IN VITRO EFFICACY AND SAFETY OF A SYSTEM FOR SORBENT-ASSISTED PERITONEAL DIALYSIS

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Abstract

Background: A system for sorbent-assisted peritoneal dialysis (SAPD) was designed to continuously recirculate dialysate *via* a tidal mode using a single lumen peritoneal catheter with regeneration of spent dialysate by means of sorbent technology. We hypothesize that SAPD treatment will maintain a high plasma-to-dialysate concentration gradient and increase the mass transfer area coefficient of solutes. Thereby, the SAPD system may enhance clearance while reducing the number of exchanges. Application is envisaged at night as a bedside device (12 kg, nighttime system). A wearable system (2.0 kg, daytime system) may further enhance clearance during the day.

Methods: Urea, creatinine and phosphate removal was studied with the day- and nighttime system (n=3 per system) by recirculating 2 L of spent peritoneal dialysate *via* a tidal mode (mean flow rate: 50 and 100 ml/min, respectively) for 8 h *in vitro*. Time-averaged plasma clearance over 24 h was modeled assuming one 2-L exchange per day, an increase in MTAC and 0.9 L ultrafiltration per day.

Results: Urea, creatinine and phosphate removal was 33.2±4.1 mmol, 5.3±0.5 mmol, and 6.2±1.8 mmol, respectively, with the daytime system, and 204±28 mmol, 10.3±2.4 mmol and 11.4±2.1 mmol, respectively, with the nighttime system. Time-averaged plasma clearances of urea, creatinine and phosphate were 9.6±1.1 mL/min, 9.6±1.7 mL/min and 7.0±0.9 mL/min, respectively, with the nighttime system and 10.8±1.1 mL/min, 13.4±1.8 mL/min, 9.7±1.6 mL/min, respectively, with the day- and nighttime system.

Conclusions: SAPD treatment may improve removal of uremic toxins compared with conventional PD, provided that peritoneal mass transport will increase.

1. Introduction

Worldwide, approximately 3.4 million patients receive life-sustaining dialysis treatment of which ~88% are treated with in-center hemodialysis (HD) and ~11% are treated with peritoneal dialysis (PD) at home [17]. However, existing dialysis techniques have important disadvantages. In both PD and HD, removal of waste solutes and excess water is inadequate, contributing to severe health problems, high mortality (15-20% per year [15]) and poor quality of life [1]. Although PD has several advantages compared to HD, such as a survival advantage during the early years of dialysis [28], prolonged maintenance of residual kidney function [23, 25, 30], and a blood free access; it also has several important disadvantages such as a relatively low clearance [6, 7, 14] and limited technique survival due to structural and functional deterioration of the peritoneal membrane as a result of the high incidence of recurrent peritonitis [31] and chronic exposure to hypertonic glucose-based dialysis solutions [46].

We have developed a system for sorbent-assisted peritoneal dialysis (SAPD) to improve the existing shortcomings of conventional PD. SAPD treatment is based on continuous recirculation of peritoneal dialysate *via* a single lumen peritoneal catheter with regeneration of spent dialysate by means of sorbent technology. The first aim of the system is to increase solute clearance *via* two mechanisms. First, the continuous flow of fluid along the peritoneal membrane may enhance the mass transfer area coefficient (MTAC) as observed with continuous flow peritoneal dialysis (CFPD), presumably *via* reduction of diffusion resistances, renewal of stagnant fluid layers at the tissue surface and an increase of the effective membrane area [3, 10, 16, 18, 37]. Second, continuous purification of the dialysate will prevent saturation with toxins, maintaining a high plasma-to-dialysate concentration gradient across the peritoneal membrane that drives diffusive solute transport. In contrast, with conventional PD, the diffusion rate of toxins across the peritoneal membrane decreases during a static dwell due to equilibration of the intraperitoneal fluid with plasma.

The second aim is to improve technique survival by prolonging maintenance of the peritoneal membrane in two ways. Since glucose is easily absorbed across the peritoneal membrane, very high initial glucose concentrations are required with conventional PD to maintain an osmotic gradient up to the end of the dwell for adequate ultrafiltration. Chronic exposure to high glucose concentrations is harmful for the peritoneal membrane and may result in functional decline of the membrane and eventually ultrafiltration failure [11, 35, 45]. The SAPD system is designed to continuously release glucose at a constant rate, maintaining a constant osmotic gradient and a constant ultrafiltration rate, therewith avoiding the need for very high initial glucose concentrations. In this way, SAPD treatment may preserve integrity of the peritoneal membrane for a longer period of time. Second, instead of performing (time-consuming) 4-6 exchanges per day, the SAPD system uses one filling that is continuously purified. In

addition, by reducing the number of exchanges and (dis)connections of the peritoneal catheter, SAPD treatment may lower peritonitis rates [12], the leading cause of PD technique failure.

The first aim of the present study was to study efficacy of the SAPD system *in vitro* in terms of uremic toxin removal, base release to neutralize daily nonvolatile acid production, and stable glucose release for osmotic fluid removal. The second aim was to evaluate biocompatibility (cytotoxicity and genotoxicity) of the SAPD system *in vitro* [21, 22].

2. Methods

2.1 Materials

The SAPD system was built and kindly provided by Nanodialysis (Oischot, The Netherlands). It comprises a wearable sorbent based device (Fig. 1A "the SAPD daytime system") that is combined with a 9-L dialysate reservoir (provided in a trolley on wheels) during the night (Fig. 1B "the SAPD nighttime system"). The sorbent cartridge comprises 100 g (dry weight) of polystyrene beads modified with iron oxide hydroxide (FeOOH) and 200 g (dry weight) of activated carbon for removal of phosphate and organic waste solutes, respectively. The SAPD nighttime system is intended to be used for 8 h per night on a daily basis to allow for sufficient urea and potassium removal. Optionally, patients may continue treatment during the day with the wearable device to further enhance clearance of non-urea organic waste solutes and phosphate.

[Insert Figure 1]

2.2 Efficacy testing

Two different experimental set-ups were used to evaluate efficacy of the SAPD system *in vitro*. First, removal (or release) of urea, creatinine, phosphate, sodium, chloride, calcium, magnesium, bicarbonate, lactate and glucose, was evaluated by recirculating 2 L of spent peritoneal dialysate *via* a tidal mode, i.e. alternate in- and efflux of dialysate into- and out of the SAPD system in a closed-loop system, for 8 h (Figure 1, n=3 for daytime system, n=3 for nighttime system). In this set-up however, base and glucose release could not be evaluated due to accumulation in the 2-L reservoir. Therefore, additional experiments (n=6) were performed with the SAPD nighttime system in single-pass configuration to maintain constant solute concentrations in dialysate entering the SAPD system, simulating equilibration of the intraperitoneal and intravascular compartment *in vivo* (Fig. 3).

[Insert Figure 2]

[Insert Figure 3]

Experimental procedures: recirculation experiments with the day- and nighttime system

Two liters of spent peritoneal dialysate (Extraneal 7.5%) were collected one day prior to the experiment from three different patients after an intraperitoneal dwell time of 12 h, and stored at 4°C until use. Patients with peritonitis were excluded. Prior to start of the experiments, the peritoneal dialysate was pooled and split into three sterile 2-L bags. Mean effective dialysate flow rate (Qd, Formula 1) was 50 mL/min with the daytime system and 100 mL/min with the nighttime system. The sorbents of the daytime system were prerinsed with 6 L of Extraneal ([icodextrin] 7.5%, [Na^+] 133 mmol/L, [Ca^{2+}] 1.75 mmol/L, [Mg^{2+}] 0.25 mmol/L, [Cl^-] 96 mmol/L, [lactate] 40 mmol/L], pH 5.5; Baxter GmbH, Germany) and sorbents of the nighttime system were prerinsed with a solution containing [Na^+] 134 mmol/L, [Ca^{2+}] 1.25 mmol/L, [Mg^{2+}] 0.50 mmol/L, [Cl^-] 100.5 mmol/L and [lactate] 35 mmol/L] at pH 7.0. Of note, lactate concentrations were equal in the in- and effluent after this procedure. The dialysate reservoir of the nighttime system contained StaySafe® Balance ([glucose] 1.5%; [Na^+] 134 mmol/L, [Ca^{2+}] 1.25 mmol/L, [Mg^{2+}] 0.50 mmol/L, [Cl^-] 100.5 mmol/L, [lactate] 35 mmol/L], pH 7.0; Fresenius Medical Care GmbH, Bad Homburg, Germany) peritoneal dialysis solutifon. To simulate transport of uremic toxins from the intravascular space into the peritoneal cavity, urea, creatinine and (tripotassium) phosphate were spiked hourly into the 2-L dialysate reservoir. creatinine and a 1.3-fold (Qd: 50 mL/min) and 1.8-3.2-fold (Qd: 100 mL/min) increase in MTAC Spike amounts were modeled assuming a 1.2-fold (Qd: 50 mL/min) and 1.4-3.2-fold (Qd: 100 mL/min) increase in MTAC urea, a 1.3-fold (Qd: 50 mL/min) and 1.9-3.9-fold (Qd: 100 mL/min) increase in MTAC phosphate with continuous flow peritoneal dialysis (CFPD) based on [16, 18, 37] (Table 1). In addition, with the daytime system, we assumed saturation of activated carbon with urea after 1 h. Of note, phosphate was spiked as potassium salt (and not as sodium salt) to allow evaluation of influences of the system on sodium balance. Dialysate samples were taken from the 2-L dialysate reservoir before start and up- and downstream of the SAPD system after 10 min, 1 h, 2 h, 4 h, 6 h and 8 h of treatment for measurement of urea (mmol/L), creatinine (µmol/L), phosphate (mmol/L), bicarbonate (mmol/L), lactate (mmol/L), sodium (mmol/L), chloride (mmol/L), calcium (mmol/L), magnesium (mmol/L), and glucose (mmol/L) concentrations. Hydrogen chloride (1.2 mmol/L) was spiked into the reservoir if pH exceeded 8.0 to prevent calcium carbonate and calcium phosphate precipitations (assuming that *in vivo* OH^- and lactate, released from the phosphate sorbent (FeOOH beads) in exchange for phosphate, would distribute across the peritoneal membrane into a larger volume and have less effect on pH of the peritoneal dialysate).

[Insert Table 1]

Experimental procedures: single-pass experiments with the nighttime system

A volume of 36 L of dialysate was prepared using acid concentrate for hemodialysis (Dirinco, 874), sodium bicarbonate (Sigma-Aldrich) and demineralized water. Varying concentrations of potassium, calcium, magnesium, bicarbonate and lactate were applied to evaluate removal (or release) of these solutes for a range of clinically relevant values. Phosphate 2 mmol/L was spiked because calcium and magnesium can be removed *via* binding to negatively charged phosphate that is bound to FeOOH. The dialysate was circulated single-pass *via* a tidal mode at a Qd of 75 mL/min through the SAPD nighttime system into a waste reservoir for 8 h (n=6). Dialysate samples were taken from the waste reservoir hourly. The dialysate reservoir of the nighttime system contained Physioneal 35 ([Na^+] 132 mmol/L, [Ca^{2+}] 1.75 mmol/L, [Mg^{2+}] 0.25 mmol/L, [Cl^-] 101 mmol/L, [bicarbonate] 25 mmol/L, [lactate] 10 mmol/L, pH 7.4; Baxter) peritoneal dialysis solution with varying glucose concentrations (1.36-2.27%) to study glucose release. Physioneal 35 was selected because use of a combined bicarbonate/lactate buffer is associated with improved biocompatibility *in vitro* and *in vivo* compared with solutions that only use lactate [2, 24, 33, 36, 51]. The sorbents were prerinsed with 6 L of [Na^+] 132 mmol/L, [Cl^-] 97 mmol/L, [bicarbonate] 30 mmol/L, [lactate] 10 mmol/L and pH 7.0. Of note, the rinsing fluid no longer contained calcium, magnesium and glucose to prevent calcium and magnesium carbonate precipitations during storage, and the formation of toxic glucose degradation products during steam sterilization and storage, respectively. Equilibration was performed at relatively low pH (7.0) to maintain a physiologic pH (~7.4) in the effluent of the device which releases alkaline anions (OH^-, bicarbonate and/or lactate) in exchange for phosphate.

2.3 Calculations

Mean effective dialysate flow rate was calculated using the following formula:

$$\textit{Formula } 1: \qquad Qd = \frac{TV}{tIN + tOUT}$$

Where Qd = mean effective dialysate flow rate, t_{IN} = time of the inflow phase, t_{OUT} = time of the outflow phase, and TV = tidal volume.

Recirculation experiments

Cumulative solute removal (or release) by the SAPD system from dialysate was calculated using the following formula:

$$\textit{Formula } 2: \qquad A(t1 \rightarrow t2) = \frac{(CdIN - CdOUT)t1 + (CdDIN - CdOUT)t2}{2} \times Qd \times t$$

Where $A_{(t1 \rightarrow t2)}$ = amount removed by the SAPD system between t1 and t2, Cd_{IN} = dialysate concentration in the ingoing line (i.e. upstream of the dialysate reservoir and/or sorbent cartridge), Cd_{OUT} = dialysate

concentration in the outgoing line (downstream of the sorbent cartridge), Qd = mean effective dialysate flow rate and t = time between two consecutive measurements (t2-t1).

To get an impression of saturation of the system the percentage reduction in urea, creatinine and phosphate concentration in the 2-L dialysate reservoir between two consecutive measurements was calculated as follows:

$$Formula\ 3: \qquad Percentage\ reduction\ t2 = \frac{(CdIN)t1 - (CdIN)t2}{(CdIN)t1} \times 100\%$$

Where Cd_{IN} = dialysate concentration in the ingoing line (i.e. upstream of the dialysate reservoir and/or sorbent cartridge), t1 = immediately after the spiking of solutes, t2 = prior to the spiking of solutes.

Based on the observed removal *in vitro*, time-averaged plasma clearances per 24 h (mL/min) were modeled for an 8-h treatment per day with the nighttime system (Formula 3), and for combined treatment with the day- and nighttime system (8 h per system per day) (Formula 4), applying one 2-L exchange in the morning, a partial drain in the evening prior to start of treatment with the SAPD nighttime system (aiming at ~1 L residual intraperitoneal volume according to the intended use), and assuming an ultrafiltration volume of 0.9 L per day [34] and an increase in the MTAC as described above.

$$Formula\ 4: \qquad Cl = \frac{((Anight + (Vt0 \times Cdt0) + (Vtx\ \times Cdtx))/\ 1440}{Cp}$$

$$Formula\ 5: \qquad Cl = \frac{((Aday\ + Anight + (Vt0 \times Cdt0) + (Vtx\ \times Cdtx))\ /\ 1440}{Cp}$$

Where Cl = time averaged plasma clearance per 24 h (mL/min), A_{day} = cumulative removal with daytime system, A_{night} = cumulative removal with the nighttime system, V_{t0} = volume of the partial drain prior to start of treatment with the nighttime system (was assumed to be 1.4 L, including 0.4 L ultrafiltration during the day dwell), C_{dt0} = concentration in the partial drain, V_{tx} = intraperitoneal volume at the end of treatment with the nighttime system (was assumed to be 1.5 L, including 0.5 L ultrafiltration during treatment with the nighttime system), and Cp = plasma concentration (was assumed to be equal to the mean plasma concentration in PD patients [34, 49]).

Single-pass experiments

Cumulative solute removal (or release) by the SAPD system from dialysate was calculated using the following formula:

$$Formula\ 6: \qquad A(t1 \rightarrow t2) = (CdIN\ -\ CdOUT) \times Qd\ \times t$$

Where $A_{(t1 \rightarrow t2)}$ = amount removed by the SAPD system between t1 and t2, Cd_{IN} = dialysate concentration in the 36-L dialysate reservoir, Cd_{OUT} = dialysate concentration in the dialysate waste reservoir, Qd = mean effective dialysate flow rate and t = time between two consecutive measurements (t2-t1).

Glucose adsorption (mmol/h) by the sorbents (activated carbon) from dialysate during experiments with the SAPD nighttime system was calculated based on the difference in total glucose release by the SAPD system and glucose release by the 9-L dialysate using the following formula:

$$Formula\ 7: \qquad Aads(t1 \rightarrow t2) = \frac{A(t1 \rightarrow t2) - \big((Cdt2 - Cdt1) \times V\big)}{t}$$

Where $Aads_{(t1 \rightarrow t2)}$ = amount adsorbed by the sorbents between t1 and t2, $A_{(t1 \rightarrow t2)}$ = amount released by the SAPD system between t1 and t2, Cd = glucose concentration in the 9-L dialysate reservoir of the SAPD system, V = volume of the dialysate reservoir of the nighttime system (i.e. 9 L) and t = time between t1 and t2 in hours.

2.4 *In vitro* cytotoxicity and genotoxicity

To assess *in vitro* cytotoxicity of the SAPD system, cell morphology, expression of epithelial and mesenchymal cell markers, cell apoptosis and proliferation, oxidative stress (quantification of reactive oxygen species), cell migration (wound healing assay), lactate dehydrogenase release, and inflammation (release of vascular endothelial growth factor (VEGF), interleukin 6 (IL-6) and transforming growth factor β1 (TGF-β1)), were evaluated after exposure of human peritoneal mesothelial cells (virus-transformed MeT-5A cells from ATCC) to SAPD-treated spent peritoneal dialysate and untreated spent peritoneal dialysate (control). Genotoxicity was assessed by performing a bacterial reverse mutation assay ("Ames test") and a mouse lymphoma assay. Testing was performed in accordance with ISO 10993 series of standards "Biological evaluation of medical devices"[20]. The concise procedures for test sample preparations and assay methods are described in the Supplementary materials (URL: https://figshare.com/s/1cb9febefe32a9970b58 DOI: 10.6084/m9.figshare.11912430), section 1 "Methods *in vitro* biocompatibility".

2.5 Statistical analysis

One-way ANOVA for repeated measures with post-hoc Tukey test was used to analyze the difference between untreated spent peritoneal dialysate (T0) and spent peritoneal dialysate treated by the SAPD system for 8 h (T8) and 16 h (T16). The generalized Extreme Studentitized Deviate method (Grubbs' test) was used to identify significant outliers which were excluded from analysis. A P value < 0.05 was considered statistically significant. Analyses were performed with GraphPad Prism 7.04 (GraphPad Software, La Jolla, CA, USA).

3. Results

3.1 Efficacy testing

Recirculation experiments

Cumulative removal of urea, creatinine and phosphate with the day- and nighttime system and the modeled time-averaged cumulative removal and plasma clearance per 24 h with the SAPD day- and nighttime system are presented in Table 2. Reduction ratios between two consecutive measurements for urea, creatinine and phosphate are presented in Figure 4. Cumulative removal (or release) of sodium, chloride, calcium, magnesium, bicarbonate, lactate and glucose with the day- and nighttime system is presented in Table 3. Of note, potassium removal is not reported since high dialysate potassium concentrations due to spiking of K_3PO_4 yielded high removal rates, not representative for the *in vivo* situation.

[Insert Table 2]

[Insert Figure 4]

[Insert Table 3]

Single-pass experiments

Base and glucose release by the nighttime system were evaluated in single-pass configuration to maintain constant solute concentrations in dialysate entering the SAPD system, simulating equilibration of the intraperitoneal and intravascular compartment *in vivo* (Table 4). Potassium removal was also determined at dialysate potassium concentrations representative for the *in vivo* situation (Table 5). Remarkably, despite the absence of a cation exchanger, a limited amount of cations was removed by the sorbents (Table 3 and 5), probably *via* binding to negatively charged phosphate that was bound to FeOOH. Glucose release increased at higher glucose concentrations in the Physioneal 35 dialysate reservoir (Table 4, Figure 5A). Stable dialysate glucose concentrations were achieved downstream of the sorbents (Figure 5B).

[Insert Figure 5]

[Insert Table 4]

[Insert Table 5]

3.2 *In vitro* cytotoxicity and genotoxicity

The results of the *in vitro* cytotoxicity and genotoxicity assays are summarized in Table 6 and are presented in detail in the Supplementary materials (https://figshare.com/s/1cb9febefe32a9970b58), section 2 “Results *in vitro* biocompatibility”.

[Insert Table 6]

4. Discussion

In the present study, we demonstrate the efficacy and biocompatibility of a novel system for sorbent-assisted peritoneal dialysis for continuous flow peritoneal dialysis *in vitro*.

Clinically relevant removal of urea, creatinine, phosphate and potassium from peritoneal dialysate by the SAPD system was observed *in vitro* as compared to a daily urea and creatinine production of ~240-470 mmol [39, 44] and 8-17 mmol [27], respectively, and phosphate and potassium intake of ~15 and ~45 mmol [29, 41, 43], respectively, in dialysis patients, and an average dialysate removal of ~325-360 mmol [6, 14], ~6.4 mmol [47] , ~6.5-8.0 mmol [9] and ~29-41 mmol [32, 50] for urea, creatinine, phosphate and potassium, respectively, in conventional PD. Of note, absolute urea removal in the present study was relatively low compared with conventional PD because urea concentrations in the 2-L dialysate reservoir (representing patient's plasma concentration at the time of equilibration between plasma and dialysate urea concentration) varied between 2.9-26.8 mmol/L due to non-continuous spiking, whereas patients had a relatively constant plasma urea concentration of ~40 mmol/L [6, 14]. However, maximum urea removal capacity of the nighttime system was not yet achieved after 8 h of treatment, indicating that removal will be increased at higher urea concentrations entering the SAPD system.

For the nighttime system maximum removal capacity was not achieved, especially for creatinine that did not show any decrease in reduction ratio over 8 h. Estimated time-averaged plasma clearances with the nighttime system (applying one exchange per day and assuming an increase of MTAC with CFPD as reported [16, 18, 37] and 0.9 L ultrafiltration [34]) suggest superior performance compared to conventional PD [6, 7, 14, 34]. The modeled time-averaged plasma clearances of urea, creatinine and phosphate would increase by a factor ~1.5, ~2.1 and ~1.9, respectively, compared with APD/CAPD [6]. Combined use of the day- and nighttime system may further enhance plasma clearance, especially for creatinine and phosphate (3.0-fold and 2.7-fold, respectively, *vs* APD/CAPD [6]), which may allow for a more liberal diet and reduction of phosphate binders. Since most organic waste solutes bind efficiently to activated carbon similar to creatinine [4, 48], we expect that clearance of these solutes may also increase. Moreover, also in case of minimal adsorption to the sorbents, the continuous flow along the peritoneal membrane, increasing peritoneal mass transport, in combination with the dialysate reservoir may theoretically enhance the clearance of any solute.

The SAPD nighttime system comprises a dialysate reservoir to remove urea by dilution in addition to a small amount of urea that is removed by activated carbon (~30 mmol in 8 h). As a result, miniaturization of PD technology is not achieved. Currently, no efficient urea sorbent is available for

application in a wearable artificial kidney [42]. As the affinity of urea for activated carbon is relatively low (0.1-0.2 mmol/g), a relatively large amount of activated carbon (1.2-4.7 kg) would be required to remove the daily urea production [42]. Htay et al. report use of enzymatic hydrolysis of urea by urease for dialysate regeneration in a wearable artificial kidney for CFPD, a system of <2 kg using 3 cartridges and 3 exchanges of 2 L per day [5, 19].

Urea removal by urease was first applied in the REcirculation DialYsis (REDY) sorbent system in HD [8]51]. Although a urease-based sorbent system may allow miniaturization of the system to wearable proportions, the technology is complex and has several disadvantages. Toxic ammonium is generated during hydrolysis of urea that must be removed almost completely from dialysate by zirconium phosphate (cation exchanger), that binds ammonium in exchange for sodium and hydrogen, risking sodium release into the patient and acid base disturbances, respectively [42]. In addition, zirconium phosphate binds calcium, magnesium and too much potassium which must be re-infused from a separate reservoir. In contrast to the complex urease-based sorbent system, the SAPD system is simple and of low-risk but rather bulky. It makes use of simple sorbents, activated carbon and FeOOH, that are both being used as oral adsorbents in clinical practice, and a dialysate reservoir to remove urea and potassium by dilution. The dialysate reservoir eliminates the need for a cation exchanger and therewith the related disadvantages of sodium and/ or hydrogen release and calcium and magnesium removal.

Remarkably, despite the absence of a cation exchanger, we observed removal of a limited amount of cations by the sorbents, probably *via* binding to negatively charged phosphate that is bound to FeOOH. For the nighttime system, calcium removal could be prevented by application of a relatively high calcium concentration (1.75 mmol/L) in the dialysate reservoir. Similarly, to prevent magnesium removal, a higher magnesium concentration could be applied in the dialysate reservoir.

Base release by the SAPD nighttime system seemed adequate. To compensate for daily non-volatile acid production, ~70 mmol of net base (sum of bicarbonate and lactate) must be released into the patient during dialysis treatment to prevent severe metabolic acidosis as a consequence of impaired renal acid excretion in dialysis patients [26, 38]. By using a combined lactate/bicarbonate buffer (10/25 mmol/L) in the dialysate reservoir, the single-pass experiments show that the SAPD system may release 77 mmol of lactate, provided that rapid equilibration of lactate occurs between dialysate and plasma so that the intraperitoneal lactate concentration remains low. Additional bicarbonate release will depend on the degree of metabolic acidosis.

The single-pass experiments show that the sorbents (activated carbon) adsorb glucose, in particular in the beginning of the experiment, resulting in a much lower initial glucose concentration in the effluent of the system than in the dialysate reservoir and rather stable effluent glucose concentrations throughout the whole experiment. The hypothesis is that *in vivo* activated carbon will serve as a glucose

buffer and will adsorb glucose particularly during the first part of treatment and may release glucose during the second part of the treatment, depending on the glucose concentration in the 9-L reservoir and the MTAC for glucose. This will result in rather constant glucose concentrations in the device effluent during the whole treatment without the very high initial glucose concentrations. With conventional PD, very high initial dialysate glucose concentrations are needed to maintain an osmotic gradient and some net ultrafiltration at the end of the dwell, since glucose is rapidly absorbed from the dialysate. Exposure of the peritoneal membrane to high glucose concentrations, and related advanced glycation end products and glucose degradation products, causes inflammation, apoptosis and necrosis and may eventually lead to pathological changes in peritoneal membrane structure (neoangiogenesis and fibrosis) and function (ultrafiltration failure) [11, 35, 45]. Although we did not measure icodextrin concentrations in the present study, icodextrin adsorption was quantified in a separate series of static experiments, during which the SAPD system removed a very limited amount of icodextrin (~5 g, i.e. 3% of the amount present at the start of the experiment). Thus, the remaining icodextrin of the Extraneal dwell during the day may contribute to ultrafiltration during SAPD treatment as well.

With the SAPD system, exposure of the peritoneal membrane to very high glucose concentrations may be prevented and peritoneal integrity may be preserved for a longer period of time. Kinetic modeling by Gotch *et al.* [18], based on patient data with CFPD, shows that maintaining an intraperitoneal glucose concentration of 1 % will yield a constant ultrafiltration rate of ~0.2 L/h, more than sufficient for an 8-h SAPD treatment per day. To achieve this, we estimate that the SAPD system should gradually release ~480 mmol of glucose during an 8-h treatment, assuming an MTAC of glucose of ~0.02 L/min [18]. In the present study, a dose-response was observed, with higher glucose release when using higher glucose concentrations in the dialysate reservoir. *In vivo* studies and treatment of individual patients should further define glucose concentrations in the 9-L reservoir and give more information on ultrafiltration rates with varying intraperitoneal glucose concentrations and the long-term effect of reduced glucose concentrations on peritoneal integrity.

Testing for cytotoxicity and genotoxicity *in vitro* in accordance with the ISO 10993 Standards for the biological evaluation of medical devices [21, 22], showed that SAPD-treated spent peritoneal dialysate did not compromise mesothelial cell viability, or induce epithelial to mesenchymal transition, oxidative stress or inflammation compared with untreated spent peritoneal effluent, and was not mutagenic. Testing for acute and (sub)chronic toxicity in a uremic animal model will be performed to confirm the safety of SAPD treatment *in vivo* prior to testing in humans.

This study has several limitations. First, for estimation of time-averaged plasma clearance based on cumulative solute removal achieved *in vitro*, we assumed that the MTAC of solutes will increase *in vivo* as reported in several patient studies with CFPD using two single lumen peritoneal catheters [10, 13,

16, 37, 40],while the SAPD system uses tidal flow via a single lumen catheter. We assumed that – independent of the direction of the flow that changes every 3-6 minutes- the continuous high laminar flow along the peritoneal membrane will enhance mass transport across the peritoneal membrane. However, patient studies are needed to confirm that the MTAC of solutes is indeed increased with this setup. Second, the effect of CFPD on MTAC is variable among patients and may be more pronounced in patients with a high transport status [16]. Patient studies should evaluate which parameters determine the efficacy of CFPD. Third, during the single-pass experiments, glucose concentrations upstream of the system were kept constant (44 mM) while *in vivo* intraperitoneal glucose concentrations will be different with different glucose concentrations in the 9-L reservoir (namely higher intraperitoneal glucose concentrations with higher glucose concentrations in the 9-L reservoir and vice versa) which is expected to result in larger differences in glucose concentrations in the effluent of the system *in vivo*.

In conclusion, the uremic toxin removal capacity of the SAPD system *in vitro* suggests superior performance compared with conventional PD, provided that peritoneal mass transport will increase. Evaluation of the SAPD system in a uremic large animal model is now indicated to study plasma solute clearance, ultrafiltration and safety *in vivo*.

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6. Conflict of interest

The authors declare no conflict of interest.

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Figure captions

Figure 1. A**:** The SAPD daytime system (2.0 kg) comprises the sorbent cartridge and electronics. B: The SAPD nighttime system (12 kg) combines the daytime system with a dialysate reservoir.

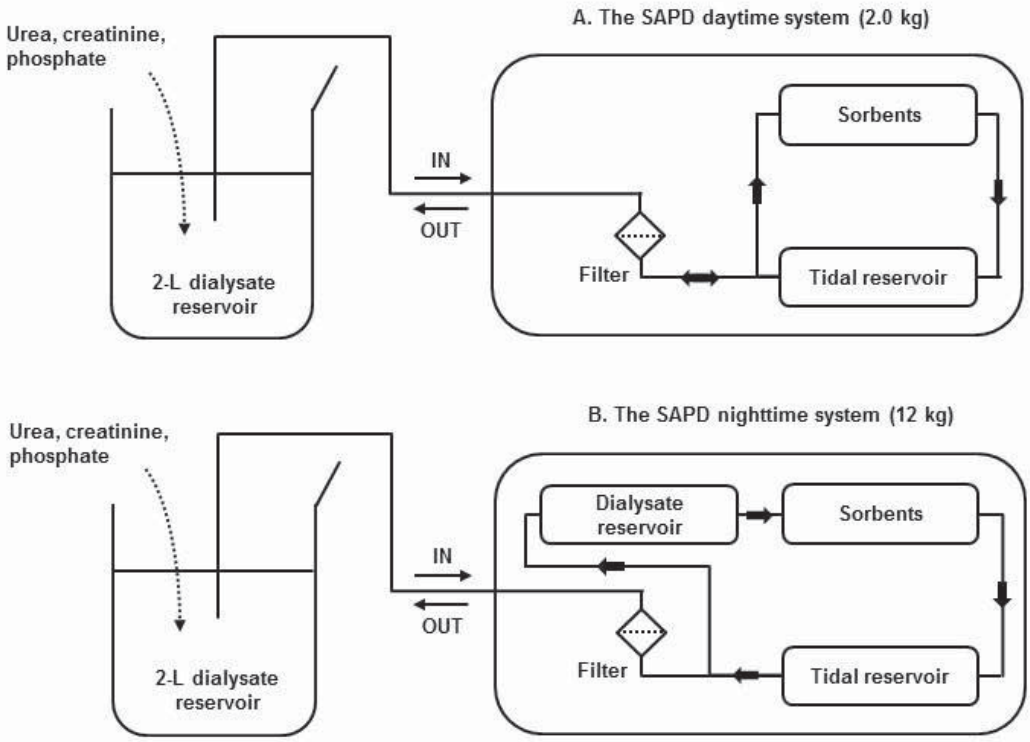
Figure 2. Experimental set-up for recirculation experiments with the SAPD day- (A) and nighttime (B) system (n=3 per system). 2 L of spent peritoneal dialysate (Extraneal 7.5%, mix of 3 patients) is continuously recirculated *via* a tidal mode, i.e. alternate in- and efflux of dialysate into- and out of the SAPD system, for 8 h. To simulate the *in vivo* situation, urea, creatinine and phosphate are spiked hourly into the 2-L reservoir (that represents the patient's peritoneal cavity). The nighttime system combines the daytime system with a dialysate reservoir. A filter is placed between the dialysate regeneration circuit and dialysate line to the 2-L dialysate reservoir, to prevent particles from entering the dialysate reservoir (i.e. peritoneal cavity).

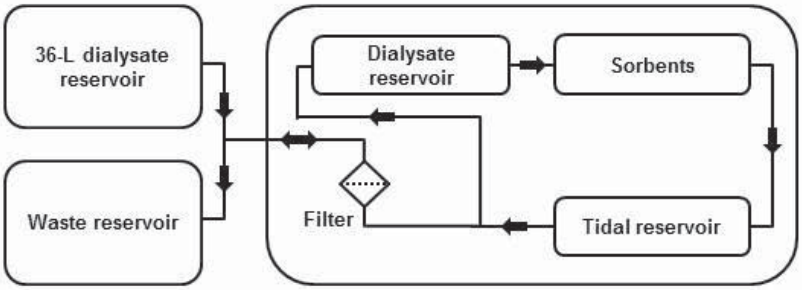
Figure 3. Experimental set-up adapted for single-pass experiments with the SAPD nighttime system (n=6). Dialysate is circulated from the 36-L dialysate reservoir through the SAPD nighttime system into a waste reservoir *via* a tidal mode for 8 h.

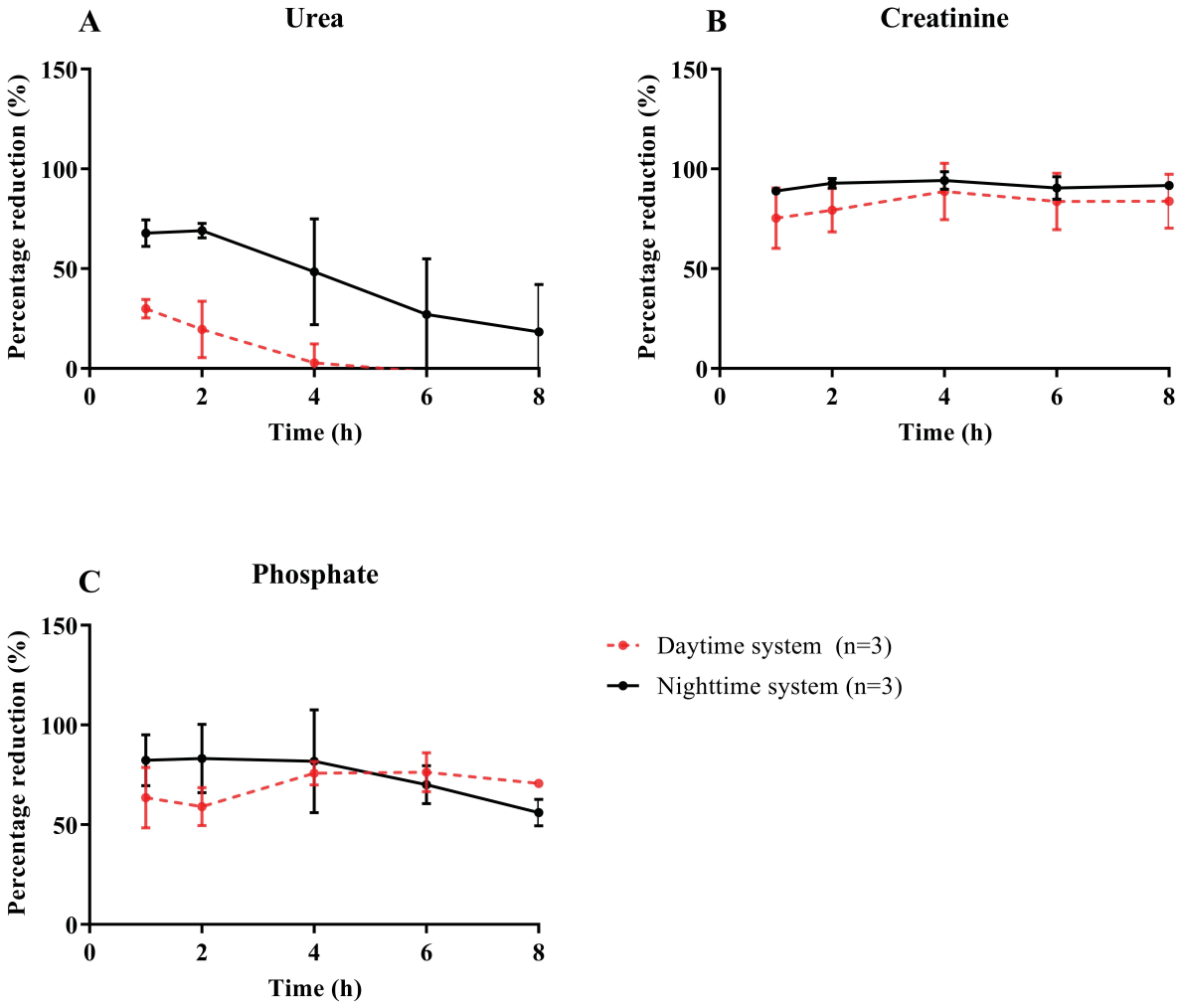
Figure 4. Percentage reduction (%) of urea (A), creatinine (B) and phosphate (C) in the 2-L dialysate reservoir between two consecutive measurements is presented for recirculation experiments with the SAPD day- and nighttime system (n=3 per system).

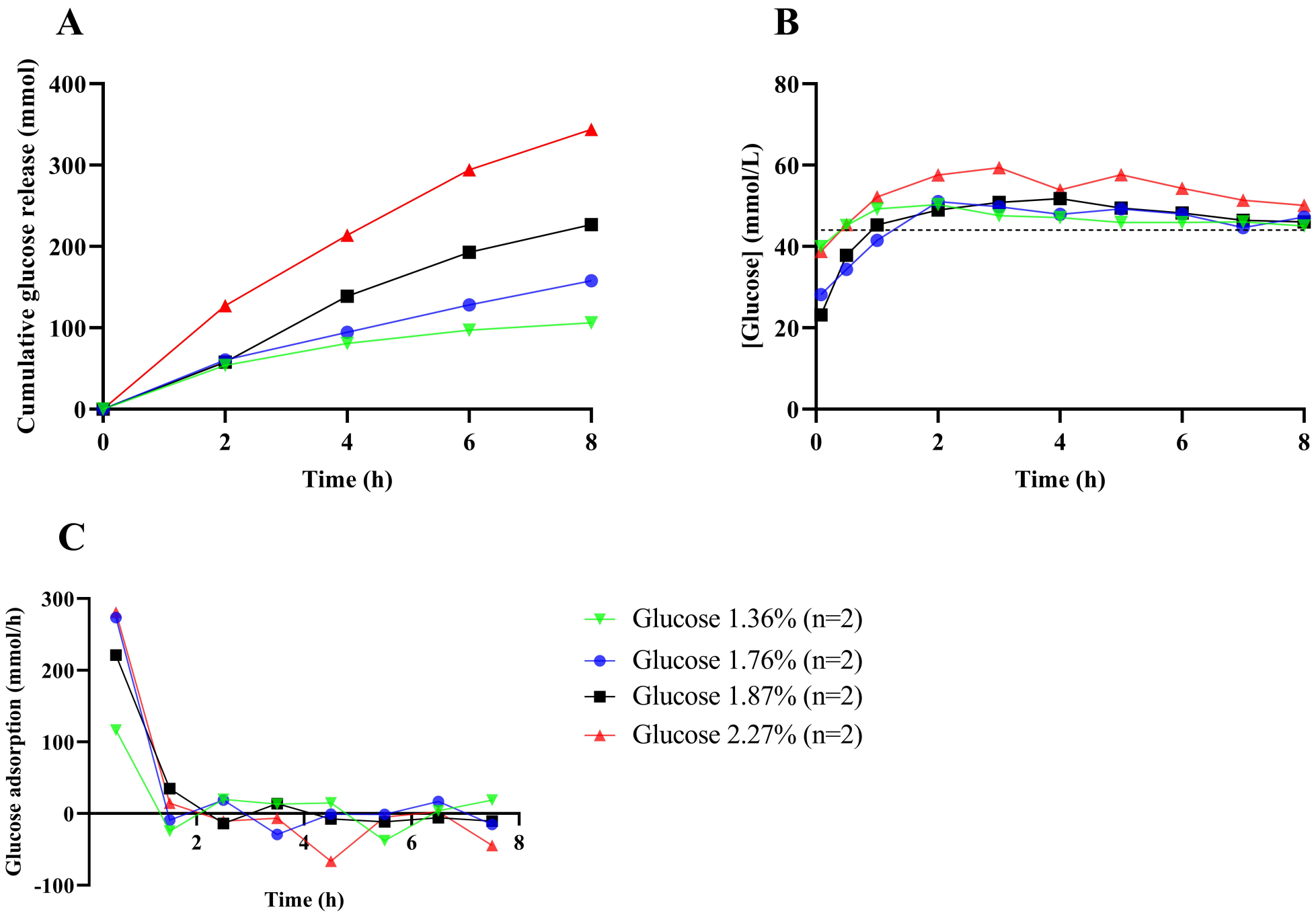
Figure 5. A: Glucose release (mmol) by the SAPD nighttime system in single-pass configuration for different glucose concentrations (1.36% (n=2), 1.76% (n=2), 1.87% (n=2) and 2.27% (n=2)) in the dialysate reservoir. B: Glucose concentrations (mmol/L) downstream of the sorbents for different glucose concentrations in the dialysate reservoir of the nighttime system. The dashed line represents the glucose concentration (44 mmol/L) in the 36-L dialysate reservoir upstream of the SAPD system. C: Glucose adsorption (mmol/h) by the sorbents with the SAPD nighttime system. Each graph represents the mean of two experiments.

A. The SAPD daytime system (2.0 kg) B. The SAPD nighttime system (12 kg)









Tables

Table 1. Total spike amounts (mmol) into the 2-L dialysate reservoir.

	Daytime system (n=3)	**Nighttime system (n=3)**
Urea	24-30	165-210
Creatinine	4.2	6.3-10.5
Phosphate	10.8-14.4	23.1-31.5

Table 2. Cumulative removal of urea, creatinine and phosphate *in vitro* and the modeled time-averaged cumulative removal and plasma clearance per 24 h with the SAPD day- and nighttime system.

	Cumulative removal *in vitro* (mmol)		**Nighttime system + 1 exchange**		**Day- and nighttime system + 1 exchange**	
Solute	**Daytime system (n=3)**	**Nighttime system (n=3)**	**Cumulative removal (mmol)**	**Cl (mL/min)**	**Cumulative removal (mmol)**	**Cl (mL/min)**
Urea	33.2 ± 4.1	204 ± 28	258 ± 28	9.6 ± 1.1	292 ± 30	10.8 ± 1.1
Creatinine	5.3 ± 0.5	10.3 ± 2.4	13.0 ± 2.4	9.6 ± 1.7	18.3 ± 2.4	13.4 ± 1.8
Phosphate	6.2 ± 1.8	11.4 ± 2.1	15.9 ± 2.1	7.0 ± 0.9	22.2 ± 3.7	9.7 ± 1.6

Mean ± standard deviation is presented (n=3 per system). Cumulative removal and time-averaged plasma clearance (Cl) were modeled for an 8-h treatment per day with the nighttime system, and for the day- and nighttime system combined (8 h per system per day), based on the observed removal *in vitro*, assuming an increase of the MTAC with CFPD, one 2-L exchange per day, an ultrafiltration volume of 0.9 L per day, and a urea, creatinine and phosphate plasma concentration of 18.8 mmol/L, 946 µmol/L and 1.58 mmol/L, respectively [34, 49].

Table 3. Cumulative removal (positive values) and release (negative values) of solutes with the SAPD day- and nighttime system with dialysate recirculation (n=3 per system).

	Cumulative removal/ release (mmol)	
Solute	**Daytime system (n=3)**	**Nighttime system (n=3)**
Sodium	1.7 ± 8.3	-16.4 ± 3.9
Chloride	5.0 ± 10.9	-2.5 ± 3.2
Calcium	2.10 ± 1.64	1.59 ± 1.64
Magnesium	0.68 ± 0.10	0.23 ± 0.29
Bicarbonate	17.4 ± 2.3	41.1 ± 5.2
Lactate	-28.0 ± 4.8	-60.1 ± 4.1
Glucose	32.3 ± 19.1*	-90.2 ± 22.5

Mean ± standard deviation is presented. *Unexpectedly, glucose concentrations in the 2-L dialysate reservoir at the start of the experiment were relatively high (11.3, 13.2 and 33.0 mmol/L).

Table 4. Cumulative removal (positive values) and release (negative values) of solutes with the SAPD nighttime system in single-pass configuration (n=6).

Solute	**No. of experiments**	**Cd 36-L reservoir (mmol/L)***	**Cd dialysate reservoir (mmol/L)†**	**Removal / release (mmol)**
Potassium	2	3.0	0	17.7; 27.8
	3	4.5	0	35.7 ± 5.8
	3	6.0	0	53.5 ± 0.9
Sodium	8	132	132	1.1 ± 11.7
Chloride	8	111	101	144.1 ± 23.8
Phosphate	8	2.0	0	22.5 ± 2.9
Calcium	4	1.10	1.75	-3.04 ± 0.57
	4	1.32	1.75	-1.30 ± 0.75
Magnesium	4	0.50	0.25	2.36 ± 0.33
	4	0.70	0.25	3.79 ± 0.33
Bicarbonate	4	17	25	-82.2 ± 3.6
	4	24	25	-20.0 ± 11.8
Lactate	8	0	10	-77.0 ± 6.6
Glucose	2	44 (0.80%)	76 (1.36%)	-89.9; -123.1
	2	44 (0.80%)	98 (1.76%)	-141.3; -168.6
	2	44 (0.80%)	104 (1.87%)	-206.1; -247.5
	2	44 (0.80%)	126 (2.27%)	-323.7; -364.2

Mean ± standard deviation is presented. In case of n=2, the results per experiment are presented separated by a semicolon. Cd, dialysate concentration.
*Concentrations in the 36-L dialysate reservoir upstream of the SAPD system.
†Concentrations in the dialysate reservoir of the SAPD nighttime system that contained Physioneal 35.

Table 5. Cation removal by the sorbents with the SAPD nighttime system in single-pass configuration (n=8).

Solute	**Removal (mmol)**
Potassium	2.96 ± 1.60
Calcium	2.31 ± 0.96
Magnesium	0.64 ± 0.40

Table 6. Results of the *in vitro* cytotoxicity and genotoxicity assays

	Test duration (h)	Outcome*
Cytotoxicity†		
Cell morphology	24 h	Mesothelial cell morphology and ability to form a confluent monolayer is maintained.
Epithelial and mesenchymal cell markers	72 h	Epithelial expression of cytokeratin 8+18 is maintained. No increase in expression of mesenchymal marker FSP-1.
Cell apoptosis and proliferation	24-72 h	No increase in cell death. Cell proliferation is not impaired.
Oxidative stress (ROS)	6-24 h	No increase in intracellular ROS levels.
Cell migration (wound healing assay)	24-72 h	No difference in wound healing capacity.
LDH release	24 h	No increase in LDH activity in cell media.
Inflammatory response	24 h	No increase in VEGF, IL-6 or TGF-β1 levels in cell media.
Genotoxicity		
Bacterial reverse mutation assay (Ames)		No induction of bacterial mutations.
Mouse lymphoma assay		No induction of mammalian cell mutations.

FSP-1, fibroblast specific protein-1; IL-6, interleukin 6; LDH, lactate dehydrogenase; ROS, reactive oxygen species; TGF-β1, transforming growth factor β1; VEGF, vascular endothelial growth factor. *Spent peritoneal dialysate treated by the SAPD daytime system for 8 h and 16 h was compared with untreated spent peritoneal dialysate. †All cytotoxicity assays were performed using a human peritoneal mesothelial cell (HPMC) line (virus-transformed MeT-5A cells from ATCC, ATCC® CRL9444™).