car could be regenerated under mild conditions using 0.1 M $NaHCO_3$ (desorption/adsorption ratio 0.86±0.05).

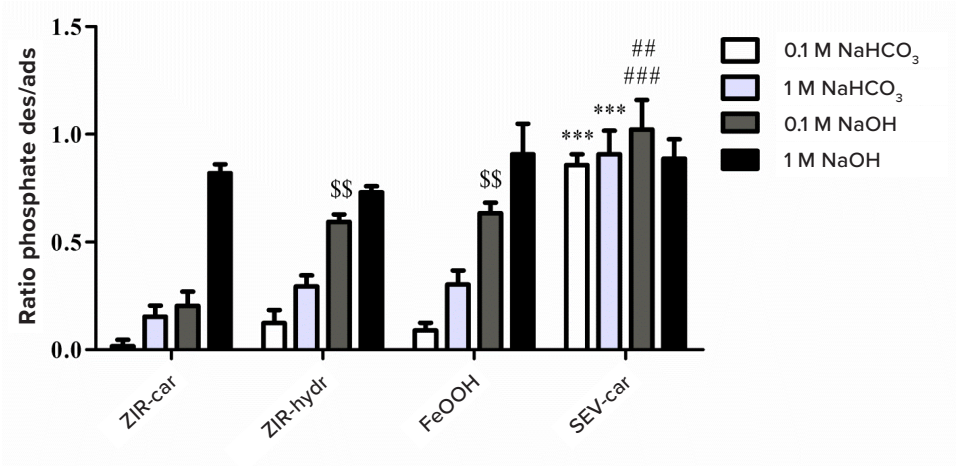

FIGURE 5. Regenerability of phosphate adsorbents: phosphate desorption/adsorption ratio (mmol/mmol, n=3). *** p<0.001 versus ZIR-car, ZIR-hydr and FeOOH 0.1 M and 1.0 M $NaHCO_3$; ### p<0.001 versus ZIR-car and ZIR-hydr 0.1 M NaOH, ## p<0.01 versus FeOOH 0.1 M NaOH; $$ p<0.01 versus ZIR-car 0.1 M NaOH.

Sorbents incorporated in our prototype of a wearable artificial kidney device.

Further testing was performed using RES-A beads and FeOOH beads since these ion exchangers were available to us in a spherical form that could be incorporated in a dialysis device. Noteworthy is that the calcium/phosphate adsorption ratio of the FeOOH beads was much lower than that of FeOOH in powder form (0.25±0.11 vs. 0.66±0.14 mmol/mmol respectively, p<0.001), presumably because of reduced accessibility of the adsorbed phosphate groups in the porous structure for calcium binding due to steric hindrance. Release of iron by the FeOOH beads was not detected.

Dynamic testing with bovine blood

Cumulative potassium adsorption (figure 6.A) from bovine blood amounted to 10.72±2.06 mmol in 3 hours. Adsorption in the first hour was significantly more than in the third hour (5.85±1.73 mmol versus 2.67±0.22 mmol respectively, p<0.05). Phosphate adsorption showed a similar pattern (figure 6.B). Cumulative adsorption after 3 hours was 4.73±0.53 mmol. There was a decline in phosphate adsorption during the experiment but this did not reach statistical significance (2.15±0.54 mmol in the first hour versus 1.44±0.07 mmol in the third hour, p=0.09). In exchange for potassium adsorption, sodium was released by the RES-A beads resulting in cumulative sodium release of 10.67±4.16 mmol in 3 hours.

The experiment with the highest starting concentrations of potassium and phosphate (experiment 1, $[K^+]$ 6.29 mmol/L and $[PO_4^{3-}]$ 2.40 mmol/L) showed the highest cumulative

adsorption of both electrolytes, whereas experiment 2, with the lowest starting concentrations ([K^+] 4.05 mmol/L and [PO_4^{3-}] 1.99 mmol/L), showed the lowest cumulative adsorption. These observations indicate concentration-dependency of adsorption by the sorbents.

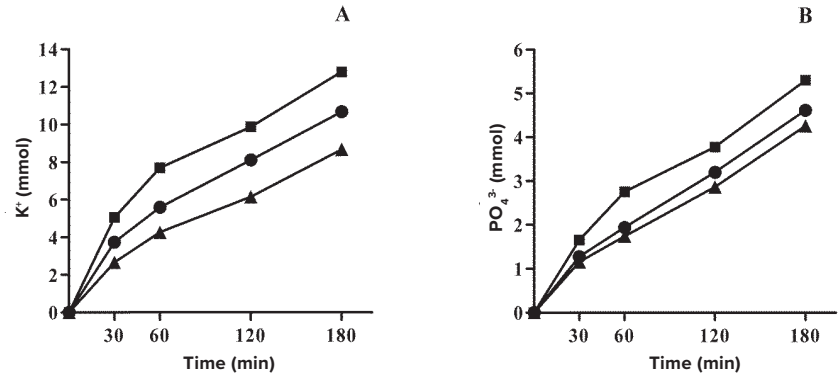

FIGURE 6. Dynamic testing with bovine blood. A: Cumulative potassium adsorption (mmol, n=3). B: Cumulative phosphate adsorption (mmol, n=3). Experiment 1 (squares), 2 (triangles) and 3 (circles) plotted separately.

Considerable calcium and magnesium adsorption was observed. This could be prevented by preloading the sorbents with calcium and magnesium chloride without affecting potassium and phosphate adsorption (figure 7.A and 7.B).

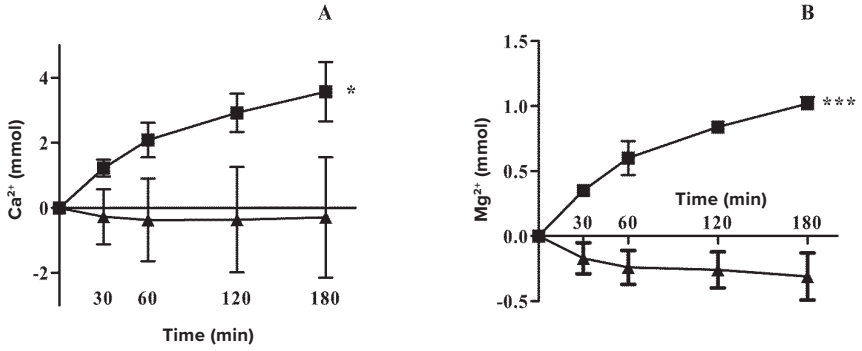

FIGURE 7. Dynamic testing with bovine blood. A: Cumulative calcium adsorption (mmol, n=3). B: Cumulative magnesium adsorption (mmol, n=3). With (triangles) and without (squares) preloading. *** $p<0.001$, * $p<0.05$ versus with preloading.

DISCUSSION

In the present study, we screened a number of ion-exchange sorbents with respect to their potential to bind potassium and phosphate, respectively. In addition, we studied the possible release of unwanted substances as well as sorbent regenerability. The latter is an important aspect for the future application of these binders in miniaturized wearable or portable artificial kidney devices.

TABLE 1. Characteristics of potassium sorbents

K^+ sorbents	K^+ adsorption	Ca^{2+} adsorption	Na^+ release	pH	Regenerability
ZEO	++[1]	- - -	-	⬆	++[2] / +++[3]
RES-A	+	- - -	- -	=	+++ / +++
ZIR-phos	+++	-	+	⬇⬇⬇	+ / ++

[1] positive (+) and negative (-) characteristics; [2] with 0.1 M NaCl; [3] 1 M NaCl

As summarized in the semi-quantitative overview of our key potassium results in table 1, ZIR-phos was the most potent potassium sorbent with the advantage that adsorption is based on exchange for sodium as well as hydrogen [11,19]. This even resulted in some net sodium removal, but a potential disadvantage is the induction of metabolic acidosis due to proton release. ZIR-phos showed limited regenerability and therefore appears mainly suitable for application in a non-regenerable system that uses replaceable cartridges. This approach has been successfully applied in the REDY system [11,12]. However, economically a regenerable system is preferable to a non-regenerable system [20]. In this respect, both ZEO and RES-A are more promising. ZEO showed ~50% regenerability with 0.1 M sodium regeneration fluid, but was a somewhat less potent potassium sorbent than ZIR-phos. In contrast to ZIR-phos, ZEO released some sodium in exchange for potassium, but there was a small and theoretically favourable increasing effect on pH. A potential disadvantage of ZEO, which is an aluminosilicate mineral, is the release of aluminum (data not shown). Under the conditions of the batch studies, RES-A was the least effective potassium sorbent, albeit with neutral pH effects and good regenerability, even with a 0.1M NaCl solution. A disadvantage is the considerable sodium release in exchange for potassium, which may complicate patient fluid management. However, downward adjustments of the sodium content of the dialysate could be applied to prevent sodium loading of patients. Finally, all sorbents showed some degree of calcium adsorption, probably necessitating some form of calcium loading of the sorbents in clinical applications.

TABLE 2. Characteristics of phosphate sorbents

PO_4^{3-} sorbents	PO_4^{3-} adsorption	Ca^{2+} adsorption	pH	Regenerability
ZIR-car	+++[1]	- - -	↓	-[2] / +[3] / +[4] / +++[5]
ZIR-hydr	+++	- - -	=	+ / + / ++ / +++
FeOOH	++	- -	=	+ / + / ++ / +++
SEV-car	+	-	↑↑	+++ / +++ / +++ / +++

[1] positive (+) and negative (-) characteristics; [2] with 0.1 M $NaHCO_3$; [3] 1 M $NaHCO_3$; [4] 0.1 M NaOH; [5] 1 M NaOH

The summary of our key phosphate results in table 2 shows that ZIR-car and ZIR-hydr were the most potent phosphate sorbents. Regenerability was similar to that of FeOOH, which also showed reasonable phosphate adsorption. By far the best regenerability was demonstrated by SEV-car which, despite being an effective oral phosphate binder in clinical practice, was the least effective phosphate binder in the setting of our batch experiments. Remarkably, all phosphate sorbents showed some calcium binding that was proportional to phosphate adsorption, again probably necessitating some form of calcium loading of the sorbents in clinical applications. We presume that this is possibly due to calcium binding to adsorbed phosphate via charge interaction. Whereas most phosphate binders had minimal effects on pH, SEV-car had a considerable alkalizing effect. This might be of use to compensate for the acidifying effects of some potassium binders, such as ZIR-phos, by combining these sorbents.

For dynamic testing, sorbent beads instead of powders have to be used because the resistance to fluid flow in the sorbent cartridge is inversely related to particle size. Since only RES-A and FeOOH were available to us as beads at the time of the experiments, these sorbents were used for the proof of principle experiments. The sorbent cartridge, containing 111 g of RES-A and 55 g of FeOOH beads, was remarkably effective during the 3 hr dynamic experiments using a miniaturized dialysis module. In this period, the cartridge removed approximately 10 and 5 mmol of potassium and phosphate, respectively. These preliminary findings clearly show that it will become feasible to remove a very considerable part of the daily load of potassium and phosphate by sorbent technology incorporated in miniaturized dialysis devices. However, additional investigation is required into optimal sorbent content of the cartridge, its rate of saturation and the frequency of regeneration dictated by this.
Notably, a cartridge containing RES-A combined with FeOOH not only appears to be surprisingly effective, but may also be more readily acceptable to clinicians and regulatory agencies as far as safety and toxicology aspects are concerned compared with the other sorbents investigated in the present study. RES-A has been applied for decades as oral potassium sorbent and safety risks seem limited [21]. Furthermore, although iron release was not detected using the FeOOH beads, release of small amounts of iron may not harm

dialysis patients as a considerable part of them suffers from iron deficiency [22].

In the batch binding studies, calcium was adsorbed by all potassium and phosphate exchangers. Net calcium and magnesium adsorption was successfully eliminated in our dynamic setup by preloading the sorbents with these cations. This might abolish the need for post-cartridge infusion of these electrolytes, as applied in the well-known (modified) REDY sorbent system [10,12,14,23], containing activated carbon, urease, zirconium phosphate, zirconium oxide and zirconium carbonate. However, to control for calcium and magnesium levels in the wearable device, implementation of online sensors for both electrolytes shall probably be necessary.

The zirconium compounds were not available to us in bead form that could be incorporated into the prototype. Hypothetically, even less sorbent would be required using zirconium compounds because of their high adsorptive capacity for both potassium (ZIR-phos) and phosphate (ZIR-car and ZIR-hydr).

Zirconium has been safely incorporated in all kinds of (bio)medical applications [24]. It is known for its high chemical stability supporting their use in dialysis patients [25], although an increase in oxidative metabolism has been reported after long-term exposure [26]. SEV-car, which was also not available to us in bead form, still remains an interesting compound because of its excellent regenerability and the fact that it has a well-known safety profile in humans. Analogous to RES-A and FeOOH, this may the enhance acceptance of incorporation of this compound in dialysis devices by clinicians and regulatory agencies.

Conclusions

Adequate potassium and phosphate adsorption from dialysate can be achieved by the use of modest amounts of RES-A and FeOOH. RES-A shows excellent and FeOOH acceptable regenerability under mild conditions. Use of zirconium carbonate and zirconium oxide hydroxide may further increase phosphate adsorption but may compromise sorbent regenerability. Use of polymeric amines for phosphate adsorption may enhance sorbent regenerability. Calcium and magnesium adsorption can be limited by preloading these sorbents with these electrolytes. Future research should elucidate whether these sorbents can be applied safely in *in vivo* conditions, e.g. in a wearable artificial kidney device for continuous dialysis.

REFERENCES

1. Mittal SK, Ahern L, Flaster E, Maesaka JK, Fishbane S. Self-assessed physical and mental function of haemodialysis patients. *Nephrol Dial Transplant* 2001; 16: 1387-1394
2. Mittal SK, Ahern L, Flaster E, Mittal VS, Maesaka JK, Fishbane S. Self-assessed quality of life in peritoneal dialysis patients. *Am J Nephrol* 2001; 21: 215-220
3. Locatelli F, Canaud B. Dialysis adequacy today: a European perspective. *Nephrol Dial Transplant* 2012; 27: 3043-3048
4. Wolfe RA, Ashby VB, Milford EL *et al*. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med* 1999; 341: 1725-1730
5. Achinger SG, Ayus JC. The role of daily dialysis in the control of hyperphosphatemia. *Kidney Int Suppl* 2005; S28-S32
6. Ayus JC, Achinger SG, Mizani MR *et al*. Phosphorus balance and mineral metabolism with 3 h daily hemodialysis. *Kidney Int* 2007; 71: 336-342
7. King RS, Glickman JD. Electrolyte management in frequent home hemodialysis. *Semin Dial* 2010; 23: 571-574
8. Lacson E Jr, Xu J, Suri RS *et al*. Survival with three-times weekly in-center nocturnal versus conventional hemodialysis. *J Am Soc Nephrol* 2012; 23: 687-695
9. Walsh M, Manns BJ, Klarenbach S, Tonelli M, Hemmelgarn B, Culleton B. The effects of nocturnal compared with conventional hemodialysis on mineral metabolism: A randomized-controlled trial. *Hemodial Int* 2010; 14: 174-181
10. Davenport A, Gura V, Ronco C, Beizai M, Ezon C, Rambod E. A wearable haemodialysis device for patients with end-stage renal failure: a pilot study. *Lancet* 2007; 370: 2005-2010
11. Agar JW. Review: understanding sorbent dialysis systems. *Nephrology (Carlton)* 2010; 15: 406-411
12. Ash SR. Sorbents in treatment of uremia: a short history and a great future. *Semin Dial* 2009; 22: 615-622
13. Gura V, Beizai M, Ezon C, Polaschegg HD. Continuous renal replacement therapy for end-stage renal disease. The wearable artificial kidney (WAK). *Contrib Nephrol* 2005; 149: 325-333
14. Lee DB, Roberts M. A peritoneal-based automated wearable artificial kidney. *Clin Exp Nephrol* 2008; 12: 171-180
15. McGill RL, Bakos JR, Ko T, Sandroni SE, Marcus RJ. Sorbent hemodialysis: clinical experience with new sorbent cartridges and hemodialyzers. *ASAIO J* 2008; 54: 618-621
16. Murisasco A, Baz M, Boobes Y *et al*. A continuous hemofiltration system using sorbents for hemofiltrate regeneration. *Clin Nephrol* 1986; 26 Suppl 1: S53-S57
17. Ronco C, Fecondini L. The Vicenza wearable artificial kidney for peritoneal dialysis (ViWAK PD). *Blood Purif* 2007; 25: 383-388
18. Yu Z, Qi T, Qu J, Wang L, Chu J. Removal of Ca(II) and Mg(II) from potassium chromate solution on Amberlite IRC 748 synthetic resin by ion exchange. *J Hazard Mater* 2009; 167: 406-412
19. Rosenbaum BP, Ash SR, Wong RJ, Thompson RP, Carr DJ. Prediction of hemodialysis sorbent cartridge urea nitrogen capacity and sodium release from in vitro tests. *Hemodial Int* 2008; 12: 244-253
20. Drukker W, Doorn van A. *Replacement of Renal Function by Dialysis: A Textbook of Dialysis*. In:

Maher J, ed. *Dialysate regeneration.* Springer, Houten, The Netherlands 1989; 417-438

21. Putcha N, Allon M. Management of hyperkalemia in dialysis patients. *Semin Dial* 2007; 20: 431-439
22. Fishbane S, Maesaka JK. Iron management in end-stage renal disease. *Am J Kidney Dis* 1997; 29: 319-333
23. Fuchs C, Dorn D, Rieger J, van Doorn AW, Striebel JP, Scheler F. Capabilities of the Redy cartridge for regeneration of hemofiltrate. *Artif Organs* 1979; 3: 279-280
24. Lee DB, Roberts M, Bluchel CG, Odell RA. Zirconium: biomedical and nephrological applications. *ASAIO J* 2010; 56: 550-556
25. Ghosh S, Sharma A, Talukder G. Zirconium. An abnormal trace element in biology. *Biol Trace Elem Res* 1992; 35: 247-271
26. Olmedo DG, Tasat DR, Evelson P, Rebagliatti R, Guglielmotti MB, Cabrini RL. In vivo comparative biokinetics and biocompatibility of titanium and zirconium microparticles. *J Biomed Mater Res A* 2011; 98: 604-613

3

A regenerable potassium and phosphate sorbent system to enhance dialysis efficacy and device portability: a study in awake goats

Maarten Wester, Karin G. Gerritsen, Frank Simonis, Walther H. Boer, Diënty H. Hazenbrink, Koen R. Vaessen, Marianne C. Verhaar and Jaap A. Joles

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ABSTRACT

Background

Patients on standard intermittent hemodialysis suffer from strong fluctuations in plasma potassium and phosphate. Prolonged dialysis with a wearable device, based on continuous regeneration of a small volume of dialysate using ion exchangers, could moderate these fluctuations and offer increased clearance of these electrolytes. We report *in vivo* results on the efficacy of potassium and phosphate adsorption from a wearable dialysis device. We explore whether equilibration of ion exchangers at physiological [Ca^{2+}], [Mg^{2+}] and hypotonic [NaCl] can prevent calcium/magnesium adsorption and net sodium release, respectively. Effects on pH and [HCO_3^-] were studied.

Methods

Healthy goats were instrumented with a central venous catheter and dialyzed. Potassium and phosphate were infused to achieve plasma concentrations commonly observed in dialysis patients. An adsorption cartridge containing 80g sodium poly(styrene-divinylbenzene) sulphonate and 40g iron oxide hydroxide beads for potassium and phosphate removal, respectively, was incorporated in a dialysate circuit. Sorbents were equilibrated and regenerated with a solution containing NaCl, $CaCl_2$ and $MgCl_2$. Blood was pumped over a dialyzer and dialysate was recirculated over the adsorption cartridge in a countercurrent direction.

Results

Potassium and phosphate adsorption was 7.7**±**2.7 and 4.9**±**1.3 mmol in 3h, respectively. Adsorption capacity remained constant during consecutive dialysis sessions and increased with increasing [K^+] and [PO_4^{3-}]. Equilibration at physiological [Ca^{2+}] and [Mg^{2+}] prevented net adsorption, eliminating the need for post-cartridge calcium and magnesium infusion. Equilibration at hypotonic [NaCl] prevented net sodium release. [Fe^{2+}] and arterial pH did not change. Bicarbonate was adsorbed which could be prevented by equilibrating at [HCO_3^-] 15 mM.

Conclusion

We demonstrate clinically relevant, concentration-dependent, pH-neutral potassium and phosphate removal *in vivo* with small volumes of regenerable ion exchangers in our prototype wearable dialysis device. Application of the selected ion exchangers for potassium and phosphate removal in a wearable dialysis device appears to be effective with a low risk profile.

INTRODUCTION

Removal of potassium and phosphate is inadequate with conventional thrice-weekly hemodialysis [1,2]. Due to the intermittency of the treatment, both pre-dialysis hyperkalemia and post-dialysis hypokalemia are common in dialysis patients, which may lead to arrhythmias. Insufficient phosphate removal causes bone and cardiovascular problems [3,4]. Substantial rebound of plasma potassium and phosphate occurs following dialysis [1,2]. Strict dietary potassium and phosphate restrictions as well as potassium- and phosphate-binding drugs are often required to limit interdialytic increase of these ions. Increasing dialysis dose, by increasing dialysis time and frequency, has been shown to improve potassium and phosphate removal and reduce the need for diet restrictions and oral binders [5-7].
A miniature dialysis device that can be used at home would facilitate longer and more frequent dialysis or ideally allow continuous hemodialysis, and may thereby improve potassium and phosphate balance. The miniaturization can be achieved by continuous regeneration of a small volume of dialysate by adsorption [8-13]. Previously, we showed that *in vitro* removal of potassium and phosphate could achieve clinically relevant levels by use of the ion-exchangers poly(styrene-divinylbenzene)sulphonate and iron oxide hydroxide (FeOOH) incorporated in a miniature artificial kidney device [14]. Here, we study *in vivo* efficacy of potassium and phosphate removal by the device in healthy goats and investigated whether removal was dependent on plasma concentrations. Potassium and phosphate were infused to achieve plasma concentrations close to the ranges observed in dialysis patients. Firstly, we studied the effect on plasma calcium and magnesium concentrations because we observed *in vitro* that the sorbents bind both calcium and magnesium; we could prevent this problem by prerinsing the sorbents with a solution containing calcium and magnesium which we now test here *in vivo*. Secondly, we observed *in vitro* equimolar exchange of potassium for sodium when prerinsing the sorbents with sodium chloride 154 mM; we now explore whether sodium release can be reduced by prerinsing at lower sodium concentration. Thirdly, because we used an iron-containing sorbent to bind phosphate, we determined whether *in vivo* exposure to this sorbent affected plasma iron concentration. Finally, we studied the effect on plasma pH and [HCO_3^-] to confirm the neutral acid-base properties of the sorbents that we observed *in vitro* [14].

MATERIALS AND METHODS

Materials

A miniature dialysis device of 1.5 kg (plus battery pack of 250 g for 3 hours of dialysis) was built and provided by Nanodialysis BV (Oirschot, The Netherlands) as described [14]. 80 g Poly(styrene-divinylbenzene)sulphonate beads and 40g FeOOH beads (together 120 g in a dry and 161 g in a wet state) were incorporated in the adsorption cartridge of the device for respective potassium and phosphate removal [14]. High-flux dialyzers (Polyfluxâ 2H 0.2 m2; Gambro Dialysatoren GmbH, Hechingen, Germany) were used to separate blood and dialysate. Thomas pumps (Gardner Denver Thomas, Sheboygan, WI, USA) were applied [14]. A 72 cm double lumen central venous catheter (CVC) was selected for hemodialysis (Palindrome Chronic Dialysis Catheter, Covidien Netherlands). Unfractionated heparin was used for anticoagulation.

Methods

Animals

In vivo experiments were approved by the Animal Experiments Committee (Utrecht, The Netherlands) and performed in accordance with national guidelines for the care and handling of animals. Healthy Dutch White goats (N=2) were selected since these animals are docile, have easily accessible neck veins, and body weights (70-90kg) and distribution volumes comparable to humans.

Sterilization and regeneration procedure

In each experiment new sterile tubing and dialyzer were used. Prior to each experiment the sorbents were sterilized in sterile environment by 1h exposure of the internal fluidic circuit to 1 L 5% hydrogen peroxide (m/m) and 0.2% peracetic acid (m/m) in NaCl 120 mM/$CaCl_2$ 1.2 mM/$MgCl_2$ 0.45 mM, followed by 12h exposure to 1 L NaOH 120 mM/ethanol 20% (v/v). This procedure was shown to be effective in eliminating bacteria and endotoxins. Subsequently, pH was adjusted to a physiological range using 1 L NaCl 120mM/HCl 50mM, followed by rinsing and equilibration of the sorbents with 3L rinsing solution (pH 7.4-8) containing NaCl, $CaCl_2$ and $MgCl_2$ and in 7 experiments also $NaHCO_3$. Equilibration was achieved by recirculating the rinsing solution in the blood circuit at 110 mL/min while draining the solution from the dialysate circuit at 40mL/min and measuring calcium in the effluent (equilibration of calcium is slower than that of other relevant electrolytes). The rinsing procedure was continued until in- and outlet calcium concentrations levelled (usually after circulation of 3L of rinsing solution). Sodium concentration of the rinsing solution was 120mM based on prior *in vitro* experiments, showing no net sodium release at this concentration. $CaCl_2$ and $MgCl_2$ concentrations were optimized during the experiments of the

current study. Net changes in total plasma calcium and magnesium were minimal at [Ca^{2+}] 1.2 mM and [Mg^{2+}] 0.40-0.45 mM, respectively (Supplemental Figures 1 and 2). Data shown in the results section on calcium and magnesium balance are obtained with these rinsing solution concentrations (N=6 experiments for [Ca^{2+}] 1.2mM and N=7 for [Mg^{2+}] 0.40-0.45 mM). In the first 7 of 14 experiments no bicarbonate was added to the rinsing solution. Only in the last 2 of these 7 experiments bicarbonate was measured downstream of the dialyzer. Since net bicarbonate adsorption across the dialyzer occurred, $NaHCO_3$ was added to the rinsing solution in the last 7 experiments to obtain a [HCO_3^-] of 15 mM (thus, in the first 7 experiments NaCl 120 mM was applied and in the last 7 NaCl 105 mM/$NaHCO_3$ 15 mM). Supplemental table 1 shows the composition of the rinsing solution per experiment.

Experimental procedure

Fourteen experiments were performed in 2 goats (N=6 and 8, respectively). Goats were temporarily sedated (with detomidine and propofol) to insert a CVC in the jugular vein. A catheter was placed in the auricular artery for arterial pressure measurement and withdrawal of arterial blood gases. The dialysate circuit was filled with ~100 mL rinsing solution. Blood lines were connected and blood was pumped (110 mL/min) over the dialyzer (Figure 1: Experimental setup).

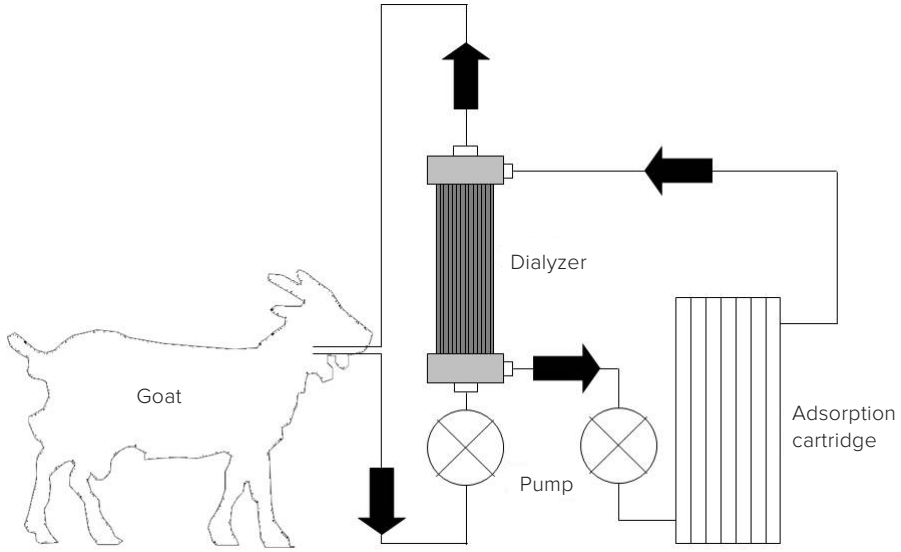


FIGURE 1. Experimental setup.

After 30 minutes habituation of the goat and equilibration of blood with the dialysate compartment, the dialysate pump was started and dialysate was recirculated (40 mL/min)

during 3 h over the adsorption cartridge (Figure 2: Experimental procedure) in countercurrent direction (prior experiments showed that higher pump speeds did not improve potassium and phosphate removal; thus, 40 mL/min was applied to keep energy consumption low). Blood and dialysate samples were taken hourly from the blood circuit (down- and upstream of the dialyzer), from the dialysate circuit (down- and upstream of the adsorption cartridge) and from the arterial catheter. [K^+], [PO_4^{3-}], [total Ca], [total Mg], [Fe^{2+}], [albumin], [Na^+], [HCO_3^-] and arterial pH were monitored. At the end of each experiment the catheters were removed. In one experiment no arterial blood gases were drawn because placement of the catheter in the auricular artery failed. Of note, during six experiments, electro-oxidation was applied for urea removal [15]. Electro-oxidation influenced neither potassium/phosphate adsorption nor the acid-base findings reported below (data not shown).

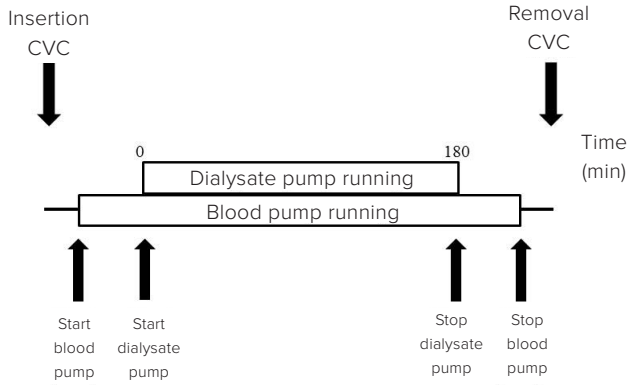


FIGURE 2. Experimental procedure. CVC = central venous catheter.

Spontaneous systemic potassium and phosphate concentrations in the goats were 3.9 ±0.3 mM and 1.5 ±0.3 mM, respectively. To achieve higher potassium and phosphate plasma concentrations closer to the range commonly observed in dialysis patients, we infused potassium and phosphate [15] using a 1 M KCl solution at 0-45 mL/h and a 0.05 M Na_2HPO_4/0.25 M KH_2PO_4 solution at 0-25 mL/h in eight experiments. Infusion rates were adjusted depending on plasma potassium and phosphate concentrations, aiming for [K^+] 4-5.5 mM and [PO_4^{3-}] 1.6-3.0 mM, respectively [16] (Supplemental Figure 3)**.** To test regenerability, nine consecutive experiments were performed with the same adsorption cartridge while sterilizing, regenerating and equilibrating the cartridge between the experiments (see 2.2).

Anticoagulation strategy

After insertion of the CVC, a bolus of unfractionated heparin was given (10,000 IU), followed by continuous infusion of ~3,500 IU/h. The activated clotting time (ACT) was mea-

sured every hour and the heparin dose adjusted, aiming for an ACT >500 s. One hour before removal of the CVC, heparin infusion was stopped to allow coagulation to recover.

Laboratory measurements

All electrolyte measurements were performed by the laboratory of the UMC Utrecht. Potassium, phosphate, sodium, calcium, magnesium, venous bicarbonate, albumin and iron concentrations were analyzed with an AU 5800 routine chemistry analyzer (Beckman Coulter, Brea, California) using ion selective electrodes and photometry. Plasma bicarbonate down- and upstream of the dialyzer and dialysate bicarbonate down- and upstream of the sorption unit was only measured in nine experiments. Arterial blood gas analyses were performed using a blood gas analyzer (Rapidlab type 1265; Siemens Medical Solutions Diagnostics B.V., Breda, The Netherlands).

Calculations, analyses and statistics

Removal/release of electrolytes to or from plasma was calculated using the following formula:

$$A_p = ((C_i - C_o)_{t1} + (C_i - C_o)_{t2})/2 \times Q \times \Delta t \times (1\text{-}Ht)$$

and removal/release to or from dialysate:

$$A_d = ((C_i - C_o)_{t1} + (C_i - C_o)_{t2})/2 \times Q \times \Delta t$$

where A = amount removed (or released) (mmol) from (or into) plasma (A_p) or dialysate (A_d), C_i = inlet plasma concentration (i.e. upstream of dialyzer) or inlet dialysate concentration (i.e. upstream of adsorption unit) (mmol/L), C_o = outlet plasma or dialysate concentration (mmol/L), t = time after start of dialysate pump (min) (see Figure 2), Q = blood or dialysate flow (L/min), Δt = t2-t1 and Ht = hematocrit.

Removal data are presented as average removal from plasma and dialysate (A):

$$A = (A_p + A_d)/2$$

Plasma calcium levels were corrected for albumin:

$$[Ca] = C_i + 0.02 \times (\text{normal albumin} - \text{plasma albumin})$$

Change in plasma iron levels across the dialyzer (measurements performed at the start and end of each experiment) were quantified:

$$\Delta C = ((C_i - C_o)_{t1} + (C_i - C_o)_{t2})/2$$

For investigation of the concentration-dependency of potassium and phosphate removal, cumulative 3 h removal was related to the average inlet plasma concentrations during the 3h experiment.
Data are shown as mean ± standard deviation. Linear interpolation was used for 13% of the acid-base measurements (7 out of 52) where data were missing. Missing data were all at 120 min, 1 per experiment for 7 experiments.

Statistical significance was determined using (paired) t-tests and linear regression analysis. For comparison of absolute removal, 1-way ANOVA testing with post hoc correction using Tukey's multiple comparisons test was applied.

RESULTS

Potassium and phosphate removal

Cumulative removal in 3h was 7.7±2.7 mmol potassium (N=14; 96±34 µmol/gram sorbent) at plasma [K^+] 4.1±0.9 mM (Figure 3.A) and 4.9±1.3 mmol phosphate (N=14; 123±32 µmol/gram sorbent) at plasma [PO_4^{3-}] 2.1±0.5 mM (Figure 3.B). Adsorption rate decreased gradually with an absolute potassium removal of 4.5±1.8 mmol in the 1st hour, 2.4±1.0 mmol in the 2nd hour and 0.8±0.7 mmol in the 3rd hour ($p<0.001$) and an absolute phosphate removal of 2.4±0.7 mmol in the 1st hour, 1.6±0.5 mmol in the 2nd hour and 0.8±0.4 mmol in the 3rd hour ($p<0.001$).

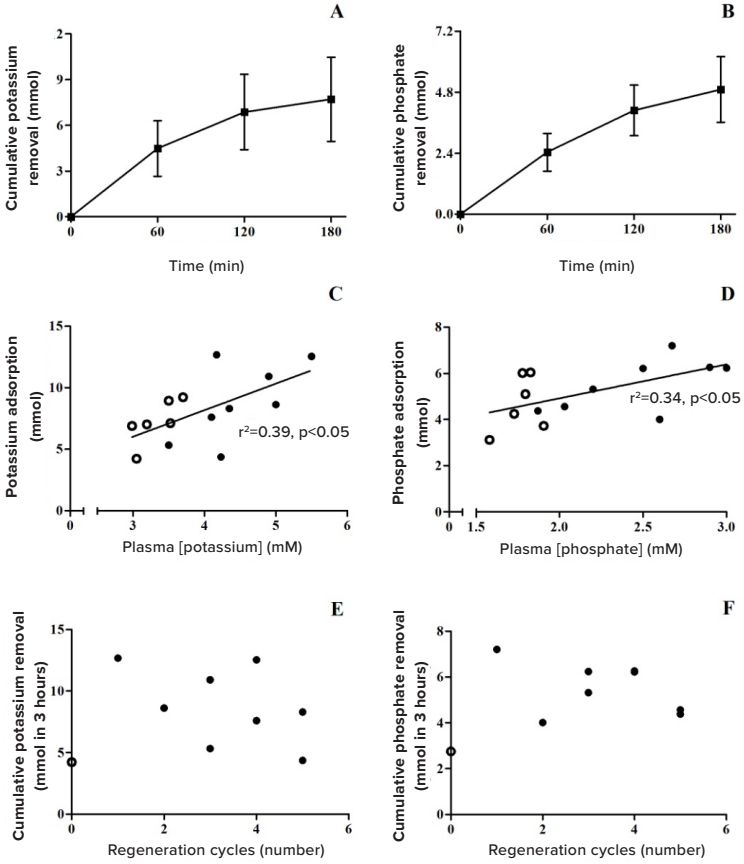


FIGURE 3. *In vivo* potassium and phosphate removal. Experiments with (solid dots) and without (open dots) potassium and phosphate infusion.A: Cumulative potassium removal (N=14, mean ±SD). B: Cumulative phosphate removal (N=14, mean ±SD). C: Cumulative potassium removal in 3h versus average plasma [K^+] upstream of the dialyzer (N=14). D: Cumulative phosphate removal in 3h versus average plasma [PO_4^{3-}] upstream of the dialyzer (N=14). E, F: Cumulative potassium (E) and phosphate (F) removal in 3h versus the number of regeneration cycles (N=9, p=0.96 with linear regression analysis for potassium, p=0.62 for phosphate).

Adsorption of both potassium and phosphate was clearly concentration-dependent (Figure 3.C,D). Linear regression analysis shows that with every mmol/L increase of plasma [K^+] or plasma [PO_4^{3-}] upstream of the dialyzer, 3 h removal increases with roughly 2.6 mmol potassium or 2.1 mmol phosphate, respectively.

Overall, 3 h adsorption capacity during consecutive dialysis sessions did not show a significant decline after regeneration and re-use of the same adsorption cartridge (Figure 3.E, F), although variation in potassium and phosphate concentrations might mask a small decrease in binding capacity with increasing regeneration cycles.

Influence on calcium and magnesium concentrations and balance

When equilibrating the sorbents at [Ca^{2+}] 1.2 mM (N=6) and [Mg^{2+}] 0.40-0.45 mM (N=7), systemic plasma concentrations of calcium and magnesium measured upstream of the dialyzer remained stable (Figure 4.A). A limited release of calcium was measured across the dialyzer (Figure 4.B: 0.52±0.46 mmol calcium in 3 h), although no significant differences were observed between average calcium concentrations up- and downstream of the dialyzer (plasma) or adsorption cartridge (dialysate) (Figure 4.C)**.** No net change in magnesium balance was detected in 3 h (Figure 4.B, D).

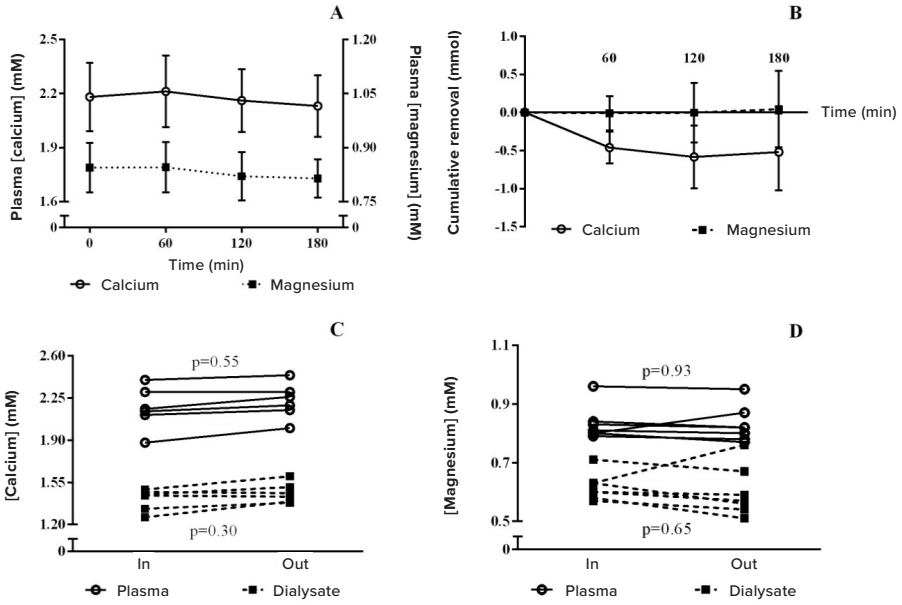


FIGURE 4. Calcium (N=6) and magnesium (N=7). A: Systemic plasma concentrations (mean ±SD, calcium: solid line, magnesium: dashed line) measured upstream of the dialyzer (p=0.54 with linear regression analysis for calcium, p=0.29 for magnesium). B: Cumulative removal (mean ±SD; calcium (solid line): p=0.02 versus zero with linear regression analysis**;** magnesium (dashed line): p=0.80 versus zero with linear regression analysis). C, D: Plasma and

dialysate calcium (C) and magnesium (D) concentrations averaged during the 3 h experiment up- and downstream ('In' and 'Out') of the dialyzer (plasma, solid line) and adsorption cartridge (dialysate, dashed line).

Influence on plasma sodium concentrations and balance

Rinsing solution with a sodium concentration of 120 mM (N=14), did not significantly change the systemic plasma [Na^+] during the experiments, and no net sodium release was detected (Figure 5.A-D).

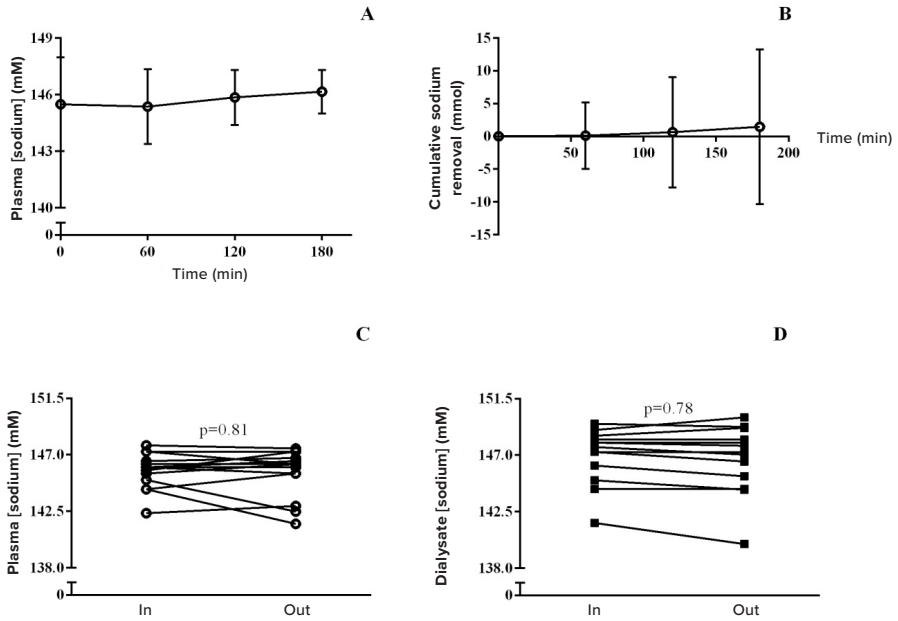


Figure 5. Sodium (N=14). A: Systemic plasma concentration, measured upstream of the dialyzer (mean ±SD, p=0.25 with linear regression analysis). B: Cumulative removal (mean ±SD, p=0.59 with linear regression analysis). C: Plasma concentrations averaged during the 3 h experiment up- and downstream ('In' and 'Out') of the dialyzer. D: Dialysate concentrations averaged during the 3 h experiment up- and downstream ('In' and 'Out') of the adsorption cartridge.

Influence on plasma iron concentrations and balance

There was no net adsorption of iron across the dialyzer and also no release from the iron-containing phosphate sorbent (average concentration change across the dialyzer (downstream minus upstream): -0.44±1.58 µmol/L, p=0.81). No iron was detected in the dialysate.

Influences on acid base status

The average arterial blood pH at the start of the experiments was 7.44±0.04 (N=13). Linear regression analysis did not show a significant change of arterial pH and plasma bicarbonate concentrations in time (Figure 6.A, B). While there was a net bicarbonate adsorption across the dialyzer when prerinsing without bicarbonate in the solution (supplemental Figure 4), no net release or adsorption was detected when the dialyzer was prerinsed with 15 mM bicarbonate (Figure 6.C, D, N=7).

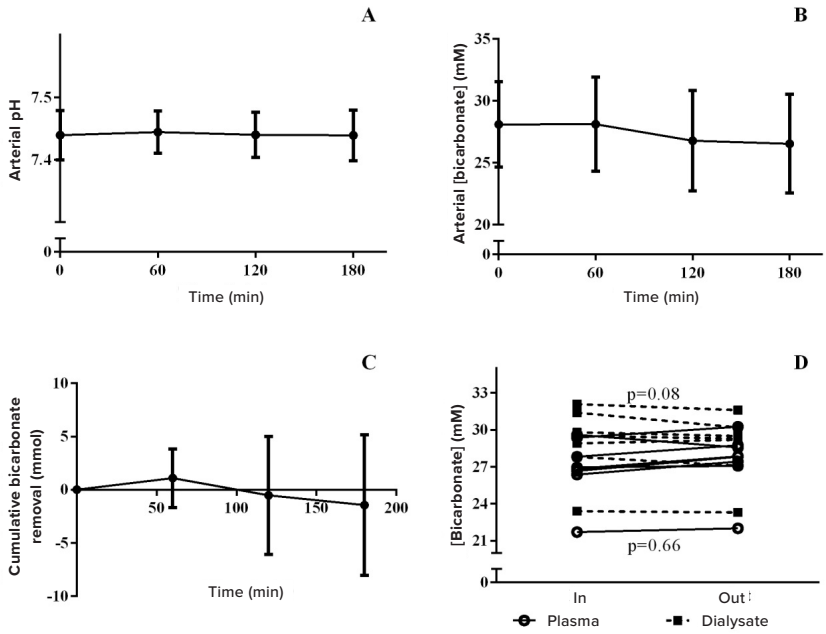


FIGURE 6. Acid-base effects. A: Arterial pH (N=13, mean±SD; p=0.89 with linear regression analysis). B: Arterial bicarbonate concentration (N=13, mean±SD; p=0.20 with linear regression analysis). C: Cumulative bicarbonate removal at [HCO_3^-] 15mM in the rinsing solution (N=7, mean±SD; p=0.43 with linear regression analysis). D: Bicarbonate concentrations averaged during the 3 h experiment up- and downstream ('In' and 'Out') of the dialyzer (plasma, solid line) and adsorption cartridge (dialysate, dashed line) at [HCO_3^-] 15 mM in the rinsing solution (N=7).

Vital parameters before, during and after dialysis

Other than a parallel rise in blood pressure and pulse rate, which was probably due to increasing sympathetic tone in the restrained goats during the course of the experiment (while the effect of the sedatives was wearing off), no relevant adverse effects were observed during the experiments (Supplemental table 2: Vital parameters before, during and after dialysis).

DISCUSSION

In this study, we demonstrate clinically relevant, plasma concentration-dependent removal of potassium and phosphate by a miniature dialysis device in awake non-uremic goats. Potassium and phosphate removal was achieved through adsorption from a dialysate circuit by a cartridge containing ion-exchangers (poly(styrene-divinylbenzene) sulphonate and FeOOH for potassium and phosphate removal, respectively). Net adsorption of calcium and magnesium by the ion exchangers was prevented by prerinsing with a calcium- and magnesium-containing solution. No relevant adverse effects were observed during the experiments.

Our study shows that a cartridge containing a mixture of 80 grams of poly(styrene-divinylbenzene) sulphonate and 40 grams of FeOOH beads can remove 7.7**±**2.7 mmol potassium (96±34 µmol/gram sorbent) at an average plasma [K^+] of 4.1±0.9 mM, and 4.9**±**1.3 mmol phosphate (123±32 µmol/gram sorbent) of phosphate at an average plasma [PO_4^{3-}] of 2.1 ±0.5 mM during three hours *in vivo*. These numbers are comparable to previously reported *in vitro* removal [14] (97±19 µmol potassium/gram sorbent at [K^+] 3.8±1.0 mM**;** 86 ±10 µmol phosphate/gram sorbent at [PO_4^{3-}] 1.8±0.4 mM)**.** Of note, we assumed a linear decrease in adsorption rate during the interval between two sampling times and might therefore have overestimated the adsorbed amount to some extent. Note that the 14 experiments reported in this study were performed on 2 goats (6 in one and 8 in the other). Saturation of the sorbents occurred, resulting in a potassium and phosphate removal of only ~1/5th and ~1/3rd in the third hour as compared to the first hour of treatment. To remove the daily load of potassium and phosphate (45 and 15 mmol respectively, assuming that the intake of patients dependent on dialysis is 25% less than the intake of healthy individuals [17-19]), extending the length of dialysis with the same adsorption cartridge will therefore not be very effective. Consequently, the amount of sorbent would have to be increased to achieve removal of the daily load. To compete with intermittent (3-weekly), 4-hours hemodialysis with a potassium and phosphate removal of ~63-80 and ~22-38 mmol per treatment [20,21], respectively, the total weight of the sorbents in the dialysis device would have to be increased to ~830-1160 g. It is of note that we have shown *in vitro* that saturated cartridges containing poly(styrene-divinylbenzene) sulphonate and FeOOH can be regenerated [14]. The present study indicates that repetitive use of the same adsorption cartridge also does not compromise removal capacity *in vivo*, thereby facilitating reuse and limiting costs. To reduce device weight, saturated intermediate size adsorption cartridges could be replaced by regenerated cartridges several times per day.

Currently, we use a rather large volume of aqueous solutions for the sterilization and re-

generation procedure (~6-6.5 L for sterilization, regeneration and dialysate), but this volume might be considerably reduced by using alternative sterilization techniques, optimizing the equilibration procedure and centralizing the procedure. The optimization of this procedure is the focus of future research and is also dependent on other components of the device (e.g. the currently applied electronic sensors are not compatible with gamma radiation). Nevertheless, there is already a large difference between the volumes currently needed for our device and those used in conventional hemodialysis (~120 L of dialysate per session plus varying amounts of fluid used for rinsing of the dialysis machine and reject fluid resulting from preparation of reverse osmosis water). Our methodology will be beneficial in terms of ecological and economic perspective for situations when water is scarce or expensive (e.g. in some developing countries).

Importantly, the binding capacity of the sorbents is highly concentration-dependent: per mmol plasma potassium and phosphate increment, the 3 h removal increases with 2.6 mmol and 2.1 mmol, respectively. This concentration-dependency is a valuable safety characteristic of the device, since this implies that the lower the concentration, less is removed and vice versa. Consequently, risks of extreme levels of potassium and phosphate are attenuated. Based upon this predictive relationship, expected removal and its effect on plasma concentrations can be calculated at the start of each dialysis session, making this an intrinsically safe modus of operation.

Both poly(styrene-divinylbenzene) sulphonate and FeOOH can bind significant amounts of calcium and magnesium. Equilibrating the sorbents with a rinsing solution containing relatively high calcium and magnesium concentrations was shown to prevent this absorption *in vitro*, even inducing some release of these electrolytes [14]. In the current *in vivo* study, equilibrating the sorbents with a rinsing solution with lower calcium and magnesium concentrations (close to the respective ionized plasma concentrations) resulted in negligible calcium release and prevention of net magnesium release and adsorption. Interestingly, our results suggest that the net balance of calcium and magnesium could be regulated in an individualized fashion by altering their concentration in the rinsing solution. For example, a net negative calcium balance may be induced in patients using calcium-containing phosphate binders by using a rinsing solution with a low calcium concentration. *In vitro* experiments showed that calcium- and magnesium-prerinsing did not affect potassium-binding capacity of the sorbents (data not shown).

In vitro, we found that poly(styrene-divinylbenzene) sulphonate binds potassium in exchange for sodium in an equimolar fashion [14]. Equilibrating the adsorption cartridge at a low sodium concentration resulted in no net measurable change of in- and outlet plasma

and dialysate sodium concentrations during the 3 h *in vivo* experiments; the mechanism is unclear. We hypothesize that net sodium release, resulting from exchange of potassium for sodium, is prevented by simultaneous exchange of sodium for hydrogen. Hydrogen may subsequently combine with hydroxyl ions (released in exchange for phosphate from the FeOOH beads) to form water, or with bicarbonate to form water and carbon dioxide. The latter would partly explain bicarbonate removal (prevented by prerinsing with bicarbonate) [8-10,22].

The phosphate sorbent FeOOH could theoretically release iron into the dialysate and the patient. The findings that the plasma dialyzer inlet and outlet iron concentrations did not change and no iron was detected in the dialysate argue against significant iron release by the adsorption cartridge. Whether significant changes in iron stores occur after long-term use of the sorbent and exposure to trace concentrations of iron remains to be determined. Some of the potassium or phosphate sorbents tested in our *in vitro* experiments had either marked acidifying (zirconium phosphate and carbonate) or alkalizing (zeolite, sevelamer carbonate) effects whereas the two sorbents used in the current study were shown to be acid-base neutral [14]. However, the *in vivo* study showed that bicarbonate was adsorbed, probably by FeOOH, which was not detected in the *in vitro* studies because bicarbonate-containing solutions were not applied in the static binding tests. The bicarbonate removal could be prevented by equilibrating the sorbents with bicarbonate 15 mM.

Our sorbent unit may have some advantages over the (modified) REDY system, that is currently the only dialysate regenerating system applied in trials with wearable dialysis devices [8-10,12,13,22]. Although the binding capacity of the REDY sorbents (zirconium salts) is higher than that of our sorbents, our sorbents can be regenerated, which reduces overall costs of the miniature dialysis device. As shown in our *in vitro* experiments, the sorbents of the REDY system cannot be easily regenerated [14]. Furthermore, because zirconium phosphate removes all calcium and magnesium, the REDY system relies on post-cartridge infusion of these electrolytes to maintain stable plasma levels. As described, there is no adsorption of calcium and magnesium in our device when prerinsing with these electrolytes and thus no need for reinfusion.

To offer a realistic alternative for regular dialysis, additional techniques would have to be incorporated in this miniature dialysis device to remove other toxins, such as activated carbon for organic waste solutes and possibly electro-oxidation for urea removal [15]. In addition, it will require much effort to translate these promising *in vivo* results to safe application of the selected sorbents in a wearable dialysis device in patients. The familiarity of clinicians with the nature of the sorbents with a low toxicity profile in the current study

should facilitate acceptance for clinical purposes [14,23].

Conclusions

Clinically relevant potassium and phosphate removal from plasma was achieved *in vivo* by use of 80 grams of poly(styrene-divinylbenzene) sulphonate and 40 grams of iron oxide hydroxide beads. Removal is concentration-dependent which can be regarded as an intrinsic safety feature of the device, facilitating introduction in maintenance dialysis. Net calcium and magnesium adsorption can be prevented by prerinsing the sorbents with these electrolytes, thereby eliminating the need for extra infusion. Importantly, this miniature dialysis system shows no sodium release *in vivo* and there are no effects on arterial pH and bicarbonate levels if the sorbents are prerinsed with bicarbonate.

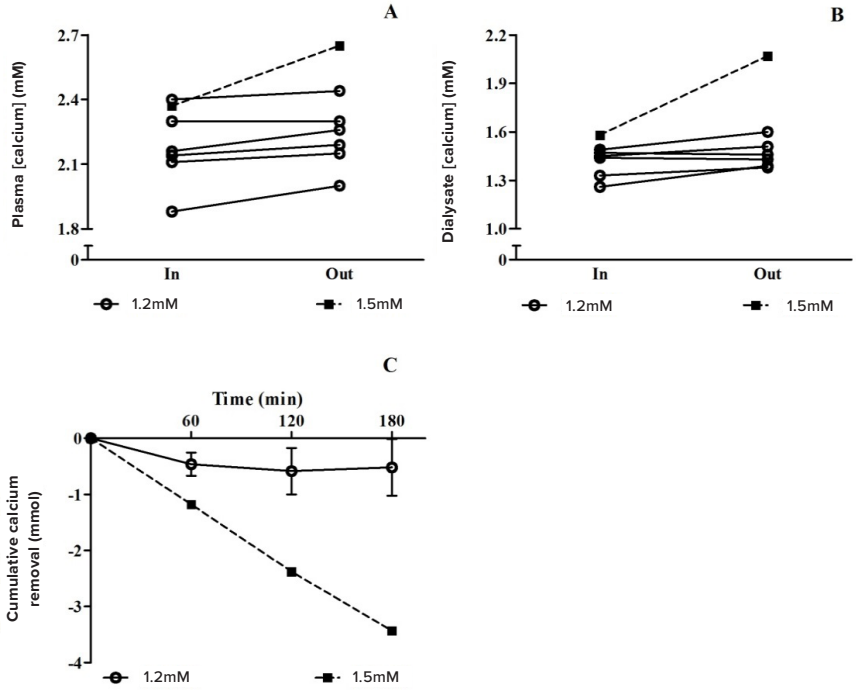
REFERENCES

1. Blumberg A, Roser HW, Zehnder C, Muller-Brand J. Plasma potassium in patients with terminal renal failure during and after haemodialysis; relationship with dialytic potassium removal and total body potassium. *Nephrol Dial Transplant* 1997; 12: 1629-1634
2. Haas T, Hillion D, Dongradi G. Phosphate kinetics in dialysis patients. *Nephrol Dial Transplant* 1991; 6 Suppl 2: 108-113
3. Cupisti A, Kalantar-Zadeh K. Management of natural and added dietary phosphorus burden in kidney disease. *Semin Nephrol* 2013; 33: 180-190
4. Fouque D, Horne R, Cozzolino M, Kalantar-Zadeh K. Balancing nutrition and serum phosphorus in maintenance dialysis. *Am J Kidney Dis* 2014; 64: 143-150
5. Tentori F, Zhang J, Li Y *et al*. Longer dialysis session length is associated with better intermediate outcomes and survival among patients on in-center three times per week hemodialysis: results from the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Nephrol Dial Transplant* 2012; 27: 4180-4188
6. Mucsi I, Hercz G, Uldall R, Ouwendyk M, Francoeur R, Pierratos A. Control of serum phosphate without any phosphate binders in patients treated with nocturnal hemodialysis. *Kidney Int* 1998; 53: 1399-1404
7. Achinger SG, Ayus JC. The role of daily dialysis in the control of hyperphosphatemia. *Kidney Int Suppl* 2005; S28-S32
8. Ash SR. Sorbents in treatment of uremia: a short history and a great future. *Semin Dial* 2009; 22: 615-622
9. Davenport A, Gura V, Ronco C, Beizai M, Ezon C, Rambod E. A wearable haemodialysis device for patients with end-stage renal failure: a pilot study. *Lancet* 2007; 370: 2005-2010
10. Lee DB, Roberts M. A peritoneal-based automated wearable artificial kidney. *Clin Exp Nephrol* 2008; 12: 171-180
11. McGill RL, Bakos JR, Ko T, Sandroni SE, Marcus RJ. Sorbent hemodialysis: clinical experience with new sorbent cartridges and hemodialyzers. *ASAIO J* 2008; 54: 618-621
12. Murisasco A, Baz M, Boobes Y *et al*. A continuous hemofiltration system using sorbents for hemofiltrate regeneration. *Clin Nephrol* 1986; 26 Suppl 1: S53-S57
13. Ronco C, Fecondini L. The Vicenza wearable artificial kidney for peritoneal dialysis (ViWAK PD). *Blood Purif* 2007; 25: 383-388
14. Wester M, Simonis F, Gerritsen KG *et al*. A regenerable potassium and phosphate sorbent system to enhance dialysis efficacy and device portability: an in vitro study. *Nephrol Dial Transplant* 2013; 28: 2364-2371
15. Wester M, Simonis F, Lachkar N *et al*. Removal of urea in a wearable dialysis device: a reappraisal of electro-oxidation. *Artif Organs* 2014; 38: 998-1006
16. Beal AM, Budtz-Olsen OE, Clark RC, Cross RB, French TJ. Renal and salivary responses to infusion of potassium chloride, bicarbonate and phosphate in Merino sheep. *Q J Exp Physiol Cogn Med Sci* 1973; 58: 251-265
17. Mente A, O'Donnell MJ, Rangarajan S *et al*. Association of urinary sodium and potassium excretion with blood pressure. *N Engl J Med* 2014; 371: 601-611
18. Therrien M, Byham-Gray L, Denmark R, Beto J. Comparison of dietary intake among women on

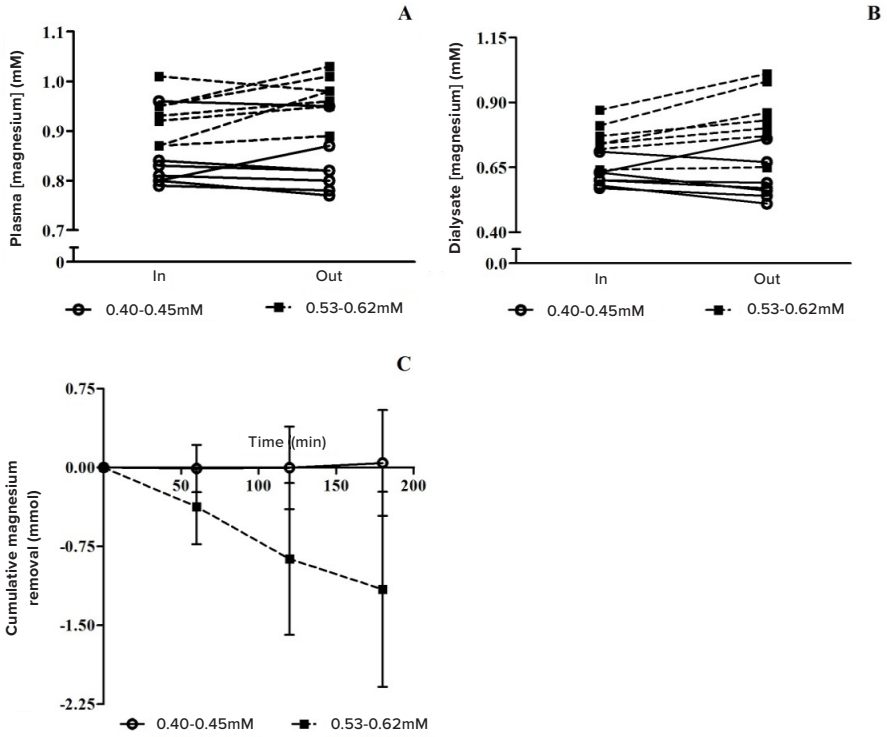
maintenance dialysis to a Women's Health Initiative cohort: results from the NKF-CRN Second National Research Question Collaborative Study. *J Ren Nutr* 2014; 24: 72-80

19. Vervloet MG, van Ittersum FJ, Buttler RM, Heijboer AC, Blankenstein MA, ter Wee PM. Effects of dietary phosphate and calcium intake on fibroblast growth factor-23. *Clin J Am Soc Nephrol* 2011; 6: 383-389
20. De NL, Bellizzi V, Minutolo R *et al*. Effect of dialysate sodium concentration on interdialytic increase of potassium. *J Am Soc Nephrol* 2000; 11: 2337-2343
21. Wang M, Li H, Liao H *et al*. Phosphate removal model: an observational study of low-flux dialyzers in conventional hemodialysis therapy. *Hemodial Int* 2012; 16: 363-376
22. Fuchs C, Dorn D, Rieger J, van Doorn AW, Striebel JP, Scheler F. Capabilities of the Redy cartridge for regeneration of hemofiltrate. *Artif Organs* 1979; 3: 279-280
23. Floege J, Covic AC, Ketteler M *et al*. Long-term effects of the iron-based phosphate binder, sucroferric oxyhydroxide, in dialysis patients. *Nephrol Dial Transplant* 2015; 30: 1037-1046

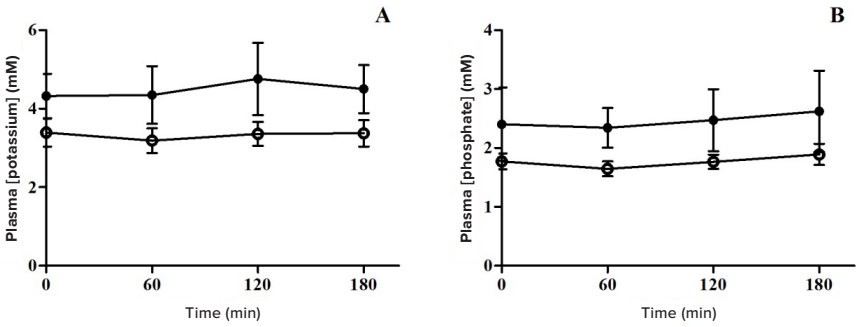
SUPPLEMENTAL FIGURES



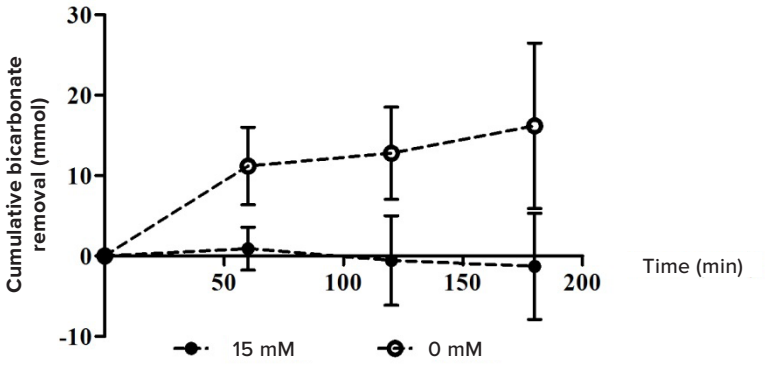
SUPPLEMENTAL FIGURE 1. Influence on calcium concentrations and removal/release at [Ca^{2+}] 1.2 mM and 1.5 mM in the rinsing solution. A, B: Plasma and dialysate concentrations averaged during the 3 h experiment up- and downstream ('In' and 'Out') of the dialyzer (plasma, A) and adsorption cartridge (dialysate, B). C: Cumulative removal/release (mean ±SD).



SUPPLEMENTAL FIGURE 2. Influence on magnesium concentrations and removal/release at [Mg^{2+}] 0.40-0.45 mM and 0.53-0.62 mM in the rinsing solution. A, B: Plasma and dialysate concentrations averaged during the 3h experiment up- and downstream ('In' and 'Out') of the dialyzer (plasma, A) and adsorption cartridge (dialysate, B). C: Cumulative removal/release (mean ±SD).



SUPPLEMENTAL FIGURE 3. *In vivo* plasma potassium and phosphate concentrations upstream of the dialyzer (mean ±SD). Experiments with (solid dots) and without (open dots) potassium and phosphate infusion. A: Potassium (N=8 with, N=6 without potassium infusion). B: Phosphate (N=8 with, N=6 without phosphate infusion).



SUPPLEMENTAL FIGURE 4. Cumulative HCO_3^- removal without HCO_3^- (open dots) and with [HCO_3^-] 15 mM (solid dots) in the rinsing solution (mean ±SD).

SUPPLEMENTAL TABLES

SUPPLEMENTAL TABLE 1. Composition of the rinsing solution per experiment. Sorbent batch 1 was used for experiments 1-5 and sorbent batch 2 for experiments 6-14.

Experiment	Composition rinsing solution (mM)			
	[NaCl]	[$NaHCO_3^-$]	[$CaCl_2$]	[$MgCl_2$]
1-6	120	0	1.5	0.53-0.62
7	120	0	1.5	0.45
8-14	105	15	1.2	0.40-0.45

SUPPLEMENTAL TABLE 2. Vital parameters before, during and after dialysis.

	Pre-dialysis	During dialysis	After dialysis
SBP (mmHg)	114±17	119±12 *	124±10 *
DBP (mmHg)	75±12	84±10 ***	87±11 **
Pulse rate (beats/min)	70±16	85±27 ***	115±21 $$$
Oxygen saturation (%)	97±4	98±3	97±5

Vital parameters (mean±SD) before (pre-dialysis), during and after dialysis.

SBP = systolic blood pressure. DBP = diastolic blood pressure. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ versus pre-dialysis; $$$ $p<0.001$ versus pre-dialysis and versus during dialysis.

PART II

Urea removal by electro-oxidation

4

Removal of urea in a wearable dialysis device: a reappraisal of electro-oxidation

Maarten Wester, Frank Simonis, Nadia Lachkar, Will K. Wodzig, Frank Meuwissen, Jeroen P. Kooman, Walther H. Boer, Jaap A. Joles and Karin G. Gerritsen

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ABSTRACT

Background

A major challenge for a wearable dialysis device is removal of urea, since urea is difficult to adsorb while daily production is very high. Electro-oxidation (EO) seems attractive since electrodes are durable, small and inexpensive. We studied efficacy of urea oxidation, generation of chlorine by-products and their removal by activated carbon (AC).

Methods

EO-units were designed. Three electrode materials (platinum, ruthenium oxide and graphite) were compared in single pass experiments using a urea in saline solution. Chlorine removal by AC in series with EO by graphite electrodes was tested. Finally, urea-spiked bovine blood was dialyzed and dialysate was recirculated in a dialysate circuit with AC in series with an EO-unit containing graphite electrodes.

Results

Platinum electrodes degraded more urea (21±2 mmol/h) than ruthenium oxide (13±2 mmol/h) or graphite electrodes (13±1 mmol/h). Chlorine generation was much lower with graphite (13±4 mg/h) than with platinum (231±22 mg/h) or ruthenium oxide electrodes (129±12 mg/h). Platinum and ruthenium oxide electrodes released platinum (4.1 (3.9–8.1) umol/h) and ruthenium (83 (77–107) nmol/h), respectively. AC potently reduced dialysate chlorine levels to <0.10 mg/L. Urea was removed from blood by EO at constant rate (9.5±1.0 mmol/h).

Conclusion

EO by graphite electrodes combined with AC shows promising urea removal and chlorine release complying with AAMI standards and may be worth further exploring for dialysate regeneration in a wearable system.

INTRODUCTION

The concept of a wearable dialysis device is based on a closed-loop system that continuously regenerates a small volume of dialysate. A major challenge is removal of urea, the main nitrogenous waste product and the toxin with the highest daily production. Although urea is often considered a relatively harmless uremic retention solute, there is ample evidence that urea can exert toxic effects [1-11]. Consequently, effective urea removal is crucial for successful dialysate regeneration.

Electro-oxidation technology (EO) seems attractive for urea removal in a wearable dialysis device since it can be miniaturized, needs no regeneration and is inexpensive. Urea can be oxidized either directly at the anode (Figure 1, reaction 1) [12,13], or indirectly in the bulk solution by anodically generated hypochlorite (reactions 2-4). However, toxic chlorine species, comprising free and bound chlorine, may accumulate during EO. Free chlorine is formed by the reaction of chlorine with water, the most prominent being hypochlorite. Bound chlorine is formed by the reaction of chlorine with organic molecules or ammonia, the most prominent being chloramines (i.e. chlorine bound to nitrogen).

$$(NH_2)_2CO + H_2O \longrightarrow N_2 + CO_2 + 6H^+ + 6e^- \quad (1)$$

$$2Cl^- \longrightarrow Cl_2\uparrow + 2e^- \quad (2)$$

$$Cl_2 + H_2O \longrightarrow HOCl + HCl \quad (2)$$

$$(NH_2)_2CO + 3OCl^- \longrightarrow N_2\uparrow + CO_2\uparrow + 3Cl^- + 2H_2O \quad (2)$$

FIGURE 1. Chemical equations involved in complete oxidation of urea during electro-oxidation of dialysate. Reaction 1: direct oxidation at the anode. Reaction(s) 2: indirect oxidation in the bulk solution. Oxidation of chloride ions at the anode yields chlorine gas [44]. Chlorine gas reacts with water yielding hypochlorous acid (HOCl). Urea is oxidized by hypochlorite (OCl^-) [3].

In the 1970's and 80's several groups explored electrochemical dialysate regeneration *in vitro* and demonstrated efficient urea degradation [12,14-22]. Remarkably, although results of these studies and one later study [13] seemed promising, the method has never been applied in clinical practice. The reason for discontinuation of this line of research has not been reported, but may be related to generation of toxic chlorine species, metal leaching from electrodes, or development of acidosis [15].

In the present study, we re-explored electrochemical urea degradation in dialysate regeneration. First, we performed a direct comparison of different electrode materials, not

only with respect to their urea degradation capacity, but also regarding their potential to generate toxic chlorine species. Platinum, ruthenium and graphite electrodes were selected because they have demonstrated effective urea degradation [12-15,17-23]. Second, we studied the influence of dialysate urea concentration and current applied in the EO unit on the rate of urea degradation and chlorine generation. Third, we tested whether dialysate chlorine levels can be reduced by use of activated carbon (AC), a potent electron donor and therefore potential reductor of these species [24]. Finally, to simulate the *in vivo* situation, we designed a prototype dialysis device and tested its efficacy of urea removal and its effect on the acid base and redox state in bovine blood spiked with urea.

MATERIALS AND METHODS

Platinum-coated titanium (platinum) electrodes and ruthenium mixed metal oxide on titanium (ruthenium) electrodes were purchased from Magneto Special Anodes (Vlaardingen, The Netherlands). Graphite electrodes were provided by Nanodialysis (Oirschot, The Netherlands). 'EO units' were designed, each containing ten electrodes with a cumulative electrode surface of 585 cm^2 per EO unit. The units also contained poly(styrene-divinylbenzene) sulphonate and iron oxide hydroxide beads for respective removal of potassium and phosphate from dialysate as described [25]. Static tests showed that these beads did not bind urea and that replacing them by chemically inert beads (poly(styrene-divinylbenzene) beads; Chem-Impex International Inc., Wood Dale, IL) did not affect urea removal (experiments not shown). A degassing unit and activated carbon filters were provided by Nanodialysis. Polyflux 2H dialyzers (0.2 m2) were purchased from Gambro Dialysatoren GmbH (Hechingen, Germany) and Thomas peristaltic pumps from Gardner Denver Thomas (Sheboygan, WI). Urea was obtained from Sigma (Zwijndrecht, The Netherlands).

Comparison of electrode materials

A saline solution containing 20 mM urea was pumped at 50 mL/min in a single pass configuration through the units equipped with either platinum, ruthenium or graphite electrodes (Figure 2.A). A current of 3 A was applied because previous research showed that this leads to minimal hypochlorite accumulation [13]. Samples were drawn downstream of the EO unit after 60 min. Pilot studies demonstrated that urea degradation rate stabilized within five minutes and remained constant thereafter. Urea and chlorine concentrations were measured. In experiments with the metal electrodes, platinum and ruthenium concentrations were also measured. Urea removal was calculated as follows:

$$\text{Amount removed (mmol/h)} = (C_r - C_e) \times S \quad (1)$$

Urea removal was also expressed in mmol/hour/dm^2/A for comparison with previous reports, where dm^2 is electrode surface and A is current through the whole system.
Platinum and ruthenium release was calculated as follows:

$$\text{Amount released (nmol/h)} = (C_e - C_r) \times S \quad (2)$$

where C_r and C_e are the concentrations (mM) before and after the EO unit respectively; S is the pump speed (L/h).

Free and bound chlorine release was calculated as follows:

$$\text{Amount measured (mg/h)} = C_c \times S \quad (3)$$

where C_c is the concentration (mg/L) after the EO unit; S is the pump speed (L/h).

Concentration and current dependency

Graphite electrodes were selected for further testing as motivated in the discussion section. To assess whether urea removal and chlorine generation were dependent on input urea concentration or current, the experiments described above were repeated at different urea concentrations (5, 10, 20 and 30 mM) and at different currents (2, 3, 4 and 5 A). Efficiency was defined as the ratio of the amount of urea that was removed by EO to the total amount of urea that was delivered to the electrodes.

Removal of chlorine by activated carbon

At 20 mM urea and 3 A, five AC filters, each containing 15 mL (7.5 g) of AC, were placed in series downstream of the EO unit to remove chlorine. Each AC filter consists of a tube with a diameter of 1.7 cm and a length of 12.0 cm containing AC granules with a bead diameter of 0.1-0.5 mm. The dry and wet weight of each AC column were 12.5 and 22 g respectively. Chlorine concentrations were measured downstream of the EO unit and after each AC filter at t= 60 min.

Urea removal in urea-spiked bovine blood

To simulate the *in vivo* situation, a prototype dialysis device was designed (Figure 2.B) consisting of a blood and a dialysate circuit (200 mL) separated by a dialyzer. The dialysate circuit contained an EO unit with graphite electrodes and two 50 mL (~25 g) AC columns in series, downstream of the EO unit. Each AC filter consists of a tube with a diameter of 3.0 cm and a length of 11.5 cm. An additional 60 mL (~30 g) AC was incorporated in the EO unit. A pressure-dependent degassing unit downstream of the EO unit allowed gasses formed during EO to escape. Three batches of bovine blood (2.0-3.0 L) spiked with urea to a uremic concentration of ~20 mM were re-circulated over the dialyzer (~110 mL/min) for 3 hours. The reservoir was stirred continuously with a magnetic stirrer. Dialysate was pumped in counter current direction through the dialyzer (~50 mL/min). A current of 3 A was applied to the EO unit. Every 30 min the electrode polarity was inversed to eliminate deposits (e.g. Mg salts) that may form on the electrodes [13]. Experiments were also performed without EO (current of 0 A) to determine whether adsorption to AC contributed to urea removal. Samples were drawn directly from the reservoir at t= 0, 60, 120 and 180 min. At 60 and 120 min the estimated amount of removed urea was added to the reservoir to mimic movement of urea from tissue compartments to blood plasma. Total urea removed per hour was calculated as follows:

$$\text{Amount removed (mmol)} = (C_b - C_s) \times V \qquad (4)$$

C_b and C_s are the concentrations (mM) in the reservoir at the start and the end, respectively, of each hour during the experiment; V is the batch volume (L).

Chlorine levels were measured hourly downstream of the AC cartridge in the dialysate circuit when EO was applied. pH, pCO_2, bicarbonate and oxidation reduction potential (ORP) were measured hourly in blood samples from the reservoir. All experiments were performed at room temperature.

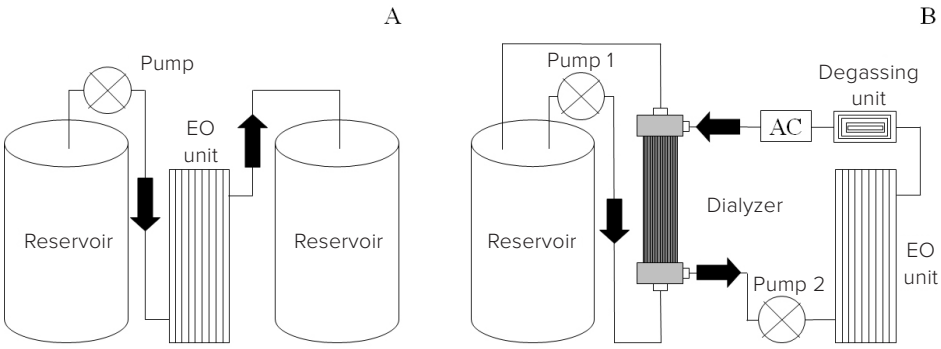


FIGURE 2. A: Dialysis circuit in single pass configuration, consisting of 2 3 L reservoirs, 1 pump (50 mL/min) and an electro-oxidation (EO) unit containing 10 electrodes. B: Dialysis circuit in recirculation configuration, consisting of a 3 L reservoir, 2 pumps (speed of pump 1: 110 mL/min and of pump 2: 50 mL/min), a dialyzer, an EO unit, a degassing unit and 5 activated carbon filters (AC) in series, each containing 15 mL (7.5 g) AC.

Analyses

Free and total chlorine concentrations were measured using a ChloroSense Chlorine Meter (CS 100 Palintest Ltd, Gateshead, UK). Bound chlorine levels were defined as the calculated difference between free and total chlorine. Urea was determined with Urea CT FT (DiaSys, Holzheim, Germany). Platinum and ruthenium were measured by Inductive Coupled Plasma - Mass Spectrometry (XSeries 2 ICP-MS; ThermoFisher Scientific, Breda, The Netherlands). pH, pCO_2 and bicarbonate were measured using a blood gas analyzer (Rapidlab type 1265; Siemens Medical Solutions Diagnostics B.V., Breda, The Netherlands). Oxidation-reduction potential (ORP) was measured using an Ultrameter II (6Psi; Myron L Company, Carlsbad (CA)).

Statistical analysis

All experiments were performed in triplicate. Data are shown as mean ± standard deviation. Statistical significance was determined using Sigmastat 11.0 one-way ANOVA for grouped analyses and (paired) t-tests for subgroup analyses.

RESULTS

Comparison of electrode materials

Platinum electrodes degraded more urea (21.3±2.4 mmol/h; 7.1±0.8 mmol/h/dm^2/A) than ruthenium (13.3±2.1 mmol/h; 4.4±0.7 mmol/h/dm^2/A) or graphite electrodes (13.2±1.2 mmol/h; 4.4±0.4 mmol/h/dm^2/A). Bound chlorine release was ten-fold lower with graphite electrodes (13±4 mg/h; 1.0±0.3 mg/mmol urea) than with ruthenium electrodes (129±12 mg/h; 9.8±0.8 mg/mmol urea) (Figure 3). Platinum electrodes caused the highest bound chlorine release (231±22 mg/h; 10.9±0.7 mg/mmol urea). Free chlorine release showed a similar pattern (21.5±0.8, 30±0.0 and 0.6±0.2 mg/h for platinum, ruthenium and graphite electrodes, respectively). Both platinum and ruthenium electrodes released metal ions, (4.1 (3.9 – 8.1) µmol/h of platinum and 83.1 (77.2 – 106.9) nmol/h of ruthenium, respectively). Graphite electrodes were selected for further testing.

FIGURE 3. Urea removal (mmol/h; left y-axis) and chlorine release (mg/h; right y-axis) at 3 A and 20 mM urea inlet concentration.**p<0.01 (urea), ***p<0.001 (free and bound chlorine) versus ruthenium and graphite; ###p<0.001 versus graphite (free and bound chlorine).

Concentration and current dependency

Urea degradation rate increased with increasing inlet urea concentration (up to 20 mM) while efficiency decreased (Figure 4.A). Urea removal efficiency did not exceed 35% in any of the experiments. Bound chlorine release was not affected by inlet urea concentration (Figure 4.B) resulting in a decrease in the ratio of bound chlorine release to urea degradation with increasing urea concentration (up to 20 mM). Free chlorine release decreased

with increasing urea concentration (up to 20 mM).

Higher current resulted in increased urea degradation rate and efficiency and higher free and bound chlorine release (Figure 4.C, D). The ratio of free as well as bound chlorine release to urea degradation was lowest at 2-3 A.

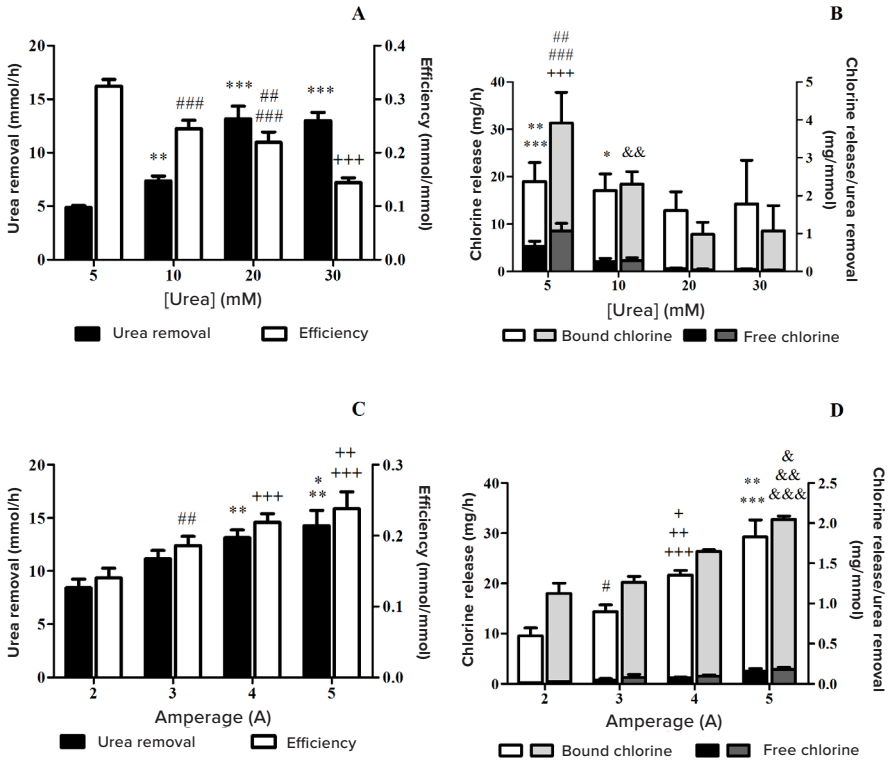


FIGURE 4. Influence of dialysate urea concentration and current on urea removal rate and chlorine release. A: Urea removal with increasing inlet concentration of urea at 3 A (left y-axis) and urea removal efficiency (mmol urea removed/mmol urea delivered per hour; right y-axis). ***p<0.001 versus 5 and 10 mM; **p<0.01 versus 5 mM; +++p<0.001 versus 5, 10 and 20 mM; ###p<0.001, ##p<0.05 versus 5 mM. B: Free and bound chlorine release with increasing inlet concentrations of urea at 3 A (left y-axis) and ratio of chlorine release versus urea removal (right y-axis). Free chlorine: ***p<0.001, *p<0.05 versus 20 and 30 mM; **p<0.01 versus 10 mM; +++p<0.001 versus 10, 20 and 30 mM; Bound chlorine: ###p<0.001, &&p<0.01 versus 20 and 30 mM; ##p<0.01 versus 10 mM. C: Urea removal with increasing electrical current at 20 mM urea inlet concentration (left y-axis) and urea removal efficiency (mmol urea removed/mmol urea delivered per hour; right y-axis). **p<0.01 versus 2 A; *p<0.05 versus 3 A; +++p<0.001 versus 2 A; ++p<0.05 versus 3 A; ##p<0.05 versus 2 A. D: Free and bound chlorine release with increasing electrical current at 20 mM urea inlet concentration (left y-axis) and ratio of chlorine release versus current (right y-axis). ***p<0.001 versus 2 and 3 A (free and bound chlorine); **p<0.01 versus 4 A (free and bound chlorine); +++p<0.001

versus 2 A (bound chlorine); ++p<0.01 versus 3 A (bound chlorine), +p<0.05 versus 2 A (free chlorine); #p<0.05 versus 2 A (bound chlorine); &&&p<0.001 versus 2 A (free and bound chlorine), versus 3 A (bound chlorine); &&p<0.01 versus 3 A (free chlorine), versus 4 A (bound chlorine); &p<0.05 versus 4 A, @@p<0.01 versus 3 A (bound chlorine), @p<0.05 versus 2 A (free chlorine).

Chlorine removal by activated carbon

Bound chlorine levels were significantly reduced by AC (Figure 5). With 60 mL (~30 g) AC, bound chlorine levels in the dialysate were reduced from 3.26±0.56 to 0.08±0.08 mg/L (p<0.001) and free chlorine from 0.21±0.10 to 0.10±0.02 mg/L (p=0.13). Additional amounts of AC did not further reduce chlorine levels.

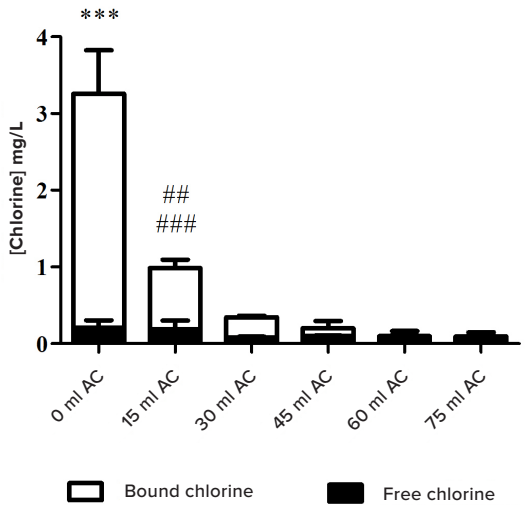


FIGURE 5. Chlorine concentration by use of increasing amounts of activated carbon (AC) filters in series at 20 mM urea inlet concentration. Bound chlorine: ***p<0.001 versus 15, 30, 45, 60 and 75 mL AC; ###p<0.001 versus 60 and 75 mL AC; ##p<0.01 versus 30 and 45 mL AC.

Urea removal from urea-spiked bovine blood

The urea degradation rate was constant at 9.5±1.0 mmol/h, which is 71% of the amount removed in the single pass experiments. Substantial urea adsorption by AC was only observed in the first hour (8.7±1.0 mmol in the 1st h, 9.1±1.6 mmol in 3 hrs; Figure 6.A). Chlorine levels in the dialysate downstream of the AC cartridge did not exceed 0.10 mg/L. Blood pH decreased from an initial value of 7.31±0.10 to 7.02±0.04 after 3 hours (Figure 6.B,

p=0.01). According to the Henderson-Hasselbalch equation, the pH drop during EO could be fully accounted for by measured increase in pCO_2. There was no significant change in blood bicarbonate concentration (+0.33±2.6 mmol/L at 180 versus 0 minutes, p=0.85). The blood oxidation-reduction potential decreased ~200 mV during the first hour and stabilized thereafter (Figure 6.C).

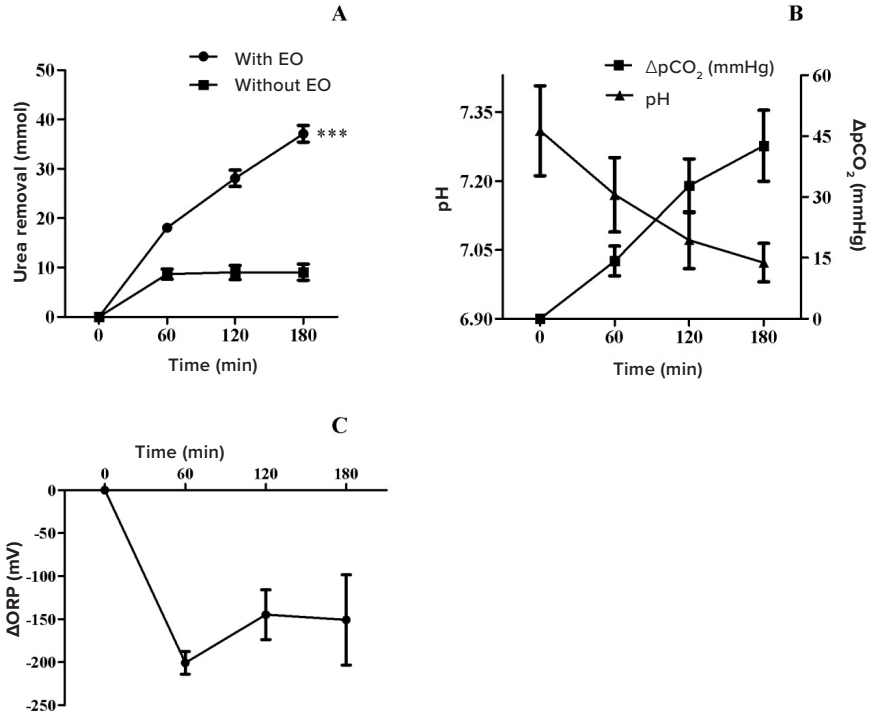


FIGURE 6. Electro-oxidation (EO) by graphite electrodes in combination with activated carbon in urea-spiked bovine blood. A: Cumulative urea removal (mmol) with EO (3 A) and without EO. ***p<0.001 versus without EO. B: pH and pCO_2 effects of EO at 3 A. C: Difference between measured and initial oxidation-reduction potential (Δ ORP) of bovine blood during EO (3 A).

DISCUSSION

In this study, we demonstrate that EO using graphite electrodes incorporated in a miniature dialysis system designed to regenerate dialysate showed clinically relevant urea removal from urea-spiked blood *in vitro*. The addition of an AC cartridge in the dialysate circuit reduced toxic chlorine levels to below the recommended maximum for conventional hemodialysis and prevented any detrimental effect of EO on the redox state of the blood.

Effective urea removal is crucial for successful dialysate regeneration when designing portable or wearable dialysis systems, since it is the main nitrogenous waste product in humans and the uremic toxin with the highest daily production (~350-550 mmol in healthy subjects, ~200 mmol in patients with advanced chronic kidney disease (CKD)) [26-28]. Urea is often considered a harmless substance, but at high plasma levels it undoubtedly has toxic effects [1-11]. Gradually increasing urea to above 50 mM in dialysis patients, by adding urea to dialysate, induced uremic symptoms [3]. High urea concentrations can disturb protein function [6], inhibit Na-K-2Cl co-transport in human erythrocytes [4], inhibit inducible nitric oxide synthase in macrophages [5] and induce oxidative stress in adipocytes leading to insulin resistance [2]. High urea influx into the gastrointestinal tract contributes to CKD-induced disruption of the intestinal barrier [7,8]. It seems therefore important to pursue an adequate urea removal strategy.

Unfortunately, in contrast to other organic waste compounds, urea binds poorly to sorbents (affinity for AC ~0.1 mmol/g) [29]. As an alternative for removal of urea by adsorption, the hydrolysis of urea by urease into ammonium and carbonate has been applied in the REDY system (REcirculating DialYsis) [30-32]. A disadvantage of this system is that large amounts of cation exchange material (>0.5 kg) are required to remove the generated ammonium (~0.8 moles/day) [30]. In addition, ammonium is partially exchanged for sodium, resulting in high sodium release (~2-4 g/day) [33], which complicates body fluid and blood pressure management. Furthermore, urease cartridges showed aluminum release in the past and are costly [34,35]. Consequently, this system has found only very limited clinical application so far.

Electro-oxidation has been explored several decades ago as a urea removal strategy for dialysate regeneration [12,14-20]. Although the *in vitro* results were promising, this line of research came to an abrupt halt and did not reach clinical application for unreported reasons. This suggests technical or toxicological problems that were insurmountable at the time. In view of the ongoing need for efficient urea removal, we reappraised several aspects of EO.

First, different electrode materials used in previous studies were compared directly with respect to urea removal, toxic chlorine generation and the release of metal ions. Platinum electrodes showed the highest urea removal of 7.10 mmol/hour/dm^2/A. Ruthenium and graphite electrodes yielded acceptable urea removal of 4.43 and 4.40 mmol/hour/dm^2/A, respectively. These results are in line with earlier reports (2.81-7.08mmol/hour/dm^2/A for platinum [13,14,17,18,23], 3.28-5.75 mmol/hr/dm^2/A for ruthenium [19,23] and 4.25-5.19 mmol/hour/dm^2/A for graphite foil electrodes [12]). An important new finding is that chlorine release was 10- to 18-fold higher with ruthenium and platinum electrodes than with graphite electrodes indicating less chloride oxidation by graphite electrodes. Notably, virtually all previous studies used platinum or ruthenium electrodes [13,14,17,18,23], except one using graphite foil electrodes [12]. Quantification of chlorine release during electrochemical degradation of urea was reported in only one study with platinum electrodes [17], showing high hypochlorite release varying from ~15 mg/L at 10W up to ~110 mg/L at 80 W and 8 mM dialysate urea concentration. In addition to this, we detected release of platinum and ruthenium ions from the metal electrodes. Although no detailed information on *in vivo* toxicity of platinum or ruthenium ions is available, prolonged use of such metal electrodes may result in accumulation of these ions and additional measures would be required to remove them. In view of the findings reported above, graphite electrodes are probably to be preferred for further application in electrochemical dialysate regeneration and were consequently used in the experiments reported below.

Second, we studied the effect of the urea concentration of the dialysate entering the EO unit and the current applied on urea removal and chlorine release by graphite electrodes. Although urea removal efficiency was higher with lower inlet urea concentrations, absolute urea removal per liter was lower. This implies that at lower urea concentrations, e.g. with continuous dialysis by a wearable dialysis device, the EO capacity of the miniaturized dialysis device must be increased to remove the daily produced amount of urea. In addition, lower urea concentrations resulted in higher chlorine release per removed mole of urea, which is in accordance with previous reports [13,23]. This increase in chloride oxidation is probably due to less competitive direct urea oxidation at the anode surface [23] and may necessitate more stringent measures to remove toxic chlorine species in patients with lower urea concentrations. As was observed before [23], higher current led to higher urea removal but unfortunately also to higher chlorine release. Chlorine release per removed mole of urea was lowest at 2-3 A (0.3-0.5A/dm^2). Grinval'd et al. [13] observed lowest hypochlorite accumulation at 0.5 A/dm^2. Since urea removal efficiency was considerably higher at 3 A than at 2 A (Figure 4.C), a current of 3 A (0.5 A/dm^2) seems a good compromise between efficient urea degradation and limited chlorine release.

Third, to reduce the release of toxic chlorine species to the dialysate, application of AC downstream of the EO unit was tested. Activated carbon is a potent electron donor that effectively removes chlorine from aqueous solutions by reducing chlorine to chloride ions [36]. We found that use of graphite electrodes combined with AC (60 mL (~30 g) in the experiments with spiked saline solution and 160 mL (~80 g) in the experiments with spiked bovine blood) resulted in dialysate chlorine concentrations below the maximum allowable levels as defined in the AAMI standards for dialysate used in standard hemodialysis (bound chlorine <0.10 mg/L, free chlorine <0.50 mg/L [37]). Because, as outlined above, continuous dialysis with a wearable dialysis device will result in lower plasma urea concentrations and consequently more chlorine release, additional measures may be required to remove chlorine species, such as extra AC or infusion of reductive agents (e.g. vitamin C or glutathione).

Finally, in proof-of-principle experiments using a prototype wearable dialysis device with bovine blood and recirculation and regeneration of a small volume of dialysate, the urea degradation rate was ~71% of that in the single pass experiments with a saline/urea solution. This is probably due to competitive oxidation of other organic compounds present in whole blood but not in the test solution. Blood pH dropped, which was fully explained by the rise in pCO_2 due to urea oxidation (Figure 1). Complete oxidation by EO of the amount of urea produced daily would result in an estimated production of ~400 mmol CO_2. This is less than 5% of the average daily metabolic CO_2 production of approximately 15.000 mmol [38] and only a minor increase in alveolar ventilation would be needed to remove this additional CO_2 load to prevent acidosis. However, since N_2 is also generated and oxidation of other organic compounds may also yield CO_2, it seems necessary to include an efficient degassing system in the dialysis circuit. Theoretically, EO could lower the plasma bicarbonate concentration by the production of organic acids from oxidation of other substrates than urea (e.g. carbohydrates) [14,15] or the oxidation of bicarbonate itself [39]. The finding that plasma bicarbonate did not fall is an indirect but strong indication that this did not occur to a significant extent. A potential disadvantage of EO could be the generation of large quantities of oxidative by-products, other than free and bound chlorine, that could negatively affect the redox state of the blood. Indeed, preliminary experiments (supplementary Figure 1) using an AC-free EO unit showed high oxidation-reduction potential (ORP) values immediately downstream of the EO unit, representing strong oxidizing conditions in the dialysate. Downstream of the AC cartridge, however, ORP values were low, probably due to the reductive capacity of carbon. This resulted in a net decrease in blood ORP values, indicating that AC even somewhat improved the total oxidative of the blood treated by EO.

The current prototype dialysis device reduced the dialysate urea concentration from 20

mM to 16 mM in the single pass experiments and the system was somewhat less effective in the proof-of-principle experiments. Obviously, for *in vivo* application a substantial increase in efficiency is required. Increasing the dialysate temperature from 25 °C to 37 °C increases urea removal by EO by 22% [17]. As we performed our studies at room temperature, increasing the dialysate temperature to 37 °C may therefore markedly increase urea removal with our device. Efficiency may be further improved by increasing the electrode surface [14]. Increasing the current will also improve efficiency, although this will also result in higher chlorine release. The benefit of increasing dialysate flow rate is uncertain, since reduced dialysate electrode contact time may compromise efficiency [18].

Although our studies show that EO by graphite electrodes combined with AC results in low chlorine release, only volatile acid production and favorable oxidative status of the dialysate, major question remains whether EO can be safely applied *in vivo*. In theory, many other toxic degradation products may be formed besides chlorine species, such as N_xO_x compounds from oxidation of nitrogen-containing organic solutes [23,40], carbonyl compounds from carbohydrates [41] and quinones from aromatic compounds [42]. Preliminary *in vivo* safety studies by Schuenemann et al. using EO combined with AC, showed that regenerated hemofiltrate obtained in humans administered to rats by daily intraperitoneal injection for 20 days was well tolerated [18]. Hemofiltration treatment of healthy minipigs for a median of 14 days did not show differences in clinical parameters when either filtrate regenerated by EO or prepared solutions were used for substitution, except for a tendency to metabolic acidosis with filtrate regeneration [18]. Although these preliminary results are reassuring, more elaborate biocompatibility testing, e.g. analysis of degradation products, cell toxicity assays and long-term animal testing, is warranted to get a definite answer whether electrochemical dialysate regeneration is safe.

In conclusion, EO by graphite electrodes combined with AC may be worth further exploration as a method for urea removal from dialysate in a wearable closed-loop dialysis system. Future research is warranted to elucidate whether EO can be applied safely *in vivo*.

REFERENCES

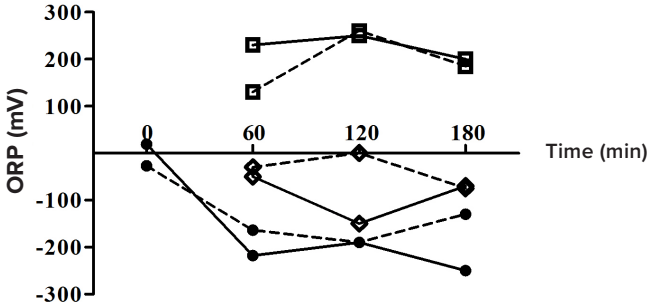
1. Barton KN, Buhr MM, Ballantyne JS. Effects of urea and trimethylamine N-oxide on fluidity of liposomes and membranes of an elasmobranch. Am J Physiol 1999; 276: R397-R406
2. D'Apolito M, Du X, Zong H, Catucci A, Maiuri L, Trivisano T et al. Urea-induced ROS generation causes insulin resistance in mice with chronic renal failure. J Clin Invest 2010; 120: 203-213
3. Johnson WJ, Hagge WW, Wagoner RD, Dinapoli RP, Rosevear JW. Effects of urea loading in patients with far-advanced renal failure. Mayo Clin Proc 1972; 47: 21-29
4. Lim J, Gasson C, Kaji DM. Urea inhibits NaK2Cl cotransport in human erythrocytes. J Clin Invest 1995; 96: 2126-2132
5. Moeslinger T, Friedl R, Volf I, Brunner M, Baran H, Koller E et al. Urea induces macrophage proliferation by inhibition of inducible nitric oxide synthesis. Kidney Int 1999; 56: 581-588
6. Singh LR, Dar TA, Ahmad F. Living with urea stress. J Biosci 2009; 34: 321-331
7. Vaziri ND, Yuan J, Rahimi A, Ni Z, Said H, Subramanian VS. Disintegration of colonic epithelial tight junction in uremia: a likely cause of CKD-associated inflammation. Nephrol Dial Transplant 2012; 27: 2686-2693
8. Vaziri ND, Yuan J, Norris K. Role of urea in intestinal barrier dysfunction and disruption of epithelial tight junction in chronic kidney disease. Am J Nephrol 2013; 37: 1-6
9. Wu JW, Wang ZX. New evidence for the denaturant binding model. Protein Sci 1999; 8: 2090-2097
10. Zhang Z, Dmitrieva NI, Park JH, Levine RL, Burg MB. High urea and NaCl carbonylate proteins in renal cells in culture and in vivo, and high urea causes 8-oxoguanine lesions in their DNA. Proc Natl Acad Sci U S A 2004; 101: 9491-9496
11. Zou Q, Habermann-Rottinghaus SM, Murphy KP. Urea effects on protein stability: hydrogen bonding and the hydrophobic effect. Proteins 1998; 31: 107-115
12. Koster K, Wendt H, Gallus J, Krisam G, Lehmann HD. Regeneration of hemofiltrate by anodic oxidation of urea. Artif Organs 1983; 7: 163-168
13. Grinval'd V, Leshchinskii GM, Rodin VV, Strelkov SI, Yakovleva AA. Development and Testing of a Unit for Electrochemical Oxidation of Products of Hemodialysis. Biomedical Engineering 2003; 37: 67-72.
14. Fels M. Recycle of dialysate from the artificial kidney by electrochemical degradation of waste metabolites: small-scale laboratory investigations. Med Biol Eng Comput 1978; 16: 25-30
15. Fels M. Recycle of dialysate from the artificial kidney by electrochemical degradation of waste metabolites: continuous reactor investigations. Med Biol Eng Comput 1982; 20: 257-263
16. Hintzen K, Stiller S, Brunner H, Rautenbach R, Mann H. Electrodialysis and reverse osmosis as a regeneration system for hemofiltrate. Artif Organs 1983; 7: 169-175
17. Keller Jr R, Yao S, Brown J, Wolfson S, Zeller M. Electrochemical Removal of Urea from Physiological Buffer as the Basis for a Regenerative Dialysis System. Bioelectrochemistry and Bioenergetics 1980; 7: 469-485
18. Schuenemann B, Quellhorst E, Kaiser H, Richter G, Mundt K, Weidlich E et al. Regeneration of filtrate and dialysis fluid by electro-oxidation and absorption. Trans Am Soc Artif Intern Organs 1982; 28: 49-53

19. Wright J, Michaels AS, Appleby AJ. Electrooxidation of Urea at the Ruthenium Titanium Oxide Electrode. AIChE Journal 1986; 32: 1450-1458.
20. Yao SJ, Wolfson SK, Jr., Ahn BK, Liu CC. Anodic oxidation of urea and an electrochemical approach to de-ureation. Nature 1973; 241: 471-472
21. Yao S, Wolfson Jr S, Tokarsky J, Ahn B. De-ureation by Electrochemical Oxidation. Bioelectrochemistry and Bioenergetics 1974; 1: 180-186
22. Yao S, Wolfson Jr S, Krupper M, Wu K. Controlled-potential controlled-current electrolysis: In vitro and in vivo electrolysis of urea. Bioelectrochemistry and Bioenergetics 1984; 13: 15-24
23. Simka W, Piotrowski J, Robak, A, Nawrat G. Electrochemical treatment of aqueous solutions containing urea. J Appl Electrochem 2009; 39: 1137-1143.
24. Meyer MA, Klein E. Granular activated carbon usage in chloramine removal from dialysis water. Artif Organs 1983; 7: 484-487
25. Wester M, Simonis F, Gerritsen KG, Boer WH, Wodzig WK, Kooman JP et al. A regenerable potassium and phosphate sorbent system to enhance dialysis efficacy and device portability: an in vitro study. Nephrol Dial Transplant 2013; 28: 2364-71.
26. Walser M, Bodenlos LJ. Urea metabolism in man. J Clin Invest 1959; 38: 1617-1626
27. Long CL, Jeevanandam M, Kinney JM. Metabolism and recycling of urea in man. Am J Clin Nutr 1978; 31: 1367-1382
28. Walser M. Urea metabolism in chronic renal failure. J Clin Invest 1974; 53: 1385-1392
29. Stephens RL, Jacobsen SC, Atkin-thor E, Kolff W. Portable/wearable artificial kidney (WAK) - initial evaluation. Proc Eur Dial Transplant Assoc 1976; 12: 511-518
30. Agar JW. Review: understanding sorbent dialysis systems. Nephrology (Carlton) 2010; 15: 406-411
31. Ash SR. Sorbents in treatment of uremia: a short history and a great future. Semin Dial 2009; 22: 615-622
32. Fuchs C, Dorn D, Rieger J, van Doorn AW, Striebel JP, Scheler F. Capabilities of the Redy cartridge for regeneration of hemofiltrate. Artif Organs 1979; 3: 279-280
33. Rosenbaum BP, Ash SR, Wong RJ, Thompson RP, Carr DJ. Prediction of hemodialysis sorbent cartridge urea nitrogen capacity and sodium release from in vitro tests. Hemodial Int 2008; 12: 244-253
34. Culpepper CP, Cummings R, Westervelt FB, Savory J, Wills MR. Aluminum kinetics of the REDY system: a study of the impact of deferoxamine therapy. Trans Am Soc Artif Intern Organs 1983; 29: 76-80
35. Drukker W, Doorn van A. Replacement of Renal Function by Dialysis: A Textbook of Dialysis. In: Maher J, ed. Dialysate regeneration. Houten (The Netherlands): Springer, 1989
36. Li Y, Zhang X, Shang C. Effect of reductive property of activated carbon on total organic halogen analysis. Environ Sci Technol 2010; 44: 2105-2111
37. AAMI standards for hemodialysis systems. ANSA/AAMI (RD 5). 1981
38. Rose B, Post T. Clinical physiology of acid-base and electrolyte disorders. New York: McGraw Hill, 2000
39. Zhang H, Joseph J, Felix C, Kalyanaraman B. Bicarbonate enhances the hydroxylation, nitration, and peroxidation reactions catalyzed by copper, zinc superoxide dismutase. Intermediacy of car-

bonate anion radical. J Biol Chem 2000; 275: 14038-14045

40. Jara C, Martinez-Huitle C, Torres-Palma RA. Distribution of nitrogen ions generated in the electrochemical oxidation of nitrogen containing organic compounds. Portugaliae Electrochimica Acta 2009; 27: 203-213
41. Miyata T, Inagi R, Asahi K, Yamada Y, Horie K, Sakai H et al. Generation of protein carbonyls by glycoxidation and lipoxidation reactions with autoxidation products of ascorbic acid and polyunsaturated fatty acids. FEBS Lett 1998; 437: 24-28
42. Jurva U. Electrochemistry on-line with mass spectrometry Instrumental methods for in vitro generation and detection of drug metabolites [dissertation]. Groningen (The Netherlands): Rijksuniversiteit Groningen; Department of Mathematics and Physics; 2004

SUPPLEMENTARY FIGURE



SUPPLEMENTARY FIGURE 1. Oxidation-reduction potential (ORP) during electro-oxidation of urea-spiked bovine blood (EO) at 3 A using a carbon-free EO unit and 50 mL (~25 g) of activated carbon (AC). Dotted line: experiment 1; solid line: experiment 2. Squares: Effluent of the EO unit, upstream of AC filter; prisms: effluent of AC filter; circles: blood.

5

Removal of urea by electro-oxidation in a miniature dialysis device: a study in awake goats

Maarten Wester, Maaike K. van Gelder, Jaap A. Joles, Frank Simonis, Diënty H. Hazenbrink, Theo W. van Berkel, Koen R. Vaessen, Walther H. Boer, Marianne C. Verhaar and Karin G. Gerritsen

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ABSTRACT

Background

The key to success in developing a wearable dialysis device is a technique to safely and efficiently regenerate and re-use a small volume of dialysate in a closed-loop system. In a hemodialysis model in goats, we explored whether urea removal by electro-oxidation (EO) could be effectively and safely applied *in vivo*.

Methods

A miniature dialysis device was built, containing 1 or 2 'EO unit(s)', each with 10 graphite electrodes, with a cumulative electrode surface of 585 cm^2 per unit. The units also contained poly(styrene-divinylbenzene) sulphonate beads, FeOOH beads and activated carbon for respective potassium, phosphate and chlorine removal. Urea, potassium and phosphate were infused to create 'uremic' conditions.

Results

Urea removal was dependent on total electrode surface area (removal of 8±1 and 16±2 mmol/h and clearance of 12±1 and 20±3 mL/min with 1 and 2 EO units, respectively) and plasma urea concentration, but not on flow rate. Extrapolating urea removal with 2 EO units to 24 h would suffice to remove daily urea production, but for intermittent dialysis additional units would be required. EO had practically no effects on potassium and phosphate removal or electrolyte balance. However, slight ammonium release was observed and some chlorine release at higher dialysate flow rates. Minor effects on acid-base balance were observed, possibly partly due to infusion of chloride. Mild hemolysis occurred, which seemed related to urea infusion.

Conclusions

In conclusion, clinically relevant urea removal was achieved *in vivo* by electro-oxidation. Efficacy and safety testing in a large animal model with uremia is now indicated.

INTRODUCTION

For decades, strategies to develop a wearable dialysis device have been explored, that could offer continuous or more frequent dialysis resulting in more adequate toxin removal, steadier electrolyte concentrations and less abrupt fluid removal [12,17,23,29,30]. The key to success in developing such a device is to invent a technique to safely and efficiently regenerate and re-use a small volume of dialysate in a closed-loop system. Thus far, predominantly sorbent cartridges containing adsorbing particles for electrolyte removal and urease for enzymatic degradation of urea have been tested [3]. A major drawback of urea degradation by urease is the production of ammonium that in turn must be adsorbed. However, development of an alternative urea removal strategy is challenging since the daily production of urea, the main waste product of nitrogen metabolism, is very high (250-400 mmol/day) [13,31,37] and urea is very difficult to adsorb [18,32,40].

Previously, we explored electro-oxidation (EO) *in vitro* using graphite electrodes incorporated in a sorbent cartridge containing sorbent particles for potassium and phosphate removal [39]. In this technology, a current is applied to the dialysate by which urea is converted into nitrogen, carbon-dioxide and water, either directly at the anode or indirectly in the bulk solution via the intermediate EO-product hypochlorite. A disadvantage is the generation of chlorinated byproducts. However, these can be neutralized by activated carbon [AC]. We achieved clinically relevant urea degradation in blood (9.5±1.0 mmol/h) with an EO unit in series with AC [39]. Chlorine release was below maximum acceptable levels as defined by the Association for the Advancement of Medical Instrumentation (AAMI) standards [1].

In the present study, we explored whether urea removal by EO could be effectively and safely applied *in vivo*, in a hemodialysis (HD) model in goats. We incorporated an EO unit containing graphite electrodes and sorbent particles for potassium and phosphate removal in the dialysate circuit of a prototype miniature dialysis device. First, we studied efficacy of urea removal and explored whether doubling of the electrode surface area (by using 2 EO units) and increasing the dialysate flow could increase urea removal efficiency. Removal of creatinine, potassium and phosphate by the device was also quantified. Second, we evaluated biocompatibility of EO by monitoring influence on vital parameters, calcium, magnesium, sodium, hemolysis parameters, acid-base status, iron and glucose and by measuring release of chlorine species and ammonium.

METHODS

Materials

A miniature dialysis device of ~1.5 kg (plus battery pack of 250 g for 3 h of dialysis) was built and provided by Nanodialysis BV (Oirschot, The Netherlands). 1 or 2 'EO unit(s)' (weight: 250 g per unit) were incorporated in the dialysate circuit, each containing 10 graphite electrodes with a cumulative electrode surface of 585 cm^2 per EO unit [39]. Each unit contained 80 g (when using 1 unit) or 90 g (when using 2 units) poly(styrene-divinylbenzene) (PS-DVB) sulfonate beads and 40 g (when using 1 unit) or 30 g (when using 2 units) FeOOH beads for respective potassium and phosphate removal (the amounts were adjusted after the experiments with 1 unit to achieve the target potassium/ phosphate removal ratio of ~2-3:1). A degassing unit and AC filters (25 g per EO unit) were placed downstream of the EO unit(s). High-flux dialyzers (Polyfluxâ 2H 0.2 m2; Gambro Dialysatoren GmbH, Hechingen, Germany) were used to separate blood and dialysate. Thomas pumps (Gardner Denver Thomas, Sheboygan, WI, USA) were applied. A 72 cm double lumen central venous catheter (CVC) was selected for blood access (Palindrome Chronic Dialysis Catheter, Covidien, Medtronic, Minneapolis, MN, USA). Unfractionated heparin was used for anticoagulation.

Methods

Animals

In vivo experiments were approved by the Animal Experiments Committee (Utrecht, The Netherlands) and performed in accordance with national guidelines for the care and handling of animals. Healthy Dutch White goats (N=2) were selected since these animals are docile, have easily accessible neck veins, and body weights (70-90 kg) and distribution volumes comparable to humans [38].

Sterilization and regeneration procedure

In each experiment, a new sterile dialyzer and tubing were used. Prior to each experiment the EO units were sterilized by 1 h exposure of the internal fluidic circuit to 1 L 5% hydrogen peroxide (m/m) and 0.2% peracetic acid (m/m) in NaCl 120 mM/ $CaCl_2$ 1.2 mM/ $MgCl_2$ 0.45 mM, followed by 12 h exposure to 1 L NaOH 120 mM/ ethanol 20% (v/v), in sterile environment throughout. This procedure was shown to be effective in eliminating bacteria and endotoxins. Subsequently, pH was adjusted to 7.4-8.0 using 1 L NaCl 120 mM/ HCl 50 mM, followed by rinsing and equilibration of the EO units with 3 L rinsing solution (pH 7.4-8.0) containing NaCl 105 mM (1 unit) or 95 mM (2 units), $NaHCO_3$ 15 mM (1 unit) or 25 mM (2 units), $CaCl_2$ 1.2 mM and $MgCl_2$ 0.45 mM. These concentrations were based on prior *in vitro* and *in vivo* experiments, showing no net electrolyte release at these concentrations [39]. Equilibration was achieved by recirculating the rinsing solution in the blood circuit at 110

mL/min while draining the solution from the dialysate circuit at 40 mL/min and measuring calcium in the effluent (equilibration of calcium is slower than that of other relevant electrolytes). The rinsing procedure was continued until in- and outlet calcium concentrations were equal (usually after circulation of 3 L of rinsing solution).

Experimental procedure

Sixteen experiments were performed in 2 goats (8 per goat). Goats were temporarily sedated (with detomidine and propofol) to insert a CVC in the jugular vein. A catheter was placed in the auricular artery for arterial pressure measurement and withdrawal of arterial blood gases.

To prevent a decrease of systemic potassium and phosphate plasma concentrations we infused 1 M KCl and 0.05 M Na_2HPO_4/0.25 M KH_2PO_4 solution as described [38]. To achieve higher urea concentrations, we administered an oral bolus of 30 g urea (dissolved in syrup) to the goats the evening and morning prior to the experiment in the first 3 experiments. However, the goats progressively disliked urea and therefore we switched to intravenous administration of urea via the venous lumen of the CVC in the remaining experiments (200 mmol/h (0.67 L of urea 0.3 M)) during the first hour prior to starting dialysis followed by continuous infusion of 100 mmol/h (0.33 L/h of urea 0.3 M during dialysis). Spontaneous urea concentrations were 6.5±1.4 mM. After respectively dietary or intravenous administration of urea, urea concentrations were 11.0±2.2 and 13.1±1.9 mM respectively (Figure 1).

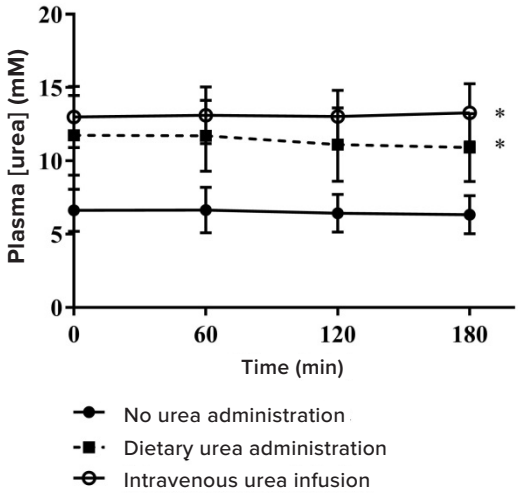

FIGURE 1. Plasma urea concentration without urea administration (n=16), after dietary urea administration (n=3) and after intravenous urea infusion (n=13). *p<0.0001 vs. no urea administration.

NaCl 0.65-0.9% was infused to balance urea-induced osmotic diuresis (NaCl 0.65% in the experiments with 1 EO unit and in 4 of 9 experiments with 2 EO units, and NaCl 0.9% in 5 of 9 experiments with 2 EO units). The dialysate circuit was filled with ~100 mL rinsing solution. Blood lines were connected, and blood was pumped (110 mL/min) across the dialyzer (Figure 2: Experimental setup).

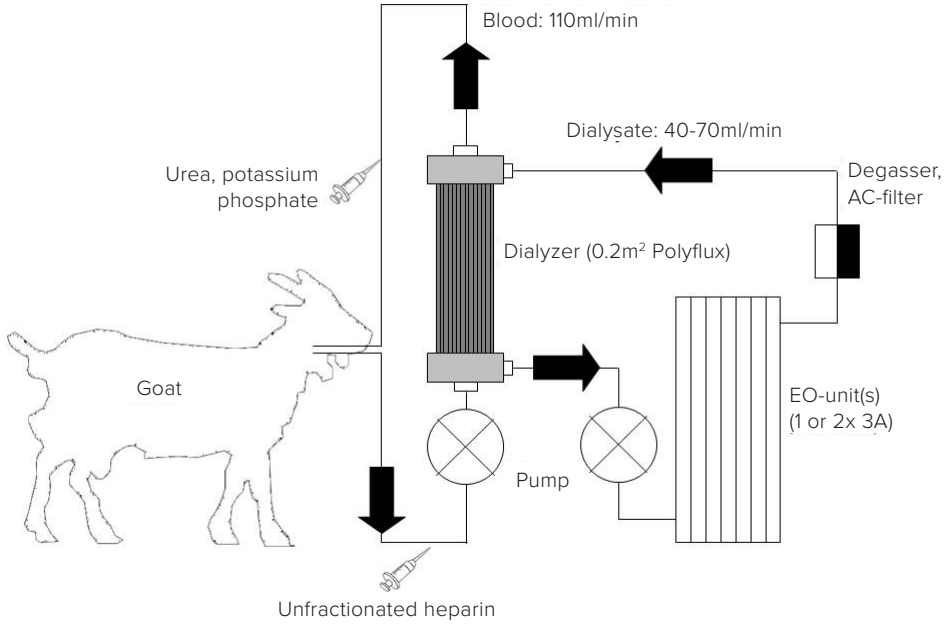

FIGURE 2. Experimental setup (adapted from [13]). AC = activated carbon. EO = electro-oxidation.

After 30 minutes habituation of the goat and equilibration of blood with the dialysate compartment, the dialysate pump was started, and dialysate was recirculated during 3 h over the EO unit(s) in counter-current direction with blood in the dialyzer [38].

To assess whether urea removal was dependent on cumulative electrode surface or dialysate flow, we performed experiments with 1 EO unit (n=7) at a dialysate flow of 40 mL/min and 2 EO units in parallel at a dialysate flow of 40 mL/min/unit (n=5) or 70 mL/min/unit (n=4) (note, 70 mL/ min was the maximal dialysate flow that could be achieved with the pumps). A current of 3 A was applied per unit because previous research showed that this leads to efficient urea degradation while keeping chlorine release low [39]. A voltage of 3.5 V was applied across each EO unit. Every 30 min the electrode polarity was inversed to eliminate

deposits (e.g. Mg salts) that may form on the electrodes [11]. Blood and dialysate samples were taken hourly from the blood circuit (down- and upstream of the dialyzer), from the dialysate circuit (down- and upstream of the EO unit(s)) and from the arterial line. [Urea], [Creatinine], [K^+], [PO_4^{3-}], [total Ca], [total Mg], [Na^+], [HCO_3^-] and [glucose] were monitored in plasma and dialysate. [LDH] was only measured in plasma. [NH_4^+] was only measured in plasma in the last 6 experiments at the start and end of the experiment. In the first 2 experiments dialysate [Fe^{2+}] and [albumin] appeared to be below detection limit and were therefore only measured in plasma in the remaining experiments ([Fe^{2+}] at the start and end of the experiment and [albumin] hourly). Hemoglobin was monitored hourly and leukocytes and thrombocytes at the start and end of the experiment (note, leukocyte and thrombocyte counts at the start and end were lacking in 2 and 4 experiments, respectively). Blood gasses were taken hourly except in one experiment where the arterial line could not be placed. Chlorine measurements were performed hourly in dialysate in the experiments with 2 EO units. At the end of each experiment the catheters were removed. Before, during and after the experiments vital parameters (heart rate, blood pressure, temperature, blood oxygen saturation) were monitored [38].

Anticoagulation strategy

After insertion of the CVC, a bolus of unfractionated heparin was given (10,000 IU), followed by continuous infusion of ~3,500 IU/h. The activated clotting time (ACT) was measured every hour and the heparin dose adjusted, aiming for an ACT >500s. One hour before removal of the CVC, heparin infusion was stopped [38].

Laboratory measurements

All electrolyte measurements were performed at the hospital laboratory of the UMC Utrecht. Urea, creatinine, potassium, phosphate, sodium, calcium, magnesium, bicarbonate, albumin, ammonium, lactate dehydrogenase (LDH) and iron concentrations were analyzed with an AU 5800 routine chemistry analyzer (Beckman Coulter, Brea, CA, USA) using ion selective electrodes and spectrophotometry. Arterial blood gas analyses were performed using a blood gas analyzer (Rapidlab type 1265; Siemens Medical Solutions Diagnostics B.V., Breda, The Netherlands). Free and total chlorine concentrations were measured using a ChloroSense Chlorine Meter (CS 100 Palintest Ltd, Gateshead, UK) [38].

Calculations, analyses and statistics

Removal from plasma was calculated using the following formula:

$$(A_p)_{t1\rightarrow t2} = ((C_{pi} - C_{po})_{t1} + (C_{pi} - C_{po})_{t2})/2 \times Q \times \Delta t \times (1\text{-}Ht)$$

and removal from dialysate:

$$(A_d)_{t1\rightarrow t2} = ((C_{di} - C_{do})_{t1} + (C_{di} - C_{do})_{t2})/2 \times Q \times \Delta t$$

Removal is presented as the mean value of removal from plasma and dialysate:

$$A_m = (A_p + A_d)/2$$

Plasma clearance was calculated using the following formulas:

For urea: $Cl_{t1\rightarrow t2} = (A_d)_{t1\rightarrow t2}/(((C_{pi})_{t1} + (C_{pi})_{t2})/2)$

For creatinine: $Cl_{t1\rightarrow t2} = (A_m)_{t1\rightarrow t2}/(((C_{pi})_{t1} + (C_{pi})_{t2})/2)$

Where A = amount removed from plasma (A_p) or dialysate (A_d) or average removal from plasma and dialysate (A_m),Cl = plasma clearance (ml/min), C_{pi} = inlet plasma concentration (i.e. upstream of dialyzer), C_{di} = inlet dialysate concentration (i.e. upstream of the EO unit), C_{po} = outlet plasma concentration (i.e. downstream of dialyzer), C_{do}= outlet dialysate concentration (i.e. downstream of the EO unit), t = time after start of dialysate pump (min), Q = blood or dialysate flow (L/min), Δt = t2-t1, Ht = hematocrit.

Note, urea, potassium and phosphate removal were calculated using only dialysate data and urea clearance was calculated using only removal from dialysate since some of the outlet plasma concentrations were influenced by their infusion via the venous lumen of the CVC. Moreover, urea is also removed from the cell compartment to an unknown extent [8].
Total plasma calcium concentration (mM) was corrected for albumin (g/L):

$$[Ca]_{corrected} = [Ca]_{measured} + 0.02 \times (\text{normal} - \text{measured plasma [albumin]})$$

Anion gap (mEq/L) was calculated using $[Na^+]$, $[Cl^-]$ and $[HCO_3^-]$ (mM) and corrected for albumin (g/L) using the following formula:

$$\text{Anion gap} = [Na^+] - ([Cl^-] + [HCO_3^-]) + 0.25x \text{ (normal-measured plasma [albumin])}$$

(Note, for normal albumin concentration average albumin concentration in the goats before intervention (34.2 g/L) was used).

Data are shown as mean ± standard deviation. Linear interpolation was used for 12% of the arterial blood gas measurements (7 out of 60) where data were missing.
Statistical significance was determined using Student's (paired) t-test, Wilcoxon matched-pairs signed rank tests and linear regression analysis as appropriate. For comparison of absolute removal 2-way ANOVA for repeated measures with post hoc correction using Tukey's multiple comparisons test, was applied.

RESULTS

Efficacy

Urea removal

Urea removal and clearance were 8.4±1.3 mmol/h and 11.9±1.1 mL/min, respectively, using 1 EO unit (dialysate flow 40 mL/min; Figure 3.A, B) and remained stable during consecutive hours. Use of 2 units in parallel doubled the removal of urea (15.9±2.2 mmol/h) and caused a 1.6-fold increase in urea clearance (19.5±2.8 mL/min). Increase of the dialysate flow to 70 mL/min per unit did not increase urea removal (15.8±3.4 mmol/h; p=0.77 versus flow 2x40 mL/min) or urea clearance (21.0±2.5 mL/min; p=0.53 versus flow 2x40 mL/min). Urea removal was dependent on urea plasma concentration (Figure 3.C, D) while there was no relation between urea concentration and clearance.

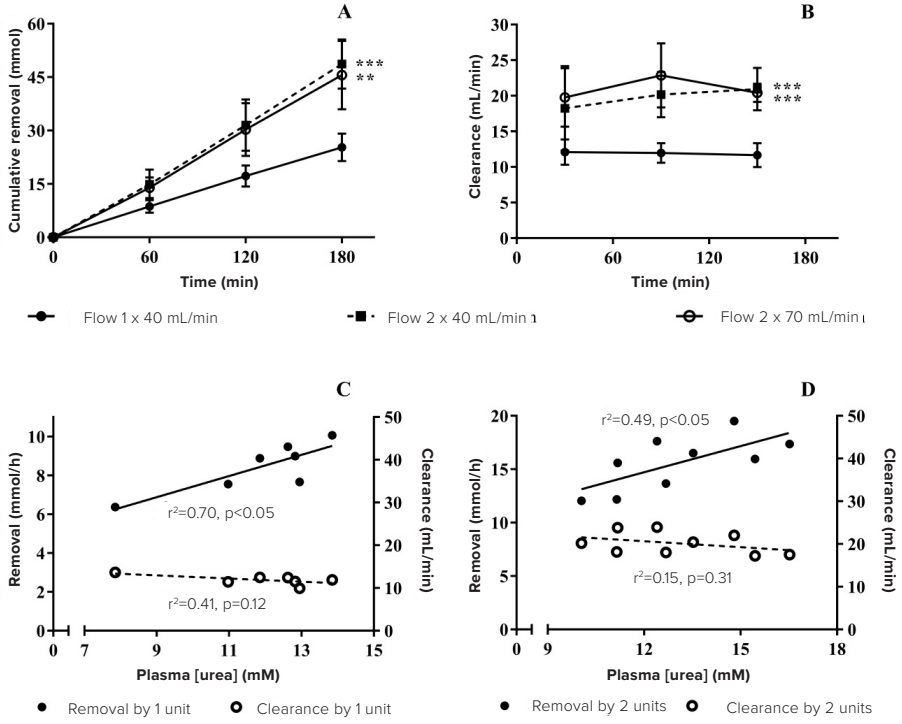

FIGURE 3. Urea removal using 1 or 2 EO units at a dialysate flow of 1x40 (n=7), 2x40 (n=5) or 2x70 (n=4) mL/min. A: Cumulative urea removal in 3 h. B: Urea clearance. C/D: Relation between cumulative urea removal and plasma urea concentration (solid line) and between urea clearance and plasma urea concentration (dashed line) using 1 unit (C) or 2 EO units (D). ***p<0.001, **p=0.003 versus flow 1x40 mL.

Creatinine removal

Creatinine removal and clearance were 97±6 µmol/h (at plasma creatinine concentrations of 59±4 µM) and 27.9±2.5 mL/min, respectively, using 1 EO unit (dialysate flow 40 mL/min; Figure 4.A, B) and remained stable during consecutive hours. Doubling the electrode surface by using 2 units in parallel caused a 1.5-fold increase in creatinine removal (155±16 µmol/h at plasma creatinine concentrations of 60±7 µM) and clearance (42.8±3.4 mL/min). In contrast to urea removal and clearance, creatinine removal and clearance were dialysate flow dependent. Increase of the dialysate flow to 70 mL/min resulted in a creatinine removal of 210±40 µmol/h and clearance of 62.6±6.0 mL/min.

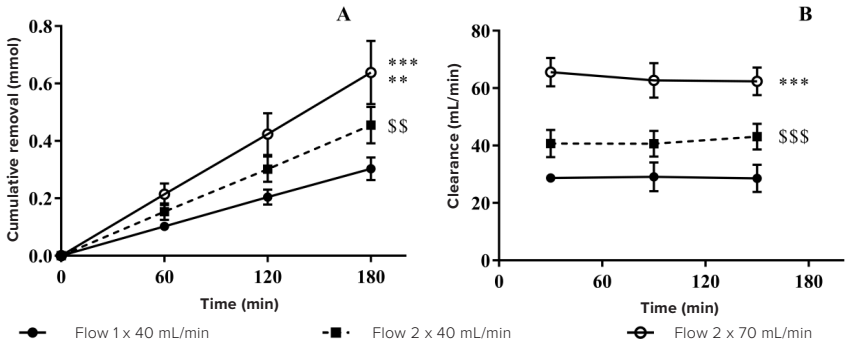

FIGURE 4. Creatinine removal using 1 or 2 EO units at a dialysate flow of 1x40 (n=7), 2x40 (n=5) or 2x70 (n=4) mL/min. Cumulative creatinine removal in 3 h. B: Creatinine clearance. ***p<0.001, $$p=0.006 versus flow 1x40 mL/min; $$$p<0.001, **p= 0.004 versus flow 2x40 mL/min.

Potassium and phosphate removal

Details on potassium and phosphate removal have been discussed in our previous publication [38]. Novel in the present study is that we also applied 2 EO units. Cumulative potassium removal in 3 h was 7.8±3.1 mmol (98±39 µmol/gram sorbent) using 1 EO unit (Figure 5.A), at average plasma potassium concentrations of 4.5±0.6 mM. When using 2 units in parallel, cumulative potassium removal in 3 h was 13.2±1.2 mmol (73±7 µmol/gram sorbent) at a dialysate flow of 40 mL/min and 22.5±4.9 mmol (125±27 µmol/gram sorbent) at 70 mL/min, at average plasma potassium concentrations of 4.7±0.2 mM and 4.6±0.1 mM, respectively. Cumulative phosphate removal in 3 h was 5.3±1.0 mmol (132±25 µmol/gram sorbent) using 1 unit (Figure 5.B) at average plasma phosphate concentrations of 2.5±0.5 mM. When using 2 units in parallel, cumulative phosphate removal in 3 h was 6.5±1.0 (108±17 µmol/gram sorbent) at a dialysate flow of 40 mL/min and 10.0±1.4 mmol (166±23

µmol/gram sorbent) at 70 mL/min, at average plasma phosphate concentrations of 2.1±0.6 mM and 2.0±0.3 mM, respectively.

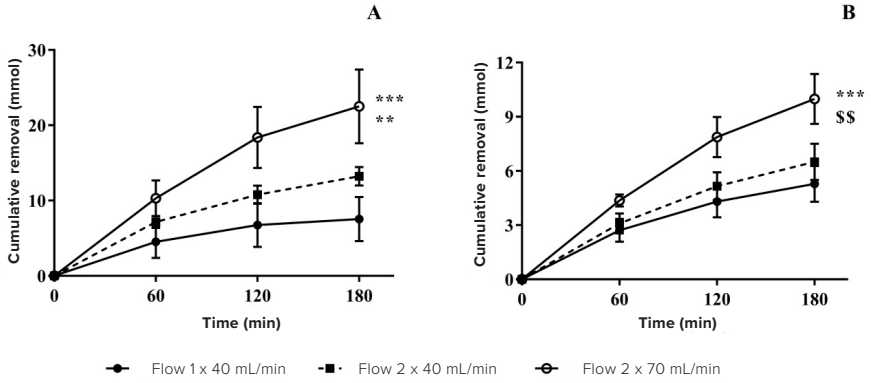

FIGURE 5. A: Cumulative potassium removal in 3 h using 1 or 2 EO units at a dialysate flow of 1x40 (n=7), 2x40 (n=3) or 2x70 mL/min (n=3). B: Cumulative phosphate removal in 3 h using 1 or 2 EO units at a dialysate flow of 1x40 (n=7), 2x40 (n=3) or 2x70 mL/min (n=3). ***p<0.001 versus flow 1x40 mL/min; **p=0.027, $$p=0.01 versus flow 2x40 mL/min.

Safety analysis

Influences on vital parameters

Vital parameters remained practically stable except for a rise in heart rate (Table 1).

TABLE 1. Vital parameters before, during and after dialysis.

	Before dialysis	During dialysis	After dialysis
SBP (mmHg)	119 ± 22	123 ± 14	119 ± 19
DBP (mmHg)	76 ± 14	87 ± 14*	84 ± 13
Heart rate (beats/min)	67 ± 26	84 ± 24**	98 ± 24***
Oxygen saturation (%)	95 ± 4	97 ± 5	97 ± 2

Mean±SD. SBP = systolic blood pressure. DBP = diastolic blood pressure. * p<0.05, ** p<0.01, *** p<0.001 versus after dialysis.

Calcium, magnesium and sodium

Plasma calcium concentrations showed a limited decrease of on average 0.04±0.08 mM (p=0.04) across the dialyzer while dialysate calcium concentrations remained stable (Figure 6.A, B). Systemic plasma calcium concentrations decreased from 2.2±0.2 to 2.1±0.2 mM (p=0.003) (Figure 6.C). Plasma and dialysate magnesium concentrations showed a limited decrease of on average 0.04±0.04mM (p=0.002) across the dialyzer and 0.02±0.05 mM (p=0.006) across the EO unit, respectively (Figure 6.D, E). Systemic magnesium concentra-

tions decreased from 0.84±0.06 to 0.78±0.06 mM (p=0.003; Figure 6.F). Plasma sodium concentrations were stable across the dialyzer (Figure 6.G), but dialysate sodium concentrations slightly decreased across the EO unit (0.67±0.40 mM, p<0.001; Figure 6.H). Systemic plasma sodium concentration increased from 146±1.9 to 148±2.5 mM (p=0.03; Figure 6.I).

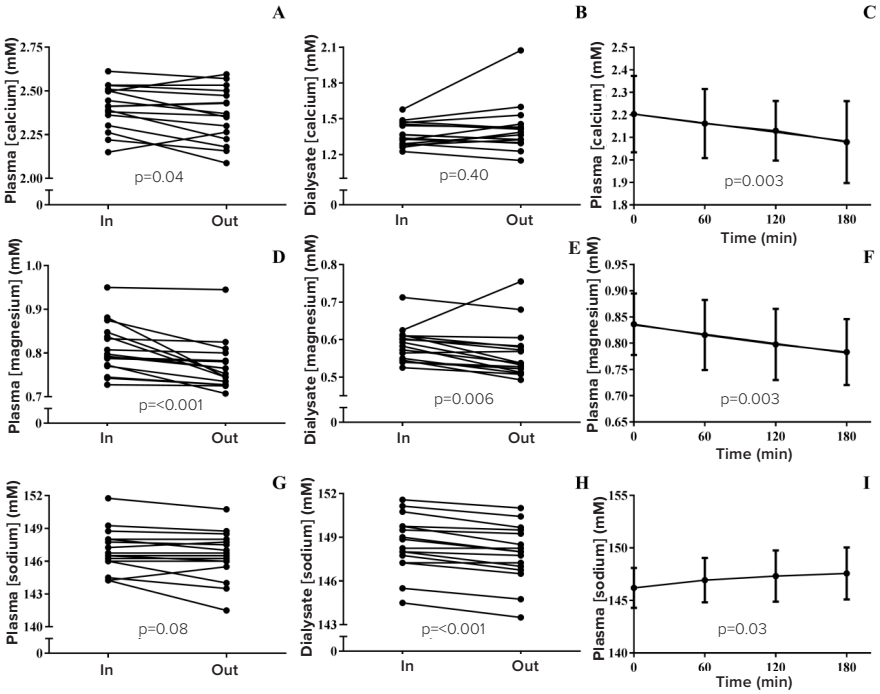

FIGURE 6. Influences on calcium, magnesium and sodium concentration at the inlet (In) and outlet (Out) of the dialyzer (plasma), and up- (In) and downstream (Out) of the EO unit (dialysate) (n=16). Measurements were performed each hour and per experiment the concentrations (In) and (Out) were averaged. A, B: Plasma and dialysate calcium concentration. C: Plasma calcium concentration. D, E: Plasma and dialysate magnesium concentration. F: Plasma magnesium concentration. G, H: Plasma and dialysate sodium concentration. I: Plasma sodium concentration.

Influences on hemolysis parameters, leukocytes and thrombocytes

Hb across the dialyzer and systemic Hb remained stable during the experiments (Figure 7.A, B). LDH did not change across the dialyzer (Figure 7.C), but increased systemically in the experiments where urea was infused (Figure 7.D; p=0.05). In 2 of these experiments visible hemolysis occurred with pink discoloration of plasma. Of note, we also observed visible hemolysis in one of two experiments where only urea was infused, and no dialysis (or EO) was performed. Leukocyte number increased during the experiment from 7.7±2.0 to 8.9±1.8 x10^9/L (Figure 7.E; p=0.002). Thrombocyte number remained stable (Figure 7.F).

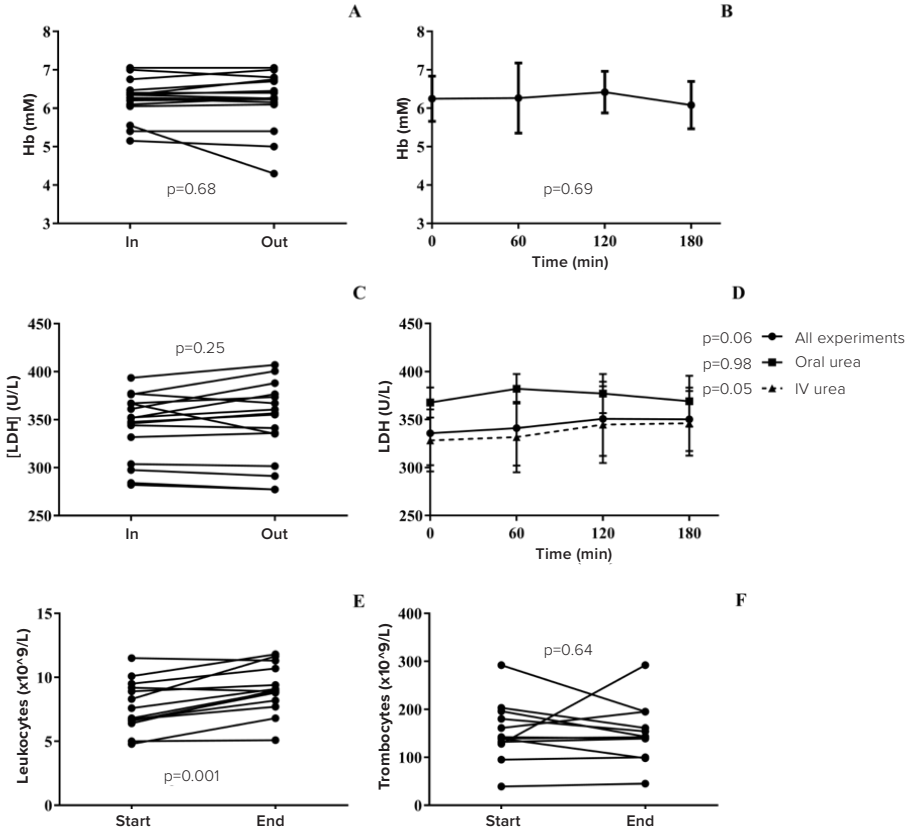

FIGURE 7. A: Hb concentration at the inlet (In) and outlet (Out) of the dialyzer (n=16).* B: Hb concentration (n=16). C: Plasma LDH concentration at the inlet (In) and outlet (Out) of the dialyzer (n=16).* D: Plasma LDH concentration (n=16 all experiments, n=3 oral urea, n=13 IV urea). E: Leukocyte number at the start and end of the experiment (n=14).* F: Thrombocyte number at the start and end of the experiment (n=12).*

*Measurements were performed each hour and per experiment the concentrations (In) and (Out) or (Start) and (End) were averaged.

Influences on acid-base status

Arterial blood pH at the start of the experiments was 7.41±0.03 (1 unit) and 7.43±0.05 (2 units) and did not change during the experiments (Figure 8.A). Plasma bicarbonate concentrations showed a (tendency) to gradually decrease (from 25.7±2.7 to 23.3±1.1 mM (p=0.04) for arterial bicarbonate and from 27.6±3.1 to 25.6±2.5 mM (p<0.001) for venous bicarbonate using 1 unit; from 26.8±2.2 to 25.4±2.0 mM (p=0.13) for arterial bicarbonate and from 28.0±2.2 to 26.0±2.2 mM (p=0.08) for venous bicarbonate using 2 units) (Figure 8.B, C). The systemic decrease in bicarbonate concentration was accompanied by a de-

crease in pCO_2 from 40.6±3.4 to 34.6±5.0 mmHg (p=0.03 using 1 unit) and from 40.2±5.7 to 37.3±3.9 mmHg (p=0.01 using 2 units) (Figure 8.D). Plasma bicarbonate concentrations remained stable across the dialyzer using 1 unit and increased across the dialyzer from 26.9±2.1 to 27.8±2.5 mM using 2 units (p<0.001, Figure 8.E). Dialysate bicarbonate concentrations showed a (tendency to) decrease across the EO unit from 28.3±2.5 to 27.6±2.4 mM using 1 unit (p=0.08) and from 29.1± 2.1 to 28.5±2.0 mM using 2 units (p=0.01; Figure 8.E, F).

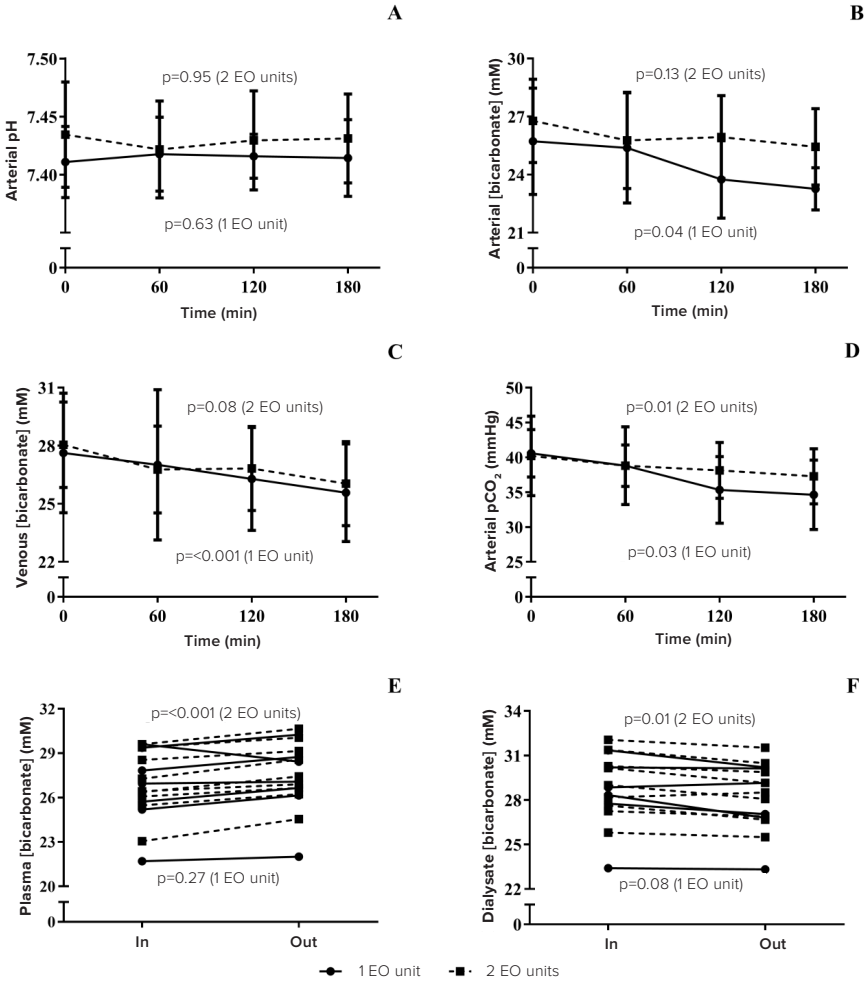

FIGURE 8. Influences on acid-base status. A: Arterial pH (1 EO unit: n=7, 2 EO units: n=8). B: Arterial bicarbonate (1 EO unit: n=7, 2 EO units: n=8). C: Venous bicarbonate (1 EO unit: n=7, 2 EO units: n=9). D: Arterial pCO_2 (1 EO unit: n=7, 2 EO units: n=8). E: Plasma bicarbonate at the inlet (In) and outlet (Out) of the dialyzer (1 EO unit: n=7, 2 EO units: n=9).* F: Dialysate bicarbonate concentration upstream (In) and downstream (Out) of the EO unit (1 EO unit: n=7, 2 EO units: n=9).*
*Measurements were performed each hour and per experiment the concentrations (In) and (Out) were averaged.

Plasma anion gap showed a tendency to increase in time in the systemic circulation (p=0.18 using 1 unit and p=0.07 using 2 units; Figure 9.A), while a (tendency to) decrease was observed across the dialyzer (p=0.06 for 1 unit and p<0.001 for 2 units; Figure 9.B). Lactate did not increase systemically (Figure 9.C). Plasma chloride concentration showed a (tendency to) gradually increase in the systemic circulation (from 109±3 to 111±2 mM (p=0.005) using 1 unit, and from 109±2 to 112±3 mM (p=0.08) using 2 units; Figure 9.D) and across the dialyzer using 2 units (from 112±1.5 to 111±1.5 (p=0.03); Figure 9.E). Plasma chloride concentration remained stable across the dialyzer using 1 unit (Figure 9.E). Dialysate chloride concentration increased across the EO unit (from 120±3 to 121±3 mM (p=0.03) using 1 unit and from 121±2 to 122±2 mM (p=0.03) using 2 units (Figure 9.F)).

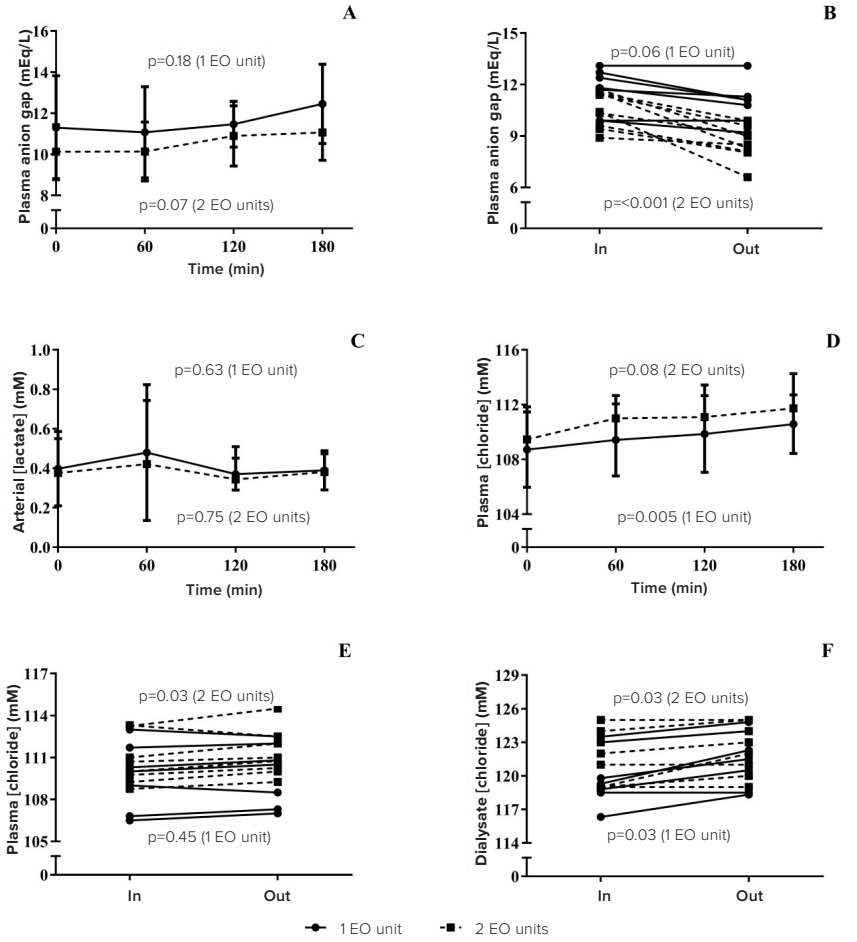

FIGURE 9. Influences on acid-base status. A: Venous anion gap (1 EO unit: n=7, 2 EO units: n=9). B: Plasma anion gap at the inlet (In) and outlet (Out) of the dialyzer (1 EO unit: n=7, 2 EO units: n=9).* C: Arterial lactate concentration (1 EO unit: n=7, 2 EO units: n=8). D: Venous chloride concentration (1 EO unit: n=7, 2 EO units: n=9). E: Plasma chloride

concentration at the inlet (In) and outlet (Out) of the dialyzer (1 EO unit: n=7, 2 EO units: n=9).* F: Dialysate chloride concentration at the inlet (In) and outlet (Out) of the dialyzer (1 EO unit: n=7, 2 EO units: n=9).*

*Measurements were performed each hour and per experiment the concentrations (In) and (Out) were averaged.

Iron

Iron did not significantly change across the dialyzer or systemically during the experiments (Figure 10).

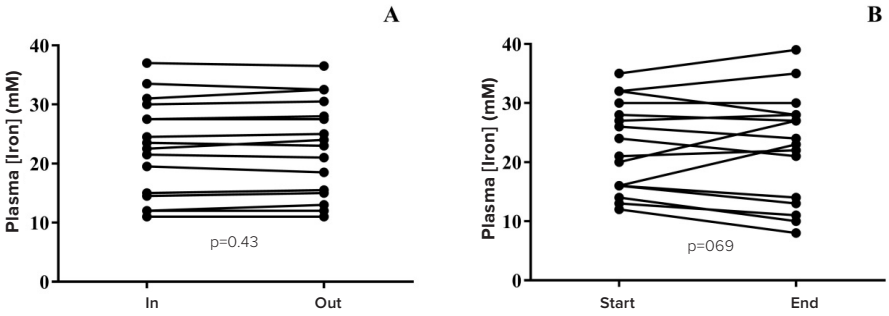

FIGURE 10. A: Plasma iron concentration at the inlet (In) and outlet (Out) of the dialyzer (n=16). Measurements were performed at the start and end of each experiment and per experiment the concentrations (In) and (Out) were averaged. B: Plasma iron concentration at the start and end of each experiment (n=16).

Glucose

Plasma and dialysate glucose concentrations decreased across the dialyzer from 5.5±1.6 to 5.0±1.6 mM ($p<0.001$; Figure 11.A) and EO unit from 5.7±1.6 to 5.2±1.7 mM ($p<0.001$; Figure 11.B), respectively. Systemic plasma glucose concentration remained stable during the experiment (Figure 11.C).

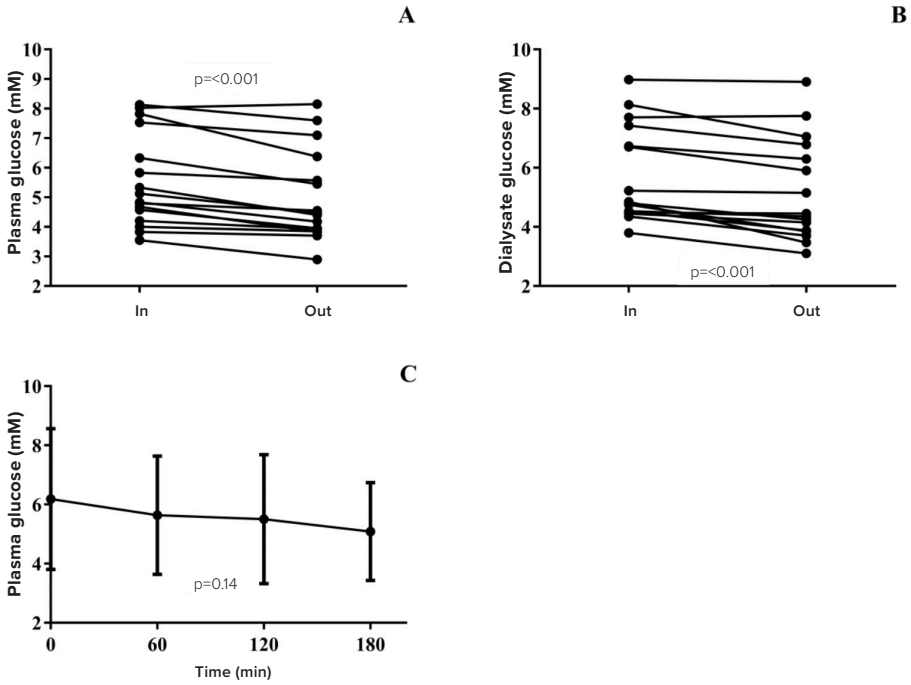

FIGURE 11. A: Influences on plasma and dialysate glucose concentration at the inlet (In) and outlet (Out) of the dialyzer (n=16).* B: Influences on dialysate glucose concentration upstream (In) and downstream (Out) of the EO unit (n=16).* C: Plasma glucose concentration (n=16).

*Measurements were performed each hour and per experiment the concentrations (In) and (Out) were averaged.

Chlorine species

Chlorine concentrations in the dialysate downstream of the AC filter were below the maximum allowed level of 0.1 mg/L (according to AAMI standards for conventional (HD) [1] at dialysate flow rates of 40 mL/min. At dialysate flow rates of 70 mL/min (4 experiments), total dialysate chlorine was >0.1 mg/L in 0, 2, 4 and 4 out of 4 measurements per experiment with a maximal bound chlorine of 0.22 mg/L and maximal free chlorine of 0.05 mg/L.

Ammonium

Limited release of ammonium into plasma was observed (Figure 12.A; 54±30 μM at the inlet and 387±68 μM at the outlet of the dialyzer; p=0.03; 148±48 μmol ammonium per removed mmol urea per hour). This did not result in a systemic increase in plasma ammonium during the experiment (Figure 12.B; 36±22 μM at the start vs. 58±31 μM at the end; p=0.25).

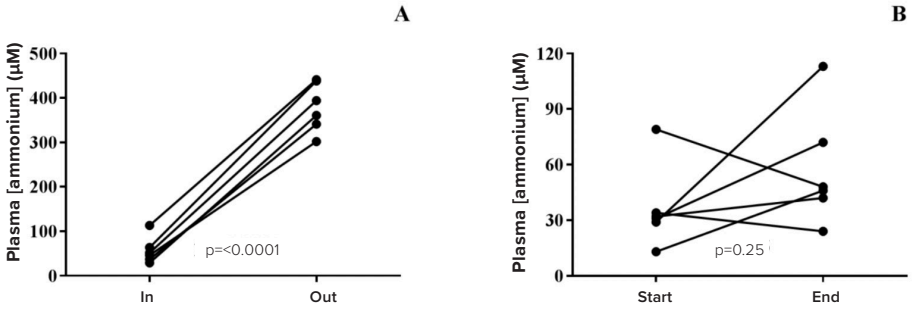

FIGURE 12. A: Plasma ammonium concentration at the inlet (In) and outlet (Out) of the dialyzer (n=6). Measurements were performed at the start and end of each experiment and per experiment the concentrations (In) and (Out) were averaged. B: Plasma ammonium concentration at the start and end of each experiment (n=6).

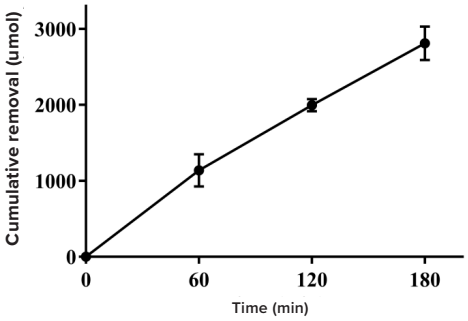

FIGURE 13. Cumulative creatinine removal *in vitro* circulating uremic plasma (n=3; mean [creatinine] 734±132 µM) at a blood flow of 110 mL/min and a dialysate flow of 40 mL/min using 1 EO unit.

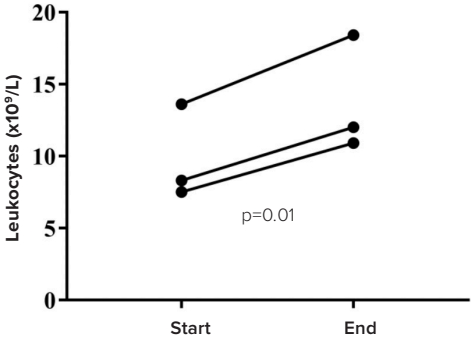

FIGURE 14. Leukocyte number at the start and end of treatment with a conventional hemodialysis machine (n=3).

DISCUSSION

In this study, clinically relevant urea removal was achieved with a miniature dialysis device in awake non-uremic goats by use of EO. The biocompatibility of this technique is a point of concern, as ammonium release was detected and dialysate chlorine concentrations were above acceptable levels at higher dialysate flow rates.

Our study shows that a miniature dialysis device containing an EO unit with graphite electrodes can remove ~8.4 mmol urea/h at a plasma [urea] of ~12 mM. This amount is comparable to that reported previously *in vitro* (9.5 mmol/h at a higher blood [urea] of 18.6 mM) [39]. As shown in our *in vitro* report, absolute urea removal increased with increasing urea concentration. In our *in vitro* report we suggested several measures to increase urea removal. First, we hypothesized that increasing the electrode surface area would result in more urea removal. In the present study we showed that doubling the electrode surface area indeed doubled the urea removal rate (15.9 mmol/h). Second, we suggested that a higher dialysate flow rate would result in more urea removal. However, the current experiments showed that increasing the dialysate flow rate from 40 to 70 mL/min did not increase urea removal (15.8 mmol/h). We submit that reduced dialysate electrode contact time, resulting from an increased dialysate flow rate, compromised the urea oxidation efficiency and thereby overall urea degradation [30,39]. Third, increasing the applied current is expected to increase urea removal. However, in our *in vitro* report we showed that a current of 3 A (0.5 A/dm^2 electrode), as applied in the current study, resulted in the lowest release of chlorine species per removed mole of urea [39].

To compensate for the total production of urea from catabolism of dietary protein, ~250-400 mmol of urea will have to be removed daily [13,31,37]. Extrapolation of the urea removal that we obtained by using two EO units in parallel during 3 h, would result in removal of ~380 mmol per day if the device would be used continuously 24 h/day. To achieve similar time averaged urea plasma clearance as thrice weekly 4-h HD (12.3 mL/min [9]) or continuous ambulatory peritoneal dialysis (PD) (9 mL/min [10]), the 2-unit device (urea clearance: 19.5 mL/min) should be used for at least ~15 h/day or 11 h/day 7 days per week, respectively. However, several factors preclude the use of our device for an extended time up to 24 h/day. First, our two unit device weighs ~2 kg (including battery), which may be too heavy for frail patients. Second, the lack of a reliable vascular access that can be used continuously is a bottleneck since conventional long-term venous access solutions, such as tunneled CVCs, carry the risk of considerable blood loss and air embolism in case of accidental disconnection and are associated with a high incidence of bloodstream infections [15]. To allow the use of EO for intermittent HD, it will be necessary to further increase the cumulative

electrode surface of the EO units. For example, for daily 8 h nocturnal HD, the electrode surface area would have to be increased by 2-3 fold.

Creatinine removal showed a different pattern than urea removal. Creatinine was completely removed from the dialysate when one unit or two units were used, also at a higher dialysate flow rate, and was therefore flow dependent (in contrast to urea removal). Creatinine removal and clearance were primarily determined by equilibration of creatinine across the dialyzer membrane, which was not complete using a dialyzer with a surface area of 0.2 m^2 (dialysate concentrations downstream of the dialyzer were lower than plasma concentrations upstream of the dialyzer at all dialysate flow rates), and may therefore be further increased by using a dialyzer with a higher surface area. Of note, the absolute amount of creatinine that was removed is relatively low (at maximum 210 µmol/h) compared to the daily creatinine production (~8-17 mmol [19]) due to low (non-uremic) plasma creatinine concentrations (range: 45-76 µM) in the healthy goats. *In vitro* studies performed earlier with the single unit device showed that creatinine removal up to ~1 mmol/h could be achieved at 'uremic' concentrations (unpublished observation, Figure 13). To match time-averaged plasma clearances for conventional HD and PD (9.4 mL/min and 4.3 mL/min [10], respectively), the 2 unit device (50 g of AC) would need to be used for at least 25 h/week or 12 h/week, respectively. Alternatively, the number of units and the cumulative dialysate flow could be increased.

Clinically relevant potassium and phosphate removal was achieved with 2 units (17.9 mmol/3 h and 8.2 mmol/3 h, respectively, as compared to a required daily removal of ~45 mmol potassium and ~15 mmol phosphate) [21,33,35]. In comparison, potassium and phosphate removal with conventional HD is ~53 mmol [2] and ~15-30 mmol per 4-h session [22,36], respectively, and with PD ~29-42 mmol/day [24,41] and ~7.2 mmol/day [6], respectively. Potassium and phosphate removal using 1 EO unit were in the same range as the previously reported removal using sorbent units without EO [38]. Consequently, potassium and phosphate removal appear not to be influenced by EO. Increasing the amount of potassium sorbent from 80 to 180 g (x 2.25) and the amount of phosphate sorbent from 40 to 60 g (x 1.50) resulted in a comparable increase in removal (a 2.29-fold increase in potassium removal and a 1.55-fold increase in phosphate removal). To match the potassium and phosphate removal during a conventional HD session, the amounts of the potassium and phosphate sorbent should be further increased to ~533 g and ~110-220 g, respectively, and to match PD, only the amount of the potassium sorbent should be increased to ~292-422 g.

Although these *in vivo* results showed that clinically relevant removal of urea, potassium

and phosphate can be achieved with our prototype miniature device that combines EO and sorbent technology, several adverse effects were noted. They can be divided into EO related events, sorbent induced changes and effects probably induced by the specific animal model.

Specific EO induced adverse effects included the generation of chlorine by-products and ammonium. Although dialysate chlorine concentrations remained well below the maximum levels defined in the AAMI standards for dialysate (bound chlorine <0.10 mg/L, free chlorine <0.50 mg/L) at a dialysate flow rate of 40 mL/min, concentrations exceeding these levels were detected at a dialysate flow rate of 70 mL/min. This should be avoided, as increased levels of chlorine by-products may cause hemolysis [34]. The explanation for the increased chlorine levels at higher dialysate flow rates is probably that the contact time of the dialysate with the AC was too short for sufficient adsorption to occur [14]. To counteract this problem, the amount of AC, that functions as an electron donor for reductive dechlorination) [39], could be increased. Alternatively, the current density (i.e. current per unit surface area of the electrode) could be decreased while increasing the total electrode surface area to maintain urea removal rate constant, since we observed *in vitro* that this strategy virtually abolishes chlorine release (unpublished observation).

Ammonium was also generated in the EO unit, probably due to hydrolysis of urea [20], and released into the dialysate at a rate of 148±48 µmol ammonium per removed mmol urea per hour. At a urea removal rate of ~16 mmol per hour this would amount to a production of ~7 mmol during a 3 h treatment. This amount is low compared to the daily amount of ammonia/-um that is converted into urea by the liver (~500-800 mmol), and resembles the production by healthy kidneys under basal conditions (~60-80 mmol/day) of which ~50% is secreted and the remaining enters the systemic circulation [37]. Ammonium release did not result in a systemic increase in ammonium concentration, presumably because of fast hepatic conversion into urea and possibly also increased ammonium excretion by the healthy kidneys [25,26]. For proper evaluation of the influence of EO-induced ammonia/-um release on systemic ammonium concentrations in end stage kidney disease, experiments should be repeated in animals with end stage kidney disease.

Nevertheless, the direct release of ammonium into the systemic circulation may be harmful, in particular for the brain, although other organs and tissues may also be affected [7], and should preferably be avoided. Consequently, strategies should be developed to prevent the generation of ammonium in the EO unit or to prevent its release into the circulation by incorporating additional cation exchanger in the circuit downstream of the EO unit.

Changes induced by the sorbents included minor decreases in systemic plasma calcium and magnesium concentrations. As both the plasma calcium and magnesium concentrations decreased slightly after flowing through the dialyzer, some adsorption of these substances must have occurred by the PS-DVB sulfonate beads contained in the EO/sorbent unit. This can be easily prevented by prerinsing the sorbents at somewhat higher calcium and magnesium concentrations. Of note, sodium release, which could in theory occur due to exchange of sodium for other cations by the PS-DVB sulfonate beads, was prevented by prerinsing the sorbents at [Na^+] 120 mM, as previously reported [38]. A minimal decrease in sodium concentration (< 1 mmol) occurred across the dialyzer, suggesting even minimal sodium adsorption by the PS-DVB sulfonate beads. Consequently, we should use a slightly higher sodium concentration for prerinsing the sorbents. Plasma and dialysate glucose concentrations decreased moderately across the dialyzer and EO/sorbent unit, respectively, while systemic glucose concentration did not change during the experiments. Removal of glucose by the device is probably due to adsorption by activated carbon which may be prevented by prerinsing at a physiological glucose concentration. *In vitro* experiments have shown that electro-oxidation of glucose is negligible (data not shown).

Several changes in plasma chemistry could not be explained by parallel changes occurring in plasma flowing through the dialyzer, and they must therefore be related to the animal model using non-uremic goats. First, the systemic plasma sodium concentration increased, despite a tendency of the plasma sodium concentrations to decrease across the dialyzer. This very mild systemic increase in plasma sodium concentration may be related to a high electrolyte-free water clearance inherent to urea-induced osmotic diuresis [5,19,27]. Second, a small but significant decrease in plasma bicarbonate concentration of 1-2 mM was observed which contrasted with a minimal (<1 mM) increase in plasma bicarbonate across the dialyzer. This reduction in plasma bicarbonate concentration was accompanied by an increase in plasma chloride of a similar magnitude. The most likely explanation for this combination (hyperchloremic metabolic acidosis) is the intravenous infusion of solutions that exclusively contained chloride as an anion [16], although minor chloride release across the dialyzer (only in experiments with 2 units) probably related to the exchange of phosphate and organic acids for chloride in the FeOOH beads, may have played a role. No influence on pH was observed, which was due to a coinciding decrease in arterial pCO_2, probably due to stress-induced hyperventilation which increased during the experiment while the effect of the sedatives wore off. Third, hemolysis was observed *in vivo*, This could be attributed to urea infusion, since visible hemolysis and LDH increase occurred exclusively in experiments with urea infusion. Importantly, hemolysis also occurred in an experiment with urea infusion in which no dialysis and EO were performed, indicating that hemolysis was not EO-related. In agreement with our observation in goats, it has been

shown that urea infusion also induces hemolysis in humans [4,27]. Finally, the leukocyte number increased during the experiments. This may be due to 'catecholamine-induced leukocytosis', a phenomenon characterized by a rapid and transient increase in leukocytes observed during acute stress, presumably reflecting demargination of leukocytes from the marginal pool [39]. In agreement with such an acute stress reaction, diastolic blood pressure and heart rate increased during treatment in these awake animals. Similar leukocyte and cardiovascular responses were observed in animals treated by the device without activating EO [38] and in HD experiments using a conventional HD machine (unpublished observation, Figure 14), suggesting that this stress reaction was not induced by EO, the sorbents or other materials in the device. However, it has not been excluded that dialyzable substances derived from the device may provoke an acute inflammatory response with a presentation indistinguishable from that of an acute stress reaction. To exclude this, the pro-inflammatory capacity of dialysate treated by the device should be studied *in vitro*.

In summary, the present study shows that a device combining EO, sorbents and AC can remove relevant amounts of urea, potassium and phosphate in a non-uremic large animal model. However, many challenges have to be overcome to reach the stage of application of the device in humans. First, a uremic large animal model has to be developed, as the current model involves infusion of relatively large volumes of solutions containing electrolytes and urea that obscure the true effects of the device on electrolyte and acid-base parameters and hemocompatibility. Second, little is known about potentially toxic oxidative by-products other than chlorines and ammonium that may be formed during electro-oxidation [38]. Research is underway to define the chemical constituents of dialysate of uremic subjects exposed to electro-oxidation and to test the biocompatibility of such fluids in *in vitro* experiments [28]. Third, the changes in the concentration of bicarbonate in plasma flowing through the dialyzer are small and equivocal. Apparently, very little bicarbonate is released from the current device, which would prevent correction of the metabolic acidosis present in uremic patients needing dialysis, and this problem remains to be solved. Finally, the system would require upscaling in order to develop a portable device that can be used for nocturnal or daily home dialysis. However, this process will also increase the generation of known toxic by-products of electro-oxidation such as chlorines or ammonium, and strategies will have to be developed to prevent the production or release of these substances.

REFERENCES

1. AAMI standards for hemodialysis systems. ANSA/AAMI (RD 5).
2. Agar BU, Culleton BF, Fluck R, and Leypoldt JK. Potassium kinetics during hemodialysis. *Hemodialysis international International Symposium on Home Hemodialysis* 19: 23-32, 2015.
3. Agar JW. Review: understanding sorbent dialysis systems. *Nephrology (Carlton)* 15: 406-411, 2010.
4. Bensinger TA, Glader BE, and Conrad ME. Intravascular hemolysis associated with intravenous urea infusions in normal individuals. *Blood* 41: 461-464, 1973.
5. Bodonyi-Kovacs G, and Lecker SH. Electrolyte-free water clearance: a key to the diagnosis of hypernatremia in resolving acute renal failure. *Clinical and experimental nephrology* 12: 74-78, 2008.
6. Courivaud C, and Davenport A. Phosphate Removal by Peritoneal Dialysis: The Effect of Transporter Status and Peritoneal Dialysis Prescription. *Peritoneal dialysis international : journal of the International Society for Peritoneal Dialysis* 36: 85-93, 2016.
7. Dasarathy S, Mookerjee RP, Rackayova V, Rangroo Thrane V, Vairappan B, Ott P, and Rose CF. Ammonia toxicity: from head to toe? *Metab Brain Dis* 32: 529-538, 2017.
8. Descombes E, Perriard F, and Fellay G. Diffusion kinetics of urea, creatinine and uric acid in blood during hemodialysis. Clinical implications. *Clin Nephrol* 40: 286-295, 1993.
9. Eknoyan G, Beck GJ, Cheung AK, Daugirdas JT, Greene T, Kusek JW, Allon M, Bailey J, Delmez JA, Depner TA, Dwyer JT, Levey AS, Levin NW, Milford E, Ornt DB, Rocco MV, Schulman G, Schwab SJ, Teehan BP, and Toto R. Effect of dialysis dose and membrane flux in maintenance hemodialysis. *The New England journal of medicine* 347: 2010-2019, 2002.
10. 10. Evenepoel P, Meijers BK, Bammens B, Viaene L, Claes K, Sprangers B, Naesens M, Hoekstra T, Schlieper G, Vanderschueren D, and Kuypers D. Phosphorus metabolism in peritoneal dialysis- and haemodialysis-treated patients. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 31: 1508-1514, 2016.
11. Grinval'd V LG, Rodin VV. Development and Testing of a Unit for Electrochemical Oxidation of Products of Hemodialysis. Biomedical Engineering 37: 67-72, 2013.
12. Gura V, Beizai M, Ezon C, and Polaschegg HD. Continuous renal replacement therapy for end-stage renal disease. The wearable artificial kidney (WAK). *Contrib Nephrol* 149: 325-333, 2005.
13. Ikizler TA, Greene JH, Yenicesu M, Schulman G, Wingard RL, and Hakim RM. Nitrogen balance in hospitalized chronic hemodialysis patients. *Kidney Int Suppl* 57: S53-56, 1996.
14. Kochany J, and Lipczynska-Kochany E. Catalytic destruction of chloramine to nitrogen using chlorination and activated carbon--case study. *Water Environ Res* 80: 339-345, 2008.
15. Kooman JP, Joles JA, and Gerritsen KG. Creating a wearable artificial kidney: where are we now? *Expert Rev Med Devices* 12: 373-376, 2015.
16. Kraut JA, and Kurtz I. Treatment of acute non-anion gap metabolic acidosis. *Clinical kidney journal* 8: 93-99, 2015.
17. Lee DB, and Roberts M. A peritoneal-based automated wearable artificial kidney. *Clin Exp*

Nephrol 12: 171-180, 2008.

18. Lehmann HD, Marten R, and Gullberg CA. How to catch urea? Considerations on urea removal from hemofiltrate. *Artif Organs* 5: 278-285, 1981.
19. Lindner G, Schwarz C, and Funk GC. Osmotic diuresis due to urea as the cause of hypernatraemia in critically ill patients. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* 27: 962-967, 2012.
20. Lu F BG. Ammonia Generation via a Graphene-Coated Nickel Catalyst. *Coatings* 7: 2017.
21. Mente A, O'Donnell MJ, Rangarajan S, McQueen MJ, Poirier P, Wielgosz A, Morrison H, Li W, Wang X, Di C, Mony P, Devanath A, Rosengren A, Oguz A, Zatonska K, Yusufali AH, Lopez-Jaramillo P, Avezum A, Ismail N, Lanas F, Puoane T, Diaz R, Kelishadi R, Iqbal R, Yusuf R, Chifamba J, Khatib R, Teo K, and Yusuf S. Association of urinary sodium and potassium excretion with blood pressure. *The New England journal of medicine* 371: 601-611, 2014.
22. Mucsi I, Hercz G, Uldall R, Ouwendyk M, Francoeur R, and Pierratos A. Control of serum phosphate without any phosphate binders in patients treated with nocturnal hemodialysis. *Kidney international* 53: 1399-1404, 1998.
23. Murisasco A, Baz M, Boobes Y, Bertocchio P, el Mehdi M, Durand C, Reynier JP, and Ragon A. A continuous hemofiltration system using sorbents for hemofiltrate regeneration. *Clin Nephrol* 26 Suppl 1: S53-57, 1986.
24. Musso CG. Potassium metabolism in patients with chronic kidney disease. Part II: patients on dialysis (stage 5). *Int Urol Nephrol* 36: 469-472, 2004.
25. Olde Damink SW, Deutz NE, Dejong CH, Soeters PB, and Jalan R. Interorgan ammonia metabolism in liver failure. *Neurochemistry international* 41: 177-188, 2002.
26. Owen EE, Johnson JH, and Tyor MP. The effect of induced hyperammonemia on renal ammonia metabolism. *The Journal of clinical investigation* 40: 215-221, 1961.
27. Ravin MB, Garber V, and Gibson EL. Intravascular hemolysis following urea administration during hypothermia. *Anesthesiology* 25: 576-580, 1964.
28. Renal Replacement Technology Grant "Safety analysis and optimization of electrochemical dialysate regeneration" (Dutch Kidney Foundation N, 2013-2018).
29. Ronco C, and Fecondini L. The Vicenza wearable artificial kidney for peritoneal dialysis (ViWAK PD). *Blood Purif* 25: 383-388, 2007.
30. Schuenemann B, Quellhorst E, Kaiser H, Richter G, Mundt K, Weidlich E, Loeffler G, Zachariae M, and Schunk O. Regeneration of filtrate and dialysis fluid by electro-oxidation and absorption. *Trans Am Soc Artif Intern Organs* 28: 49-53, 1982.
31. Shinaberger CS, Kilpatrick RD, Regidor DL, McAllister CJ, Greenland S, Kopple JD, and Kalantar-Zadeh K. Longitudinal associations between dietary protein intake and survival in hemodialysis patients. *Am J Kidney Dis* 48: 37-49, 2006.
32. Stephens RL, Jacobsen SC, Atkin-thor E, and Kolff W. Portable/wearable artificial kidney (WAK) - initial evaluation. *Proc Eur Dial Transplant Assoc* 12: 511-518, 1976.
33. Therrien M, Byham-Gray L, Denmark R, and Beto J. Comparison of dietary intake among women

on maintenance dialysis to a Women's Health Initiative cohort: results from the NKF-CRN Second National Research Question Collaborative Study. *Journal of renal nutrition : the official journal of the Council on Renal Nutrition of the National Kidney Foundation* 24: 72-80, 2014.

34. Tipple MA, Shusterman N, Bland LA, McCarthy MA, Favero MS, Arduino MJ, Reid MH, and Jarvis WR. Illness in hemodialysis patients after exposure to chloramine contaminated dialysate. *ASAIO Trans* 37: 588-591, 1991.
35. Vervloet MG, van Ittersum FJ, Buttler RM, Heijboer AC, Blankenstein MA, and ter Wee PM. Effects of dietary phosphate and calcium intake on fibroblast growth factor-23. *Clinical journal of the American Society of Nephrology : CJASN* 6: 383-389, 2011.
36. Wang M, Li H, Liao H, Yu Y, You L, Zhu J, Huang B, Yuan L, Hao C, and Chen J. Phosphate removal model: an observational study of low-flux dialyzers in conventional hemodialysis therapy. *Hemodialysis international International Symposium on Home Hemodialysis* 16: 363-376, 2012.
37. Weiner ID, Mitch WE, and Sands JM. Urea and Ammonia Metabolism and the Control of Renal Nitrogen Excretion. *Clinical journal of the American Society of Nephrology : CJASN* 10: 1444-1458, 2015.
38. Wester M, Gerritsen KG, Simonis F, Boer WH, Hazenbrink DH, Vaessen KR, Verhaar MC, and Joles JA. A regenerable potassium and phosphate sorbent system to enhance dialysis efficacy and device portability: a study in awake goats. *Nephrol Dial Transplant* 32: 951-959, 2017.
39. Wester M, Simonis F, Lachkar N, Wodzig WK, Meuwissen FJ, Kooman JP, Boer WH, Joles JA, and Gerritsen KG. Removal of urea in a wearable dialysis device: a reappraisal of electro-oxidation. *Artif Organs* 38: 998-1006, 2014.
40. Yatzidis H. Charcoal for the treatment of endogenous and exogenous intoxications. Its use as an effective artificial kidney. Proc Eur Dialysis Transplant Assoc 1:83.
41. Yu HL, Lu XH, Su CY, Tang W, and Wang T. Potassium metabolism in continuous ambulatory peritoneal dialysis patients. *Ren Fail* 36: 748-754, 2014.

PART III

Uremic toxin removal by mixed matrix membranes

6

A novel approach for blood purification: mixed-matrix membranes combining diffusion and adsorption in one step

Marlon S. Tijink, Maarten Wester, Junfen Sun, Anno Saris, Lydia Bolhuis-Versteeg, Jabar Saiful, Jaap A Joles, Zandrie Borneman, Matthias Wessling, Dimitrios Stamatialis

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ABSTRACT

Hemodialysis is a commonly used blood purification technique in patients requiring kidney replacement therapy. Sorbents could increase uremic retention solute removal efficiency but, because of poor biocompatibility, their use is often limited to the treatment of patients with acute poisoning. This paper proposes a novel membrane concept for combining diffusion and adsorption of uremic retention solutes in one step: the so-called mixed-matrix membrane (MMM). In this concept, adsorptive particles are incorporated in a macro-porous membrane layer whereas an extra particle-free membrane layer is introduced on the blood-contacting side of the membrane to improve hemocompatibility and prevent particle release. These dual-layer mixed-matrix membranes have high clean-water permeance and high creatinine adsorption from creatinine model solutions. In human plasma, the removal of creatinine and of the protein-bound solute para-aminohippuric acid (PAH) by single and dual-layer membranes is in agreement with the removal achieved by the activated carbon particles alone, showing that under these experimental conditions the accessibility of the particles in the MMM is excellent. This study proves that the combination of diffusion and adsorption in a single step is possible and paves the way for the development of more efficient blood purification devices, excellently combining the advantages of both techniques.

INTRODUCTION

The prevalence of end-stage renal disease (ESRD) was ~535,000 in the USA in 2008. Of these patients, ~355,000 were treated with hemodialysis. Despite the high health care costs of dialysis treatment (over €50,000 per patient per year), hemodialysis is only partially successful in the treatment of patients with ESRD. Mortality (15–20% per year) and morbidity of these patients remain excessively high, whereas their quality of life is generally low [1]. This is reflected in the expected remaining life years, which are 25.0 years for the general US population, 15.7 for ESRD patients with a kidney transplant and 5.6 years for ESRD patients receiving dialysis treatment [2].

In the last three decades, sorbent technology has been applied in the treatment of severe intoxication and to increase the efficiency of hemodialysis, or replace it, and as a treatment for fulminant hepatic failure. In hemoperfusion (or plasma perfusion), blood (or plasma) is purified by extracorporeal passage through a column containing the adsorbent which can remove or neutralize the substance of interest. Hemoperfusion cannot fully substitute hemodialysis because it does not remove urea and excess fluid. Sorbents used in hemoperfusion help to remove uremic toxins; however, direct blood contact with the adsorbent often causes hemocompatibility issues, especially on the long term [3]. Activated carbon (AC) has a long record as a sorbent in blood purification in the case of intoxications, acute and chronic renal failure as well as liver failure [3-5]. Uncoated activated carbon is a strong adsorbent for uremic toxins [6] whereas polymeric coatings of activated carbon might help to improve hemocompatibility. However, coated activated carbon could still release carbon fragments, even after careful washing, and a double coating process is needed to overcome this problem [7]. In conventional hemoperfusion columns, optimal distribution of blood flow within the packed sorbent bed is very important for adequate use of the adsorption capacity, especially with rather viscous and complex solutions like blood or plasma. Channelling within the column leads to suboptimal adsorption and can induce blood coagulation. Furthermore, micro-particles that can be released into the circulation and can cause emboli are always a concern related to hemoperfusion.

It is obvious that a combination of the strengths of dialysis membranes with the adsorption power of high surface area sorbents can be very beneficial for the blood purification efficacy [8]. In fact, in the late 1970s so-called sorbent membranes were developed. These membranes were even on the market for a certain period, produced by Enka [9-15]. However, due to their quick saturation, manufacturing difficulties, reduced patient convenience and lack of adsorbents with high purity [16-19], they were quickly abandoned. More recently, membrane filtration and adsorption columns are often combined as two separate steps in

wearable artificial kidneys [20,21].

In this paper, we propose a novel membrane concept for combining diffusion and adsorption of uremic retention solutes in one step: the so-called mixed-matrix membrane (MMM). In this concept, adsorptive particles are incorporated in a macro-porous membrane matrix. A particle-free membrane layer is introduced on the blood contacting side of the membrane, aiming to improve membrane hemocompatibility and prevent particle release into the circulation and hence emboli formation (see Figure 1).

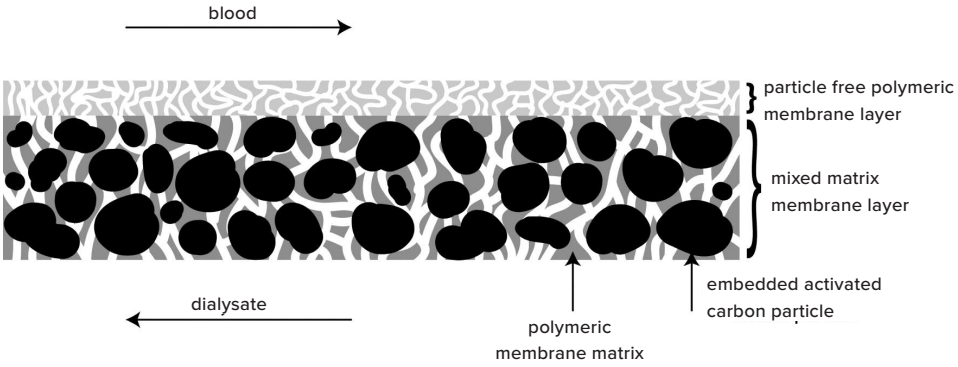

FIGURE 1. Concept of dual-layer mixed-matrix membranes for blood purification.

Mixed-matrix membranes have been proposed as an alternative for traditional chromatographic columns [22,23]. Compared to conventional columns, they have low flow resistance, which allows the use of smaller particles, resulting in an improved adsorption capacity and adsorption kinetics [24,25]. Furthermore, the particles can be homogenously distributed by embedding them in the matrix, leading to optimal adsorption efficiencies and preventing quick saturation.

Here, for the proof of concept, we prepare and investigate flat sheet MMMs using materials with an excellent record in blood purification. A polyethersulfone (PES)/polyvinylpyrrolidone (PVP) polymer blend is used for the preparation of the macro-porous membrane matrix (PES as a membrane-forming polymer blended with PVP to improve hydrophilicity) and activated carbon is used as adsorptive particle. Creatinine, a small-molecular-weight uremic retention solute, often used as a marker of kidney function, is used as model water soluble solute. The para-aminohippuric acid (PAH) which belongs to the family of hippurates, and is often used as a marker for organic anion transport because of tubular secretion, is used in this study as a model protein-bound solute [26-29].

The study investigates the combination of diffusion and adsorption in a single step, which probably leads to more efficient blood purification devices and will prevent issues related to the use of conventional hemoperfusion columns.

MATERIALS AND METHODS

Materials

Polyethersulfone (PES) (ULTRASON, E6020P, BASF, the Netherlands) was used as membrane material. Polyvinylpyrrolidone (PVP) (K90), (MW ≈ 360,000, Fluka, Sigma–Aldrich, Germany) and extra pure N-Methylpyrrolidone (NMP) (Acros organics, Belgium) were used as additive and solvent, respectively. Ultrapure water, prepared with a Millipore purification unit, was used as non-solvent in the coagulation bath. Activated carbon (Norit A Supra EUR, Norit Netherlands B.V., the Netherlands) was sieved with a 45 µm sieve (Fritsch GmbH, Germany) and used as adsorbent particles (median size 27 µm). The following chemicals were purchased from Fluka, Sigma–Aldrich. Creatinine was dissolved in Tyrode's buffer (pH 7.4) composed of 137 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 11.9 mM $NaHCO_3$ and 5.5 mM glucose in ultrapure water.

Membrane preparation

The particle-free membrane layer was prepared using a 15 wt.% PES and a 7 wt.% PVP in NMP solution which was stirred at a roller bank overnight at room temperature. For the MMM, first a mixture of 14 wt.% PES and 1.4 wt.% PVP in NMP solution was prepared and stirred at a roller bank overnight at room temperature, then different amounts of dry activated carbon particles were added. Loadings of 50, 60 and 70 wt.% activated carbon in relation to the amount of PES in the mixed-matrix membrane layer were applied, calculated as:

$$\text{Loading } (\%) = \frac{W_{AC}}{W_{AC} + W_{PES}} \text{ x } 100 \quad (1)$$

where W_{AC} is the dry weight of activated carbon particles (g) and W_{PES} is the dry weight of PES (g). The mixtures were stirred at least overnight and ultrasound was applied for at least 15 min to break down possible particle clusters. After degassing overnight, all the membranes were prepared by immersion precipitation.

Solutions were cast on a glass plate using a casting knife. A slit of 300 µm and 150 µm for single-layer MMMs and single particle-free membranes were used respectively. An adjustable co-casting knife was used for dual-layer MMMs, see Figure 2. The heights of the slits of the first and second knife were 300 and 450 µm respectively. Casting was immediately followed by immersion into the coagulation bath, containing 60 wt.% NMP in ultrapure water at room temperature. After the membrane formation process, the membranes were rinsed with ultrapure water to remove residual solvent traces, and stored in ultrapure water upon further use.

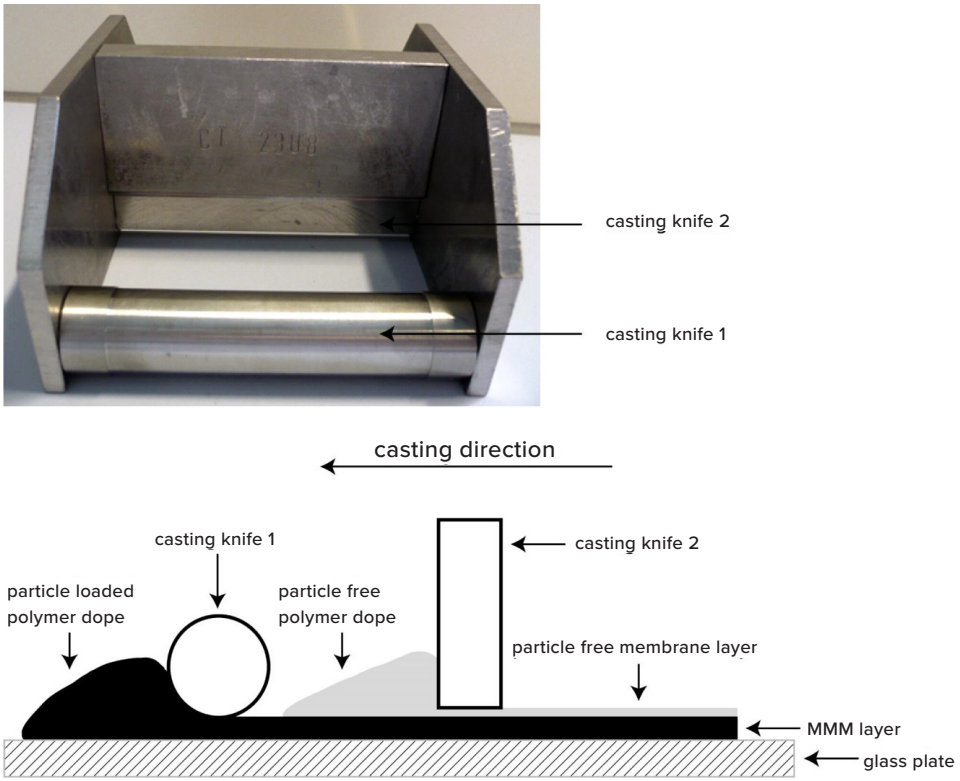

FIGURE 2. Picture and schematic drawing of the co-casting knife. It consists of two attached casting knifes with 300 and 450 µm slits. The particle-free polymer dope is cast by casting knife 2 on top of the particle-loaded polymer dope casted by casting knife 1 to form dual-layer membranes.

Membrane characterization

Scanning electron microscopy

For scanning electron microscopy (SEM), membranes were dried in air at room temperature and cryogenically broken in liquid nitrogen. The obtained cross-sections were dried overnight under vacuum at 30 °C and gold coated using a BalzersUnion SCD 040 sputter coater (OerlikonBalzers, Belgium). Coated membrane samples were examined using a JEOL JSM-5600LV scanning electron microscope (JEOL, Japan).

Membrane transport properties

Clean-water fluxes of the membranes were tested at room temperature using a nitrogen pressurized dead-end "Amicon type" ultrafiltration cell and ultrapure water. Flat membranes with an active surface area of 8.04 cm^2 were used. First, membranes were pre-pressurized for at least 0.5 h at the highest applicable pressure, which was 1.00 bar. Subsequently,

pressures of 0.25, 0.5, 0.75 and 1.00 bar were applied for at least 20 min and the clean-water flux at each pressure was determined. The membrane permeance was calculated from the slope of the linear part of the flux vs. transmembrane pressure relation.

The BSA sieving coefficient of the single and dual-layer membranes was measured at room temperature using a nitrogen pressurized dead-end Amicon ultrafiltration cell. BSA was dissolved in ultrapure water with an initial concentration of 1 mg $ml^{-1}$and was pressurized through the membranes (with active surface area of 12.57 cm^2) at 0.5 bar for 30 min. The BSA sieving coefficient (SC) was calculated using the equation:

$$SC = \frac{C_p}{C_f} \tag{2}$$

where C_p and C_f are the BSA concentrations in the permeate and feed solution, respectively.

The BSA concentrations were determined by spectrophotometric analysis (Varian, Cary 300 Scan UV–visible spectrophotometer) at 278 nm. The student **t**-test was used for statistical testing ($p < 0.05$).

Creatinine adsorption capacity

The creatinine adsorption capacity of the prepared membranes was determined by batch adsorption experiments with model creatinine solutions. Known amounts of dry membranes were equilibrated in solutions with different creatinine concentrations in a shaking water bath at 37 °C for 24 h. The equilibrium creatinine concentration (C) was determined by spectrophotometric analysis (Varian, Cary 300 Scan UV–visible spectrophotometer) at 230 nm with 2 mm quartz cuvettes at 25 °C. Via the mass balance the amount of adsorbed creatinine was calculated from the depleted amount of creatinine in the solution. The adsorption capacity (q) was expressed as mg adsorbed creatinine per g of adsorptive particle. For this the proportion of activated carbon particles in the membrane was estimated (see Supplementary for details). Dry membrane weight was multiplied by this proportion and the obtained amount of activated carbon particles in the membrane was used to relate with the amount of adsorbed creatinine. Origin 7.5 was used for non-linear curve fitting of the isotherm in order to obtain a Langmuir fit according to the following equation:

$$q = \frac{q_m \times C}{K_d + C} \tag{3}$$

In which q is the adsorption capacity (mg g^{-1} AC), **C** is the equilibrium concentration of creatinine (mg ml^{-1}) in the solution, q_m is the maximum adsorption capacity (mg g^{-1} AC) and K_d is the dissociation constant (mg ml^{-1}).

Adsorption from human blood plasma

Human plasma was obtained from six patients who underwent plasma exchange because of acute renal disease. 25 mg activated carbon, MMM and dual-layer MMM, which contained ~25 mg activated carbon based on the proportion of activated carbon particles in them and a particle-free membrane of similar size as the dual-layer MMM, were incubated in 4 ml of six different plasmas. In the case of small deviations from the 25 mg sorbent weight, the amount of plasma was adjusted so that the sorbent-volume proportion would be similar to 25 mg in 4 ml. These test samples and plasmas without sorbents were incubated on a roller bank for 4 h. After incubation, samples were centrifuged at 4 °C for 10 min. The supernatant, ~3 ml per sample, was collected and stored in micro-cups at 4 °C for analysis later. Osmolarity, pH, total protein and creatinine concentrations were measured according to the protocol of manufacturer of the kit and/or device (see Table 1) whereas the PAH concentration was measured following the protocol described elsewhere [30].

TABLE 1. Test methods for analysis of blood plasma.

Parameter	Kit/Device
Osmolarity	Advanced instruments osmometer model 3320
pH	Radiometer Copenhagen PHM lab pH meter
Creatinine	Bio-Rad Microplate reader Benchmark 16-channel photometer DiaSysCreatinine PAP FS (1 1759 99 10 026)
Total protein	Bio-Rad Laboratories GmbH Protein Assay (cat# 500-0006) Bio-Rad Microplate reader Benchmark 16-channel photometer

Since the initial concentrations of creatinine and PAH in plasma were different for every patient, and to avoid large variation by taking averages of the absolute concentrations, we used relative concentrations. The absolute initial creatinine concentrations in the six different plasmas were: 495.4, 1299.2, 332.6, 44.6, 276.4 and 60.9 µmol l^{-1}. For the PAH, only two plasmas had reasonable baseline concentrations (49.8 and 72.4 µmol l^{-1}); therefore only two plasmas were used for these experiments. The relative change in concentration of the various solutes was calculated as follows:

$$\text{Relative concentration} = \frac{C_s}{C_b} \tag{4}$$

where C_s is the concentration in the plasma incubated with a sample (AC or membranes) and C_b is the concentration in the blank solution (plasma without sorbents or membranes). Likewise, the relative osmolarity and relative pH were calculated. Statistical differences were determined using a one-way ANOVA and Dunnett's test for the creatinine, pH, osmolarity and total protein concentrations. For PAH, due to the low number of plasma samples no statistical analysis was performed.

Two-compartment diffusion test

A two-compartment diffusion device [31] was used to measure diffusion and adsorption of creatinine onto dual-layer MMMs at room temperature. Creatinine is a uremic retention solute and is used as an indicator for solute removal by MMMs.The donor compartment was filled with 0.1 mg ml^{-1} creatinine in Tyrode's buffer, whereas the acceptor compartment was filled with pure Tyrode's buffer. The compartments were separated by a dual-layer MMM, with the particle-free layer facing the creatinine containing donor solution. The volume of each compartment was 65 ml and the active membrane area was 3.14 cm^2. Both solutions were stirred at room temperature. During the experiment, 600 µl samples were taken in time to determine creatinine concentrations in both compartments. The creatinine decrease in the donor compartment was considered as total removal. The amount of creatinine that appeared in the acceptor compartment was considered as creatinine which was diffused from the donor compartment. The creatinine deficiency in the mass balance was considered to be adsorbed onto the membrane. This amount was related to the dry membrane weight, which was measured after the experiment.

RESULTS AND DISCUSSION

Here we describe the characterization of the prepared mixed-matrix membranes in terms of morphology and transport properties. First we show the influence of the particle loading on membrane morphology followed by the clean-water permeance measurements and creatinine adsorption isotherms for both the optimized single and dual-layer mixed-matrix membranes. Furthermore, we show adsorption from human blood plasma. Finally, we show creatinine transport results of dual-layer mixed-matrix membranes.

Membrane particle loading optimization

Different amounts of activated carbon particles were embedded in mixed-matrix membranes. Particle loading is an important parameter: besides influencing membrane morphology, the amount of adsorptive sites in the MMM increases as the particle loading increases. High particle loading can result in too high viscosities for proper casting or result in membranes with low mechanical strength.

Figure 3 shows cross-sections of MMMs containing 50, 60 and 70 wt.% of activated carbon in relation to the amount of PES. All membranes have a porous structure and no significant loss of particles was observed during membrane fabrication. Membranes loaded with 50 wt.% and 60 wt.% particles (Figure 3.A,B,D and E) have some macro-voids which may reduce mechanical strength of the membrane and may create transport channels. Membranes loaded with 70% particles contain more adsorptive sites per gram of membrane than at lower loadings. Furthermore, membranes with 70 wt.% activated carbon particles possess an open interconnected porous sponge-like structure, without macro-voids across the entire cross-section. In fact, as the particle loading increases, the viscosity of the dope increases as well. Higher dope viscosity restricts growth of the polymer lean phase in the phase separation process, because of a higher mass flow resistance. Likewise non-solvent inflow into the polymer solution is restricted, leading in turn to a slower phase separation process. The high viscosity prevents the formation of macro-voids and leads to the formation of smaller pores [32]. Furthermore, the particles probably act as a nucleus in the phase inversion process, leading to sponge-like structures [33]. Figure 3.G,H and I presents bottom surfaces of the different membranes. An increase in particle loading can clearly be observed by the amount of particles there. Activated carbon particles are tightly held together in the porous polymer matrix and no particle clusters are observed. All the obtained membranes possess sufficient mechanical strength for handling and characterization.

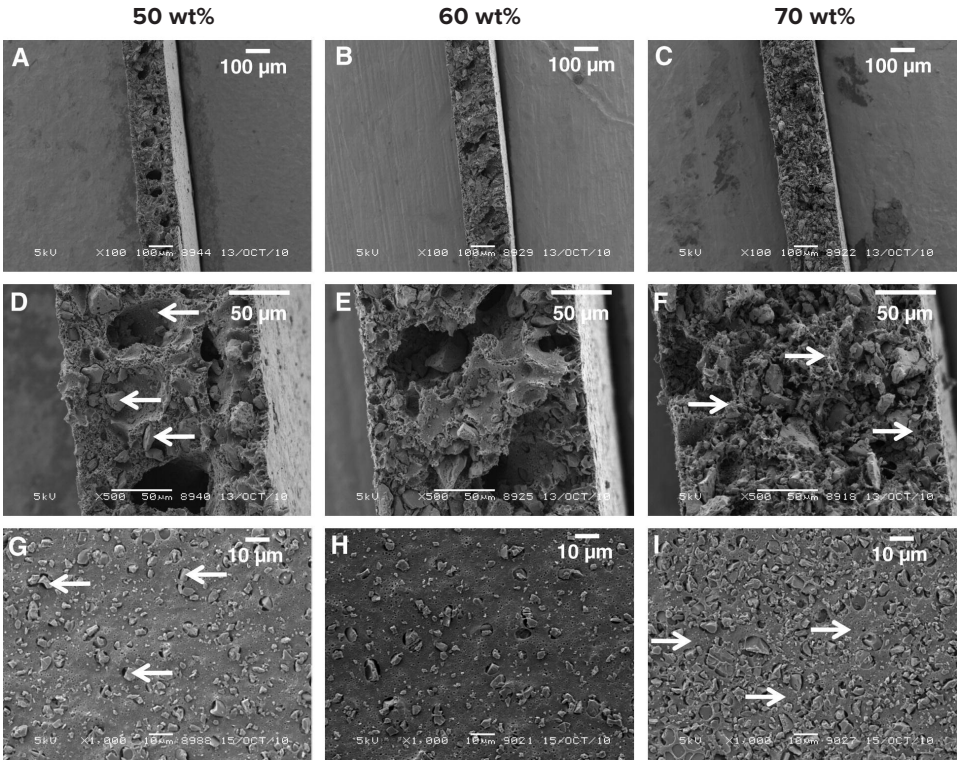

FIGURE 3. Scanning electron microscopy pictures of cross-sections (A, B, C, D, E and F) and bottom surface sections (G, H and I) of single-layer mixed-matrix membranes with loadings of 50 wt.% (A, D, G), 60 wt.% (B, E, H) and 70 wt.% (C, F, I). The arrows in D and G indicate the activated carbon particles whereas arrows in F and I indicate the porous polymeric membrane matrix.

In conclusion, membranes loaded with 70 wt.% activated carbon particles contain relatively the highest amount of adsorptive sites, show a porous interconnected structure and have sufficient mechanical strength. Therefore these membranes are selected for further characterization and development of dual-layer mixed-matrix membranes.

Dual-layer mixed-matrix membranes

To obtain dual-layer MMMs, we co-cast 70 wt.% particle-loaded polymer solution with a particle-free polymer solution. This particle-free layer will be the blood-contacting membrane side to avoid direct blood contact with the embedded sorbents during blood purification. Furthermore, it will prevent particle release into the circulation and consequent emboli formation.

Figure 4.A and B presents a single-layer MMM and dual-layer MMM respectively. The dual-layer MMM has a rather open interconnected porous structure. The particle-loaded layer of the dual-layer MMM possesses a sponge-like structure. Some small round-shaped voids are present in this layer but macro-voids through the entire cross-section are absent. The particle-free layer has a dense top layer and some macro-voids are present below this layer.

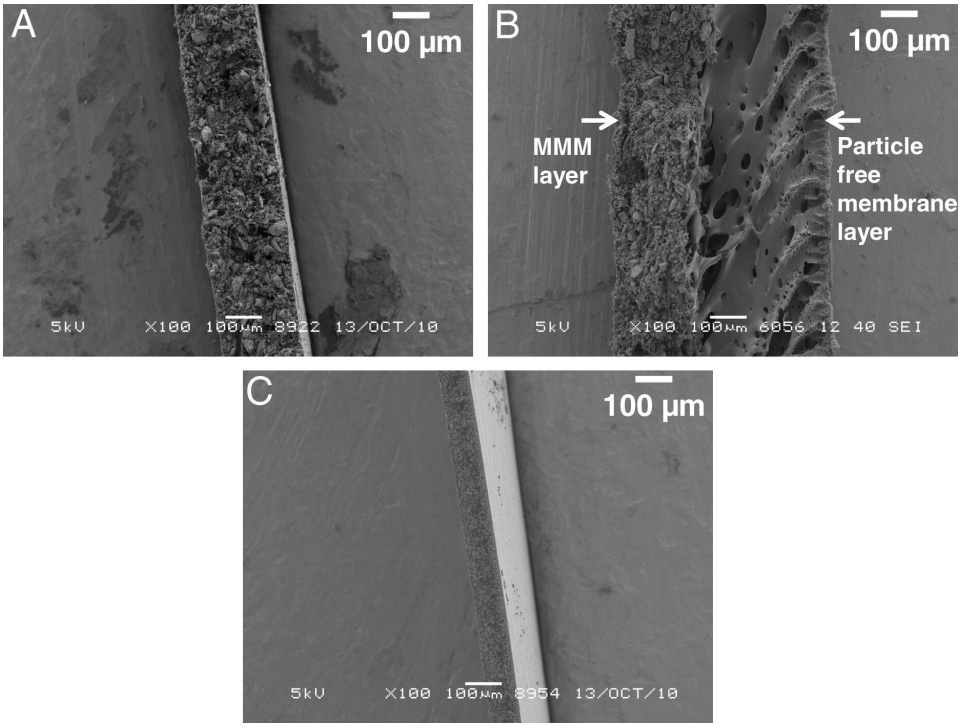

FIGURE 4. Scanning electron microscopy pictures of cross-sections of A. single-layer MMM, B. dual-layer MMM and C. particle-free membrane.

Figure 4.C presents the single particle-free membrane, which was cast directly on a glass plate. This single particle-free membrane has a homogenous sponge-like structure, whereas the particle-free layer in the dual-layer MMM has a dense sponge-like top layer but with a more open sub-layer with macro-voids. Besides, the thickness of the two layers of the dual-layer MMM is not in agreement with the casting thicknesses. Probably, the co-casting process, different viscosities of the dopes and different shrinkage of the two membrane layers during phase separation influence the final membrane structure of the dual-layer MMM.

Figure 5.A and B show photos of a single-layer mixed-matrix membrane and dual-layer mixed-matrix membrane respectively. In the dual-layer MMM, the membrane layer with the particles is black and the particle-free layer is white and completely covers the layer with the particles, see Figure 5.B. Figure 5.C shows that the layer with the particles is rather rough whereas the particle-free layer has a more smooth surface and smaller pores.

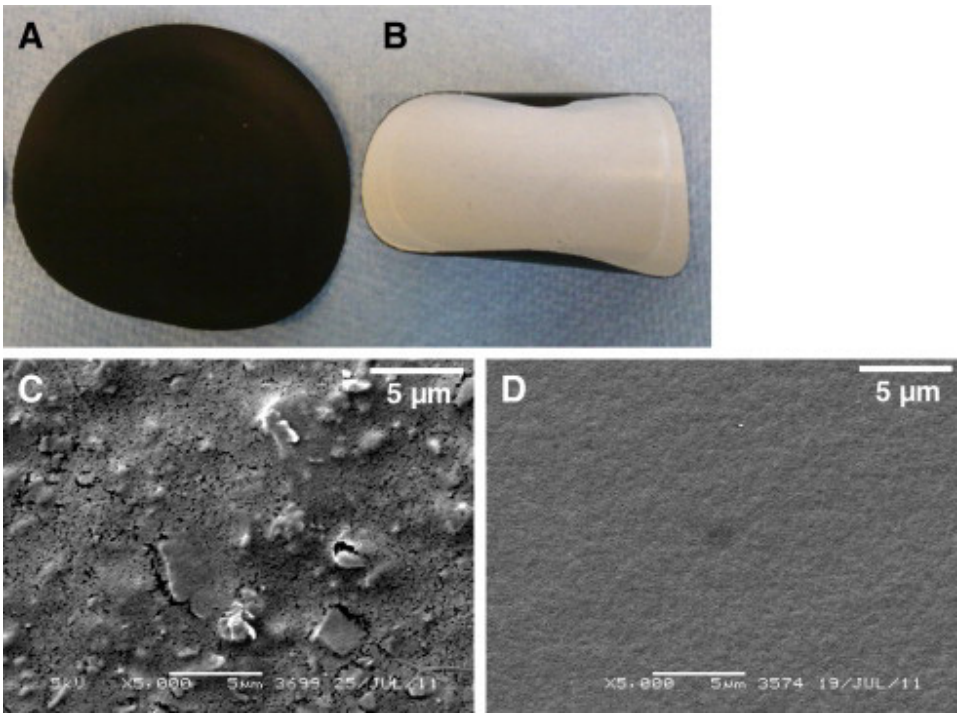

FIGURE 5. Surface area pictures and SEM pictures of A, C: a single-layer MMM and B, D: dual-layer MMM.

In dual-layer MMM, the two different layers can clearly be distinguished and are well attached to each other. In fact, no delamination of the two membrane layers was observed. These 70 wt.% loaded single-layer membranes and dual-layer membranes are further characterized.

Membrane transport properties

Figure 6 shows the clean-water flux at various transmembrane pressures. For single-layer MMMs, the clean-water permeance is 1839 ± 55 lm^{-2} h^{-1} bar^{-1} based on the slope up to 0.5 bar. Although these membranes were pre-pressurized before the measurement, the flux–transmembrane pressure relationship at higher pressures is not linear. This might be

due to membrane compaction during the measurement or possibly relocation of the particles in the matrix which might close the bigger channels. For dual-layer MMMs the permeance is significantly lower, 350.7 ± 69 lm^{-2} h^{-1} bar^{-1} ($p < 0.05$) and the flux–transmembrane pressure relationship is linear in the used pressure range. The decrease in clean-water permeance for the dual-layer MMMs is probably due to the additional particle-free layer, which has a dense sponge-like skin structure.

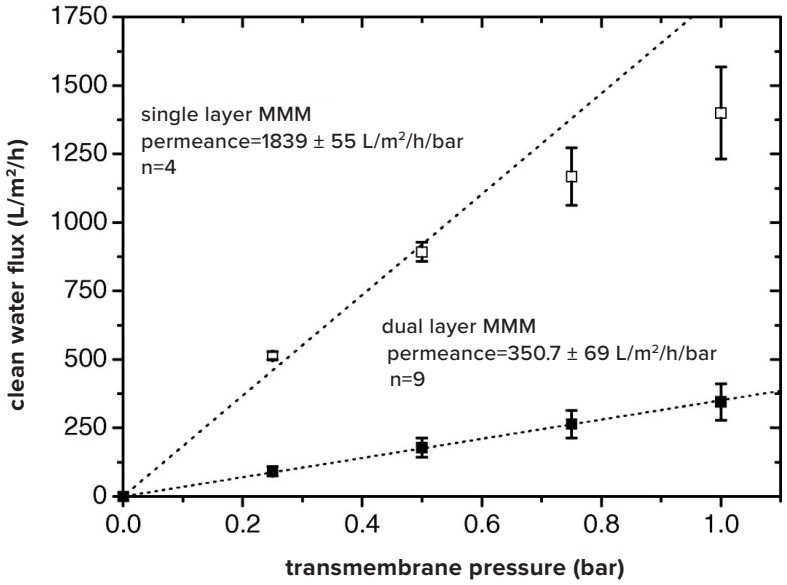

FIGURE 6. Average clean-water flux plotted against transmembrane pressure, for single-layer MMMs (white squares) and dual-layer MMMs (black squares). The error bars indicate standard deviations.

The single-layer MMMs have a BSA sieving coefficient of 0.8 ± 0.1. The dual-layer MMMs have a significantly lower sieving coefficient of 0.4 ± 0.2 ($p < 0.05$). It seems that the additional particle-free layer tailors the transport through the membrane. For future applications the molecular weight cut-off of the membrane is important and can probably be tailored by optimization of the particle-free layer.

Creatinine adsorption isotherms

For single and dual-layer MMMs, the creatinine adsorption at various concentrations was measured. The adsorption capacity is expressed in mg adsorbed creatinine per g of activated carbon. Hence the exact particle proportion in the MMM is necessary and is calculated (via the equations in the supplement) to be 0.68 and 0.53 for single- and dual-layer

MMMs respectively.

Figure 7 presents the results expressed in adsorption capacity (q) vs. the equilibrium creatinine concentration (C). The isotherms of single and dual-layer MMMs are almost identical and appear to be of Langmuir type. For the tested concentration range the best Langmuir isotherm curve fit has q_m = 234 mg g^{-1} AC and K_d = 0.351 mg ml^{-1}. The range of creatinine concentrations used here (0–0.15 mg/mL) is relevant because the concentrations are close to the creatinine levels in the normal (0.012 mg/mL) and uremic situation (0.136 mg/mL).

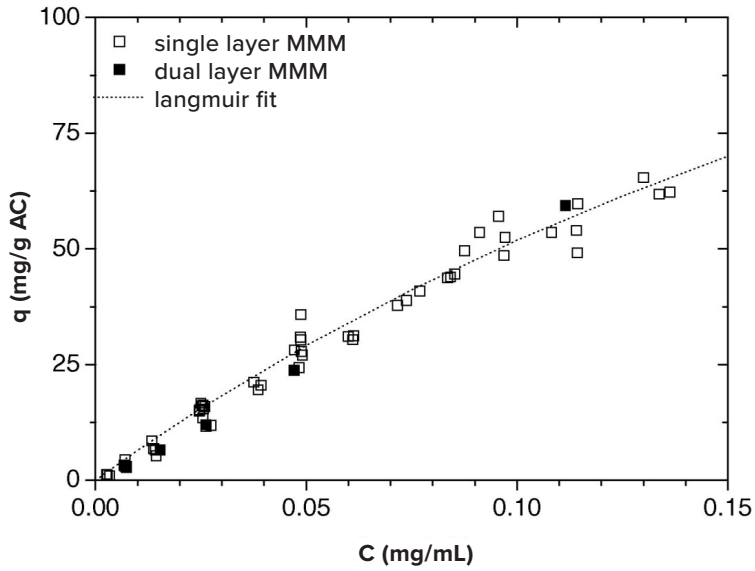

FIGURE 7. Creatinine isotherms for single (white squares) and dual-layer (black squares) MMMs. The equilibrium adsorption capacity (q) expressed in mg adsorbed creatinine per gram of activated carbon is plotted against the equilibrium creatinine concentration (C). The dotted line represents the calculated Langmuir isotherm fit.

Compared to other studies, the adsorption capacity of the mixed-matrix membranes is high. For example at an equilibrium concentration of 0.05 mg ml^{-1} our MMMs adsorb 29 mg creatinine per g of activated carbon. At the same equilibrium creatinine concentration, Deng et al. reported adsorption of 13 mg creatinine per g of activated carbon [34] for PES and activated-carbon-based hybrid beads, and Ye et al. reported adsorption of 15–20 mg creatinine per g of activated carbon or carbon nanotube [35]. In another approach using polymeric micro-spheres, the creatinine adsorption was less than 10 mg per gram of micro-sphere [36].

Human plasma adsorption

Figure 8.A presents average relative creatinine concentrations after incubation in plasma for 4 h, which is an average duration of a hemodialysis treatment. The particle-free membrane does not significantly lower the creatinine concentration. However, more than 80% of the creatinine is removed by the single- and dual-layer membranes, which is in excellent agreement with the removal achieved by the activated particles alone under these experimental conditions. This shows that the accessibility of the particles in the MMM is excellent. These creatinine adsorption results also fit very well to the isotherm obtained with model creatinine solutions (as presented in Figure 7), suggesting that even for the more complex human plasma the accessibility of our membranes is optimal.

The results of PAH removal by the membrane (see Figure 8.B) are consistent with the results of creatinine. The particle-free membrane does not seem to lower the PAH concentration, whereas the single and dual-layer MMMs seem to remove more than 80% of PAH similar to the removal obtained by the activated carbon particles alone. These results indicate that the developed MMMs can probably remove protein-bound compounds, and the accessibility of the particles there seems to be similar to the accessibility of the particles alone. Protein-bound solutes are thought to contribute to uremic toxicity and are hardly removed by hemodialysis [37,38]. Adsorption has been proposed as a way to improve removal of these toxins [39,40] and it has been shown that the addition of activated carbon to the dialysate compartment of a hemodialyzer can improve protein-bound toxin removal [41,42]. Our first results suggest that the MMM could be suitable for the improvement of protein-bound toxin removal.

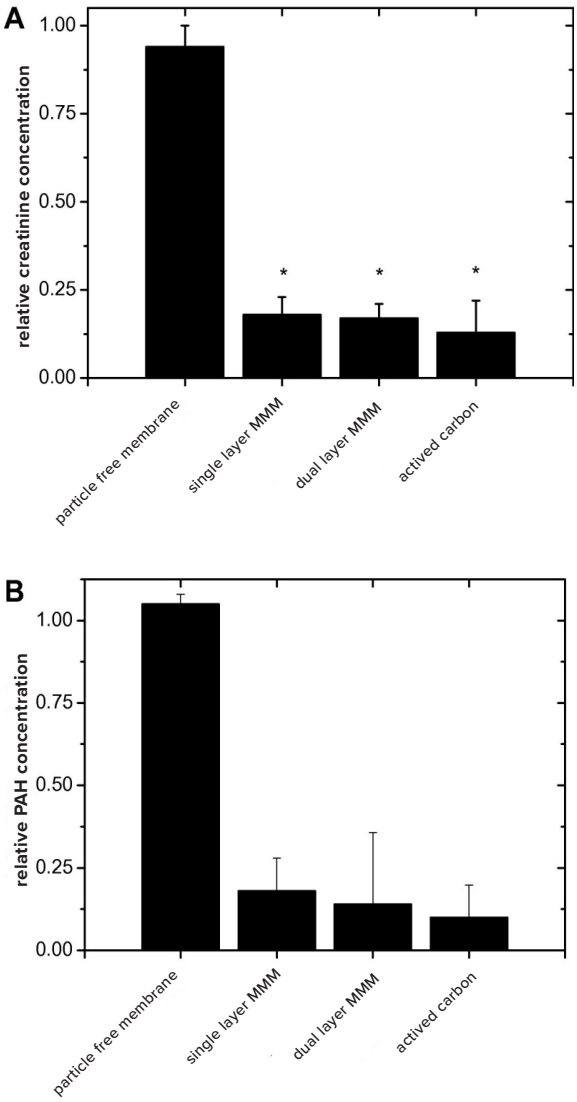

FIGURE 8. A: Average relative creatinine concentration after incubation of a particle-free membrane, single-layer MMMs, dual-layer MMMs or pure activated carbon in plasma (n = 6) for 4 h. * indicates $p < 0.05$ in comparison with a particle-free membrane. B: Average relative PAH concentration after incubation of a particle-free membrane, single-layer MMM, dual-layer MMM or pure activated carbon particles in plasma (n = 2) for 4 h.

It is also important to note that while under these experimental conditions our single- and dual-layer MMMs seem to remove more than 80% of the creatinine and PAH from the human plasma, they do not cause any significant changes to the plasma pH, osmolarity and

the total protein concentration (see Table 2). The latter indicates that undesired general protein binding is limited.

TABLE 2. Relative osmolarity, pH and total protein concentration after incubation in human blood plasma (n = 6) for 4 h.

Sample	Relative osmolarity	Relative pH	Relative total protein concentration
Activated carbon	0.99 ± 0.01	1.02 ± 0.04	0.93 ± 0.02
Single-layer MMM	1.00 ± 0.01	1.02 ± 0.02	0.94 ± 0.05
Dual-layer MMM	0.98 ± 0.01	1.00 ± 0.01	1.01 ± 0.07
Particle-free membrane	1.01 ± 0.01	1.03 ± 0.02	1.03 ± 0.06

Direct comparison of the performance of our membranes in human plasma with other literature studies is rather difficult since not all experimental conditions are the same.

In this study, 25 mg of adsorbent was incubated in 4 ml plasma. This proportion can be related to the proportion used in hemoperfusion, where ~3000 ml plasma is in contact with a hemoperfusion column containing ~300 gram of activated carbon (Adsorba 300, Gambro). This gives a sorbent–volume proportion of 100 mg per ml of plasma. In our experiments the sorbent–volume proportion is lower, 6 mg per ml of plasma. This indicates that when we would apply a similar sorbent–volume proportion as in hemoperfusion, the obtained relative removals by our MMMs could even be higher.

Perhaps the best way of comparing adsorption results is by means of isotherms at similar equilibrium concentrations of model solutions but which are unfortunately not often discussed in the literature. Nonetheless, there are a few studies with which some comparison can be done. For example, Malchesky et al. developed a blood purification device containing charcoal encapsulated in semi-permeable hollow tubing. A 35 l test solution with an initial concentration of 0.095 mg ml^{-1} creatinine was pumped through a device containing 37 mg activated carbon (sorbent–volume proportion of 0.001 mg ml^{-1}) and 30.1% of the creatinine was removed after 4 h [43]. The Vicenza Wearable Artificial Kidney for peritoneal dialysis contains less than 200 g adsorptive particles, 40% polystyrenic resins, 60% activated carbon with the latter mainly removing creatinine. A 12 l dialysate solution was pumped through the device and 94.2% of the creatinine was removed after 4 h [21].

Creatinine diffusion through MMMs

For estimating the transport of uremic retention solutes through our membranes, we tested diffusion through and adsorption onto dual-layer MMMs using a two-compartment diffusion device with a 0.1 mg ml^{-1} creatinine feed solution. This concentration is clinically relevant as it is very close to the mean/median uremic creatinine concentration measured in uremia

of 0.136 mg ml^{-1}[27] [27]. Figure 9 shows that after some time the diffused creatinine starts increasing and continues in time. Adsorption of creatinine starts almost immediately, and after more than 24 h seems to reach a plateau. The contribution of creatinine removal after 7 h by adsorption is over 80% of the total creatinine removal. No quick saturation occurs for the developed MMMs under these experimental conditions. The MMM combines uremic retention solute removal via both diffusion as well as adsorption in one single step.

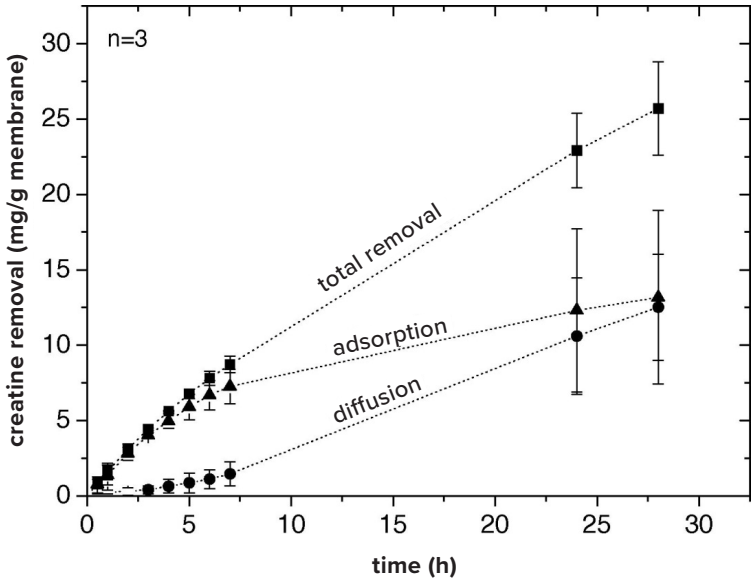

FIGURE 9. Average creatinine total removal, diffusion and adsorption plotted vs. time, n = 3. Total removal (squares) is the amount of creatinine removed from the donor compartment. This removal is mediated by diffusion (circles) and adsorption (triangles) of creatinine. Error bars indicate standard deviations. The dotted lines are plotted to guide the eye.

This is a novel and promising approach in the extracorporeal blood purification technology. In this paper we showed the first proof of concept; in the future, we foresee several opportunities for MMMs in the use of blood purification. MMMs could be used in a wearable artificial kidney (WAK). The MMM combines two methods of blood purification in one step (adsorption and membrane-based removal). This could enhance the miniaturization of the device. Besides, MMMs might be useful for dialysis with less dialysate (in a WAK), making sure that sufficient amounts of toxins can be removed. Or MMMs could be placed in a separate circuit for regeneration of the dialysate, which could be useful for a WAK system. Furthermore, the MMM may function as an adsorptive barrier for endotoxins, thereby preventing endotoxins from the dialysate entering the patients circulation.

CONCLUSIONS AND OUTLOOK

This work presents a novel approach for uremic retention solute removal combining diffusion and adsorption. Dual-layer MMMs were formed consisting of a particle-free membrane layer and a mixed-matrix membrane layer containing activated carbon. The dual-layer MMMs have a clean-water permeance of ~350 lm^{-1} h^{-1} bar^{-1}, and show rather high creatinine adsorption of ~29 mg creatinine per g of activated carbon at equilibrium concentration of 0.05 mg ml^{-1}. Moreover, both single- and dual-layer MMMs significantly reduce the creatinine concentration in human blood plasma, without general effects on osmolarity, pH and total protein concentration.

Our future plans will focus on removal of a broad range of uremic toxins including other protein-bound toxins and middle molecules since these are difficult to remove with current hemodialysis. Adsorption-based removal with MMMs might improve the clearances of these toxins as well.

We will also fabricate and optimize dual-layer mixed-matrix hollow fiber membranes with a thin particle-free inner layer. Recent literature studies have shown that it is possible to prepare dual-layer membranes and dual-layer MMMs with different characteristics (material, layer thickness, particle type, etc.) [44-49]. Our focus will be on tailoring the membrane structure by parameters like spinneret design, pumping speeds and polymer and bore liquid compositions to achieve optimal toxin removal.

REFERENCES

1. Bethesda M. US Renal Data System, USRDS 2010 Annual Data Report: Atlas of Chronic Kidney Disease and End-Stage Renal Disease in the United States National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases 2010.
2. Bethesda M. US Renal Data System, USRDS 2006 Annual Data Report: Atlas of End-Stage Renal Disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases 2006.
3. Mydlik M, Bucek J, Derzsiova K. Influence of charcoal haemoperfusion on platelet count in acute poisoning and during regular dialysis treatment. Int Urol Nephrol 1981;13:387–9.
4. Ash SR, Sullivan TA, Carr DJ. Sorbent suspensions vs. sorbent columns for extracorporeal detoxification in hepatic failure. Ther Apheresis Dial 2006;10:145–53.
5. Winchester JF et al. Sorbents in acute renal failure and end-stage renal disease: middle molecule and cytokine removal. Blood Purif 2004;22:73–7.
6. La Greca G, Brendolan A, Ghezzi PM, De Smet R, Tetta C, Gervasio R, et al. The concept of sorbent in hemodialysis. Int J Artif Organs 1998;21:303–8.
7. Nakabayashi NM, E. Preparation of Poly-Hema Coated Spherical Activated Charcoal for Direct Hemoperfusion. Hemoperfusion: kidney and liver support and detoxification 1980:57–61.
8. Davankov VA, Pavlova LA, Tsyurupa MP, Tur DR. Novel polymeric solid-phase extraction material for complex biological matrices; portable and disposable artificial kidney. J Chromatogr B: Biomed Sci Appl 1997;689:117–22.
9. Malchesky PS, Varnes W, Piatkiewicz W, Nose Y. Membranes containing sorbents for blood detoxification. Trans Am Soc Artif Intern Organs 1977;23:659–66.
10. Denti E, Walker JM, Brancaccio D, Tessore V. Evaluation of novel sorbent systems for joint hemodialysis and hemoperfusion. Med Instrum 1977;11:212–6.
11. Malchesky PS, Piatkiewicz W, Nakamoto S, Nose Y. Haemoperfusion made safe with sorbent membranes. Proc Eur Dial Transplant Assoc 1978;15:591–2.
12. Malchesky PS, Piatkiewicz W, Varnes WG, Ondercin L, Nose Y. Sorbent membranes – device designs, evaluations and potential applications. Artif Organs 1978;2:367–71.
13. Klein E, Holland FF, Eberle K. Sorbent-filled hollow fibers for hemopurification. Transactions of the American Society for Artificial Organs 1978;24:127–30.
14. Gurland HJ, Castro LA, Samtleben W, Fernandez JC. Combination of hemodialysis and hemoperfusion in a single unit for treatment of uremia. Clin Nephrol 1979;11:167–72.
15. Henne W DG, Schmitz W, Pohle R, Lawitzki F, 4267,047. Dialyzing membrane with adsorbent layer. United States 1981 May 12.
16. Chapman GV et al. evaluation of hemodiafiltration and sorbent membrane dialysis.1. Invivo and invitro dialyzer performance. Dial Transplant 1982;11:758–65.
17. Chapman GV, Hone PWE, Shirlow MJ. Evaluation of hemodiafiltration and sorbent membrane dialysis: II. Clinical, nutritional, and middle molecule assessment. Dial Transplant 1982;11:871–6.
18. Maeda K, Saito A, Kawaguchi S. Problems with

activated charcoal and alumina as sorbents for medical use. Artif Organs 1979;3:336–40.

19. Randerson DH et al. Sorbent membrane dialysis in Uremia. Contrib Nephrol 1982;29:53–64.
20. Davenport A, Gura V, Ronco C, Beizai M, Ezon C, Rambod E. A wearable haemodialysis device for patients with end-stage renal failure: a pilot study. Lancet 2007;370:2005–10.
21. Ronco C, Fecondini L. The vicenza wearable artificial kidney for peritoneal dialysis (ViWAK PD). Blood Purif 2007;25:383–8.
22. Avramescu ME, Borneman Z, Wessling M. Particle-loaded hollow-fiber membrane adsorbers for lysozyme separation. J Membr Sci 2008;322:306–13.
23. Saiful, Borneman Z, Wessling M. Enzyme capturing and concentration with mixed matrix membrane adsorbers. J Membr Sci 2006;280:406–17.
24. Avramescu ME, Borneman Z, Wessling M. Dynamic behavior of adsorber membranes for protein recovery. Biotechnol Bioeng 2003;84:564–72.
25. Avramescu ME, Gironès M, Borneman Z, Wessling M. Preparation of mixed matrix adsorber membranes for protein recovery. J Membr Sci 2003;218:219–33.
26. Smith HW, Finkelstein N, Aliminosa L, Crawford B, Graber M. The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. J Clin Invest 1945;24:388–404.
27. Vanholder R et al. Review on uremic toxins: Classification, concentration, and interindividual variability. Kidney Int 2003;63:1934–43.
28. Van Aubel RAMH, Masereeuw R, Russel FGM. Molecular pharmacology of renal organic anion transporters. Am J Physiol – Renal Physiol 2000;279:F216–32.
29. Besseghir K, Mosig D, Roch-Ramel F. Facilitation by serum albumin of renal tubular secretion of organic anions. Am J Physiol – Renal Fluid and Electrolyte Physiol 1989:256.
30. Waugh WH, Beall PT. Simplified measurement of p aminohippurate and other arylamines in plasma and urine. Kidney Int 1974;5:429–36.
31. Papenburg BJ, Vogelaar L, Bolhuis-Versteeg LAM, Lammertink RGH, Stamatialis D, Wessling M. One-step fabrication of porous micropatterned scaffolds to control cell behavior. Biomaterials 2007;28:1998–2009.
32. Smolders CA, Reuvers AJ, Boom RM, Wienk IM. Microstructures in phaseinversion membranes. Part 1. Formation of macrovoids. J Membr Sci 1992;73:259–75.
33. Husain S, Koros WJ. Macrovoids in hybrid organic/inorganic hollow fiber membranes. Ind Eng Chem Res 2009;48:2372–9.
34. Deng X, Wang T, Zhao F, Li L, Zhao C. Poly(ether sulfone)/activated carbon hybrid beads for creatinine adsorption. J Appl Polym Sci 2007;103:1085–92.
35. Ye C, Gong QM, Lu FP, Liang J. Adsorption of uraemic toxins on carbon nanotubes. Sep Purif Technol 2007;58:2–6.
36. Gao B, Yang Y, Wang J, Zhang Y. Preparation and adsorption characteristic of polymeric microsphere with strong adsorbability for creatinine. J Biochem Mol Toxicol 2008;22:166–74.
37. Bammens B, Evenepoel P, Keuleers H, Verbeke K, Vanrenterghem Y. Free serum concentrations of the protein-bound retention solute p-cresol predict mortality in hemodialysis patients. Kidney

Int 2006;69:1081–7.

38. Vanholder R, De Smet R, Lameire N. Protein-bound uremic solutes: the forgotten toxins. Kidney Int Suppl 2001;59:S266–70.
39. Winchester JF, Amerling R, Dubrow A, Feinfeld DA, Gruber SJ, Harbord N, et al. Dialysis desiderata. Hemodial Int 2007:11.
40. Ronco C. Sorbents: from bench to bedside – Can we combine membrane separation processes and adsorbent based solute removal? Int J Artif Organs 2006;29:819–22.
41. Dinh DC, Recht NS, Hostetter TH, Meyer TW. Coated carbon hemoperfusion provides limited clearance of protein-bound solutes. Artif Organs 2008;32:717–24.
42. Meyer TW, Peattie JWT, Miller JD, Dinh DC, Recht NS, Walther JL, et al. Increasing the clearance of protein-bound solutes by addition of a sorbent to the dialysate. J Am Soc Nephrol 2007;18:868.
43. Malchesky PS, Nose Y. Device and method for effecting fluid interchange functions. US Japan Foundation for Artifical Organs. Cleveland: Ohio; 1978. p. 10.
44. Li D, Chung TS, Wang R. Morphological aspects and structure control of duallayer asymmetric hollow fiber membranes formed by a simultaneous coextrusion approach. J Membr Sci 2004;243:155–75.
45. Albrecht W, Weigel T, Groth T, Hilke R, Paul D. Formation of porous bilayer hollow fibre membranes. Macromol Symp 2002;188:131–41.
46. Andre JMdS. Mixed matrix membranes, a new platform for enzymatic reactions. Enschede: University of Twente 2009.
47. Borneman Z. Particle loaded membrane chromatography. Enschede: University of Twente; 2006.
48. Widjojo N, Chung TS, Kulprathipanja S. The fabrication of hollow fiber membranes with double-layer mixed-matrix materials for gas separation. J Membr Sci 2008;325:326–35.
49. Widjojo N, Zhang SD, Chung TS, Liu Y. Enhanced gas separation performance of dual-layer hollow fiber membranes via substructure resistance reduction using mixed matrix materials. J Membr Sci 2007;306:147–58.

SUPPLEMENT

The formulas below are used for the calculation of the proportion of activated carbon particles in single layer and dual layer MMM.

$$P_{SL\,MMM} = \frac{W_{AC}}{W_{SL\,MMM}} = \frac{W_{AC}}{(W_{AC} + W_{PES} + W_{PVP})}$$

$$P_{DL\,MMM} = \frac{W_{AC}}{W_{DL\,MMM}} = \frac{W_{AC}}{(W_{MMM} + W_{PFL})} = \frac{P_{SL\,MMM} \cdot \rho_{MMM} \cdot F_{MMM} \cdot V_{MMM}}{(\rho_{MMM} \cdot F_{MMM} \cdot V_{MMM} + \rho_{PFL} \cdot F_{PFL} \cdot V_{PFL})}$$

$$F_{MMM} = \frac{(W_{PES} + W_{PVP} + W_{AC})}{(W_{PES} + W_{PVP} + W_{AC} + W_{NMP})}$$

$$F_{PFL} = \frac{(W_{PES} + W_{PVP})}{(W_{PES} + W_{PVP} + W_{NMP})}$$

PSL MMM = Proportion of activated carbon particles in the single layer MMM
WAC = Weight of activated carbon (g)
WSL MMM = Weight of single layer MMM (g)
WPES = Weight of PES (g)
WPVP = Weight of PVP (g)
PDL MMM = Proportion of activated carbon particles in the dual layer MMM
WDL MMM = Weight of dual layer MMM (g)
WPFL = Weight of particle free membrane layer (g)
WMMM = Weight of MMM layer (g)
ρMMM = Density of MMM dope (g/mL) = 1.12 g/mL
FMMM = Weight factor of MMM layer
VMMM = Volume of a 1x1 cm casted MMM film layer (mL)
ρPFL = Density of particle free polymer dope (g/mL) = 1.04 g/mL
FPFL = Weight factor of particle free membrane layer
VPFL = Volume of a 1x1 cm casted particle free film layer (mL)
WNMP = Weight of NMP (g)

7

Mixed matrix hollow fiber membranes for removal of protein-bound toxins from human plasma

Marlon S. Tijink, Maarten Wester, Griet Glorieux, Karin G Gerritsen, Junfen Sun, Pieter C Swart, Zandrie Borneman, Matthias Wessling, Raymond Vanholder, Jaap A Joles, Dimitrios Stamatialis

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ABSTRACT

In end stage renal disease (ESRD) waste solutes accumulate in body fluid. Removal of protein bound solutes using conventional renal replacement therapies is currently very poor while their accumulation is associated with adverse outcomes in ESRD. Here we investigate the application of a hollow fiber mixed matrix membrane (MMM) for removal of these toxins. The MMM hollow fiber consists of porous macro-void free polymeric inner membrane layer well attached to the activated carbon containing outer MMM layer. The new membranes have permeation properties in the ultrafiltration range. Under static conditions, they adsorb 57% p-cresylsulfate, 82% indoxyl sulfate and 94% of hippuric acid from spiked human plasma in 4 h. Under dynamic conditions, they adsorb on average 2.27 mg PCS/g membrane and 3.58 mg IS/g membrane in 4 h in diffusion experiments and 2.68 mg/g membrane PCS and 12.85 mg/g membrane IS in convection experiments. Based on the dynamic experiments we estimate that our membranes would suffice to remove the daily production of these protein bound solutes.

INTRODUCTION

Prevalence of chronic kidney disease (CKD) increases globally [1,2] due to the aging population and increasing incidence of risk factors such as diabetes mellitus [3]. Despite considerable amounts of healthcare budgets spent on renal replacement therapy [3,4], mortality of dialysis patients remains high [5,6] and their overall health related quality of life low [7]. In fact, the accumulation of uremic retention solutes plays an important role in CKD related morbidity and mortality [8-11]. Small water soluble molecules can be removed by dialysis, but middle molecules and protein bound toxins are difficult to remove with conventional renal replacement therapies. Protein bound toxins are involved in generation of reactive oxygen species and associated with cardiovascular disease, progression of CKD and mortality [9,11-17]. Although improved uremic toxin removal has been achieved by extending the duration of dialysis, and this is associated with lower mortality rates [18], the removal of protein bound toxins is not improved by extended treatment if the total blood and dialysate fluid crossing the dialyzer is kept constant, [19] or by high-flux dialysis [20]. Convective therapies such as hemodiafiltration can improve removal of the middle molecule ß2-microglobulin [6]. Post dilution online hemodiafiltration has shown to significantly lower total pre-dialysis concentrations of p-cresylsulfate and 3-carboxyl-4-methyl-5-propyl-2-furanpropionic acid (CMPF), two protein bound toxins with high protein binding. However, the effect on their total concentration was only moderate [21].

To improve protein bound toxin removal, the concentration of free toxin on the dialysate side should be low, so that there is a continuous driving force for the free fraction in the blood to diffuse to the dialysate side over the whole hemodialyzer length [22,23]. Indeed, Dinh *et al.* showed that adding powdered activated carbon into the dialysate compartment, improved the clearance of protein bound solutes by continuous binding of the diffused free fraction [24]. Furthermore, raising the dialysate flow can have a similar effect [22] and Sirich *et al.* showed *in vivo* that removal of protein bound solutes increased by raising the dialysate flow rates [25]. Another way of maintaining a high concentration gradient over the entire length of a hemodialyzer membrane, thereby probably enhancing protein bound toxin removal, could be incorporation of adsorptive particles in the membrane itself. In fact, more than 30 years ago, so called sorbent membranes were developed, in which adsorptive particles were embedded between two cuprophan membrane layers, or within a cuprophan matrix, to combine both filtering and adsorbing capacity for uremic toxins [26]. However, removal of protein bound toxins was not addressed. After a clinical trial with sorbent membranes, patients rated the treatment low and complained about increased lethargy [27]. This might be due to lack of adsorbents with high purity [28]. In addition, manufacturing difficulties and rapid saturation caused these membranes to be removed

from the market [29,30].

Previously, we showed the concept of a membrane with embedded adsorptive particles, a so-called porous mixed matrix membrane (MMM) [31]. These flat sheet MMMs consisted of a porous particle free layer attached to the mixed matrix membrane layer with embedded particles and showed high adsorption capacity of creatinine and could combine diffusion and adsorption of creatinine in one step [31]. Here, we develop a dual layer hollow fiber MMM to remove protein bound uremic toxins. Polyethersulfone (PES) was used as a membrane forming material, blended with the hydrophilic additive polyvinylpyrrolidone (PVP). This polymer blend is often used for hemodialysis membranes [32,33]. Activated carbon was selected as adsorptive particle because it adsorbs a broad range of solutes, including protein bound toxins, and it has a long track record in blood purification [24,34]. A special triple layer spinneret is designed for the spinning of a polymeric inner layer and a thicker outer MMM layer. The influence of spinning parameters such as bore liquid composition and pumping speeds are investigated. Fabricated fibers are characterized in terms of adsorptive capacities and transport properties. Creatinine, a small molecular weight uremic retention solute, often used as a marker of kidney function, was used as a model for water soluble solute. Hippuric acid, indoxyl sulphate and p-cresylsulphate, often used as representatives for the protein bound uremic toxins and associated with negative effects [12-15], are used as model for the protein bound uremic toxins. Static adsorption experiments as well as experiments under flow conditions are performed to estimate the transport properties of the new hollow fiber MMMs.

MATERIALS AND METHODS

Materials

Ultrason E 6020 polyethersulfone (PES), obtained from BASF (Ludwigshafen, Germany), and polyvinylpyrrolidone (PVP) K90 (360 000 g/mole) (Fluka, Sigma-Aldrich Chemie Gmbh Munich, Germany) were used as membrane forming materials. N-methylpyrrolidone (NMP) (Acros Organics, Geel, Belgium) was used as solvent. Ultra-pure water was used as non-solvent in the bore liquid and distilled water was used as non-solvent in the coagulation bath. Norit A Supra EUR (European pharmacopoeia grade) (Norit Netherlands BV, Amersfoort, The Netherlands) was sieved in a 45 µm sieve (Fritsch GmbH, Idar-Oberstein, Germany) to a median particle size of 27 µm and were used as activated carbon (AC) particles for incorporation in the MMM and tested separately as pure particles.

The chemicals needed for Tyrode's buffer and the dialysate solution were obtained from Fluka, Sigma Aldrich: 5.4 mM KCl, 137 mM NaCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 11.9 mM $NaHCO_3$ and 5.5 mM glucose were dissolved in ultra-pure water to obtain Tyrode's buffer (pH 7.4). For the dialysate solution, 2 mM KCL, 140 mM NaCl, 1.5 mM $CaCl_2$, 0.25 mM $MgCl_2$, 35 mM $NaHCO_3$ and 5.5 mM glucose were dissolved in ultra-pure water. Creatinine, indoxyl sulfate (IS) and hippuric acid (HA) were purchased from Sigma Aldrich. p-cresylsulfate (PCS) is not commercially available and was synthesized as described before [35].

Polyethylene tubes (Rubber BV, Hilversum, The Netherlands) were used for module fabrication and two component glue (Bison kombi snel rapide, Eriks, Almelo, The Netherlands) was used for potting of the modules. Single fiber modules were applied for the creatinine diffusion experiment, while four fiber modules were used for the plasma diffusion and plasma convection experiments.

Uremic human plasma was obtained from six patients who received a plasma exchange treatment because of acute kidney injury and human blood plasma was obtained from six healthy donors from Sanquin (Amsterdam, The Netherlands) in compliance with local ethical guidelines.

Hollow fiber MMM fabrication

For the adsorptive layer, a dope solution (MMM1) was prepared containing 14 wt% PES and 1.4 wt% PVP K90 dissolved in NMP. The AC particles were added to this dope and after mixing on a roller bank for at least 48 hours, a homogenous dope was obtained with a loading of 60 wt% activated carbon particles in relation to the amount of PES. This solution was degassed for at least 48 hours. The polymer dope of the particle free inner layer (IL1)

consisted of 15 wt% PES and 10 wt% PVP dissolved in NMP. After 24 hour mixing on a roller bank the solution was filtered using a Bekipor ST AL3 15 µm filter (Bekaert, Kortrijk, Belgium) and allowed to degas for at least 24h. The following bore liquids were prepared by 24h mixing: B1) 5 wt% PVP in ultra-pure water, B2) 5 wt% PVP and 60 wt% NMP in ultra-pure water and allowed to degas for at least 24h. For the single layer MMM spinneret 1 was used and for the dual layer HF MMM, spinneret 2 was designed (see Table 1 and the appendix of this chapter). The dimensions of the spinnerets were chosen because of practical reasons so that dual layer hollow fibers could be spun for the proof of concept of this study.

TABLE 1. Spinneret dimensions

	Spinneret 1	Spinneret 2
Inner diameter needle (mm)	1.2	0.26
Outer diameter needle (mm)	1.5	0.46
Inner diameter first orifice (mm)	2.2	0.66
Outer diameter first orifice (mm)		0.96
Inner diameter second orifice (mm)		1.66

All hollow fiber membranes were fabricated by dry wet spinning via immersion precipitation. The MMM dope was pressurized, while the particle free inner layer dope and the bore liquid were pumped through the spinneret. After a 3 cm air gap, the nascent hollow fiber was immersed into a water coagulation bath at room temperature and the hollow fiber was formed by phase separation.

TABLE 2. Spinning conditions

	SL	DL1	DL2	DL3
Bore liquid	B1	B1	B1	B2
Inner layer polymer dope	-	IL1	IL1	IL1
Mixed matrix membrane layer dope	MMM1	MMM1	MMM1	MMM1
Bore liquid pumping speed (mL/min)	2.7	2.7	2.7	2.7
Inner layer dope pumping speed (mL/min)	-	2.03	0.9	0.9
Pressurized mixed matrix membrane dope speed (mL/min)	8.4	3.9	3.9	3.2
Pulling wheel speed (m/min)	-	4.4	4.4	7
Spinneret	1	2	2	2

Bore liquid B1 contains 5 wt% PVP in ultra pure water, bore liquid B2 contains 5 wt% PVP and 60 wt% NMP in ultra pure water. Inner layer polymer dope IL 1 contains 15 wt% PES and 10 wt% PVP in NMP. Mixed matrix membrane layer dope MMM1 contains 14 wt% PES, 1.4 wt% PVP in NMP and 60 wt% AC in relation to the amount of PES.

During hollow fiber spinning, several parameters were varied, Table 2 describes the spinning conditions. For the single layer MMM (SL), the pulling wheel was not constantly used to collect the hollow fiber, for the other fibers the applicable pulling wheel speeds are presented in Table 2. The pulling wheel speed was adjusted so that the fiber was continuously

picked up by the wheel. The pressurized MMM dope speed was aimed to be the same in DL1, DL2, and DL3. But because of practical difficulties with the pressure regulator the pressurized MMM dope speeds was a little bit lower for DL3. Dual layer HF MMMs were collected during at least three succeeding periods of five minutes of spinning. The collected fibers were washed in ultra-pure water to remove any remaining solvent, and stored in ultra-pure water upon further use. The AC proportion in the membrane was estimated using the content of AC particles, PES and PVP in de dopes, density and pumping speeds of the dopes similar as described before [31].

Membrane characterization

Scanning electron microscopy (SEM)

To investigate their surface, the fibers were cut open to expose the inner surface and glued on a sample holder. To examine cross sections, the hollow fiber membranes were dried in air followed by fracturing in liquid nitrogen and were clamped in a cross section sample holder. Then, the samples were dried under vacuum at 30°C and subsequently gold coated using a BalzersUnion SCD 040 sputter coater (OerlikonBalzers, Balzers, Liechtenstein) and examined using a JEOL JSM-5600LV Scanning Electron Microscope (JEOL, Tokyo, Japan). Cross sections of fibers DL1, DL2 and DL3 collected in succeeding periods of five minutes during the spinning were examined. Inner diameter (I.D.), outer diameter (O.D.) and membrane layer thicknesses were determined using SEM pictures originating from all collecting periods.

Clean water permeance

Hollow fiber membranes were dried in air and single fiber modules were prepared by gluing the fiber in a tube with a Festo T-connection (Eriks, The Netherlands). After potting, both ends were cut open. The modules were equilibrated in ultra-pure water for at least seven days. Before testing, modules were pre-pressurized at 2 bar for 1 hour, then trans membrane pressures of 0.5, 1.0, 1.5 and 2.0 bar were applied and the amount of permeated ultra-pure water was measured over time. The clean water permeance (L_p) ($L/m^2/h/bar$) was determined by calculating the slope of a linear fit of the flux versus pressure graph. Fibers from each succeeding five minute period during the spinning were tested for clean water permeance.

Adsorption isotherms

Air dried hollow fiber membranes were cut in pieces of 4 cm length and were incubated in 5 mL solutions containing different concentrations of creatinine, IS or HA in Tyrode's buffer in a shaking water bath at 37°C. The range of creatinine concentrations was close to the creatinine levels present in the normal (0.012 mg/mL) and uremic state (0.136 mg/mL) [36]. Also the range of IS and HA concentrations were close to the average uremic

concentrations 0.053 mg/mL and 0.247 mg/mL and highest reported uremic concentrations 0.236 mg/mL and 0.471 mg/mL respectively [36], although recent data suggests lower concentrations [8].

After a 72, 48 or 24 hour incubation period, the creatinine, IS and HA concentrations were measured by photo spectrometric analysis at 230, 278 and 228 nm at 25°C in a 2, 2 and 10 mm quartz cuvette respectively. The adsorptive capacity for each uremic retention solute is expressed in mg adsorbed per gram of embedded activated carbon. For the isotherms, the equilibrium adsorption capacity (q) is plotted against the equilibrium concentration of the component (C). The exact particle proportion in DL3 is necessary for this and is calculated to be 0.47. A Langmuir curve fit was obtained as described before [31] and the maximum adsorption capacity (q_m) and dissociation constant (K_d) were estimated.

Static adsorption from human blood plasma

Uremic plasma was used for measurements on creatinine, osmolarity, pH, and total protein. Frozen human plasma from three healthy donors was thawed and spiked with PCS, IS and HA to obtain either similar total concentrations as in uremic patients [8] or higher total concentrations. The spiked plasma solutions were allowed to mix for 10 minutes prior to the start of the experiment. This plasma was tested for concentrations of PCS, IS, HA, creatinine, urea, total protein, Na^+, K^+, Ca^2 and osmolarity and pH.

A PES/PVP particle free flat sheet membrane was used as reference (was made as described before as a single particle free membrane [31]). Approximately 25 mg activated carbon particles, DL3 containing approximately 25 mg activated carbon and approximately 25 mg flat sheet home-made PES/PVP particle free membrane were incubated in approximately 4 mL of different plasmas. The amount of added plasma was adjusted to the amount of incubated material so that the material – plasma relation was always similar to 25 mg material in 4 mL plasma.

All tubes were incubated on a roller bank. After the incubation time, tubes were centrifuged at 3500 rpm for 10 min, and supernatant was collected and one part was stored at 4°C and another part was directly frozen and stored in -80°C. Osmolarity, pH, Na^+, K^+, Ca^{2+}, total protein, urea and creatinine concentrations are measured using the techniques described in Table 3. Furthermore, free and total PCS, IS and HA concentrations were analyzed as described before [37,38]. The absolute value of a sample was always related to the value of the control plasma at that time point as follows:

$$Relative\ concentration = \frac{C_t}{C_0}$$

Where C_t is the concentration in the plasma incubated with a sample (AC, DL3 or PES/PVP membranes) at that time point, and C_0 is the concentration in the blanc (plasma without sorbents or membranes). Relative osmolarities and pH were calculated in a similar way. Statistical differences were determined using a one-way ANOVA and a post-hoc Tukey test for PCS, IS, HA, osmolarity, pH, and Na^+, K^+, Ca^{2+}, total protein, urea and creatinine concentrations.

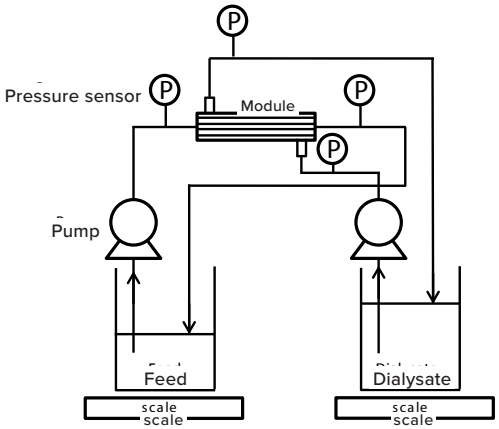
TABLE 3. Test methods for analysis of blood plasma

Parameter	Kit / Device
Osmolarity	Advanced instruments osmometer model 3320
pH	Radiometer Copenhagen PHM lab pH meter
Sodium	Corning 480 Flame Photometer
Potassium	Corning 480 Flame Photometer
Calcium	Bio-Rad Microplate reader Benchmark 16-channel photometer DiaSys Calcium CPC FS (1 1121 99 10 021)
Total protein	Bio-Rad Microplate reader Benchmark 16-channel photometer Bio-Rad Laboratories GmbH Protein Assay (cat# 500-0006)
Urea	Starrcol standard SC-60-S photometer DiaSys Urea CT FS (1 3115 99 10 026)
Creatinine	Bio-Rad Microplate reader Benchmark 16-channel photometer DiaSys Creatinine PAP FS (1 1759 99 10 026)

Cross flow measurements

Figure 1.A and B show the schematic representations of the experimental set up used for the diffusion and convection cross flow experiments respectively. Membrane modules containing one or four hollow fibers were prepared from 8 mm tubes and two Kartell T connections (VWR, Amsterdam, The Netherlands) for the experiments with model solutions and spiked human plasma respectively. Both ends were cut open after potting. Modules were equilibrated in water for at least seven days.

A



B

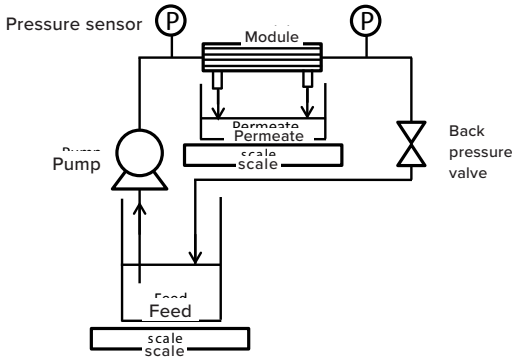


FIGURE 1. Schematic representations of experimental set up for A. diffusion experiments, B. convection experiments.

Before the start of the experiment with human plasma, clean water was pressurized through the membranes at 1 bar for at least 1 hour to check if all hollow fiber membranes were open. The modules for the diffusion and convection experiments with human plasma contained on average 96.1 ± 4.1 mg DL3. For the model solution diffusion experiment, the feed consisted of 50 mL 0.1 mg/mL creatinine in ultra-pure water solution (close to mean uremic creatinine concentration [36]) and dialysate was 100 mL ultra-pure water, while for the spiked plasma diffusion experiment the feed consisted of 50 mL spiked human plasma (spiked as described in section 3.4) and the dialysate was 100 mL dialysate buffer. For the

diffusion experiments, the feed was pumped through the lumen of the hollow fiber, while the dialysate was pumped around the fibers in the counter current direction. The feed and dialysate solutions were pumped at 5 mL/min and 31.4 mL/min respectively. Using these flow rates no transmembrane pressure could be detected.

For the spiked human plasma convection experiment the Spectrumlabs Kross Flo Research Ili system and a back pressure valve (SpectrumLabs automatic back pressure valve, JM separations, Tilburg, The Netherlands) was used in order to obtain constant trans membrane pressures during the whole experiment. The 50 mL spiked human plasma (see section 3.4) was pumped through the hollow fiber membranes at 15 mL/min and the transmembrane pressure was set at 0.25 bar.

For all cross flow experiments, at the indicated time points the pressures and the weight of the feed and dialysate or permeate compartments were measured and 2 mL samples were taken from both compartments. After the model solution diffusion experiment, creatinine concentrations were measured using photo spectrometric analysis as described in section 3.3. The amount of creatinine removed from the feed solution was defined as total removal. The amount of creatinine that appeared in the dialysate solution was considered as creatinine which was diffused from the feed solution. The creatinine deficiency in the mass balance was considered to be adsorbed onto the MMM. These amounts were related to the dry membrane weight which was estimated based on the measured active fiber length in the modules.

Plasma samples were immediately frozen in liquid nitrogen and kept at -80°C until analysis. Total protein concentration, PCS, IS and HA were analyzed as described in section 3.4. The relative total protein concentrations were calculated as described in section 3.4. In this case, the value of plasma at the start of the experiment was taken as blanc (C_0).

RESULTS AND DISCUSSION

Hollow fiber membrane fabrication and morphology

Figure 2 shows a photograph of membrane DL1. The two membrane layers can clearly be distinguished because of the black and white colors of the MMM layer with embedded black activated carbon particles and the white particle free porous polymeric inner layer, respectively.

Figure 2b shows SEM images of several fabricated hollow fiber mixed matrix membranes. In the round single layer hollow fiber MMM (SL), the AC particles are well distributed in the porous membrane matrix, no cluster formation is observed. Relatively big pores are present in the middle of the membrane structure, while close to the lumen and close to the outside surface, smaller pores are visible. No macro-voids through the complete cross section of the membrane wall are found.

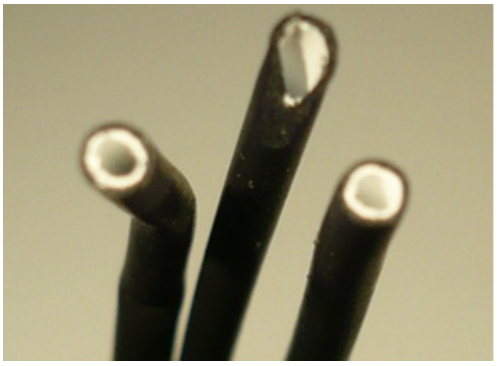


FIGURE 2. Photograph of dual layer hollow fiber MMMs DL1.

Using 5% PVP in the bore liquid and a high bore liquid pumping speed ensured formation of a fiber with a circular bore. Water as bore liquid and/or lower pumping speeds often resulted in the formation of an irregular shape of the inner contour of the fiber (data not shown). The amounts of PES and PVP in the dopes and composition of coagulation fluids were based on the literature [39] and previous experience in our lab with flat sheet membranes [31] and hollow fiber membranes (data not shown). PVP K90 is often used for hemodialysis membranes. Because of its relatively high molecular weight the viscosity of the dope solution was high, promoting formation of macro-void free membranes. Moreover this type of PVP has shown to give biocompatible macro-void free membranes with high solute permeability [40,41].

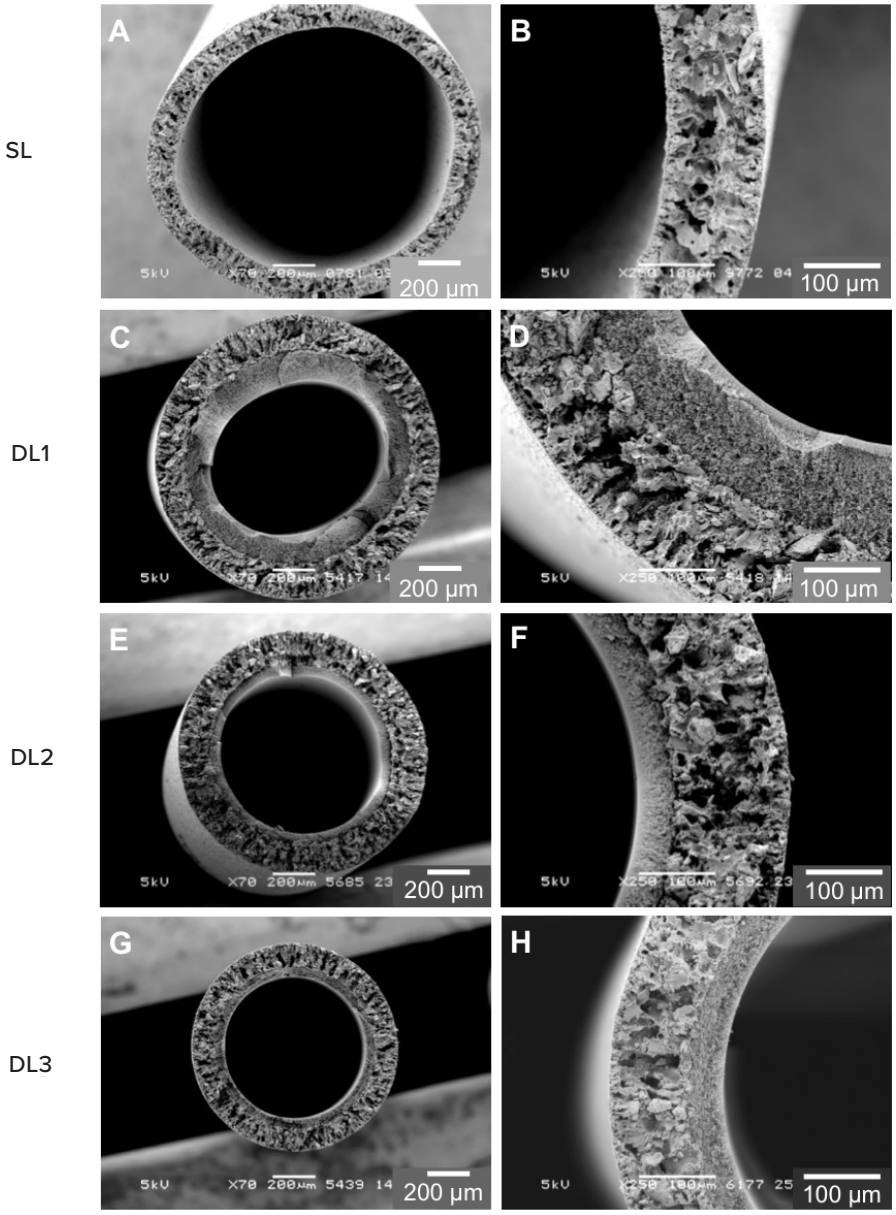


FIGURE 3. Scanning electron microscopy images of single layer hollow fiber MMM SL (A, B) and dual layer hollow fiber MMMs DL1 (C, D), DL2 (E, F), and DL3 (G, H).

Figure 3 also presents SEM pictures of DL1, a dual layer hollow fiber MMM. The two membrane layers can be clearly distinguished, and are well attached to each other. The inner

layer is a porous sponge like particle free membrane layer and seems to have a dense skin layer on the inside, whereas pores become bigger towards the outside. The outside MMM layer has a similar structure as the SL membrane. The particle free membrane layer and the MMM layer have almost the same thickness of around 140 ± 20 µm and 155 ± 20 µm (Table 4). To avoid mass transfer limitations, a thinner particle free membrane layer is desirable. Therefore, the inner layer polymer dope pumping speed was decreased and DL2 was obtained (Figure 3) having a much thinner inner membrane layer of 52 ± 3 µm. The MMM layer thickness and membrane structure is similar as in DL1. However, still a rather dense skin layer is observed on the lumen of the hollow fiber membrane.

TABLE 4. Average ± SD dimensions of hollow fiber mixed matrix membranes.

	SL	DL1	DL2	DL3
Outer diameter (O.D.) (µm)	1487 ± 38	1339 ± 14	1186 ± 37	984 ± 11
Inner diameter (I.D.) (µm)	1247 ± 21	776 ± 80	774 ± 29	669 ± 9
Inner layer thickness (µm)	-	140 ± 20	52 ± 3	49 ± 5
Mixed matrix membrane layer thickness (µm)	112 ± 4	155 ± 20	154 ± 8	111 ± 4

In general, by using bore liquids with high amounts of solvent, slower phase separation can be obtained leading to bigger pore sizes [42]. Therefore we changed the composition of the bore liquid from solvent free to a 60% solvent containing bore liquid, also based on previous experiences in flat sheet membranes [31,39]. Dual layer hollow fiber DL3 was obtained (Figure 3), and the inside layer seems to have a thinner skin layer than DL2. This can also be seen in Figure 4, no pores are visible at the inner surface of DL2 while on the inner surface of DL3 pores can be observed. The MMM layer structure of DL3 is similar as in the other dual layer hollow fiber MMM, however, the two layers seem to be better connected in DL3. Probably due to solvent containing bore liquid, slower phase separation occurs, allowing more time for the separate layers to connect. The pulling wheel speed was higher for this fiber than for DL1 and DL2. Probably, due to the slower phase separation, the nascent fiber became more extended by the pulling. This might explain the smaller dimensions of DL3 compared to DL2, see Table 4. Besides, the little decrease in pumping speed of the MMM dope may have played a role in this. The outer surfaces in Figure 4 show that the particles are well distributed in the MMM matrix.

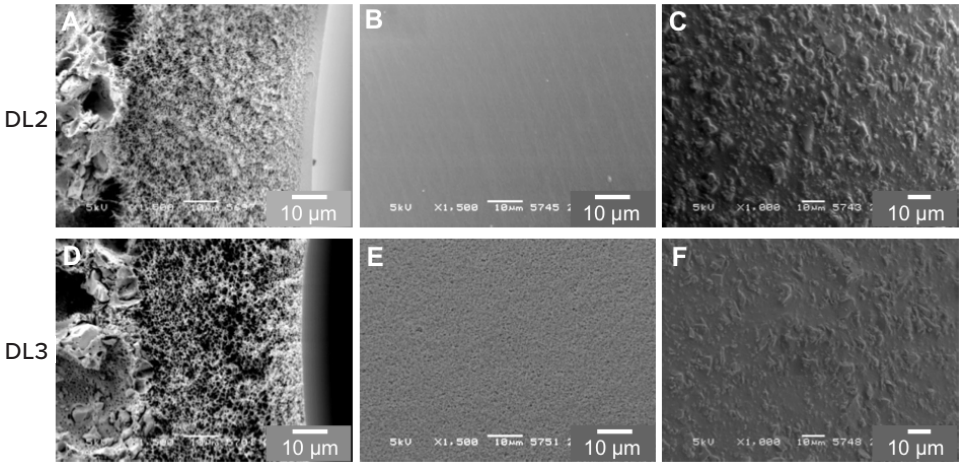


FIGURE 4. Scanning electron microscopy images of fiber DL2 (A, B, C) and DL3 (D, E, F) of cross section (A, D), inner surface (B, E) and outside surface (C, F) with magnifications of 1500x (A, B, D, E) and 1000x (C, F)

Clean water permeance

Figure 5 presents a linear clean water flux vs. pressure relationship for DL3 with a clean water permeance of 58.4 ± 9.3 L/m²/h/bar. Lower as well as higher clean water permeances have been described for polyethersulfone based hollow fibers used for hemodialysis [43,44].

Neither particle loss during the experiment nor delamination of the two membrane layers was observed. DL1 and DL2 show a clean water permeance of less than 3 L/m²/h/bar. For all hollow fibers DL 1, 2 and 3 the clean water permeance was measured for fiber samples from different collection periods and show constant clean water permeances in all cases. Because of higher water permeance, DL3 was selected for further characterization in this paper.

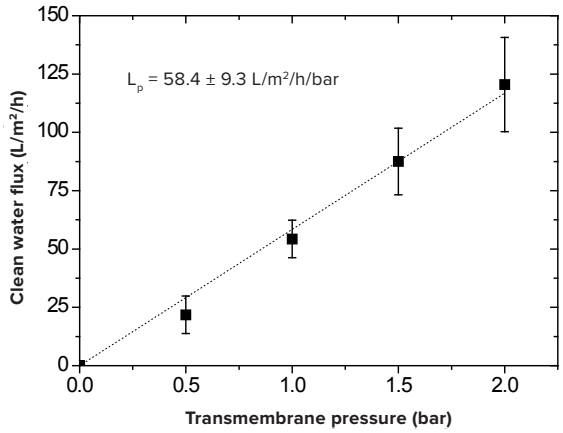


FIGURE 5. Average clean water flux versus transmembrane pressure for DL3 (n=6). Error bars indicate standard deviations.

Static adsorption

Adsorption isotherms

DL3 adsorbs creatinine, IS and HA, which is illustrated by the adsorption isotherms in Figure 6. Langmuir isotherm curve fits have been performed, even though for the tested concentration range a plateau was not reached yet in all cases. For creatinine, IS and HA, q_m= 3064 mg/g AC and K_d = 1.433 mg/mL, q_m= 350 mg/g AC and K_d=0.023 mg/mL, q_m= 134 mg/g AC and K_d=0.0195 mg/mL were obtained respectively. DL3 has much higher adsorption capacity for creatinine in comparison to flat sheet dual layer MMMs developed earlier [31]. For example, at a creatinine equilibrium concentration of 0.05 mg/mL the flat sheet dual layer MMM adsorbed approximately 29 mg creatinine per gram of activated carbon while the DL3 adsorbed around 100 mg/g AC. It is rather difficult to directly compare our results to other studies because of different experimental conditions and limited availability of *in vitro* adsorption data. In any case, we will discuss here some examples where comparison is possible. For example for creatinine adsorption, other publications with different approaches but using activated carbon as sorbent, report lower adsorption values at similar equilibrium concentrations [45-47]. Furthermore, granular and powdered carbons have been tested for adsorption of several compounds including IS. Using a sorbent/volume ratio of 2 mg/mL and an initial concentration of 0.03 mg/mL IS in a protein free solution, removal of more than 90% of the free IS was obtained after 24h. For our isotherm experiment we also used a protein free solution and free IS. Using a lower sorbent/volume ratio of 1.2 mg/mL but a longer incubation time (48h) we obtained removal of 95% at a similar initial concentration. Thus, IS adsorption by DL3 is in the same range as a commercial activated carbon [24].

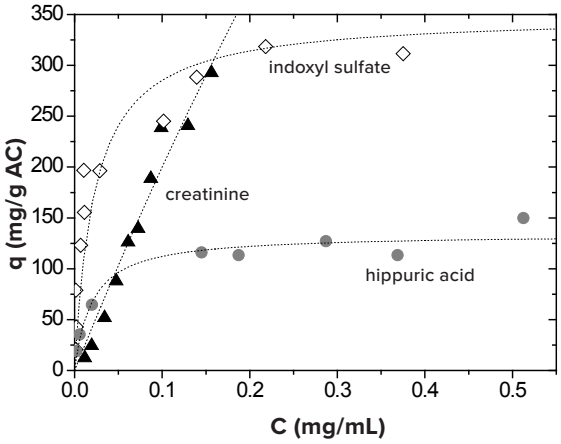


FIGURE 6. Adsorption isotherms at 37 °C for DL3. The equilibrium adsorption capacity (q) expressed in mg adsorbed creatinine (triangles), indoxyl sulfate (diamonds) or hippuric acid (circles) per gram of activated carbon (AC) embedded in DL3 is plotted against the equilibrium concentration (C) of creatinine, indoxyl sulfate and hippuric acid. The dotted lines represent the calculated Langmuir isotherm fits.

Adsorption from human blood plasma

Figure 7 shows the average relative creatinine concentrations after incubation in uremic plasma. The DL3 significantly removes creatinine compared to the particle free PES/PVP membrane, up to 83 ± 4 % is adsorbed after 4h. This approaches the creatinine removal by activated carbon particles alone, which suggests a good creatinine accessibility of the particles embedded in DL3. Relative plasma pH and osmolarity did not change significantly during the test duration. The total protein concentration is unchanged indicating that undesired protein binding is also limited (data not shown).

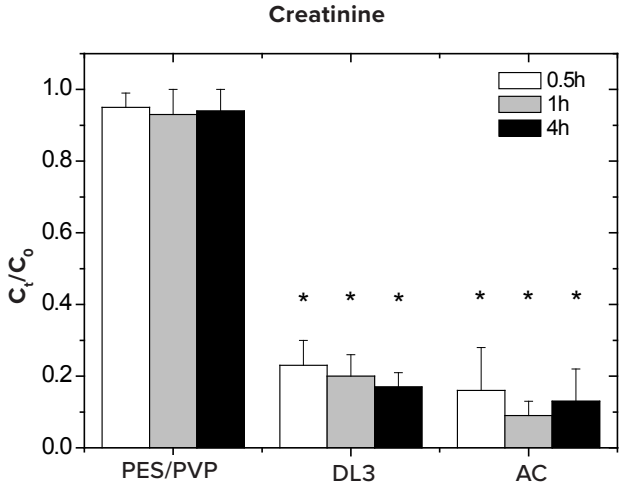


FIGURE 7. Average relative creatinine concentrations (C_t / C_0) after incubation of a PES/PVP membrane, dual layer hollow fiber mixed matrix membrane DL3 or pure activated carbon (AC) in plasma (n=6) for 0.5, 1 or 4h. * indicates a significant difference compared to particle free PES/PVP membrane (p<0.05). Error bars indicate standard deviations.

The amount of PCS, IS and HA bound to protein was on average 98%, 97% and 53% respectively and this was constant for the duration of the experiments (data not shown). Figure 8 shows the average relative PCS, IS and HA concentrations after 1h and 4h incubation with a PES/PVP membrane, DL3 or activated carbon particles for both initial spiked concentrations. DL 3 and AC both remove PCS, IS and HA significantly compared to the PES/PVP membrane. For the plasma which was spiked for uremia relevant concentrations, the relative PCS concentration is lowered to an average of 0.43 and 0.16 by DL3 and AC respectively after 4h. IS is also removed by DL3 and AC and after 4h average relative IS concentrations of 0.17 and 0.03 are obtained, respectively. For HA, the DL3 membrane and AC decrease the average relative concentrations after 4h to 0.05 and 0.01, respectively. The PES/PVP membrane does not adsorb PCS, IS and HA. Thus, even in these experimen-

tal conditions with a relatively low sorbent – plasma ratio 6.25 mg/mL (compared to ~100 mg/mL in hemoperfusion) the majority of PCS, IS and HA can be removed from the plasma within 4h by DL3. However, the activated carbon particles decreased the relative concentrations significantly more (p<0.05) than DL3 in all cases which may be due to some diffusion limitations, or reduction in adsorption capacity introduced by the membrane matrix.

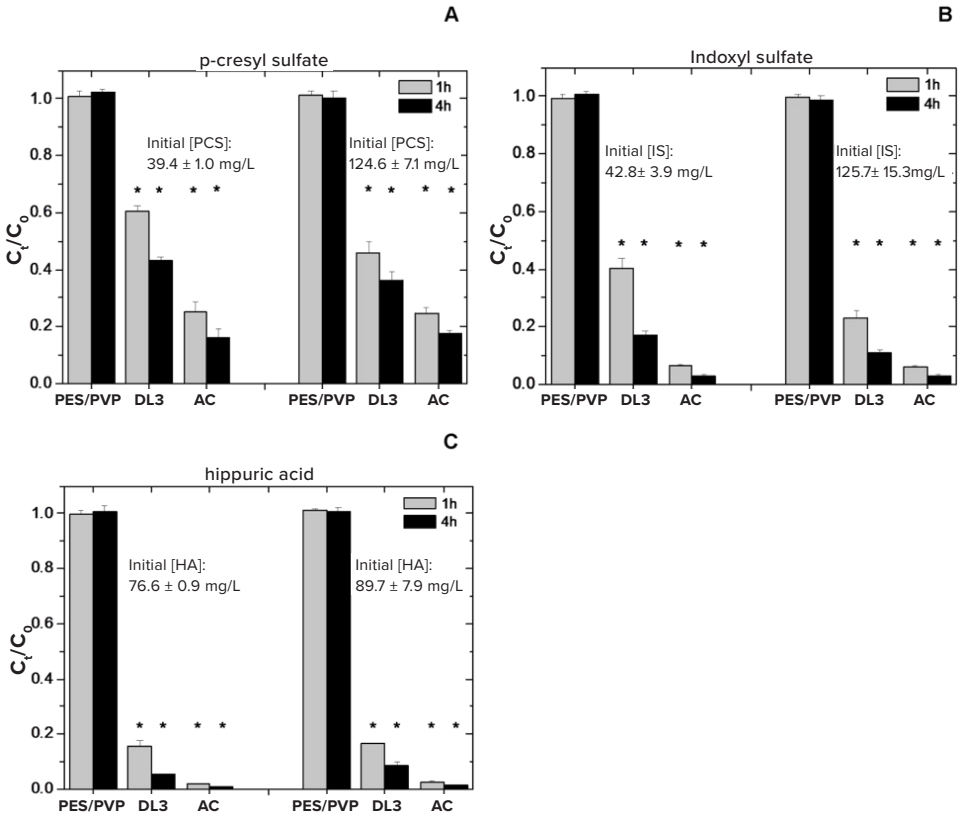


FIGURE 8. Relative concentrations after incubation of a particle free PES/PVP membrane, dual layer hollow fiber MMM DL3 and pure activated carbon particles (AC) in spiked plasma with 2 different initial concentrations for 1 or 4h b) Indoxyl sulfate, c) Hippuric acid. * indicates a significant difference compared to the particle free PES/PVP membrane (p<0.05)

One should remember however, that in this work we use small particles (median size 27 µm) which although seem to have sub optimal accessibility in the membrane matrix, but probably cannot be applied in adsorption columns which commonly have much larger particles for removal of solutes from blood or plasma. Small particles in columns could introduce high pressure drop into the system, as shown in [48], possibly resulting in protein

denaturation, and leakage of particle fragments into the patient's blood circulation. In contrast to adsorptive columns, in MMMs small particles can be used without a high pressure drop and channeling since particles are well distributed into the polymeric matrix [48]. In any case, in the future we plan to develop improved dual layer membranes with improved pore connectivity and higher particle loading.

TABLE 5. Average relative plasma values ± sd after incubation in human blood plasma of three donors for 4h. The plasma is incubated with a PES/PVP membrane, dual layer hollow fiber MMM DL3 or pure activated carbon (AC) particles alone.

	PES/PVP	DL3	AC
Relative osmolarity	1.008 ± 0.01	0.963 ± 0.01*	0.984 ± 0.01
Relative pH	1.004 ± 0.01	1.041 ± 0.07	1.046 ± 0.06
Relative Na^+ concentration	0.965 ± 0.07	0.952 ± 0.07	1.003 ± 0.02
Relative K^+ concentration	0.959 ± 0.08	0.935 ± 0.07	0.983 ± 0.01
Relative Ca^{2+} concentration	1.008 ± 0.04	1.040 ± 0.10	0.981 ± 0.07
Relative total protein concentration	1.002 ± 0.03	0.982 ± 0.05	0.996 ± 0.02
Relative urea concentration	0.986 ± 0.03	0.959 ± 0.06	0.957 ± 0.02
Relative creatinine concentration	0.946 ± 0.02	0.000 ± 0.00*	0.000 ± 0.00*

* indicates a significant difference compared with PES/PVP (p<0.05)

Table 5 presents average relative plasma values after incubation in plasma. Low levels of creatinine (compared to an uremic situation) are present in this plasma from healthy donors, and DL3 and AC completely remove creatinine within 4h (p<0.05), indicating the good adsorptive power even at low solute concentrations. There is no effect on pH or concentrations of Na^+, K^+, Ca^{2+}, total protein and urea (see Table 5) by DL3 or AC. DL3 lowered osmolarity (p<0.05 versus PES/PVP membrane), although this was not observed in the previous experiment using plasma exchange plasma.

Cross flow measurements

Creatinine diffusion cross flow measurements

As we showed before [31], dual layer MMMs can combine diffusion and adsorption in one step. Figure 9 shows that a lot of creatinine is adsorbed in the beginning and DL3 becomes more or less saturated. Whereas the diffusion of creatinine continues during the whole experiment. After 4h, which is a common duration of a hemodialysis treatment, both diffusion and adsorption equally contributed to the total creatinine removal. Almost 40 mg/g membrane creatinine was removed after 4h. Although this data cannot be directly compared to the *in vivo* situation, a rather crude comparison can be done. Assuming *in vivo* removal to be similar as in this experiment, one would require 45 gram (or 0.6 m^2) MMM to remove the daily creatinine production (~1800mg), which seems to be in a realistic range.

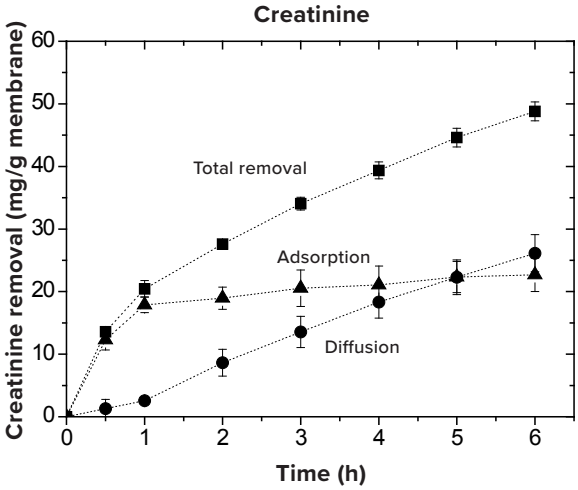


FIGURE 9. Average creatinine total removal (blocks), diffusion (circles) and adsorption (triangles) plotted vs. time, (n=3). Total removal is the amount of creatinine removed from the feed solution. This removal is mediated by diffusion and adsorption of creatinine. Error bars indicate standard deviations. The dotted lines are plotted to guide the eye.

Plasma cross flow measurements

For testing the removal of protein bound toxins PCS, IS and HA from spiked plasma, cross flow experiments were performed either in diffusion (Figure 1.A) or convection mode (Figure 1.B). The amount of PCS, IS and HA bound to protein was on average 90.0%, 86.6% and 38.1% respectively at the start of the experiments. In the diffusion experiments, albumin is retained by the membrane (relative total protein concentration in plasma after 6h: 1.000 ± 0.02) equivalent to membranes in hemodialysis. In the convection experiments, albumin partially passed through the membrane (together with a lot of fluid) with a relative total protein concentration of 2.224 ± 0.49 in the feed and 0.306 ± 0.18 in the permeate at the end of the experiment, respectively.

Figure 10.A presents the results of toxin removal in the diffusion experiments. The removal presented there corresponds to the amount of toxin depleted from the feed plasma in time. In most cases the toxin concentration in the dialysate was very low or below the HPLC detection limit. Therefore we can reasonably assume that the toxin removal here is mostly due to adsorption on the MMM. Figure 10.B presents the results of toxin adsorption onto the hollow fiber MMMs during the convection experiments. This was estimated based on the amount of toxin depleted from the feed plasma and from the collected permeate based on the mass balance. In both cases, the adsorption of the toxins on the MMMs increases in time. It seems that the amount adsorbed in the convection experiments is higher than in the diffusion experiments,

probably due to higher transport of the toxins due to the pressure difference. The removal of these hard to remove protein bound toxins by the DL3 in diffusion and convection cross flow experiments with spiked human plasma shows the potential for further development of this type of membranes. Direct comparisons of our work with other studies are rather difficult because of different experimental conditions. However, we could make a rather crude extrapolation to see if we are in a feasible range. Healthy subjects excrete 78 mg PCS and 69 mg IS in their urine in 24h [49]. Our membranes removed on average 2.27 mg PCS/g membrane and 3.58 mg IS /g membrane in 4h in the diffusion experiment and 2.68 mg/g membrane PCS and 12.85 mg/g membrane IS in the convection experiment. Assuming similar removal in the *in vivo* situation, one would need 5-35 gram (0.07-0.5 m^2) MMM for daily removal of these toxins. Comparing to hemoperfusion columns containing around 300 grams of particles, or hemodialyzers containing 1-2 m^2 membrane, these values show the good potential of our membrane. Further membrane optimization may improve the adsorption capacity of the hollow fiber membrane and its transport characteristics.

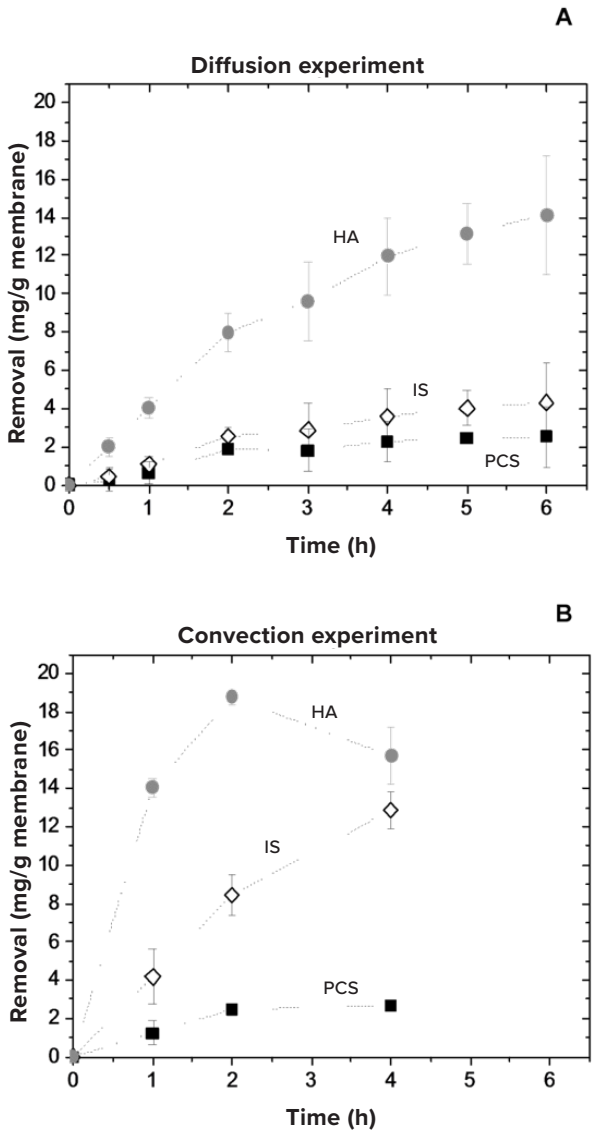


FIGURE 10. Removal of p-cresylsulfate (PCS) (blocks), indoxyl sulfate (IS) (diamonds) and hippuric acid (HA) (circles) plotted over the time. A: Diffusion experiment (n=3). B: Convection experiment (n=3). Dotted lines are plotted to guide the eye.

CONCLUSIONS AND OUTLOOK

In this work we fabricated a dual layer hollow fiber MMM with a porous macro-void free inner membrane layer that was well attached to the MMM outer layer containing AC particles. Spinning parameter as spinneret type, bore liquid composition and pumping speed of the dope solutions influence the final membrane structure of the MMM. This dual layer hollow fiber MMM adsorbs both creatinine and difficult-to-remove protein bound toxins from human plasma solutions. Furthermore, in cross flow experiments with human spiked plasma, the MMM removes protein bound toxins, indicating great potential of this new concept for removal of these difficult-to-remove type of toxins.

Future research could be focused on further membrane optimization. The inner layer thickness may be further reduced to minimize mass transport limitations. Porosity can be optimized to obtain a sharp cut off and possibly designed to avoid albumin loss and achieve optimal particle accessibility. Furthermore, the overall particle proportion of the membrane may be further increased and also other dimensions of the fiber may enhance transport properties. It would be interesting to fabricate modules of dual layer hollow fiber membranes on a bigger scale, for example comparable to hemodialysis modules in size, or comparable in terms of activated carbon content to commercially available adsorptive columns and test removal capacity of several uremic retention solutes.

REFERENCES

1. Coresh J, et al. Prevalence of chronic kidney disease in the United States. Journal of the American Medical Association 2007;298:2038-47.
2. Hamer RA, El Nahas AM. The burden of chronic kidney disease. British Medical Journal 2006;332:563-4.
3. Szczech LA, Lazar IL. Projecting the United States ESRD population: Issues regarding treatment of patients with ESRD. Kidney International, Supplement 2004;66.
4. Kerr M, Bray B, Medcalf J, O'Donoghue DJ, Matthews B. Estimating the financial cost of chronic kidney disease to the NHS in England. Nephrology Dialysis Transplantation 2012.
5. Lacson Jr E, et al. Survival with three-times weekly in-center nocturnal versus conventional hemodialysis. Journal of the American Society of Nephrology 2012;23:687-95.
6. Grooteman MPC, et al. Effect of online hemodiafiltration on all-cause mortality and cardiovascular outcomes. Journal of the American Society of Nephrology 2012;23:1087-96.
7. Mazairac AHA, et al. Changes in quality of life over time-Dutch haemodialysis patients and general population compared. Nephrology Dialysis Transplantation 2011;26:1984-9.
8. Duranton F, Cohen G, De Smet R, Rodriguez M, Jankowski J, Vanholder R, Argiles A. Normal and pathologic concentrations of uremic toxins. Journal of the American Society of Nephrology 2012;23:1258-70.
9. Bammens B, Evenepoel P, Keuleers H, Verbeke K, Vanrenterghem Y. Free serum concentrations of the protein-bound retention solute p-cresol predict mortality in hemodialysis patients. Kidney International 2006;69:1081-7.
10. Cheung AK, et al. Serum β-2 microglobulin levels predict mortality in dialysis patients: Results of the HEMO study. Journal of the American Society of Nephrology 2006;17:546-55.
11. Ito S, Osaka M, Higuchi Y, Nishijima F, Ishii H, Yoshida M. Indoxyl Sulfate Induces Leukocyte-Endothelial Interactions through Up-regulation of E-selectin. Journal of Biological Chemistry 2010;285:38869-75.
12. Adijiang A, Goto S, Uramoto S, Nishijima F, Niwa T. Indoxyl sulphate promotes aortic calcification with expression of osteoblast-specific proteins in hypertensive rats. Nephrology Dialysis Transplantation 2008;23:1892-901.
13. Lin CJ, et al. The role of protein-bound uremic toxins on peripheral artery disease and vascular access failure in patients on hemodialysis. Atherosclerosis 2012;225:173-9.
14. Wu IW, et al. P-cresyl sulphate and indoxyl sulphate predict progression of chronic kidney disease. Nephrology Dialysis Transplantation 2011;26:938-47.
15. Itoh Y, Ezawa A, Kikuchi K, Tsuruta Y, Niwa T. Protein-bound uremic toxins in hemodialysis patients measured by liquid chromatography/tandem mass spectrometry and their effects on endothelial ROS production. Anal Bioanal Chem 2012;403:1841-50.
16. Lekawanvijit S, Adrahtas A, Kelly DJ, Kompa AR, Wang BH, Krum H. Does indoxyl sulfate, a uraemic toxin, have direct effects on cardiac fibroblasts and myocytes? European Heart Journal 2010;31:1771-9.
17. Adijiang A, Higuchi Y, Nishijima F, Shimizu H, Niwa T. Indoxyl sulfate, a uremic toxin, promotes cell senescence in aorta of hypertensive rats.

Biochemical and Biophysical Research Communications 2010;399:637-41.

18. Saran R, et al. Longer treatment time and slower ultrafiltration in hemodialysis: Associations with reduced mortality in the DOPPS. Kidney International 2006;69:1222-8.
19. Basile C, et al. Removal of uraemic retention solutes in standard bicarbonate haemodialysis and long-hour slow-flow bicarbonate haemodialysis. Nephrology Dialysis Transplantation 2011;26:1296-303.
20. Dhondt A, Vanholder R, Van Biesen W, Lameire N. The removal of uremic toxins. Kidney International, Supplement 2000;58:S47-S59.
21. Meert N, et al. Prospective evaluation of the change of predialysis protein-bound uremic solute concentration with postdilution online hemodiafiltration. Artificial Organs 2010;34:580-5.
22. Meyer TW, Peattie JWT, Miller JD, Dinh DC, Recht NS, Walther JL, Hostetter TH. Increasing the clearance of protein-bound solutes by addition of a sorbent to the dialysate. Journal of the American Society of Nephrology 2007;18:868.
23. Patzer J. Principles of bound solute dialysis. Therapeutic Apheresis and Dialysis 2006;10:118-24.
24. Dinh DC, Recht NS, Hostetter TH, Meyer TW. Coated carbon hemoperfusion provides limited clearance of protein-bound solutes. Artificial Organs 2008;32:717-24.
25. Sirich TL, Luo FJG, Plummer NS, Hostetter TH, Meyer TW. Selectively increasing the clearance of protein-bound uremic solutes. Nephrology Dialysis Transplantation 2012;27:1574-9.
26. Henne W DG, Schmitz W, Pohle R, Lawitzki F, 4,267,047. Dialyzing membrane with adsorbent layer. United States, 1981 May 12.
27. Chapman GV, Hone PWE, Shirlow MJ. Evaluation of hemodiafiltration and sorbent membrane dialysis: II. Clinical, nutritional, and middle molecule assessment. Dialysis and Transplantation 1982;11:871-6.
28. Maeda K, Saito A, Kawaguchi S. Problems with activated charcoal and alumina as sorbents for medical use. Artificial Organs 1979;3:336-40.
29. Chapman GV, et al. Evaluation Of Hemodiafiltration And Sorbent Membrane Dialysis .1. Invivo And Invitro Dialyzer Performance. Dial Transplant 1982;11:758-65.
30. Vienken J, Diamantoglou M, Henne W, Nederlof B. Artificial dialysis membranes: From concept to large scale production. American Journal of Nephrology 1999;19:355-62.
31. Tijink MSL, et al. A novel approach for blood purification: Mixed-matrix membranes combining diffusion and adsorption in one step. Acta Biomaterialia 2012;8:2279-87.
32. Su BH, Fu P, Li Q, Tao Y, Li Z, Zao HS, Zhao CS. Evaluation of polyethersulfone highflux hemodialysis membrane in vitro and in vivo. Journal of Materials Science: Materials in Medicine 2008;19:745-51.
33. Zweigart C, Neubauer M, Storr M, Böhler T, Krause B. 2.13 - Progress in the Development of Membranes for Kidney-Replacement Therapy. In: Editor-in-Chief: Enrico D, Lidietta G, editors. Comprehensive Membrane Science and Engineering. Oxford: Elsevier; 2010. p. 351-90.
34. Ash SR. Sorbents in treatment of uremia: A short history and a great future. Seminars in Dialysis 2009;22:615-22.
35. Feigenbaum J, Neuberg CA. Simplified method

for the preparation of aromatic sulfuric acid esters. Journal of the American Chemical Society 1941;63:3529-30.

36. Vanholder R, et al. Review on uremic toxins: Classification, concentration, and interindividual variability. Kidney International 2003;63:1934-43.
37. Meert N, et al. Effective removal of protein-bound uraemic solutes by different convective strategies: A prospective trial. Nephrology Dialysis Transplantation 2009;24:562-70.
38. Meert N, et al. Novel method for simultaneous determination of p-cresylsulphate and p-cresylglucuronide: Clinical data and pathophysiological implications. Nephrology Dialysis Transplantation 2012;27:2388-96.
39. Boom RM. Membrane formation by immersion precipitation: the role of a polymeric additive [PhD Thesis]. Enschede: University of Twente; 1992.
40. Xu ZL, Qusay FA. Polyethersulfone (PES) hollow fiber ultrafiltration membranes prepared by PES/non-solvent/NMP solution. Journal of Membrane Science 2004;233:101-11.
41. Yang Q, Chung TS, Weber M. Microscopic behavior of polyvinylpyrrolidone hydrophilizing agents on phase inversion polyethersulfone hollow fiber membranes for hemofiltration. Journal of Membrane Science 2009;326:322-31.
42. Liu Y, Koops GH, Strathmann H. Characterization of morphology controlled polyethersulfone hollow fiber membranes by the addition of polyethylene glycol to the dope and bore liquid solution. Journal of Membrane Science 2003;223:187-99.
43. Barzin J, Feng C, Khulbe KC, Matsuura T, Madaeni SS, Mirzadeh H. Characterization of polyethersulfone hemodialysis membrane by ultrafiltration and atomic force microscopy. Journal of Membrane Science 2004;237:77-85.
44. Liao Z, Klein E, Poh CK, Huang Z, Lu J, Hardy PA, Gao D. Measurement of hollow fiber membrane transport properties in hemodialyzers. Journal of Membrane Science 2005;256:176-83.
45. Deng X, Wang T, Zhao F, Li L, Zhao C. Poly(ether sulfone)/activated carbon hybrid beads for creatinine adsorption. Journal of Applied Polymer Science 2007;103:1085-92.
46. Ye C, Gong QM, Lu FP, Liang J. Adsorption of uraemic toxins on carbon nanotubes. Separation and Purification Technology 2007;58:2-6.
47. Gao B, Yang Y, Wang J, Zhang Y. Preparation and adsorption characteristic of polymeric microsphere with strong adsorbability for creatinine. Journal of Biochemical and Molecular Toxicology 2008;22:166-74.
48. Tetala KKR, Stamatialis DF. Mixed matrix membranes for efficient adsorption of copper ions from aqueous solutions. Separation and Purification Technology 2013;104:214-20.
49. Martinez AW, Recht NS, Hostetter TH, Meyer TW. Removal of P-cresol sulfate by hemodialysis. Journal of the American Society of Nephrology 2005;16:3430-6.

APPENDIX

A triple spinneret was designed so that fibers with a thin inner layer and thicker outer layer could be fabricated. The channels for the bore liquid and inner layer polymer dope are connected as usual in spinnerets, whereas for the outer layer MMM dope a special distribution ring was designed, see Figure 1 and 2.

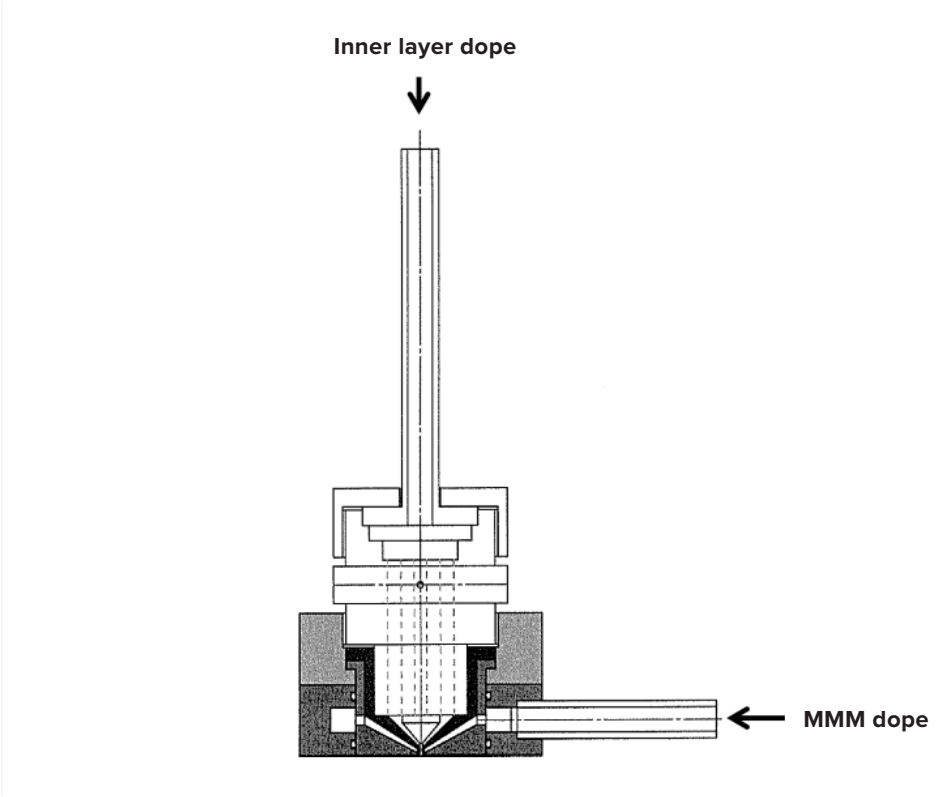


FIGURE 1. Schematic representation of triple spinneret 2

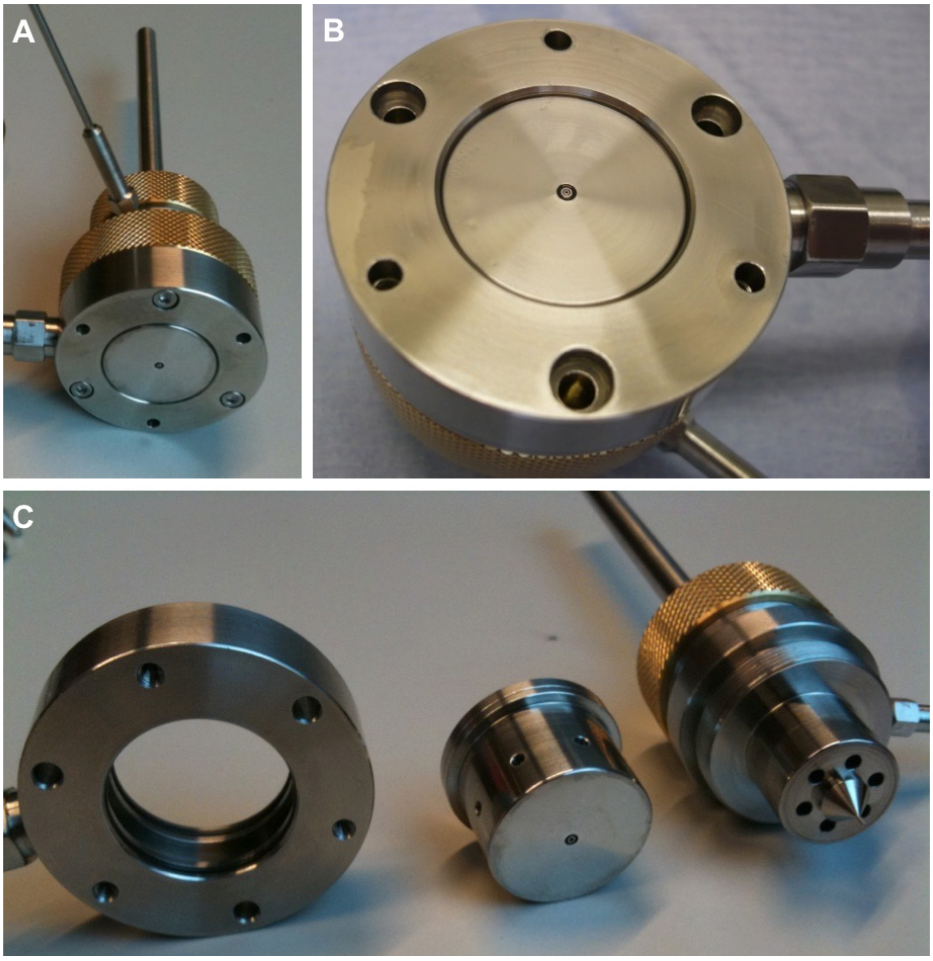
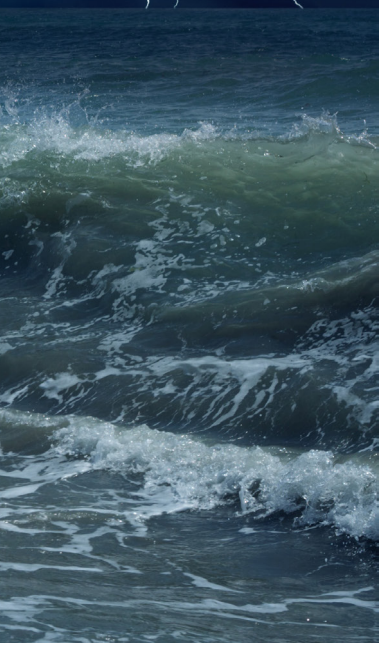


FIGURE 2. Photographs of triple spinneret 2. A: spinneret with distribution ring. B: bottom of the spinneret with distribution ring. C: Disassembled spinneret, with the distribution ring separately on the left.

PART IV

Summary, discussion and future perspectives



8

Summary, discussion and future perspectives

SUMMARY, DISCUSSION AND FUTURE PERSPECTIVES

Conventional hemodialysis (HD) and peritoneal dialysis (PD) have major shortcomings. Removal of waste molecules and excess fluid is inadequate, contributing significantly to poor life quality, high morbidity and high mortality [1-4]. Increasing the dialysis dose would be a major improvement in renal replacement therapy [3,5-10]. A miniaturized dialysis device can facilitate longer and more frequent dialysis [11]. In addition, a miniaturized design that is independent of a fixed water supply because of dialysate regeneration will offer more freedom and autonomy to the patient. In designing such a device, effective removal strategies must be developed for removal of all uremic waste products, excess sodium and water.

Currently, home HD still requires bulky dialysis machines and a large supply of dialysis fluids or an immobile water purification system. A portable home HD machine is available, but its size is rather large (38x38x46 cm, 34 kg) and its water purification system using tap water is immobile, requiring transportation of a large amount of dialysis fluid bags during traveling [12]. Further miniaturization of dialysis devices and reduction of the dialysate volume will facilitate home dialysis and increase patients' mobility and autonomy.

The concept of a miniaturized dialysis device is based on a closed-loop system that continuously regenerates a small volume of dialysate. This is radically different from conventional dialysis. In intermittent HD, fresh dialysate passes the dialyzer only once. Spent dialysate, approximately 120 L per 4 hr HD session, is not re-used, but directly discarded into the sewerage. In PD, spent dialysate is resubstituted for fresh dialysate 4 to 6 times a day, either manually (in continuous ambulatory PD) or by a machine (automated PD). Cumulatively, ~8-12 L dialysate is used per day. Efficient and sufficient regeneration of spent dialysate in HD as well as PD is thus an absolute prerequisite to succeed in developing a miniaturized dialysis device.

Regeneration of spent dialysate can be achieved by removal of waste ions (such as potassium and phosphate) and organic waste compounds (such as urea and protein bound uremic toxins (PBUTs)). Waste ions can be removed from spent dialysate using ion exchangers. Activated carbon readily adsorbs organic waste compounds except for urea (only ~0.1 mmol urea/g activated carbon). Since urea is very hard to adsorb and its daily molar production is higher than that of any other uremic solute, an efficient miniature urea removal strategy is a major challenge in the development of a miniaturized dialysis device. PBUTs are also hard to remove since only the (small) free fraction of these toxins can diffuse over the dialyzer. This is a challenge in conventional HD as well as in a miniature dialysis device.

In this thesis, we explored strategies to remove (excess) potassium, phosphate, urea and PBUTs from spent dialysate. In **part I** we evaluated *in vitro* and *in vivo* efficacy of potassium and phosphate binding sorbents and their effect on balance of other electrolytes, acid base status and vital parameters (*in vivo*). In **part II** we explored electro-oxidation as a urea removal strategy *in vitro* and *in vivo* and evaluated efficacy and effects on electrolyte balance, acid base status and vital parameters (*in vivo*). In **part III** we tested mixed matrix membranes (MMM) as a strategy to improve removal of PBUTs.

PART I – ELECTROLYTE BALANCE

The *in vitro* experiments described in **chapter 2** were performed to select potassium and phosphate sorbents with an optimal balance between binding capacity and regenerability. Based on these experiments we selected sodium poly(styrene-divinylbenzene) sulphonate beads for potassium removal and iron oxide hydroxide beads for phosphate removal. The selected sorbents were incorporated in a sorbent cartridge in spherical form since powdered sorbents would obstruct the cartridge. Both sorbents adsorbed significant amounts of the targeted electrolytes (~10 mmol potassium and 5 mmol phosphate in 3 hours by ~110 g sodium poly(styrene-divinylbenzene) sulphonate and 55 g iron oxide hydroxide) at normal plasma concentrations of these electrolytes.

The selected sorbents were also excellently regenerable. Regenerability was a major selection criterion since the possibility to regenerate spent sorbents would considerably lower exploitation costs of a miniaturized dialysis device.

A major side-effect was removal of calcium and magnesium by the potassium sorbent and remarkably also by the phosphate sorbent (probably via adsorption to the adsorbed phosphate). We showed that this could be prevented by preloading the sorbents with these electrolytes. Another side-effect was sodium release (in exchange for potassium) which may complicate fluid management in dialysis patients.

In **chapter 3** the results of our *in vitro* experiments with the selected potassium and phosphate sorbents were validated *in vivo* by testing sorbent cartridges in dialysis experiments in healthy goats. Potassium and phosphate removal were in the same range as in the *in vitro* experiments. Importantly, potassium and phosphate removal were concentration-dependent with more adsorption at higher plasma concentrations and vice versa. This may be an intrinsic safety feature of these sorbents. Sodium release, as observed during the in vitro experiments, could be prevented by equilibrating the sorbent cartridges at a low sodium concentration (120 mM).
No major adverse events were recorded. Vital parameters were stable except for a parallel rise in blood pressure and pulse rate, which was probably due to increasing sympathetic tone in the restrained goats during the experiment.

Based upon our studies, potassium and phosphate removal by the selected sorbents seems to be promising and safe. We also proved that the sorbents can be regenerated and re-used. This way costs of a miniature dialysis device can be reduced. However, efficiency needs to be increased. To compete with intermittent (3-weekly), 4-hours hemodia-

lysis with a potassium and phosphate removal of ~63-80 and ~22-38 mmol per treatment [13,14], respectively, the total weight of the sorbents in the dialysis device would have to be increased to ~693-880 g potassium sorbent and ~242-418 g phosphate sorbent. However, this might compromise wearability of the device, particularly for frail patients. Prolonged use of the device with less sorbents would not offer adequate removal since our experiments showed that the sorbents were saturated after ~3 hours dialysis. The preferred solution would be to dialyze more hours/ week to allow for more continuous removal of excess potassium and phosphate and to prevent rebound of concentrations of these electrolytes after dialysis. This could be accomplished by using sorbents cartridges with less sorbents and replacing saturated cartridges after 3 hours of dialysis. This way, patients would be able to personalize their dialysis treatment.

When compared to the well-known REDY-cartridge and derivatives of this sorbent system [15-22], our sorbents were less potent but better regenerable and showed no effect on calcium, magnesium and sodium when adequately preconditioned. The REDY system is the only sorbent dialysis system that has ever been applied in clinical practice. Its sorbents are the basis for most of the portable or wearable dialysis devices that are currently under development. It makes use of zirconium phosphate and zirconium oxide. These are very potent sorbents, but the downside of zirconium phosphate is the complete removal of calcium, magnesium and potassium which necessitates infusion of these electrolytes from a reservoir downstream of the sorbents. Consequently, the total weight of the device increases. This means that sodium poly(styrene-divinylbenzene) sulphonate as well as zirconium phosphate do not offer the ideal solution for potassium removal in a miniature dialysis device. Ideally, an ion exchanger should be developed that selectively adsorbs monovalent cations such as the relatively new selective zirconium silicate Zs9 [23].

PART II - UREA REMOVAL BY ELECTRO-OXIDATION

In part II, electro-oxidation (EO) as urea removal strategy in a wearable or portable miniature dialysis device is investigated. This technique has been explored since the '70's of last century for urea removal [24-34] but reports about EO stopped around 2004. The reason for discontinuation of this line of research has not been reported but may be related to biocompatibility issues. We think EO to be an attractive urea removal strategy since it can be miniaturized, needs no regeneration and is inexpensive.

To explore whether urea removal by EO could be a realistic strategy for urea removal in a miniature dialysis device, urea degradation efficacy and potential side effects of EO were investigated.

In **chapter 4** we compared ruthenium, platinum and graphite electrodes and quantified urea removal and generation of toxic chloride oxidation by-products. Ruthenium oxide, platinum and graphite electrodes were selected since most previous reports on EO used these electrodes [24-27,29-35].

Platinum electrodes degraded more urea (21±2mmol/hour) than ruthenium oxide (13±2mmol/hour) or graphite electrodes (13±1mmol/hour). However, generation of chloride oxidation by-products was much lower with graphite (13±4mg/hour) than with platinum (231±22mg/hour) or ruthenium oxide electrodes (129±12mg/hour). This is relevant since toxic chloride oxidation by-products can cause oxidative stress *in vivo*. Based upon these observations, graphite electrodes were chosen since they had the best ratio between urea degradation and generation of toxic chloride oxidation by-products.

Urea degradation was dependent on plasma urea concentration. Higher current resulted in increased urea degradation rate and efficiency and higher free and bound chlorine release. The ratio of free as well as bound chlorine release to urea degradation was lowest at 2-3A.

In **chapter 5** we showed *in vivo* that urea degradation was also linearly dependent on electrode surface. Increasing the dialysate flow did not result in increased urea removal. This may be because higher dialysate flow results in reduced dialysate electrode contact time and a reduced urea oxidation efficiency. Efficiency was sufficient for continuous HD (since extrapolation of our results to 24 hour per day would result in removal of ~380 mmol urea per day). However, for intermittent HD efficiency should be increased, for example for daily 8 hour nocturnal HD the electrode surface area would have to be increased by 200-300%.

To achieve similar time averaged urea plasma clearance as thrice weekly 4 hour HD (12.3 mL/min [36] or continuous ambulatory peritoneal dialysis (PD) (9 mL/min) [37], the 2-unit device (urea clearance: 19.5 mL/min) should be used for at least ~15 hour/day or 11 hour/day 7 days per week, respectively.

To allow the use of EO for intermittent HD, it will be necessary to further increase the cumulative electrode surface of the EO units. For example, for daily 8 hour nocturnal HD, the electrode surface area would have to be increased by 200-300%.

We documented potential toxic side effects of EO and tested measures to correct these by effects. First, release of toxic chloride by-products was measured. This could be reduced by use of activated carbon (AC). Activated carbon is a potent electron donor that effectively removes chlorine from aqueous solutions by reducing chlorine to chloride ions [38]. In **chapter 4** we showed that limited amounts of AC (< 100g) can be used to bring the levels of toxic chloride by-products species below maximum acceptable values according to AAMI standards. However, it should be noted that the amount of AC that is needed depends on several variables such as the plasma urea concentration (at lower plasma urea values more toxic chloride by-products are released), the amperage that is applied (with higher release of toxic chloride by-products at higher amperages) and dialysate flow (higher release of toxic chloride by-products at higher dialysate flow). Obviously, the more AC is used, the more the wearability of the device is compromised. So, when optimizing EO conditions, in addition to efficacy the amount of toxic chloride by-products that is generated and the measures that are needed to neutralize these by-products are important determinants.
Second, ammonium was generated by the EO unit, probably due to hydrolysis of urea. The amounts that were released were limited: ~7 mmol during a 3 hour dialysis session at a urea removal rate of 16 mmol/hour, compared to a daily amount of 500-800 mmol of ammonium that is converted into urea by the liver. Nevertheless, strategies should be developed to further reduce the generation of ammonium in the EO-unit or to remove ammonium from the dialysate downstream of the EO-unit.

In addition to these EO-related by-effects, we also observed several changes in plasma chemistry that are probably related to our experimental setup in which we used urea infusion in healthy goats to mimic uremic conditions. Systemic plasma sodium and chloride concentrations increased, and bicarbonate decreased probably due to infusion of solutions that exclusively contained chloride as an anion. Hemolysis was observed probably due to urea infusion, since visible hemolysis and LDH increase occurred exclusively in experiments with urea infusion. The leukocyte number increased, possibly due to 'catecholamine-induced leukocytosis'.

Summarizing, EO seems to be an interesting urea removal strategy. It seems to have several advantages over urease, that has been used for urea removal in the REDY system. Urease efficiently degrades urea but, in this process, a considerable amount of ammonium is produced. In the REDY system, a cation exchanger is incorporated to remove ammonium in exchange for sodium. To neutralize this excess amount of sodium, extra sodium-free dialysate is used. This results in a considerable increase of the total weight of the system and still holds the risk of break-through and release of ammonium into the dialysate.

However, we performed only limited safety analyses and have to take into account the possibility that further analysis of the electrochemically treated dialysate may reveal other toxic oxidation products besides chloride by-products. In addition to this, parts of the electrodes may leach into the system. Extensive biocompatibility testing, such as cytotoxicity testing, is indicated to evaluate the safety of this technology. One of the main challenges in optimizing EO is to make this technique more urea-selective. This could for instance be achieved by putting an extra AC filter upstream of the EO-unit. The extra AC adsorbs other nitrogen containing uremic retention solutes, thus preventing them from competitive exposure to EO.

PART III - UREMIC TOXIN REMOVAL BY MIXED MATRIX MEMBRANES

In part III, new dialysis membranes were developed to optimize removal of protein bound uremic toxins (PBUTs) from uremic plasma. Protein-bound solutes are thought to contribute to uremic toxicity and are hardly removed by hemodialysis because a considerable fraction of the PBUTs is protein bound and only the free fraction of these solutes can be filtered [39-45].

The so-called mixed matrix membranes (MMM) consist of a porous particle-free hemocompatible membrane layer on the blood contacting side of the membrane and an outer layer, facing the dialysate side of the membrane, containing activated carbon (AC) particles. This dual layer design is used to prevent particle release into the circulation and to prevent direct contact of blood with AC because this may result in activation of the clotting cascade. The free fraction of the PBUTs that passes the inner layer of the MMM is immediately adsorbed by the AC particles. This way a maximal concentration gradient is created which stimulates diffusion and shift of the balance protein-bound/free PBUTs towards the free fraction. Cumulatively, a more significant portion of the PBUTs is removed by combining adsorption and diffusion through these MMM.
MMM could offer additional advantages in case they are incorporated in a miniature dialysis device. They create a maximal concentration gradient for all organic solutes, including the small water-soluble solutes. This way high clearance of these solutes can be achieved, while using considerably less dialysate than in conventional dialysis (total volume of dialysate of less than 0.5 L versus 0.5 L/ minute in conventional dialysis).

In **chapter 6,** flat single and dual layer MMM were developed using a polyethersulfone (PES)/polyvinylpyrrolidone (PVP) polymer blend. Creatinine removal was quantified using creatinine as a model small water-soluble solute to assess whether the removal of small water- soluble solutes by the new membranes was preserved. Para-aminohippuric acid (PAH) was used as marker for PBUT removal. The dual layer MMM removed more than 80% of PAH and creatinine, which was similar to the removal obtained by the activated carbon particles alone. This suggests that the accessibility of the AC particles in the MMM was excellent.

In **chapter 7**, hollow fiber MMM were fabricated and removal of the PBUT's p-cresyl sulfate (PCS) and indoxyl sulfate (IS) was quantified. The MMM removed on average 2.27 mg PCS/g membrane and 3.58 mg IS/g membrane in 4h, in the diffusion experiment and 2.68 mg/g membrane PCS and 12.85 mg/g membrane IS, in the convection experiment. Assum-

ing similar removal in the in vivo situation, one would need 5-35 g (0.07-0.5 m2) MMM for removal of the daily production of these toxins (healthy subjects excrete 78 mg PCS and 69 mg IS in their urine in 24h [46].

More recently, an in vitro comparison of optimized MMM with conventional dialyzers showed a 200-300% increase in PBUT removal by the MMM compared to conventional dialyzers [47].

However, total PBUT removal by MMM is still limited. This is because only the free faction can pass the filter and the predialysis protein-bound/free fraction equilibrium is not completely restored during the treatment (the percentage protein binding increases) [48].

CONCLUSIONS AND FUTURE PERSPECTIVES

In this thesis, we showed that we were able to develop a miniature dialysis device that can remove considerable amounts of excess potassium, phosphate and urea by using sorbents for electrolyte balance and electro-oxidation for urea removal. In addition, we showed that MMM, dialysis membranes with integrated activated carbon particles, could be an asset in the optimization of protein bound uremic toxin removal in a miniature dialysis device as well as in conventional HD.

However, many challenges must be overcome to reach the stage of application of this device in humans.
First, the potassium sorbent must be optimized. Its binding capacity must be increased, and it should preferably be a selective monovalent cation binder.
The second, and to my opinion largest, challenge, is the optimization of our urea removal strategy. We will have to make EO more urea selective. This way, generation of possibly toxic by-products can be minimized, and the efficiency of this technique can be improved. Thus far, little is known about potentially toxic oxidative by-products other than chlorines and ammonium that may be formed during electro-oxidation. Research is underway to define the chemical constituents of dialysate of uremic subjects exposed to electro-oxidation and to test the biocompatibility of such fluids in *in vitro* experiments [49]. Unfortunately, alternative urea removal strategies such as urease also have several drawbacks. Should EO in the long-run not be proven to be a safe EO removal strategy that is suited for application in humans, urea sorbents may be a crucial alternative. At this moment, simultaneously to the process of optimization of EO, we are developing selective urea sorbents [50].
Third, PBUT removal in our device is (probably) limited. Integration of MMM in our device could increase PBUT removal. However, the fact that only the (small) free fraction of these toxins is removed, albeit continuously, means that cumulative removal is still relatively low. Ideally, we would simulate the active secretion of these toxins by our own kidneys. To this end, renal tubular cells would have to be incorporated in our device. In recent years, significant steps have been taken towards the development of a bioartificial kidney (BAK) but several milestones will have to be addressed before (parts of) BAK can be integrated in our device or in conventional renal replacement therapy.

Then there are additional challenges in the development and testing of our device on the road to clinical testing that are not per se related to (technical) characteristics of our device.
First, a uremic large animal model must be developed, as the current model (HD in healthy goats) involves infusion of relatively large volumes of solutions containing electrolytes and

urea that obscure the true effects of the device on electrolyte and acid-base parameters and hemocompatibility. Currently, proof-of-principle HD experiments are being performed using goats with chronic kidney disease after (partial) ablation of their kidneys followed by dose-dependent additional damage by gentamycin infusion. Subtotal renal artery embolization is performed via infusion of polyvinyl alcohol particles in branches of the renal artery. Gentamycin is used to (temporarily) aggravate uremia [51]. By using this model, several side-effects related to the experimental setup described in this thesis can be minimized.
Second, the system would require upscaling to develop a portable device that can be used for nocturnal or daily home dialysis. However, this process will also increase the generation of known toxic by-products of electro-oxidation such as chlorines or ammonium, and strategies will have to be developed to prevent the production or release of these substances. This will also compromise wearability of the device since the more the device weighs, the less likely that it will be used as a 24/7 on-body dialysis device.
Third, in case of HD, safe vascular access will have to be developed.

On our road towards a wearable, and in the end hopefully an implantable dialysis device, we are also involved in several other projects besides the projects discussed in this thesis. Currently, a portable artificial kidney (PAK [52]) is being developed for HD with urea removal by urease. Simultaneously, a miniature artificial kidney for continuous flow PD is developed [53] with urea removal by AC and dialysate. Both prototypes are still relatively heavy (over 10 kgs), thus miniaturization is required to make these devices portable or even wearable.
Finally, we will integrate the best performing technologies in a next generation prototype portable or wearable dialysis device. As discussed before, an efficient and safe urea removal strategy is probably the biggest hurdle to overcome in this endeavour. In the end, one of the possible solutions could be the integration of all sorbents in the previously discussed MMM to enable an on-body dialysis device. Our ultimate goal remains an implantable device.

Despite all these challenges, the research performed in this thesis paves the way for further testing of a miniature dialysis device and and I am confident that, in the long term, this will facilitate longer and easier dialysis at home for a larger proportion of the patients with ESKD requiring dialysis.

REFERENCES

1. Mittal SK, Ahern L, Flaster E et al. Self-assessed physical and mental function of haemodialysis patients. Nephrol Dial Transplant 2001; 16: 1387–1394.
2. Mittal SK, Ahern L, Flaster E et al. Self-assessed quality of life in peritoneal dialysis patients. Am J Nephrol 2001; 21: 215–220.
3. Locatelli F, Canaud B. Dialysis adequacy today: a European perspective. Nephrol Dial Transplant 2012; 27: 3043–3048.
4. Wolfe RA, Ashby VB, Milford EL et al. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a a first cadaveric transplant. N Engl J Med 1999; 341: 1725–1730.
5. Achinger SG, Ayus JC. The role of daily dialysis in the control of hyperphosphatemia. Kidney Int Suppl 2005; 67: S28–S32.
6. Ayus JC, Achinger SG, Mizani MR et al. Phosphorus balance and mineral metabolism with 3 h daily hemodialysis. Kidney Int 2007; 71: 336–342.
7. King RS, Glickman JD. Electrolyte management in frequent home hemodialysis. Semin Dial 2010; 23: 571–574.
8. Lacson E, Jr, Xu J, Suri RS et al. Survival with three-times weekly in-center nocturnal versus conventional hemodialysis. J Am Soc Nephrol 2012; 23: 687–695.
9. Walsh M, Manns BJ, Klarenbach S et al. The effects of nocturnal compared with conventional hemodialysis on mineral metabolism: a randomized-controlled trial. Hemodial Int 2010; 14: 174–181.
10. Bonenkamp AA, van Gelder MK, Abrahams AC et al. Home haemodialysis in the Netherlands: State of the art. Neth J Med. 2018 May;76(4):144-157.
11. Davenport A, Gura V, Ronco C et al. A wearable haemodialysis device for patients with end-stage renal failure: a pilot study. Lancet 2007; 370: 2005–2010.
12. Takahashi S. Future home hemodialysis - advantages of the NxStage System One. Contrib Nephrol. 2012;177:117-26.
13. De NL, Bellizzi V, Minutolo R et al. Effect of dialysate sodium concentration on interdialytic increase of potassium. J Am Soc Nephrol 2000; 11: 2337-2343.
14. Wang M, Li H, Liao H et al. Phosphate removal model: an observational study of low-flux dialyzers in conventional hemodialysis therapy. Hemodial Int 2012; 16: 363-376.
15. Ash SR. Sorbents in treatment of uremia: a short history and a great future. Semin Dial 2009; 22: 615-622
16. Davenport A, Gura V, Ronco C, et al. A wearable haemodialysis device for patients with end-stage renal failure: a pilot study. Lancet 2007; 370: 2005-2010.
17. Lee DB, Roberts M. A peritoneal-based automated wearable artificial kidney. Clin Exp Nephrol 2008; 12: 171-180.
18. Murisasco A, Baz M, Boobes Y et al. A continuous hemofiltration system using sorbents for hemofiltrate regeneration. Clin Nephrol 1986; 26 Suppl 1: S53-S57.
19. Ronco C, Fecondini L. The Vicenza wearable artificial kidney for peritoneal dialysis (ViWAK PD). Blood Purif 2007; 25: 383-388.
20. Fuchs C, Dorn D, Rieger J, et al. Capabilities of the Redy cartridge for regeneration of hemofiltrate. Artif Organs 1979; 3: 279-280.

21. Gura V, Beizai M, Ezon C et al. Continuous renal replacement therapy for end-stage renal disease. The wearable artificial kidney (WAK). Contrib Nephrol 2005; 149: 325–333.
22. McGill RL, Bakos JR, Ko T et al. Sorbent hemodialysis: clinical experience with new sorbent cartridges and hemodialyzers. ASAIO J 2008; 54: 618–621.
23. Ash SR, Singh B, Lavin PT et al. A phase 2 study on the treatment of hyperkalemia in patients with chronic kidney disease suggests that the selective potassium trap, ZS-9, is safe and efficient. Kidney Int. 2015 Aug;88(2):404-11.
24. Koster K, Wendt H, Gallus J, et al. Regeneration of hemofiltrate by anodic oxidation of urea. Artif Organs 1983; 7: 163-168.
25. Grinval'd V, Leshchinskii GM, Rodin VV, et al. Development and Testing of a Unit for Electrochemical Oxidation of Products of Hemodialysis. Biomedical Engineering 2003; 37: 67-72.
26. Fels M. Recycle of dialysate from the artificial kidney by electrochemical degradation of waste metabolites: small-scale laboratory investigations. Med Biol Eng Comput 1978; 16: 25-30.
27. Fels M. Recycle of dialysate from the artificial kidney by electrochemical degradation of waste metabolites: continuous reactor investigations. Med Biol Eng Comput 1982; 20: 257-263.
28. Hintzen K, Stiller S, Brunner H, et al. Electrodialysis and reverse osmosis as a regeneration system for hemofiltrate. Artif Organs 1983; 7: 169-175.
29. Keller Jr R, Yao S, Brown J, et al. Electrochemical Removal of Urea from Physiological Buffer as the Basis for a Regenerative Dialysis System. Bioelectrochemistry and Bioenergetics 1980; 7: 469-485.
30. Schuenemann B, Quellhorst E, Kaiser H, et al. Regeneration of filtrate and dialysis fluid by electro-oxidation and absorption. Trans Am Soc Artif Intern Organs 1982; 28: 49-53.
31. Wright J, Michaels AS, Appleby AJ. Electrooxidation of Urea at the Ruthenium Titanium Oxide Electrode. AIChE Journal 1986; 32: 1450-1458.
32. Yao SJ, Wolfson SK, Jr., Ahn BK, et al. Anodic oxidation of urea and an electrochemical approach to de-ureation. Nature 1973; 241: 471-472.
33. Yao S, Wolfson Jr S, Tokarsky J, et al. De-ureation by Electrochemical Oxidation. Bioelectrochemistry and Bioenergetics 1974; 1: 180-186.
34. Yao S, Wolfson Jr S, Krupper M, et al. Controlled-potential controlled-current electrolysis: In vitro and in vivo electrolysis of urea. Bioelectrochemistry and Bioenergetics 1984; 13: 15-24.
35. Simka W, Piotrowski J, Robak, A, et al. Electrochemical treatment of aqueous solutions containing urea. J Appl Electrochem 2009; 39: 1137-1143.
36. Eknoyan G, Beck GJ, Cheung AK, et. al. Effect of dialysis dose and membrane flux in maintenance hemodialysis. The New England journal of medicine 347: 2010-2019, 2002.
37. Evenepoel P, Meijers BK, Bammens B, et al. Phosphorus metabolism in peritoneal dialysis- and haemodialysis-treated patients. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association 31: 1508-1514, 2016.
38. Li Y, Zhang X, Shang C. Effect of reductive property of activated carbon on total organic halogen analysis. Environ Sci Technol 2010; 44: 2105-2111.
39. Malchesky PS, Varnes W, Piatkiewicz W, et al. Membranes containing sorbents for blood detoxification. Trans Am Soc Artif Intern Organs 1977;23:659–66.
40. Malchesky PS, Piatkiewicz W, Nakamoto S, et al. Haemoperfusion made safe with sorbent membranes. Proc Eur Dial Transplant Assoc

1978;15:591–2.

41. Malchesky PS, Piatkiewicz W, Varnes WG, et al. Sorbent membranes – device designs, evaluations and potential applications. Artif Organs 1978;2:367–71.
42. Klein E, Holland FF, Eberle K. Sorbent-filled hollow fibers for hemopurification. Transactions of the American Society for Artificial Organs 1978;24:127–30.
43. Gurland HJ, Castro LA, Samtleben W, et al. Combination of hemodialysis and hemoperfusion in a single unit for treatment of uremia. Clin Nephrol 1979;11:167–72.
44. Chapman GV et al. Evaluation of hemodiafiltration and sorbent membrane dialysis: I. In vivo and in vitro dialyzer performance. Dial Transplant 1982;11:758–65.
45. Chapman GV, Hone PWE, Shirlow MJ. Evaluation of hemodiafiltration and sorbent membrane dialysis: II. Clinical, nutritional, and middle molecule assessment. Dial Transplant 1982;11:871–6.
46. Widjojo N, Zhang SD, Chung TS, et al. Enhanced gas separation performance of dual-layer hollow fiber membranes via substructure resistance reduction using mixed matrix materials. J Membr Sci 2007;306:147–58.
47. Pavlenko D, van Geffen E, van Steenbergen MJ, et al. New low-flux mixed matrix membranes that offer superior removal of protein-bound toxins from human plasma. Sci Rep. 2016 Oct 5;6:34429.
48. Deltombe O, van Biesen W, Glorieux G, et al. Exploring protein binding of uremic toxins in patients with different stages of chronic kidney disease and during hemodialysis. Toxins (Basel). 2015;7:3933–3946.
49. Renal Replacement Technology Grant “Safety analysis and optimization of electrochemical dialysate regeneration” (Dutch Kidney Foundation, 2013-2018).
50. STW Open technology program ‘Development of a urea sorbent for dialysate regeneration’ (2015-2020).
51. Van Gelder MK, de Kort GAP, Hazenbrink DHM et al. A uremic goat model created by subtotal renal artery embolization. Poster presentation ERA-EDTA 2018.
52. http://www.neokidney.nl/index.php/nl/
53. EC-H2020-SC1-2016-2017_WEAKID_’Clinical validation of miniature wearable dialysis machine’ (2017-2019

NEDERLANDSE SAMENVATTING

In dit proefschrift beschrijven wij de resultaten van ons onderzoek naar de ontwikkeling van een draagbaar of verplaatsbaar dialyseapparaat voor patiënten bij wie de nieren niet of nauwelijks meer werken (eindstadium nierfalen).

Gevolgen nierfalen zijn groot

Als de nieren door vergaande nierschade onvoldoende werken gaat de concentratie van stikstofverbindingen zoals ureum en andere afvalstoffen in het bloed omhoog. Ook natrium, kalium en fosfaat (elektrolyten) kunnen onvoldoende worden uitgescheiden. De vochtbalans komt in gevaar: gedronken vocht kan niet makkelijk worden uitgescheiden, waardoor overvulling en een hoge bloeddruk ontstaat. Het lichaam verzuurt doordat er geen waterstofionen meer kunnen worden uitgescheiden met de urine. Door ophoping van fosfaat en tekort aan door-de-nier-geactiveerd vitamine D ontstaan problemen met de bijschildklier en ontstaan broze botten. Daarnaast verliest het lichaam waardevolle stoffen, zoals eiwitten, aminozuren en vitaminen, omdat de nieren deze niet meer vast kunnen houden (reabsorberen).

Het aantal patiënten met eindstadium nierfalen neemt overal in de wereld al jaren toe. Ten gevolge van de toenemende vergrijzing en de explosieve stijging van het aantal mensen met 'ouderdoms suikerziekte' (diabetes mellitus type 2), zal dit aantal in Nederland de komende jaren met 5-8% jaarlijks blijven stijgen. Als gevolg daarvan zullen ook steeds meer mensen voor hun overleving afhankelijk zijn van een nierfunctievervangende therapie.

Principe van nierfunctievervangende therapie

Voor hun overleving zijn patiënten met nierfalen aangewezen op nierfunctievervangende therapie, idealiter in de vorm van transplantatie van een donornier, maar veel patiënten zijn helaas nog afhankelijk van een vorm van spoeling (dialyse). Transplantatie met een donornier geeft een wezenlijke verbetering van de levensverwachting en de kwaliteit van leven, maar met name ten gevolge van een groot tekort aan geschikte donoren is deze optimale therapie helaas voor lang niet elke dialyserende patiënt beschikbaar.

In Nederland is ongeveer 83% van de dialyserende patiënten afhankelijk van bloedspoelingen (hemodialyse: samenvoeging van de Griekse woorden voor 'bloed' (*αἷμα*), 'door' (*διά*) en 'vrijkomen' (*λύειν*)). De overige 17% spoelt via een dikke slang (katheter) in de buikholte over het buikvlies ofwel peritoneum (peritoneaal dialyse; 'peritoneum' ontstond uit de samenvoeging van de Griekse woorden voor 'om' (*περί*) en 'rekken' (*τόνος*)).
Zowel bij hemodialyse als bij peritoneaal dialyse wordt gebruikt gemaakt van schone spoelvloeistof, dialysaat, om het bloed te zuiveren. Bij hemodialyse vindt zuivering van het

bloed plaats met behulp van een dialysemembraan, een speciaal soort half doorlaatbaar filter waarin 'vervuild' bloed en schoon dialysaat in tegenstroom met elkaar in contact komen via talloze dunne vezeltjes (tot wel 20.000 per dialysemembraan). Bijgevolg verplaatsen afvalstoffen overtollig vocht zich van het bloed naar het dialysaat. Bij peritoneaal dialyse fungeert het peritoneum als dialysemembraan waarover 'vervuild' bloed en schoon dialysaat afvalstoffen en overtollig vocht uitwisselen.

Bij hemodialyse is de verwijdering van afvalstoffen gebaseerd op diffusie, convectie of een combinatie van deze beide principes (hemodiafiltratie). Diffusie kun je het beste omschrijven als een natuurlijk proces waarbij deeltjes zich over de ruimte verdelen en daarbij van een gebied met een hoge concentratie van deze deeltjes naar een gebied met een lage concentratie gaan. Denk daarbij aan wat er gebeurt wanneer er een zakje thee in een (warm) glas water wordt gehangen. Diffusie wordt gedreven door de concentratieverschillen van afvalstoffen en elektrolyten tussen bloed en spoelvloeistof (dialysaat), gelimiteerd door het molecuulgewicht en de voorkeur voor vocht (hydrofiliteit) of juist vet (lipofiliteit) van de afvalstoffen. Het principe 'convectie' laat zich het best omschrijven als een proces waarbij verplaatsing van deeltjes plaatsvindt onder een drukgradiënt: deeltjes stromen van een gebied met hogere druk naar een gebied met lagere druk. Tenslotte wordt voor de verwijdering van overvloedig vocht bij hemodialyse gebruik gemaakt van druk (ultrafiltratie). Bij peritoneaal dialyse speelt alleen het principe van diffusie. Verwijdering van overvloedig vocht gebeurt onder de osmotische gradiënt die ontstaat als gevolg van de hoge concentraties van glucose, aminozuren of icodextrine (een van zetmeel afkomstig glucosepolymeer) in het dialysaat.

Grenzen aan de dialysetechniek

Helaas kan met beide nierfunctievervangende therapieën de nierfunctie slechts ten dele worden overgenomen. Met 3 keer per week 4 uur hemodialyse wordt maximaal ~15% van de normale nierfunctie behaald voor makkelijk te verwijderen afvalstoffen (voor moeilijk te verwijderen afvalstoffen soms <1%), met peritoneaal dialyse over het algemeen niet meer dan maximaal 4-6%. Hemodialyse leidt ten gevolge van het intermitterende karakter van de behandeling tot (grote) schommelingen in concentraties van afvalstoffen en elektrolyten. Voorts wordt het hart- en vaatstelsel zwaar belast door de wisselende stadia van overvulling enerzijds (tussen de dialysesessies) en ondervulling met grote bloeddrukdalingen gedurende de dialyse. Een deel van deze problemen kan worden ondervangen met thuis(hemo) dialyse waarbij langduriger kan worden gedialyseerd met bijgevolg minder grote schommelingen in zowel concentraties van afvalstoffen en elektrolyten als in de vullingsstatus.

Voor peritoneaal dialyse geldt dat er sprake is van een constantere, maar minder adequa-

te, verwijdering van afvalstoffen dan bij hemodialyse. Dit resulteert in gelijkmatigere maar hogere spiegels van afvalstoffen dan bij hemodialyse.

Peritoneaal dialyse is een behandeling die reeds zelfstandig door patiënten thuis kan worden verricht en biedt daarmee een patiënt al meer vrijheid en autonomie dan hemodialyse in een dialysecentrum. Er kunnen echter technische of persoonsgebonden redenen zijn waarom peritoneaal dialyse niet mogelijk is en patiënten zijn aangewezen op hemodialyse.

Waarom een 'draagbare' kunstnier?

Een 'draagbare kunstnier', oftewel een draagbaar hemodialyseapparaat waarmee thuis, op het werk, zelfs op reis zou kunnen worden gedialyseerd, zou een uitkomst kunnen zijn voor de vele hemodialysepatiënten die nu nog afhankelijk zijn van dialyse op de dialyseafdeling van een ziekenhuis. Een leven met dialyse is anno 2018 voor veel patiënten nog altijd overleven. Ondanks aanpassingen aan en verbeteringen van de dialyseapparatuur en wijze van dialyseren is de prognose van dialyserende patiënten, met een gemiddelde levensverwachting van 5 tot 8 jaar, nog altijd slecht.

Vooralsnog dialyseert slechts een heel klein deel van de hemodialysepatiënten thuis. Dit komt mede doordat tijdens reguliere hemodialyse de patiënt geïmmobiliseerd is omdat een dialyseapparaat zwaar (75-100 kg) en volumineus is, en omdat er een directe verbinding tussen dialysemembraan en de bloedbaan is.

Ook voor peritoneaal dialyse patiënten zou een 'draagbare' kunstnier waarmee de buikspoelingen kunnen worden gedaan, voordelen kunnen bieden in de vorm van een betere verwijdering van afvalstoffen en het gebruik van lagere concentraties glucose in het dialysaat. Op de lange termijn zal dit de overleving van het buikvlies en daarmee de mogelijkheid tot continueren van de peritoneaal dialyse verbeteren.

Eisen aan een 'draagbaar' dialyseapparaat

Een draagbaar – of op zijn minst verplaatsbaar – dialyseapparaat moet niet alleen aan dezelfde eisen voldoen als een conventioneel dialyseapparaat maar daarnaast ook dusdanig klein zijn dat het ook echt draagbaar/verplaatsbaar is. Dit betekent dat alle onderdelen van het apparaat ofwel nieuw ontwikkeld moeten worden ofwel bestaan uit geminiaturiseerde delen van bestaande apparatuur. Voor de ontwikkeling van een draagbare kunstnier kunnen dan ook verschillende eisen en voorwaarden worden opgesteld:

1. Vanzelfsprekend moet het apparaat in staat zijn een nierfunctievervangende behandeling te geven die minimaal gelijkwaardig is aan conventionele dialyse. Dit betekent dat er op zijn minst een zelfde klaring van afvalstoffen en eenzelfde mate van ultrafiltratie moeten worden bereikt.

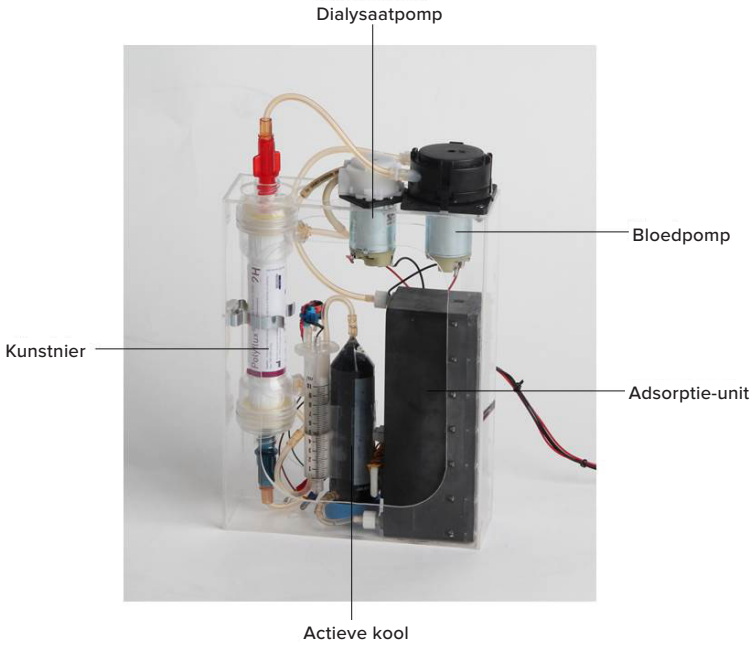
2. De hoeveelheid dialysaat moet geminimaliseerd worden. In conventionele hemodialysesessies wordt tot 120 liter dialysaat gebruikt. Om het systeem draagbaar te maken moet dit worden gereduceerd tot hooguit enkele honderden milliliters. Dit betekent dat het dialysaat hergebruikt ofwel 'geregenereerd', dus gezuiverd, moet worden.
3. Hemodialyse in het ziekenhuis vindt plaats in een veilige en gecontroleerde omgeving waarbij in geval van onvoorziene of onwenselijke voorvallen of ontwikkelingen altijd een verpleegkundige of nefroloog inzetbaar is. Omdat dit buiten het ziekenhuis lang niet altijd het geval zal zijn, zal een miniatuur dialyseapparaat uitgerust moeten worden met sensoren en veiligheidsmechanismen die deze controle (deels) kunnen overnemen. Het dialyseapparaat moet kortom voldoen aan dezelfde veiligheidseisen waaraan een conventionele thuishemodialyse machine moet voldoen. Gedacht moet worden aan lekkagesensoren, druksensoren in de aanvoerende en retournerende bloedlijnen, temperatuursensoren en, bij voorkeur, chemische sensoren die onder andere kalium- en calciumwaarden registreren. Bij afwijkende meetwaarden moeten zowel zichtbare als hoorbare alarmen worden gegenereerd die de patiënt attenderen op dreigende calamiteiten.
4. Er moet (geautomatiseerde) registratie plaatsvinden van belangrijke parameters als dialyseduur, ultrafiltratie volume, bloeddruk, gewicht en temperatuur.
5. Voor hemodialyse is een veilige vaattoegang nodig. Momenteel wordt er bij de hemodialyse gebruik gemaakt van ofwel een grote katheter (centraal veneuze lijn), welke in een vat in de hals of in de lies is ingebracht, ofwel van een door de chirurg gecreëerde verbinding tussen slagader en ader welke iedere dialyse moet worden aangeprikt. Wanneer de draagbare kunstnier wordt gebruikt voor intermitterende hemodialyse kan gebruik worden gemaakt van een conventionele vaattoegang. Voor continue hemodialyse zijn innovaties nodig voor een veilige vaattoegang. Voor peritoneaal dialyse is een geïmplanteerde katheter in de buikholte nodig.
6. Het apparaat moet een adequate energiebron bevatten die onafhankelijk van het lichtnet lang genoeg kan opereren. Daarbij moet het apparaat ook elektrisch veilig blijven.
7. Gedurende hemodialyse moet er sprake zijn van een adequate 'bloedverdunning' (antistolling).
8. Het apparaat moet ergonomisch verantwoord ontworpen worden zodat er sprake is van een optimaal draagcomfort. Uit eerdere patiëntenquêtes is gebleken dat er een lichte voorkeur lijkt te bestaan voor de variant waarbij het apparaat in meerdere delen aan een gordel om de heupen wordt gedragen ofwel als rugzak meegedragen kan worden.

Focus van dit proefschrift: hergebruik van dialysaat

Wij hebben ons in dit proefschrift gefocust op het ontwikkelen van technieken om vervuild dialysaat te hergebruiken, aangezien dit een essentiële voorwaarde is om een dialyseapparaat te kunnen miniaturiseren. Het gebruikte dialysaat wordt niet als afvalmateriaal be-

schouwd, maar juist gerecirculeerd in een gesloten systeem waarbij er continu zuivering van het vervuilde dialysaat plaatsvindt. Op die manier kan er een continue tegenstroom schoon dialysaat worden aangeboden via het dialysemembraan waardoor er een concentratiegradiënt over het dialysemembraan behouden wordt. Deze gradiënt faciliteert diffusie van afvalstoffen en elektrolyten zoals kalium over het membraan en waarborgt aldus continue zuivering van het bloed.

Hét innovatieve onderdeel van ons miniatuur dialyseapparaat is de zogenaamde adsorptie-unit. Deze unit bevindt zich in het (gesloten) dialysaatcircuit en reinigt continu het inkomende dialysaat. De unit bestaat uit een aantal plaatelektrodes in combinatie met specifieke kalium- en fosfaatadsorbers (zie figuur 1).



FIGUUR 1. proefopstelling miniatuur dialyseapparaat.

In de adsorptie-unit vindt adsorptie en oxidatie gelijktijdig plaats. Concreet betekent dit dat elke hemodialysesessie start met het vullen van het zogenaamde dialysaatcircuit met schoon dialysaat. Na passage van het dialysemembraan (en vervuilde bloed) wordt dit dialysaat gereinigd in de adsorptie-unit. Vanwege geleidelijke verzadiging van de adsorbers zullen deze periodiek vervangen moeten worden of moeten worden gereinigd met een regeneratievloeistof. Op deze manier kunnen de adsorbers opnieuw worden gebruikt in een volgende cyclus van ultrafiltratie en dialyse.

DEEL 1 – ELEKTROLYTEN BALANS

In **deel 1** van dit proefschrift (**hoofdstuk 2 en 3**) beschrijven wij het selecteren en testen van verschillende adsorberende stoffen voor de verwijdering van kalium en fosfaat uit vervuild dialysaat. Wij hebben hierbij in **hoofdstuk 2** gekeken naar de hoeveelheid kalium en fosfaat die de geteste adsorbers verwijderen, naar mogelijke nadelige effecten op andere elektrolyten (natrium, magnesium, calcium) en op de zuurgraad. Voorts hebben wij getest in hoeverre de adsorbers regenereerbaar zijn. Regenereerbaarheid van de adsorbers was een belangrijke eis voor ons omdat dat ons in staat zou stellen de adsorbers te hergebruiken met bijgevolg aanzienlijke reductie van de kosten van het apparaat. In **hoofdstuk 3** hebben wij de geselecteerde adsorbers getest in een groot diermodel waarbij wij gebruik maakten van gezonde Hollandse witte geiten. Wij hebben gekozen voor geiten in ons diermodel aangezien dit grote dieren zijn met een lichaamsgewicht dat overeenkomt met het gewicht van een gemiddelde volwassene, zij dociel zijn en oppervlakkig gelegen grote bloedvaten hebben waardoor gemakkelijk een vaattoegang kan worden gecreëerd. Aan het begin van elk experiment werd een grote katheter in de hals van de geiten aangebracht waarna zij werden aangesloten op ons miniatuur dialyseapparaat. Gedurende de experimenten werden de vitale parameters (o.a. bloeddruk, hartfrequentie, temperatuur) bijgehouden en met bloedafnames het effect op de elektrolyten en zuur-base balans gekwantificeerd.

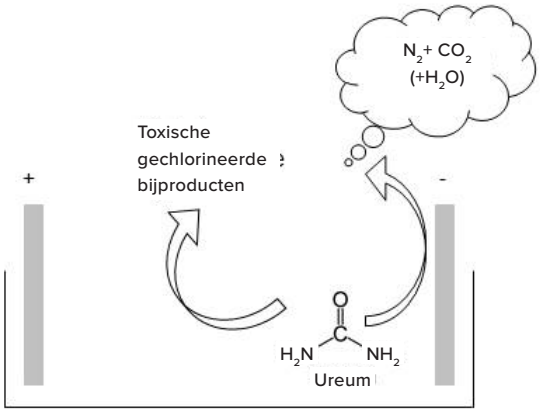
Op basis van onze experimenten bleken natrium poly(styreen-divinylbenzeen) sulfonaat voor kaliumverwijdering en ijzeroxide hydroxide voor fosfaatverwijdering geschiktste adsorbers te zijn. Met 110 gram kaliumadsorber en 55 gram ijzeradsorber kon in 3 uur ongeveer 10 mmol kalium en 5 mmol fosfaat worden verwijderd. Om eenzelfde verwijdering te behalen als bij intermitterende (3 keer per week 4 uur) hemodialyse waarbij 63-80 mmol kalium en 22-38 mmol fosfaat wordt verwijderd, zou 693-880 gram kaliumadsorber en 242-418 gram fosfaat adsorber per behandeling nodig zijn wanneer patiënten zouden besluiten het apparaat 3 keer per week gedurende 4 uur te gebruiken. Bij langdurigere of frequentere dialyse kan het gewicht aan adsorbers naar beneden worden gebracht, hetgeen de draagbaarheid of verplaatsbaarheid van het apparaat ten goede komt. Omdat de adsorbers na ongeveer 3 uur verzadigd zijn, zullen deze na 3 uur verwisseld moeten worden voor ‘schone’ adsorbers. De verzadigde adsorbers kunnen dan worden schoongespoeld, oftewel geregenereerd. Uit onze experimenten bleek dat de kaliumadsorber volledig kon worden schoongespoeld met een geconcentreerde zoutoplossing (1 molair natriumchloride) en de fosfaatadsorber vrijwel volledig met een 1 molair natriumhydroxide oplossing.

Door de adsorbers voor te spoelen met een oplossing met daarin calcium, magnesium, natrium en bicarbonaat kon het effect op de concentraties van deze elektrolyten in het bloed worden geminimaliseerd en trad er geen verstoring van de zuurbase balans op.

De dierexperimenten beschreven in **hoofdstuk 3** toonden een kalium- en fosfaatverwijdering in dezelfde orde van grootte als de experimenten in het laboratorium beschreven in **hoofdstuk 2.** Er werden geen nadelige effecten geobserveerd anders dan een stijging van zowel de bloeddruk als de hartfrequentie, mogelijk ten gevolge van de stress die de dieren ervoeren gedurende het experiment.

DEEL 2 – UREUMVERWIJDERING DOOR ELEKTRO-OXIDATIE

In **deel 2** (**hoofdstuk 4 en 5)** verkennen wij het onder elektrische spanning zetten van het dialysaat als techniek om ureum en andere stikstofhoudende afvalstoffen te verwijderen (elektro-oxidatie). Er moet per 24 uur ongeveer 25 gram ureum worden afgebroken. Bij volledige oxidatie door de elektrodes wordt dit volledig omgezet in stikstofgas en koolstofdioxidegas, wat vervolgens ontsnapt uit het dialysaat. Dit is een elegante methode, maar er ontstaan ook ongewenste toxische gechlorineerde bijproducten zoals chlooraminen (zie figuur 2). Het doel is om bij elektro-oxidatie zo min mogelijke toxische bijproducten te genereren aangezien zij oxidatieve stress en daarmee schade in het lichaam kunnen veroorzaken wanneer cellen en weefsels met deze producten in contact komen.



FIGUUR 2. Elektro-oxidatie van ureum.

In **hoofdstuk 4** worden de experimenten beschreven die wij uitvoerden om het optimale elektrodemateriaal te selecteren. Rutheniumoxide, platina en grafiet elektrodes werden getest op hun effectiviteit van ureumafbraak en de generatie van toxische gechloreerde bijproducten. Platina elektrodes toonden de grootste ureumverwijdering: 21±2 mmol/uur versus 13±2 mmol/uur door de rutheniumoxide elektrodes en 13±1 mmol/uur door de grafietelektrodes. De grafietelektrodes genereerden echter significant minder schadelijke bijproducten (13±4 mg/uur versus 231±22 mg/uur door de platina elektrodes en 129±12 mg/uur door de rutheniumoxide elektrodes). Wij kozen dan ook voor de grafietelektrodes om onze vervolgexperimenten mee uit te voeren.

Om de hoeveelheid schadelijke bijproducten verder te minimaliseren testten wij koolstoffilters met verschillende hoeveelheden actieve kool, omdat eerder onderzoek had aangetoond dat actieve kool in staat is (een groot deel van) deze bijproducten te neutraliseren. Met minder dan 100 gram actieve kool in een filter direct na de adsorptie-unit waarin ook 10 grafiet elektrodes waren verwerkt, werden concentraties van schadelijke bijproducten gemeten die ruim onder de maximaal acceptabele waarden lagen.

In **hoofdstuk 5** testten wij onze opstelling andermaal in een groot diermodel waarbij wederom gebruik werd gemaakt van gezonde Hollandse witte geiten.

Wij observeerden ureumverwijdering in dezelfde orde van grootte als in onze laboratoriumexperimenten. Er werden vooral nadelige effecten geobserveerd die het gevolg waren van ons experimentele model: omdat er gebruik werd gemaakt van gezonde geiten werden afvalstoffen en elektrolyten toegediend aan het bloed van de geiten om een geit met nierfalen na te bootsen. Ten gevolge hiervan zagen wij een toename van het gehalte natrium en chloor en juist een daling van het bicarbonaat. Daarnaast was er sprake van enige hemolyse, het kapotgaan van rode bloedcellen. Wij denken dat dit niet zal gebeuren wanneer gebruik wordt gemaakt van een geit met nierfalen omdat er dan geen noodzaak zal zijn tot toediening van afvalstoffen en elektrolyten.

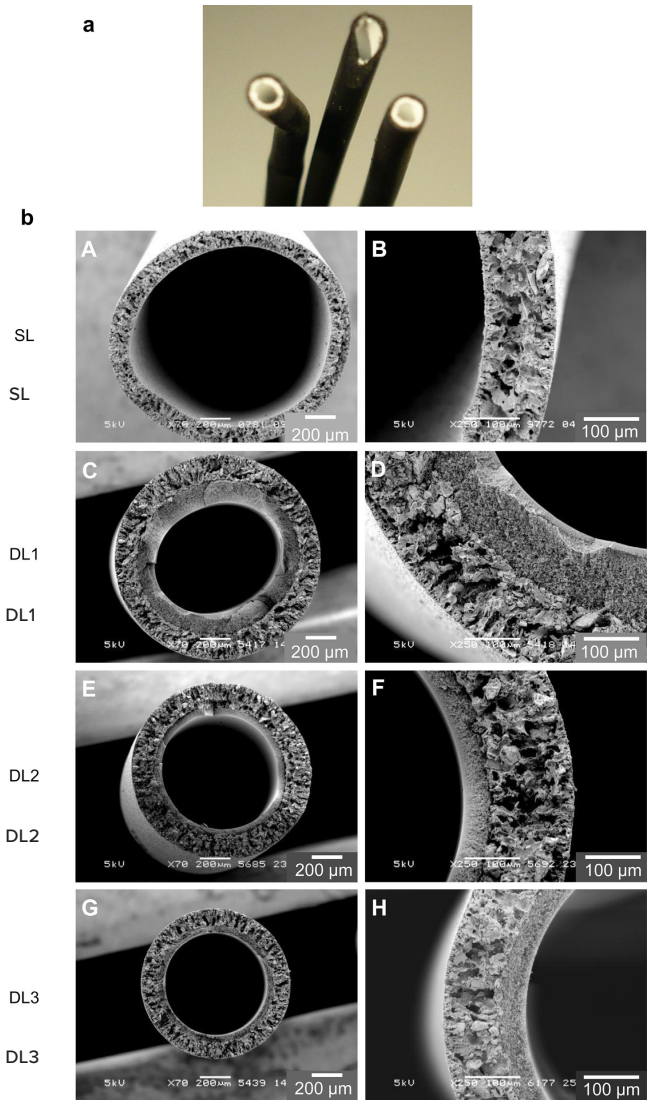
De ureumverwijdering was voldoende wanneer het dialyseapparaat 24 uur per dag zou worden gebruikt (380 mmol verwijdering bij een gemiddelde dagelijkse productie van ongeveer 400 mmol). Wanneer het dialyseapparaat minder langdurig zou worden gebruikt, zou het elektrodeoppervlak moeten worden vergroot. Voor dagelijkse nachtelijke dialyse gedurende 8 uur zou het elektrodeoppervlak bijvoorbeeld twee- tot driemaal moeten worden vergroot. Dit betekent echter niet dat het apparaat twee- tot driemaal zo groot zal worden omdat de meeste overige componenten ongewijzigd van grootte zullen zijn.

DEEL 3 – VERWIJDERING VAN UREMISCHE AFVALSTOFFEN DOOR 'MIXED MATRIX MEMBRANES'

In **deel 3** (**hoofdstuk 6 en 7)** beschrijven wij de ontwikkeling van nieuwe dialysemembranen. Deze bieden de mogelijkheid adsorptie en filtering van afvalstoffen te combineren. Om dit te bewerkstelligen is er een membraan ontwikkeld waarin adsorberende korrels zijn verwerkt, zogenaamde mixed matrix membranes (MMM's; zie figuur 3). Bepaalde actieve koolsoorten blijken in staat niet alleen kleine afvalstoffen zoals kreatinine weg te vangen maar ook sommige eiwitgebonden afvalstoffen en zogenaamde middelmoleculen, welke met de huidige dialysetechnieken moeilijk te verwijderen zijn. Van eiwitgebonden afvalstoffen en middelmoleculen zoals het moeilijk te klaren β-2-microglobuline is aangetoond dat verhoogde waarden in het bloed gerelateerd zijn aan verhoogde complicaties bij patiënten met eindstadium nierfalen. Alleen de zeer beperkte vrije fractie van eiwitgebonden afvalstoffen kan de poriën van een conventioneel dialysemembraan passeren. De eiwitgebonden fractie zal daardoor in het plasma aanwezig blijven. Experimenten met de MMM's hebben aangetoond dat deze moleculen beter kunnen worden verwijderd door actieve kooldeeltjes te verwerken in het gedeelte van het dialysemembraan dat niet blootgesteld wordt aan bloed. Hiervoor is gekozen omdat actieve kool bij direct contact met bloed leidt tot problemen met de bloedstolling. Met deze benadering zou ook conventionele hemodialyse meer effectief kunnen worden gemaakt.

In **hoofstuk 6** beschrijven wij de ontwikkeling van een enkel- en vervolgens dubbellaags dialysemembraan waarbij in de buitenste laag, welke in contact staat met het dialysaat, adsorberende koolstofdeeltjes zijn verwerkt. De verschillende lagen zijn opgebouwd uit een mix van polymeren (polyethersulfon (PES)/polyvinylpyrrolidon (PVP)). Onze experimenten toonden aan dat de membranen niet onder deden voor conventionele membranen wat betreft de verwijdering van kreatinine, waarbij kreatinine diende als vertegenwoordiger van de kleine afvalstoffen. Daarnaast bleek dat ongeveer 80% van para-aminohippuurzuur (PAH) werd verwijderd, waarbij PAH diende als vertegenwoordiger van de eiwitgebonden afvalstoffen.

In **hoofdstuk 7** werden daadwerkelijke vezels gecreëerd van bovengenoemde polymeren zodat deze op termijn ook zouden kunnen worden verwerkt in een membraan zoals bij conventionele dialysemembranen het geval is. Met deze vezels werd de verwijdering van de eiwitgebonden afvalstoffen p-cresylsulfaat (PCS) en indoxylsulfaat (IS) getest. De MMM's verwijderen gemiddeld 2.27 mg PCS/ gram membraan en 3.58 mg IS/ gram membraan in 4 uur via diffusie gevolgd door adsorptie en 2.68 mg PCS/ gram membraan en 12.85 mg IS/ gram membraan indien convectie werd toegepast. Wanneer deze resultaten zouden worden geëxtrapoleerd naar toepassing in mensen, zou 5-35 gram MMM (0.07-0.5 m2) benodigd zijn om de dagelijkse productie van deze afvalstoffen te neutraliseren.



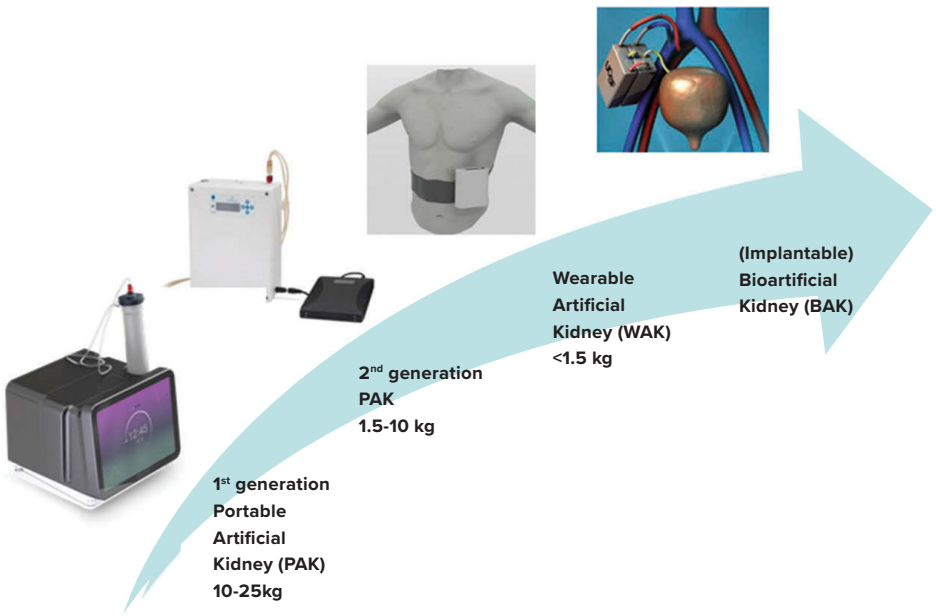
FIGUUR 3. Elektronenmicroscoop opname van 1 vezel van een mixed matrix membrane.

Vooruitzichten

In de afgelopen decennia zijn herhaaldelijk pogingen gedaan om dialyseapparatuur te miniaturiseren om op deze manier de mobiliteit, autonomie en daarmee de kwaliteit van leven van dialysepatiënten te vergroten. Helaas zijn deze pogingen tot dusver vaak gestrand omdat het niet mogelijk bleek op een veilige manier dialysaat te regenereren voor hergebruik. Zoals eerder beschreven is dit een essentiële stap om miniaturisatie van een dialyseapparaat mogelijk te maken. Wij hebben in dit proefschrift laten zien dat het mo-

gelijk is om met een combinatie van adsorbers en elektrodes dialysaat te ontdoen van kalium, fosfaat ureum en kreatinine. Voorts hebben wij laten zien dat we in staat zijn concentraties van andere elektrolyten in het bloed min of meer stabiel te houden door de adsorbers voor te behandelen. Daarnaast kunnen de geselecteerde kalium- en fosfaatadsorbers worden hergebruikt door ze te spoelen met een zoutoplossing. Wij hebben ook laten zien dat nieuwe dialysemembranen waarin adsorberende deeltjes zijn verwerkt een waardevolle toevoeging zouden kunnen zijn voor zowel een geminiaturiseerd dialyseapparaat als voor de conventionele hemodialyse.

Alvorens de stap te kunnen maken naar het testen van het miniatuur dialyseapparaat in dialysepatiënten, zal uitvoeriger moeten worden aangetoond dat de door ons gebruikte technieken daadwerkelijk veilig zijn. Hiervoor zijn aanvullende experimenten in zowel het laboratorium als met de geiten nodig. Wanneer deze experimenten geen nadelige effecten laten zien, kunnen wij verder op het pad naar ons ultieme einddoel: een op het lichaam draagbaar dialyseapparaat. De weg hiernaartoe zal stapsgewijs verlopen (zie figuur 4, waarbij we via een 'verplaatsbaar' apparaat ('PAK') toewerken naar een (op het lichaam) 'draagbaar' apparaat ('WAK'). Een stap verder zou een apparaat zijn dat zelfs in het lichaam geïmplanteerd wordt. Dit betreft een gecellulariseerd apparaat waarbij zoveel mogelijk gebruik wordt gemaakt van menselijke cellen die alle functies hebben die cellen van een 'echte nier' ook hebben. Ook hier wordt momenteel in Nederland onderzoek naar verricht waarbij hoopvol naar de toekomst wordt uitgezien!



FIGUUR 4. Ontwikkelingstraject kunstnieren.

DANKWOORD

'Ik kan het niet alleen' (De Dijk).

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CURRICULUM VITAE

Maarten Wester was born on October 2nd, 1981 in Hengelo (Overijssel), the Netherlands. In 1999 he completed the Gymnasium at the Ichthus College in Enschede. From 1999 to 2002 he studied Chemical Engineering at the University of Twente, Enschede, the Netherlands. In 2004 he obtained his Bachelor degree in Chemical Engineering. From 2002 to 2008 he studied medicine at Utrecht University, the Netherlands. During medical training, he completed an elective clinical internship tropical medicine at Hurumba Hospital, Tanzania. In the final year of his medical training he performed a scientific internship at the department of nephrology and hypertension, University Medical Center Utrecht, the Netherlands (UMCU), under the supervision of dr. J.A. Joles. This was a prelude for his Ph.D. research that led to this thesis and started in 2011 under supervision of Prof. dr. M.C. Verhaar, dr. J.A. Joles and dr. K.G.F. Gerritsen at the UMCU. In 2009 he started his residency in internal medicine at the Gelderse Vallei Hospital in Ede, the Netherlands under supervision of dr. R. Heijligenberg. June 2015 he continued his recidency internal medicine at the UMCU under supervision of Prof. dr. H.A.H. Kaasjager. September 2016 he started his nephrology training under supervision of dr. M.B. Rookmaaker.

In 2012 he married Marloes Heijstek, followed by the birth of their sons Abel (2013), Boaz (2015) and daughter Milou (2017).

LIST OF PUBLICATIONS

This thesis

Wester M, Simonis F, Gerritsen KGF, Boer WH, Wodzig WK, Kooman JP, Joles JA. A regenerable potassium and phosphate sorbent system to enhance dialysis efficacy and device portability. *Nephrol Dial Transplant. 2013 Sep;28(9):2364-71.*

Wester M, Gerritsen KGF, Simonis F, Boer WH, Vaessen K, Hazenbrink DH, Verhaar MC, Joles JA. A regenerable potassium and phosphate sorbent system to enhance dialysis efficacy and device portability: an in vivo study in awake goats. *Nephrol Dial Transplant. 2017 Jun 1;32(6):951-959*

Wester M, Simonis F, Lachkar N, Wodzig WK, Meuwissen F, Kooman JP, Joles JA, Boer WH, Gerritsen KGF. Removal of Urea in a Wearable Dialysis Device: A Reappraisal of Electro-Oxidation. *Artif Organs. 2014 Dec;38(12):998-1006.*

Wester M, van Gelder MK, Joles JA, Simonis F, Hazenbrink DHM, van Berkel TWM, Vaessen KRD, Boer WH, Verhaar MC, Gerritsen KGF. Removal of urea in a wearable dialysis device: an in vivo study in awake goats. *Am J Physiol Renal Physiol. 2018 Jul 11. doi: 10.1152/ajprenal.00094.2018. [Epub ahead of print]*

Tijink MS, Wester M, Sun J, Saris A, Bolhuis-Versteeg LA, Saiful S, Joles JA, Borneman Z, Wessling M, Stamatialis DF. A novel approach for blood purification: Mixed-matrix membranes combining diffusion and adsorption in one step. *Acta Biomater. 2012 Jul;8(6):2279-87.*

Tijink MS, Wester M, Glorieux GG, Gerritsen KGF, Sun J, Sousa R, Swart P, Borneman Z, Wessling M, Vanholder R, Joles JA, Stamatialis DF. Hollow fiber mixed matrix membranes for removal of protein-bound toxins. *Biomaterials. 2013 Oct;34(32):7819-28.*

Other publications

Pielawa L, Wester M, Simonis F, Hein A. Model-Based Physiological Data Stream Evaluation for Dialysis Therapy. *CBMS 2012.*

Wester M, Gerritsen KGF, Boer WH, Joles JA, Kooman JP. “De draagbare kunstnier”: een belofte voor de toekomst? *Nederlands Tijdschrift voor Geneeskunde. 2013;157(52):A6965.*

Tijink MS, Kooman JP, Wester M, Sun J, Saiful S, Joles JA, Borneman Z, Wessling M, Stamatialis DF. Mixed matrix membranes: a new asset to extracorporeal treatments? Blood *Purif. 2014;37(1):1-3.*

Van Gelder MK, Mihalia SM, Jansen J, Wester M, Verhaar MC, Joles JA, Stamatialis D, Masereeuw R, Gerritsen KGF. From portable dialysis to a bioengineered kidney. *Expert Rev Med Devices. 2018 May;15(5):323-336.*

LIST OF ABBREVIATIONS

AAMI	The Association for the Advancement of Medical Instrumentation
AC	Activated carbon
ACT	Activated Clotting Time
APD	Automated peritoneal dialysis
BAK	Bioartificial kidney
CHD	Conventional hemodialysis
CKD	Chronic kidney disease
CMPF	3-carboxyl-4-methyl-5-propyl-2-furanpropionic acid
CVC	Central Venous Catheter
DL	Dual layer hollow fiber mixed-matrix membrane
EO	Electro-oxidation
ESKD	End-stage kidney disease
ESRD	End-stage renal disease
HA	Hippuric acid
HD	Hemodialysis
HDF-IPIS	Haemodiafiltration at increased plasma ionic strength
HFR	Hemodiafiltration with endogenous reinfusion
I.D.	Inner diameter
IS	Indoxyl sulfate
MMM	Mixed-matrix membrane
NMP	N-Methylpyrrolidone
O.D.	Outer diameter
ORP	Oxidation reduction potential
PAH	Para-aminohippuric acid
PAK	Portable artificial kidney
PBUT	Protein-bound uremic toxins
PCS	p-Cresyl sulfate
PD	Peritoneal dialysis
PES	Polyethersulfone
PS/DVB	Poly(styrene-divinylbenzene)
PVP	Polyvinylpyrrolidone
REDY	REcirculating DialYsis
RES-A	Sodium poly(styrene-divinylbenzene) sulphonate
RRT	Renal replacement therapy
SC	Sieving coefficient

SEM	Scanning Electron Microscopy
SEV-car	Sevelamer carbonate
SL	Single layer hollow fiber mixed-matrix membrane
WAK	Wearable artificial kidney
ZEO	Zeolite
ZIR-car	Zirconium carbonate
ZIR-hydr	Zirconium oxide hydroxide
ZIR-phos	Zirconium phosphate