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

3

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40 **Keywords:**

41 Cytotoxicity tests

42 Biocompatibility

43 Kidneys, artificial

44 Peritoneal dialysis

45 Sorbent

46 **Abstract**

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

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

58 **Results:** Urea, creatinine and phosphate removal was 33.2 ± 4.1 mmol, 5.3 ± 0.5 mmol, and 6.2 ± 1.8 mmol,
59 respectively, with the daytime system, and 204 ± 28 mmol, 10.3 ± 2.4 mmol and 11.4 ± 2.1 mmol,
60 respectively, with the nighttime system. Time-averaged plasma clearances of urea, creatinine and
61 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
62 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
63 nighttime system.

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

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81 **1. Introduction**

82 Worldwide, approximately 3.4 million patients receive life-sustaining dialysis treatment of which ~88%
83 are treated with in-center hemodialysis (HD) and ~11% are treated with peritoneal dialysis (PD) at home
84 [17]. However, existing dialysis techniques have important disadvantages. In both PD and HD, removal
85 of waste solutes and excess water is inadequate, contributing to severe health problems, high mortality
86 (15-20% per year [15]) and poor quality of life [1]. Although PD has several advantages compared to HD,
87 such as a survival advantage during the early years of dialysis [28], prolonged maintenance of residual
88 kidney function [23, 25, 30], and a blood free access; it also has several important disadvantages such as a
89 relatively low clearance [6, 7, 14] and limited technique survival due to structural and functional
90 deterioration of the peritoneal membrane as a result of the high incidence of recurrent peritonitis [31] and
91 chronic exposure to hypertonic glucose-based dialysis solutions [46].

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

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

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

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

120

121 **2. Methods**

122 **2.1 Materials**

123 The SAPD system was built and kindly provided by Nanodialysis (Oischot, The Netherlands). It
124 comprises a wearable sorbent based device (Fig. 1A “the SAPD daytime system”) that is combined with a
125 9-L dialysate reservoir (provided in a trolley on wheels) during the night (Fig. 1B “the SAPD nighttime
126 system”). The sorbent cartridge comprises 100 g (dry weight) of polystyrene beads modified with iron
127 oxide hydroxide (FeOOH) and 200 g (dry weight) of activated carbon for removal of phosphate and
128 organic waste solutes, respectively. The SAPD nighttime system is intended to be used for 8 h per night
129 on a daily basis to allow for sufficient urea and potassium removal. Optionally, patients may continue
130 treatment during the day with the wearable device to further enhance clearance of non-urea organic waste
131 solutes and phosphate.

132 [Insert Figure 1]

133

134 **2.2 Efficacy testing**

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

144 [Insert Figure 2]

145 [Insert Figure 3]

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

147 Two liters of spent peritoneal dialysate (Extraneal 7.5%) were collected one day prior to the experiment
148 from three different patients after an intraperitoneal dwell time of 12 h, and stored at 4°C until use.
149 Patients with peritonitis were excluded. Prior to start of the experiments, the peritoneal dialysate was
150 pooled and split into three sterile 2-L bags. Mean effective dialysate flow rate (Qd, Formula 1) was 50
151 mL/min with the daytime system and 100 mL/min with the nighttime system. The sorbents of the daytime
152 system were prerinsed with 6 L of Extraneal ([icodextrin] 7.5%, [Na⁺] 133 mmol/L, [Ca²⁺] 1.75 mmol/L,
153 [Mg²⁺] 0.25 mmol/L, [Cl⁻] 96 mmol/L, [lactate] 40 mmol/L), pH 5.5; Baxter GmbH, Germany) and
154 sorbents of the nighttime system were prerinsed with a solution containing [Na⁺] 134 mmol/L, [Ca²⁺] 1.25
155 mmol/L, [Mg²⁺] 0.50 mmol/L, [Cl⁻] 100.5 mmol/L and [lactate] 35 mmol/L] at pH 7.0. Of note, lactate
156 concentrations were equal in the in- and effluent after this procedure. The dialysate reservoir of the
157 nighttime system contained StaySafe® Balance ([glucose] 1.5%; [Na⁺] 134 mmol/L, [Ca²⁺] 1.25 mmol/L,
158 [Mg²⁺] 0.50 mmol/L, [Cl⁻] 100.5 mmol/L, [lactate] 35 mmol/L), pH 7.0; Fresenius Medical Care GmbH,
159 Bad Homburg, Germany) peritoneal dialysis solutifon. To simulate transport of uremic toxins from the
160 intravascular space into the peritoneal cavity, urea, creatinine and (tripotassium) phosphate were spiked
161 hourly into the 2-L dialysate reservoir. creatinine and a 1.3-fold (Qd: 50 mL/min) and 1.8-3.2-fold (Qd:
162 100 mL/min) increase in MTAC Spike amounts were modeled assuming a 1.2-fold (Qd: 50 mL/min) and
163 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:
164 100 mL/min) increase in MTAC phosphate with continuous flow peritoneal dialysis (CFPD) based on
165 [16, 18, 37] (Table 1). In addition, with the daytime system, we assumed saturation of activated carbon
166 with urea after 1 h. Of note, phosphate was spiked as potassium salt (and not as sodium salt) to allow
167 evaluation of influences of the system on sodium balance. Dialysate samples were taken from the 2-L
168 dialysate reservoir before start and up- and downstream of the SAPD system after 10 min, 1 h, 2 h, 4 h, 6
169 h and 8 h of treatment for measurement of urea (mmol/L), creatinine (µmol/L), phosphate (mmol/L),
170 bicarbonate (mmol/L), lactate (mmol/L), sodium (mmol/L), chloride (mmol/L), calcium (mmol/L),
171 magnesium (mmol/L), and glucose (mmol/L) concentrations. Hydrogen chloride (1.2 mmol/L) was
172 spiked into the reservoir if pH exceeded 8.0 to prevent calcium carbonate and calcium phosphate
173 precipitations (assuming that *in vivo* OH⁻ and lactate, released from the phosphate sorbent (FeOOH beads)
174 in exchange for phosphate, would distribute across the peritoneal membrane into a larger volume and
175 have less effect on pH of the peritoneal dialysate).

176

177 [Insert Table 1]

178 *Experimental procedures: single-pass experiments with the nighttime system*

179 A volume of 36 L of dialysate was prepared using acid concentrate for hemodialysis (Dirinco, 874),
180 sodium bicarbonate (Sigma-Aldrich) and demineralized water. Varying concentrations of potassium,
181 calcium, magnesium, bicarbonate and lactate were applied to evaluate removal (or release) of these
182 solutes for a range of clinically relevant values. Phosphate 2 mmol/L was spiked because calcium and
183 magnesium can be removed *via* binding to negatively charged phosphate that is bound to FeOOH. The
184 dialysate was circulated single-pass *via* a tidal mode at a Qd of 75 mL/min through the SAPD nighttime
185 system into a waste reservoir for 8 h (n=6). Dialysate samples were taken from the waste reservoir hourly.
186 The dialysate reservoir of the nighttime system contained Physioneal 35 ([Na⁺] 132 mmol/L, [Ca²⁺] 1.75
187 mmol/L, [Mg²⁺] 0.25 mmol/L, [Cl⁻] 101 mmol/L, [bicarbonate] 25 mmol/L, [lactate] 10 mmol/L, pH 7.4;
188 Baxter) peritoneal dialysis solution with varying glucose concentrations (1.36-2.27%) to study glucose
189 release. Physioneal 35 was selected because use of a combined bicarbonate/lactate buffer is associated
190 with improved biocompatibility *in vitro* and *in vivo* compared with solutions that only use lactate [2, 24,
191 33, 36, 51]. The sorbents were prerinsed with 6 L of [Na⁺] 132 mmol/L, [Cl⁻] 97 mmol/L, [bicarbonate]
192 30 mmol/L, [lactate] 10 mmol/L and pH 7.0. Of note, the rinsing fluid no longer contained calcium,
193 magnesium and glucose to prevent calcium and magnesium carbonate precipitations during storage, and
194 the formation of toxic glucose degradation products during steam sterilization and storage, respectively.
195 Equilibration was performed at relatively low pH (7.0) to maintain a physiologic pH (~7.4) in the effluent
196 of the device which releases alkaline anions (OH⁻, bicarbonate and/or lactate) in exchange for phosphate.

198 **2.3 Calculations**

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

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203
Formula 1:
$$Qd = \frac{TV}{t_{IN} + t_{OUT}}$$

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

206 *Recirculation experiments*

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

$$\text{Formula 2: } A(t1 \rightarrow t2) = \frac{(Cd_{IN} - Cd_{OUT})t1 + (Cd_{DIN} - Cd_{OUT})t2}{2} \times Qd \times t$$

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

209 concentration in the outgoing line (downstream of the sorbent cartridge), Q_d = mean effective dialysate
210 flow rate and t = time between two consecutive measurements (t_2-t_1).

211
212 To get an impression of saturation of the system the percentage reduction in urea, creatinine and
213 phosphate concentration in the 2-L dialysate reservoir between two consecutive measurements was
214 calculated as follows:

215

$$\text{Formula 3: } \text{Percentage reduction } t_2 = \frac{(Cd_{IN})_{t_1} - (Cd_{IN})_{t_2}}{(Cd_{IN})_{t_1}} \times 100\%$$

216

217 Where Cd_{IN} = dialysate concentration in the ingoing line (i.e. upstream of the dialysate reservoir and/or
218 sorbent cartridge), t_1 = immediately after the spiking of solutes, t_2 = prior to the spiking of solutes.

219

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

$$\text{Formula 4: } Cl = \frac{((A_{night} + (V_{t0} \times C_{dt0}) + (V_{tx} \times C_{dtx}))/1440)}{C_p}$$

$$\text{Formula 5: } Cl = \frac{((A_{day} + A_{night} + (V_{t0} \times C_{dt0}) + (V_{tx} \times C_{dtx}))/1440)}{C_p}$$

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

233

234

235 *Single-pass experiments*

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

$$\text{Formula 6: } A(t_1 \rightarrow t_2) = (Cd_{IN} - Cd_{OUT}) \times Q_d \times t$$

238 Where $A_{(t_1 \rightarrow t_2)}$ = amount removed by the SAPD system between t_1 and t_2 , Cd_{IN} = dialysate concentration
239 in the 36-L dialysate reservoir, Cd_{OUT} = dialysate concentration in the dialysate waste reservoir, Q_d =
240 mean effective dialysate flow rate and t = time between two consecutive measurements (t_2-t_1).

241
242 Glucose adsorption (mmol/h) by the sorbents (activated carbon) from dialysate during experiments with
243 the SAPD nighttime system was calculated based on the difference in total glucose release by the SAPD
244 system and glucose release by the 9-L dialysate using the following formula:

$$\text{Formula 7: } A_{\text{ads}}(t_1 \rightarrow t_2) = \frac{A(t_1 \rightarrow t_2) - ((C_{dt2} - C_{dt1}) \times V)}{t}$$

245
246 Where $A_{\text{ads}}(t_1 \rightarrow t_2)$ = amount adsorbed by the sorbents between t_1 and t_2 , $A(t_1 \rightarrow t_2)$ = amount released by the
247 SAPD system between t_1 and t_2 , C_d = glucose concentration in the 9-L dialysate reservoir of the SAPD
248 system, V = volume of the dialysate reservoir of the nighttime system (i.e. 9 L) and t = time between t_1
249 and t_2 in hours.
250

251 **2.4 *In vitro* cytotoxicity and genotoxicity**

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

264 **2.5 Statistical analysis**

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

272

273 **3. Results**

274 **3.1 Efficacy testing**

275 *Recirculation experiments*

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

284
285 [Insert Table 2]

286 [Insert Figure 4]

287 [Insert Table 3]

288
289 *Single-pass experiments*

290 Base and glucose release by the nighttime system were evaluated in single-pass configuration to maintain
291 constant solute concentrations in dialysate entering the SAPD system, simulating equilibration of the
292 intraperitoneal and intravascular compartment *in vivo* (Table 4). Potassium removal was also determined
293 at dialysate potassium concentrations representative for the *in vivo* situation (Table 5). Remarkably,
294 despite the absence of a cation exchanger, a limited amount of cations was removed by the sorbents
295 (Table 3 and 5), probably *via* binding to negatively charged phosphate that was bound to FeOOH.
296 Glucose release increased at higher glucose concentrations in the Physioneal 35 dialysate reservoir (Table
297 4, Figure 5A). Stable dialysate glucose concentrations were achieved downstream of the sorbents (Figure
298 5B).

299 [Insert Figure 5]

300 [Insert Table 4]

301 [Insert Table 5]

302
303 **3.2 *In vitro* cytotoxicity and genotoxicity**

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

307
308 [Insert Table 6]

309
310 **4. Discussion**

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

313 Clinically relevant removal of urea, creatinine, phosphate and potassium from peritoneal dialysate
314 by the SAPD system was observed *in vitro* as compared to a daily urea and creatinine production of ~240-
315 470 mmol [39, 44] and 8-17 mmol [27], respectively, and phosphate and potassium intake of ~15 and ~45
316 mmol [29, 41, 43], respectively, in dialysis patients, and an average dialysate removal of ~325-360 mmol
317 [6, 14], ~6.4 mmol [47], ~6.5-8.0 mmol [9] and ~29-41 mmol [32, 50] for urea, creatinine, phosphate and
318 potassium, respectively, in conventional PD. Of note, absolute urea removal in the present study was
319 relatively low compared with conventional PD because urea concentrations in the 2-L dialysate reservoir
320 (representing patient's plasma concentration at the time of equilibration between plasma and dialysate
321 urea concentration) varied between 2.9-26.8 mmol/L due to non-continuous spiking, whereas patients had
322 a relatively constant plasma urea concentration of ~40 mmol/L [6, 14]. However, maximum urea removal
323 capacity of the nighttime system was not yet achieved after 8 h of treatment, indicating that removal will
324 be increased at higher urea concentrations entering the SAPD system.

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

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

341 application in a wearable artificial kidney [42]. As the affinity of urea for activated carbon is relatively
342 low (0.1-0.2 mmol/g), a relatively large amount of activated carbon (1.2-4.7 kg) would be required to
343 remove the daily urea production [42]. Htay et al. report use of enzymatic hydrolysis of urea by urease for
344 dialysate regeneration in a wearable artificial kidney for CFPD, a system of <2 kg using 3 cartridges and
345 3 exchanges of 2 L per day [5, 19].

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

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

363 Base release by the SAPD nighttime system seemed adequate. To compensate for daily non-
364 volatile acid production, ~70 mmol of net base (sum of bicarbonate and lactate) must be released into the
365 patient during dialysis treatment to prevent severe metabolic acidosis as a consequence of impaired renal
366 acid excretion in dialysis patients [26, 38]. By using a combined lactate/bicarbonate buffer (10/25
367 mmol/L) in the dialysate reservoir, the single-pass experiments show that the SAPD system may release
368 77 mmol of lactate, provided that rapid equilibration of lactate occurs between dialysate and plasma so
369 that the intraperitoneal lactate concentration remains low. Additional bicarbonate release will depend on
370 the degree of metabolic acidosis.

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

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

389 With the SAPD system, exposure of the peritoneal membrane to very high glucose concentrations
390 may be prevented and peritoneal integrity may be preserved for a longer period of time. Kinetic modeling
391 by Gotch *et al.* [18], based on patient data with CFPD, shows that maintaining an intraperitoneal glucose
392 concentration of 1 % will yield a constant ultrafiltration rate of ~0.2 L/h, more than sufficient for an 8-h
393 SAPD treatment per day. To achieve this, we estimate that the SAPD system should gradually release
394 ~480 mmol of glucose during an 8-h treatment, assuming an MTAC of glucose of ~0.02 L/min [18]. In
395 the present study, a dose-response was observed, with higher glucose release when using higher glucose
396 concentrations in the dialysate reservoir. *In vivo* studies and treatment of individual patients should
397 further define glucose concentrations in the 9-L reservoir and give more information on ultrafiltration
398 rates with varying intraperitoneal glucose concentrations and the long-term effect of reduced glucose
399 concentrations on peritoneal integrity.

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

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

409 16, 37, 40], while the SAPD system uses tidal flow via a single lumen catheter. We assumed that –
410 independent of the direction of the flow that changes every 3-6 minutes- the continuous high laminar flow
411 along the peritoneal membrane will enhance mass transport across the peritoneal membrane. However,
412 patient studies are needed to confirm that the MTAC of solutes is indeed increased with this setup.
413 Second, the effect of CFPD on MTAC is variable among patients and may be more pronounced in
414 patients with a high transport status [16]. Patient studies should evaluate which parameters determine the
415 efficacy of CFPD. Third, during the single-pass experiments, glucose concentrations upstream of the
416 system were kept constant (44 mM) while *in vivo* intraperitoneal glucose concentrations will be different
417 with different glucose concentrations in the 9-L reservoir (namely higher intraperitoneal glucose
418 concentrations with higher glucose concentrations in the 9-L reservoir and vice versa) which is expected
419 to result in larger differences in glucose concentrations in the effluent of the system *in vivo*.

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

424

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432

433 6. Conflict of interest

434 The authors declare no conflict of interest.

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436 References

- 437 1. Aguiar R, Pei M, Qureshi AR, *et al.* Health-related quality of life in peritoneal dialysis patients: A
438 narrative review. *Semin Dial* 2019;32(5):452-462
- 439 2. Albrektsson A, Bazargani F, Wieslander A, *et al.* Peritoneal dialysis fluid-induced angiogenesis
440 in rat mesentery is increased by lactate in the presence or absence of glucose. *ASAIO J*
441 2006;52(3):276-281
- 442 3. Amerling R, DeSimone L, Inciong-Reyes R, *et al.* Clinical experience with continuous flow and
443 flow-through peritoneal dialysis. *Semin Dial* 2001;14(5):388-390
- 444 4. Anelli A. GM, Padovese P., Colantonio G., Barbesti S., Brancaccio D. (1989) Beta-2-
445 Microglobulin (B2m) Adsorption on Activated Charcoal (AC). In: Andreucci V.E., Dal Canton

- 446 A. (eds) *Current Therapy in Nephrology*. Developments in Nephrology, vol 24. Springer, Boston,
447 MA
- 448 5. Automated Wearable Artificial Kidney (AWAK). Available online (accessed on 24 February
449 2020): <http://awak.com/product/>
- 450 6. Bammens B, Evenepoel P, Verbeke K, *et al.* Removal of middle molecules and protein-bound
451 solutes by peritoneal dialysis and relation with uremic symptoms. *Kidney Int* 2003;64(6):2238-
452 2243
- 453 7. Bammens B, Evenepoel P, Verbeke K, *et al.* Time profiles of peritoneal and renal clearances of
454 different uremic solutes in incident peritoneal dialysis patients. *Am J Kidney Dis* 2005;46(3):512-
455 519
- 456 8. Blumenkrantz MJ, Gordon A, Roberts M, *et al.* Applications of the Redy sorbent system to
457 hemodialysis and peritoneal dialysis. *Artif Organs* 1979;3(3):230-236
- 458 9. Courivaud C, Davenport A. Phosphate Removal by Peritoneal Dialysis: The Effect of Transporter
459 Status and Peritoneal Dialysis Prescription. *Perit Dial Int* 2016;36(1):85-93
- 460 10. Cruz C, Melendez A, Gotch FA, *et al.* Single-pass continuous flow peritoneal dialysis using two
461 catheters. *Seminars in dialysis* 2001;14:391-394
- 462 11. Davies SJ, Phillips L, Naish PF, *et al.* Peritoneal glucose exposure and changes in membrane
463 solute transport with time on peritoneal dialysis. *J Am Soc Nephrol* 2001;12(5):1046-1051
- 464 12. de Fijter CW, Oe PL, Nauta JJ, *et al.* A prospective, randomized study comparing the peritonitis
465 incidence of CAPD and Y-connector (CAPD-Y) with continuous cyclic peritoneal dialysis
466 (CCPD). *Adv Perit Dial* 1991;7:186-189
- 467 13. Diaz-Buxo JA, Cruz C, Gotch FA. Advances in end-stage renal diseases 2000. Continuous-flow
468 peritoneal dialysis. preliminary results. *Blood Purif* 2000;18(4):361-365
- 469 14. Evenepoel P, Meijers BK, Bammens B, *et al.* Phosphorus metabolism in peritoneal dialysis- and
470 haemodialysis-treated patients. *Nephrol Dial Transplant* 2016;31(9):1508-1514
- 471 15. Foley RN, Hakim RM. Why is the mortality of dialysis patients in the United
472 States much higher than the rest of the world? *J Am Soc Nephrol* 2009; 20(7):1432-5
- 473 16. Freida P, Issad B. Continuous flow peritoneal dialysis: assessment of fluid and solute removal in
474 a high-flow model of "fresh dialysate single pass". *Perit Dial Int* 2003;23(4):348-355
- 475 17. Fresenius Medical Care. Annual report 2018. Available online (accessed on 24 February 2020):
476 https://www.fresenius.com/media/FME_Annual-Report_2018.pdf
- 477 18. Gotch FA. Kinetic modeling of continuous flow peritoneal dialysis. *Semin Dial* 2001;14(5):378-
478 383
- 479 19. Htay H, Goa S, Jayaballa M, *et al.* Evaluation of safety of automated wearable artificial kidney
480 (AWAK) device in PD patients [poster], ISN WCN19-0817 Melbourne, Australia, 2019
- 481 20. International organisation for standardisation, biological evaluation of medical devices – part 1:
482 evaluation and testing within a risk management process, International Standard ISO 10993-1. 4th
483 edition 2009 Oct 15
- 484 21. International organisation for standardisation, biological evaluation of medical devices – part 3:
485 Tests for genotoxicity, carcinogenicity and reproductive toxicity. Revision of 2nd edition 2013
486 Mar
- 487 22. International organisation for standardisation, biological evaluation of medical devices – part 5:
488 Tests for in vitro cytotoxicity. 3rd edition 2009 June 1
- 489 23. Jansen MA, Hart AA, Korevaar JC, *et al.* Predictors of the rate of decline of residual renal
490 function in incident dialysis patients. *Kidney Int* 2002;62(3):1046-1053
- 491 24. Kuma A, Tamura M, Ishimatsu N, *et al.* Monocarboxylate Transporter-1 Mediates the Protective
492 Effects of Neutral-pH Bicarbonate/Lactate-Buffered Peritoneal Dialysis Fluid on Cell Viability
493 and Apoptosis. *Ther Apher Dial* 2017;21(1):62-70
- 494 25. Lang SM, Bergner A, Topfer M, *et al.* Preservation of residual renal function in dialysis patients:
495 effects of dialysis-technique-related factors. *Perit Dial Int* 2001;21(1):52-57

- 496 26. Lennon EJ, Lemann J Jr., Litzow JR. The effects of diet and stool composition on the net external
497 acid balance of normal subjects. *J Clin Invest* 1966;45(10):1601-1607
- 498 27. Lindner G, Schwarz C, Funk GC. Osmotic diuresis due to urea as the cause of hypernatraemia in
499 critically ill patients. *Nephrol Dial Transplant* 2012;27(3):962-967
- 500 28. Lukowsky LR, Mehrotra R, Kheifets L, *et al.* Comparing mortality of peritoneal and
501 hemodialysis patients in the first 2 years of dialysis therapy: a marginal structural model analysis.
502 *Clin J Am Soc Nephrol* 2013;8(4):619-628
- 503 29. Mente A, O'Donnell MJ, Rangarajan S, *et al.* Association of urinary sodium and potassium
504 excretion with blood pressure. *N Engl J Med* 2014;371(7):601-611
- 505 30. Moist LM, Port FK, Orzol SM, *et al.* Predictors of loss of residual renal function among new
506 dialysis patients. *J Am Soc Nephrol* 2000;11(3):556-564
- 507 31. Mujais S, Story K. Peritoneal dialysis in the US: evaluation of outcomes in contemporary cohorts.
508 *Kidney Int Suppl* 2006(103):S21-26
- 509 32. Musso CG. Potassium metabolism in patients with chronic kidney disease. Part II: patients on
510 dialysis (stage 5). *Int Urol Nephrol* 2004;36(3):469-472
- 511 33. Ogata S, Naito T, Yorioka N, *et al.* Effect of lactate and bicarbonate on human peritoneal
512 mesothelial cells, fibroblasts and vascular endothelial cells, and the role of basic fibroblast growth
513 factor. *Nephrol Dial Transplant* 2004;19(11):2831-2837
- 514 34. Paniagua R, Amato D, Vonesh E, *et al.* Effects of increased peritoneal clearances on mortality
515 rates in peritoneal dialysis: ADEMEX, a prospective, randomized, controlled trial. *J Am Soc*
516 *Nephrol* 2002;13(5):1307-1320
- 517 35. Park MS, Lee HA, Chu WS, *et al.* Peritoneal accumulation of AGE and peritoneal membrane
518 permeability. *Perit Dial Int* 2000;20(4):452-460
- 519 36. Plum J, Razeghi P, Lordnejad RM, *et al.* Peritoneal dialysis fluids with a physiologic pH based
520 on either lactate or bicarbonate buffer-effects on human mesothelial cells. *Am J Kidney Dis*
521 2001;38(4):867-8755
- 522 37. Raaijmakers R, Schroder CH, Gajjar P, *et al.* Continuous flow peritoneal dialysis: first experience
523 in children with acute renal failure. *Clin J Am Soc Nephrol* 2011;6(2):311-318
- 524 38. Scialla JJ, Asplin J, Dobre M, *et al.* Higher net acid excretion is associated with a lower risk of
525 kidney disease progression in patients with diabetes. *Kidney Int* 2017;91(1):204-215
- 526 39. Shinaberger CS, Kilpatrick RD, Regidor DL, *et al.* Longitudinal associations between dietary
527 protein intake and survival in hemodialysis patients. *Am J Kidney Dis* 2006;48(1):37-49
- 528 40. Shinaberger JH, Shear L, Barry KG. Increasing efficiency of peritoneal dialysis: experience with
529 peritoneal-extracorporeal recirculation dialysis. *Trans Am Soc Artif Intern Organs* 1965;11:76-82
- 530 41. Therrien M, Byham-Gray L, Denmark R, *et al.* Comparison of dietary intake among women on
531 maintenance dialysis to a Women's Health Initiative cohort: results from the NKF-CRN Second
532 National Research Question Collaborative Study. *J Ren Nutr* 2014;24(2):72-80
- 533 42. van Gelder MK, Jong JAW, Folkertsma L, *et al.* Urea removal strategies for dialysate
534 regeneration in a wearable artificial kidney. *Biomaterials* 2020;234:119735
- 535 43. Vervloet MG, van Ittersum FJ, Buttler RM, *et al.* Effects of dietary phosphate and calcium intake
536 on fibroblast growth factor-23. *Clin J Am Soc Nephrol* 2011;6(2):383-389
- 537 44. Weiner ID, Mitch WE, Sands JM. Urea and Ammonia Metabolism and the Control of Renal
538 Nitrogen Excretion. *Clin J Am Soc Nephrol* 2015;10(8):1444-1458
- 539 45. Witowski J, Wisniewska J, Korybalska K, *et al.* Prolonged exposure to glucose degradation
540 products impairs viability and function of human peritoneal mesothelial cells. *J Am Soc Nephrol*
541 2001;12(11):2434-2441
- 542 46. Wu HY, Hung KY, Huang TM, *et al.* Safety issues of long-term glucose load in patients on
543 peritoneal dialysis--a 7-year cohort study. *PLoS One* 2012;7(1):e30337
- 544 47. Xu Z, Murata GH, Sun Y, *et al.* Reproducibility of serial creatinine excretion measurements in
545 peritoneal dialysis. *World J Nephrol* 2017;6(4):201-208

546 48. Yamamoto S, Ito T, Sato M, *et al.* Adsorption of Protein-Bound Uremic Toxins Using Activated
547 Carbon through Direct Hemoperfusion in vitro. *Blood Purif* 2019:1-8
548 49. Yavuz A, Ersoy FF, Passadakis PS, *et al.* Phosphorus control in peritoneal dialysis patients.
549 *Kidney Int Suppl* 2008(108):S152-158
550 50. Yu HL, Lu XH, Su CY, *et al.* Potassium metabolism in continuous ambulatory peritoneal dialysis
551 patients. *Ren Fail* 2014;36(5):748-754
552 51. Zhai Y, Bloch J, Homme M, *et al.* Buffer-dependent regulation of aquaporin-1 expression and
553 function in human peritoneal mesothelial cells. *Pediatr Nephrol* 2012;27(7):1165-1177

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578 **Figure captions**

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

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

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

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

595
596 Figure 5. A: Glucose release (mmol) by the SAPD nighttime system in single-pass configuration for
597 different glucose concentrations (1.36% (n=2), 1.76% (n=2), 1.87% (n=2) and 2.27% (n=2)) in the
598 dialysate reservoir. B: Glucose concentrations (mmol/L) downstream of the sorbents for different glucose
599 concentrations in the dialysate reservoir of the nighttime system. The dashed line represents the glucose
600 concentration (44 mmol/L) in the 36-L dialysate reservoir upstream of the SAPD system. C: Glucose
601 adsorption (mmol/h) by the sorbents with the SAPD nighttime system. Each graph represents the mean of
602 two experiments.

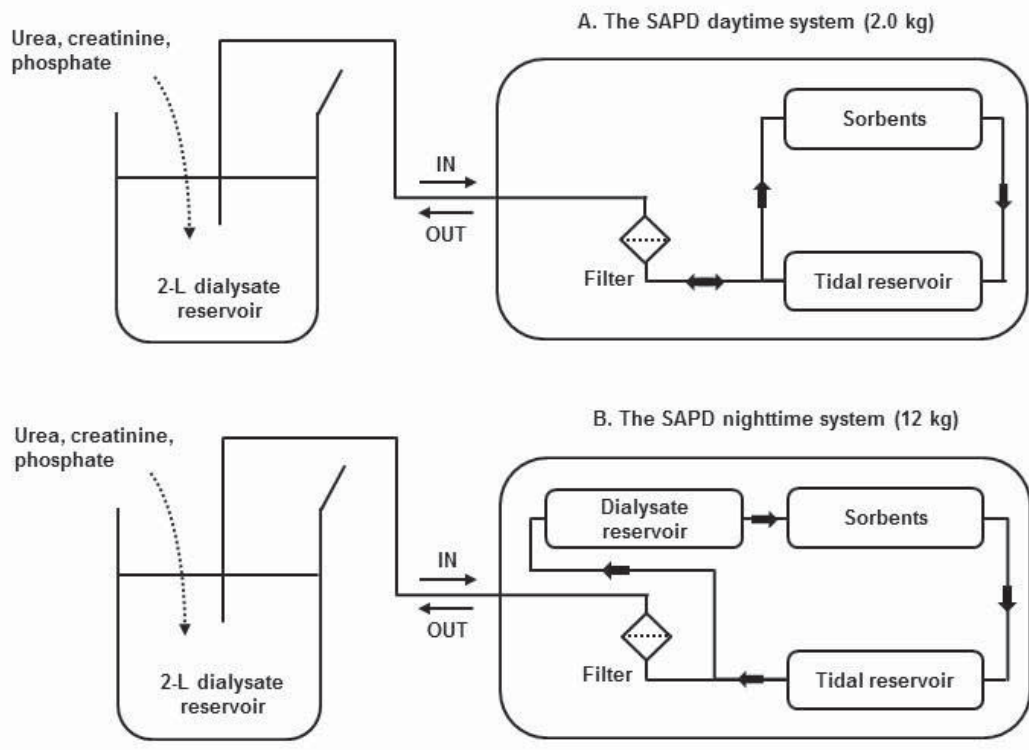
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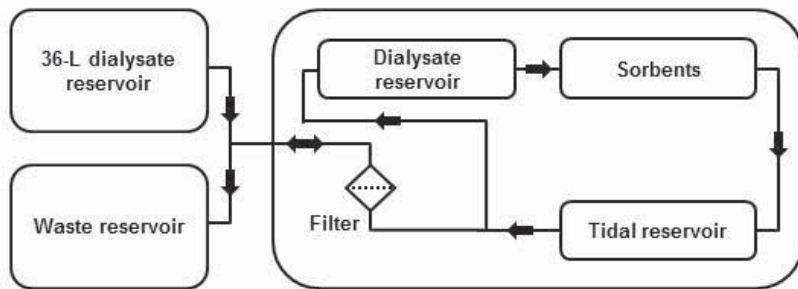


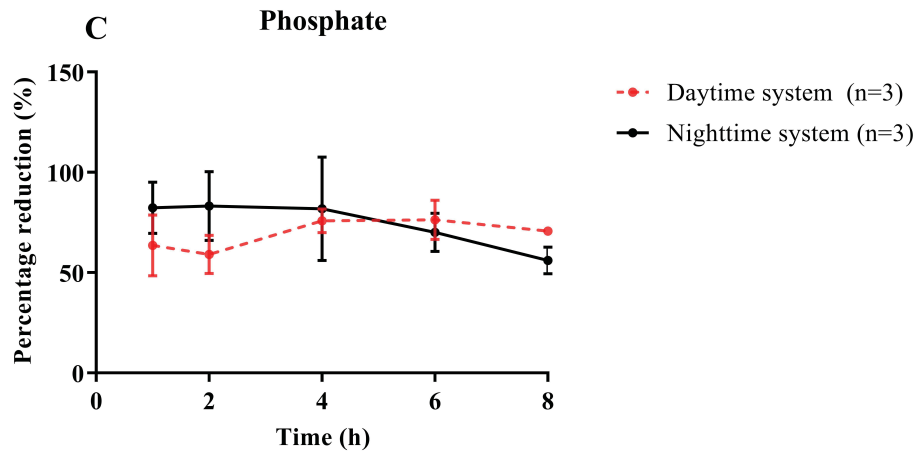
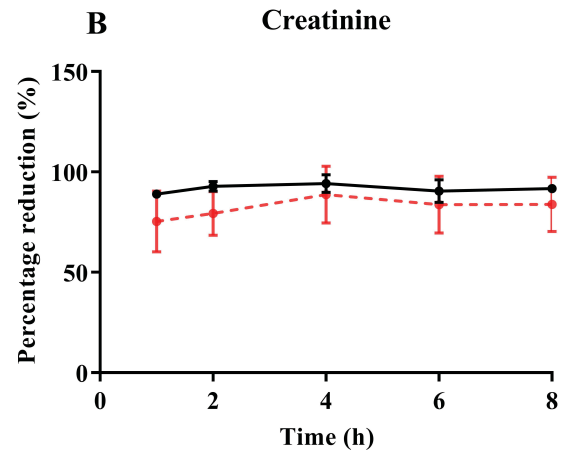
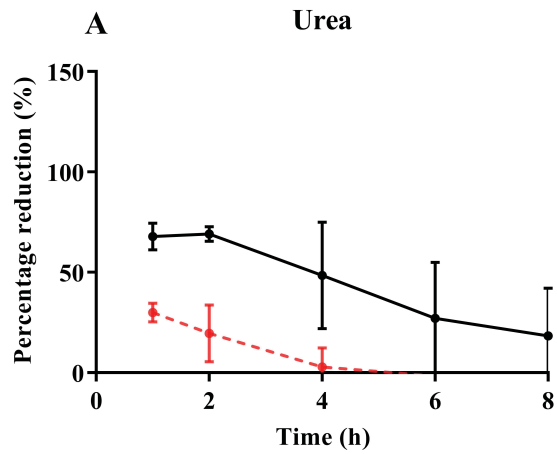
A. The SAPD daytime system (2.0 kg)

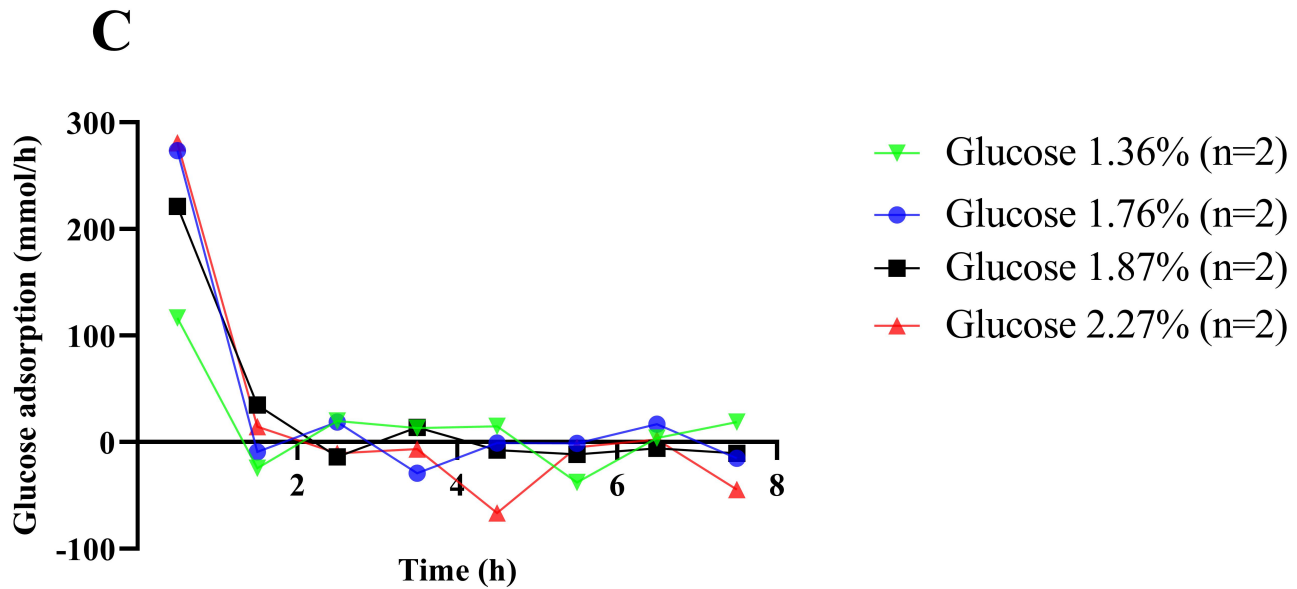
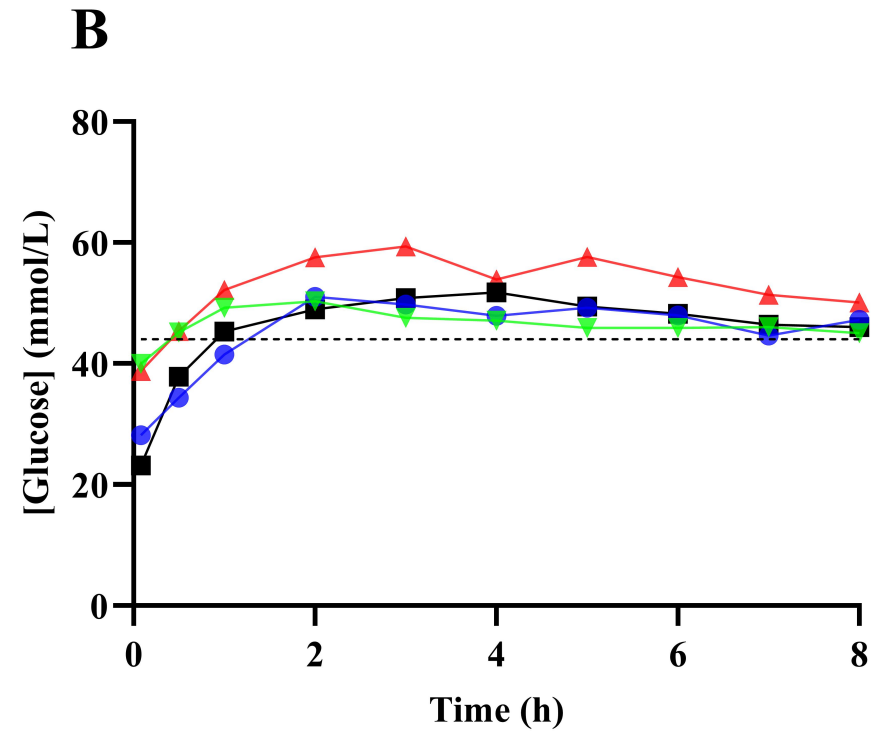
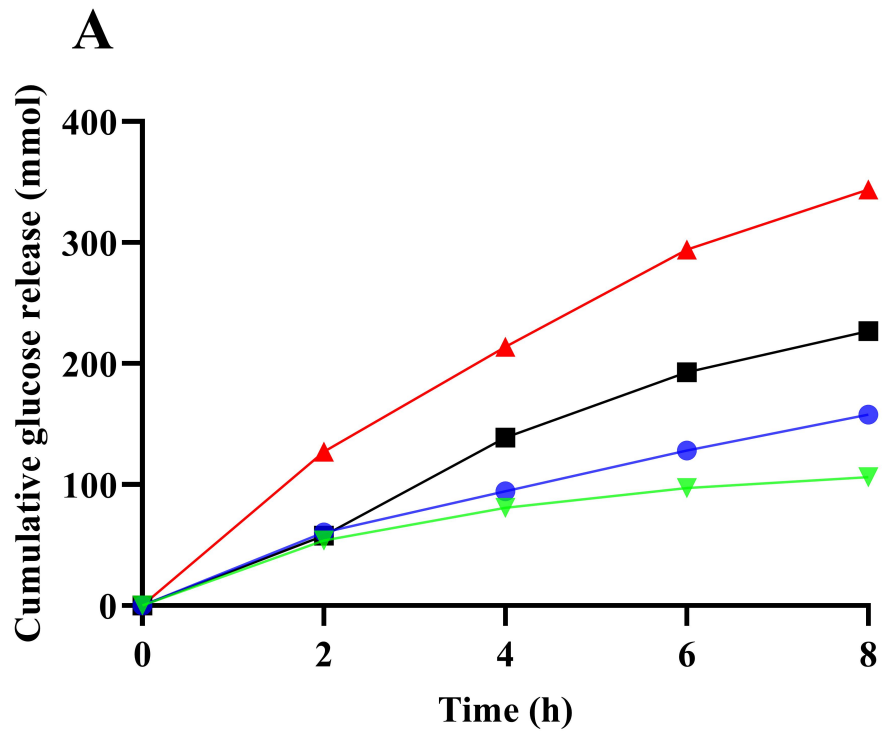


B. The SAPD nighttime system (12 kg)









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.

Solute	Cumulative removal <i>in vitro</i> (mmol)		Nighttime system + 1 exchange		Day- and nighttime system + 1 exchange	
	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).

Solute	Cumulative removal/ release (mmol)	
	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- β 1 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- β 1, transforming growth factor β 1; 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™).