ORIGINAL ARTICLES

PERITONEAL DIALYSIS CATHETER INCREASES LEUKOCYTE RECRUITMENT IN THE MOUSE PARIETAL PERITONEUM MICROCIRCULATION AND CAUSES FIBROSIS

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♦ **Background:** The objective of this study was to examine the effects of a conventional dialysis solution and peritoneal catheter on leukocyte-endothelial cell interactions in the microcirculation of the parietal peritoneum in a subacute peritoneal dialysis (PD) mouse model.

♦ **Methods:** An intraperitoneal (IP) catheter with a subcutaneous injection port was implanted into mice and, after a 2-week healing period, the animals were injected daily for 6 weeks with a 2.5% dextrose solution. Intravital microscopy (IVM) of the parietal peritoneum microcirculation was performed 4 hours after the last injection of the dialysis solution. Leukocyte-endothelial cell interactions were quantified and compared with catheterized controls without dialysis treatment and naïve mice.

♦ **Results:** The number of rolling and extravascular leukocytes along with peritoneal fibrosis and neovascularization were significantly increased in the catheterized animals compared with naïve mice but did not significantly differ between the 2 groups of catheterized animals with sham injections or dialysis solution treatment.

♦ **Conclusion:** The peritoneal catheter implant increased leukocyte rolling and extravasation, peritoneal fibrosis and vascularization in the parietal peritoneum independently from the dialysis solution treatment.

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Peritonitis is a major problem in peritoneal dialysis (PD). Chronic peritonitis drives peritoneal damage, with permeability changes and poor outcomes for patients, who may require catheter removal (1,2). Normal leukocyte effector functions and recruitment are essential for bacterial clearance in peritonitis. However, there is evidence that leukocyte responses in the peritoneum become impaired with exposure to PD fluids in humans (3,4) and in animal studies (5,6) as well as *in vitro* work (7). Leukocytes are recruited to tissues in a highly regulated multi-step process, involving leukocyte tethering and rolling along the endothelium, leukocyte adhesion to endothelial cells followed by extravasation and migration in the interstitial space. Intravital microscopy (IVM) showed that leukocyte recruitment in the visceral peritoneum microcirculation is altered with exposure to PD solutions in animal models (8–11) and the peritoneal catheter had no effect on the inflammatory microvascular responses in these studies (10,11).

Silicone PD catheter implants act as foreign bodies. In rat models of PD, the catheter implant appeared to make a large contribution to the damage of the parietal peritoneum. Changes in peritoneal structure, histology, and function were more profound when PD fluids were instilled through a catheter implant compared with intraperitoneal (IP) injections with a needle (12,13). These studies emphasize that the effects of dialysis solutions on peritoneal pathology, function, and inflammatory responses in animal models cannot be examined without careful differentiation of the effects of the PD fluid versus the peritoneal catheter.

Although the parietal peritoneal surface and the underlying microcirculation are major functional contributors to the dialysis membrane (14–17) and greater pathological changes occur in the parietal peritoneum compared with the visceral peritoneum in the same PD patients (18), reports on the effects of PD fluids and catheter on leukocyte recruitment in this layer

are lacking in the current literature. The objective of this study was to examine the effects of a conventional PD solution and the peritoneal catheter implant on leukocyte-endothelial cell interactions in the parietal peritoneum microcirculation. We developed a mouse model of the parietal peritoneum microcirculation using IVM. With this technique, we visualized the peritoneal layer, the underlying microcirculation and leukocyte recruitment in mice that were treated with PD fluids through a catheter for 6 weeks or catheterized animals that received sham injections.

MATERIALS AND METHODS

ANIMALS

The animal protocols met the regulations set by the Canadian Council of Animal Care and were approved by the McMaster University Animal Research Ethics Board (Animal Utilization Protocol #11-01-03). Six- to 8-week old male BALB/c mice were obtained from Taconic (Germantown, NY, USA). The mice were given at least 1 week to acclimatize and were housed in a specific pathogen-free facility.

NONUREMIC SUBACUTE PD MODEL

Under gaseous anesthesia (4 % Isoflurane), sterile silicone catheters (inner diameter (ID) 0.635 mm, outer diameter (OD) 1.1938 mm) that were attached to silicone injection ports (Penny MousePort; Access Technologies, Skokie, IL, USA) were implanted following aseptic techniques. Buprenorphine hydrochloride was injected subcutaneously as analgesia. The port and catheter were flushed with 10 % heparin (1,000 USP units/mL; Sandoz Canada Inc., Boucherville, QC, Canada) to maintain patency. The port was inserted subcutaneously over the back, the catheter was tunneled and a 1-cm segment was inserted into the right lower quadrant of the peritoneal cavity. The catheter was secured in the abdominal wall with a purse string stitch. To ensure catheter patency, 70 μ L of 10 % heparin solution was injected into the port 1 week after implantation.

Daily 2-mL injections of a conventional lactate-buffered dialysis solution (Dianeal PD4 CAPD solution with 2.5 % dextrose and 2.5 mEq/L calcium, approximate pH 5.2; Baxter, Mississauga, ON, Canada) were administered into the port after a 2-week catheter acclimatization period. One group of mice ($n = 4$) was dialyzed over a 6-week period. The other group of animals with catheters ($n = 4$) served as the catheter control and the mice received mock port injections with a needle. Intravital microscopy was also performed on naïve mice with no catheter implant and no dialysis solution treatment ($n = 6$). The animals were prepared for IVM of the parietal peritoneum microcirculation 4 hours after the last injection of the dialysis solution. The region of the anterior abdominal wall that was imaged was on the left side and was around the area where the intra-abdominal portion of the catheter was positioned.

INTRAVITAL MICROSCOPY

Detailed methods were previously described (19). The mice were anesthetized with a subcutaneous injection of a mixture of ketamine (200 mg/kg) and xylazine (10 mg/kg). The right internal jugular vein was cannulated with a polyethylene (PE) catheter (PE 10, ID 0.28 mm, OD 0.61 mm) for maintenance of anesthesia or administration of fluids. Skin was bluntly dissected away, and a midline incision was made along the linea alba extending inferiorly from the xiphoid process towards the left inguinal region to create a flap of musculo-peritoneum on the left side. The animals were placed in the right lateral position and the musculo-peritoneum was laid out on a microscope stage and the exposed tissue was immediately covered with plastic wrap to prevent evaporative loss.

The microcirculation underlying the left side of parietal peritoneum was observed by an inverted intravital microscope (Zeiss Inverted Axiovert 100; Carl Zeiss, Jena, Germany) under 40X objective lens and the transilluminated images were recorded for offline analysis. To minimize the inflammatory effects of tissue exposure, IVM observations were made within 10 minutes after completion of the surgical preparation (20). After completion of IVM, blood was collected via cardiac puncture and samples of the abdominal wall were extracted. Euthanasia was ensured by cervical dislocation.

OFFLINE ANALYSIS

Leukocyte-endothelial cell interactions were quantified in 4 – 6 venules per mouse. Rolling leukocytes (cells tethering to a venule with torsional motion) were counted per minute. Cells that remained stationary for at least 30 seconds were identified as adherent leukocytes. Extravascular cells were counted as perivascular leukocytes on a field of view measuring 180 μ m $\times$ 135 μ m.

LEUKOCYTE AND DIFFERENTIAL WHITE BLOOD CELL COUNTS

Systemic leukocytes were counted using a hemacytometer in blood mixed with 3% acetic acid and 1% crystal violet in a 5:44:1 ratio. Differential white blood cell counts were performed on smears of blood fixed in methanol and stained with eosin and thiazine (Harleco Hemacolor stain set; EMD Chemicals, Gibbstown, NJ, USA).

HISTOPATHOLOGIC EXAMINATION

Cross-sections of the abdominal wall were collected from the left upper quadrant and the left lower quadrant adjacent to the midline and contralateral to the catheter insertion site. The tissue samples were fixed with 10% buffered formalin, embedded in paraffin and thin-sectioned. Tissue sections were stained with hematoxylin and eosin (H&E) (Sigma-Aldrich, St. Louis, MO, USA) to measure submesothelial thickness and vascular density. For visualization of collagen, adjacent sections were stained with picro-sirius red (abcam, Cambridge, MA, USA).

Additional tissue sections were stained with resorcin-fuchsin and counterstained in van Gieson's solution and Weigert's iron hematoxylin (Electron Microscopy Sciences, Hatfield, PA, USA) for identification of elastic fibers. Microscopic examination was done using an Olympus BX41 microscope (Olympus America Inc., Center Valley, PA, USA). The thickness of the peritoneum was measured at 5 random locations in each H&E-stained section and averaged. The number of peritoneum-associated blood vessels was counted along the entire length of the peritoneum in the sections and recorded as vessels/mm of peritoneum.

STATISTICAL ANALYSIS

Data are expressed as mean $\pm$ standard error of the mean (SEM). Statistical significance was set at $p < 0.05$ and calculated using Student's *t*-test or ANOVA with Bonferroni correction for multiple comparisons using the computer software package KaleidaGraph 3.6 (Synergy Software, Reading, PA, USA).

RESULTS

ANIMAL CHARACTERISTICS

During the 2-week catheter acclimatization and the dialysis period, there were no significant changes in behavior or appearance of the animals, and skin incisions healed well with no signs of infection. There were no differences in the weights of the animals between the catheterized control group and the dialyzed group at the start (25.75 ± 0.63 g vs 25.75 ± 0.25 g, respectively) and end (29.25 ± 0.75 g vs 30.00 ± 0.91 g, respectively) of the experiment. However, 3/4 animals in the catheter control group and 4/4 animals in the dialyzed group exhibited some redness and scabbing on the skin over the port for approximately 3 weeks after surgical implantation. But this did not interfere with solution injections into the port.

LEUKOCYTE-ENDOTHELIAL CELL INTERACTIONS

The abdomen was opened via midline laparotomy and the parietal peritoneum was prepared for IVM. Some mice had adipose tissue wrapping around the catheter at the entry site on the abdominal wall, but this did not result in the occlusion of the catheter tip. Specifically, 1/4 mice had omental wrapping of the catheter and 2/4 mice had gonadal adipose tissue wrapping of the catheter in both groups. The mesothelial layer, submesothelial compact zone and underlying venules were visualized (Figure 1) and the numbers of rolling, adherent and perivascular leukocytes were determined. The regions visualized included the area that came in contact with the intra-abdominal catheter tip protruding towards the left side. The peritoneal lining appeared damaged in the catheterized groups with disorganized and hypertrophied mesothelial cells and overall hypercellularity (Figure 1, first column). Although much heterogeneity was observed, the submesothelial connective tissue in the catheterized control

and dialysis groups appeared to have less organized collagen bundles, and this was overall similar between the 2 groups (Figure 1, second column).

The number of rolling leukocytes was significantly increased in the catheterized animals compared with naïve controls (Figure 2A), while the number of adherent leukocytes was not significantly higher compared with naïve levels (Figure 2B). The number of extravascular leukocytes was significantly increased in the catheterized animals compared with naïve mice (Figure 2C). Leukocyte-endothelial cell interactions did not differ between catheterized controls and solution-treated animals with a catheter, however. Systemic leukocyte counts and differential leukocyte counts revealed monocytosis in the catheter control group (Table 1). Systemic leukocyte counts and differential leukocyte counts did not differ between the naïve mice that underwent IVM compared with baseline controls that did not undergo intravital imaging. The numbers of peritoneal leukocytes were elevated in the spent dialysate from the dialyzed animals compared with baseline peritoneal lavage levels, with $16.89 \pm 4.31 \times 10^6$ vs $3.52 \pm 0.43 \times 10^6$ total peritoneal leukocytes, respectively, but were not significantly different as determined by a Student's *t*-test, $p = 0.0526$. The spent dialysate was collected before laparotomy and tested negative for bacterial cultures grown overnight at 37°C on tryptic soy agar. These results indicate that the peritoneal catheter implant induced leukocyte rolling in the microvessels underlying the parietal peritoneum and resulted in increased accumulation of perivascular leukocytes independently from the dialysis solution treatment.

STRUCTURAL CHANGES IN THE ANTERIOR ABDOMINAL WALL

After IVM, samples of the abdominal wall were excised from the left upper quadrant and the left lower quadrant for staining and histopathologic analysis. This corresponded to the regions superior and inferior to the area in contact with the catheter. The H&E-stained sections showed similar structural changes of the peritoneal layer in mice that served as the catheterized controls and solution-treated animals (Figure 3A). Picro-sirius red-stained sections showed extensive collagen deposition in the thickened submesothelial connective tissue in both groups (Figure 3B). There were no differences in measurements of the peritoneal thickness (Figure 4A) and the vascular density (Figure 4B) between the catheterized controls and dialyzed animals. Since these samples were not directly in contact with the catheter, the structural changes observed indicate the catheter induced local responses in the peritoneum. There were no significant differences in the histologic changes between the sections taken from the upper and lower abdominal wall.

Resorcin-fuchsin-stained sections showed a network of elastic fibers lining the mesothelium in the naïve mice (Figure 5A). This elastic network was found near the base of the thickened submesothelial connective tissue in the catheterized control and dialyzed mice (Figure 5B) and no elastic fibers were observed near the mesothelial surface in these 2 groups (Figure 5C).

Figure 1 — Mice with peritoneal catheter implants had disruption of the mesothelial layer and increased leukocyte infiltration. BALB/c male mice were dialyzed with PD fluids daily over 6 weeks through a catheter implant. Four hours after the last dialysis solution (2.5 % glucose) injection, the mice were prepared for IVM and the parietal peritoneum and underlying microcirculation were imaged with transillumination technique. The mesothelial layer (first column), submesothelial compact zone (second column) and underlying venules (third column) were visualized. (A) Naïve mice had an intact mesothelial layer and had minimal leukocyte infiltration in the venules. (B) The group of mice that had catheter implants without dialysis and (C) the group that received dialysis fluids through the catheter had rounded and hypertrophied mesothelial cells and increased leukocyte infiltration in the peritoneal tissue. Representative images, $n = 4 - 6$ mice, scale bar = 20 μm . PD = peritoneal dialysis; IVM = intravital microscopy.

DISCUSSION

To determine the effects of the peritoneal catheter and dialysis solution on leukocyte recruitment, the mouse parietal peritoneum microcirculation was examined by IVM after subacute exposure to PD fluids administered through a catheter and compared with mice that had the catheter implant but no solution treatment. We observed that the number of rolling and extravascular leukocytes was significantly increased in the catheterized animals but did not significantly differ between catheter controls and solution-treated catheterized animals. Furthermore, the catheter induced fibrosis and increased vascularization of the parietal peritoneum without dialysis solution treatment. Thus, in this subacute mouse PD model, the catheter was responsible for inflammation, fibrosis and neovascularization in the parietal peritoneum.

Studies on the effects of PD solutions on leukocyte-endothelial cell interactions in the peritoneum were focused on the mesenteric microcirculation (8–11). To examine the early peritoneal response to PD fluids, rats were given IP injections of several different PD solutions with a dwell time of 5 hours. Intravital microscopy of the mesenteric venules showed that the number of rolling and adherent leukocytes was significantly increased after a single exposure to conventional PD solutions (8). Superfusion of the mesenteric venules of the small bowel with conventional PD fluids decreased leukocyte recruitment in response to *Escherichia coli* lipopolysaccharide in the rat compared with saline solution controls (9). After superfusion with the bacterial product *N*-formylmethionyl-leucyl-phenylalanine, on the other hand, injection of conventional dialysis solutions for 5 weeks increased leukocyte-endothelial cell interactions compared with naïve mice (10). In non-inflamed conditions, 5-week

Figure 2 — Catheterized mice had increased numbers of rolling and perivascular leukocytes in the parietal peritoneum microcirculation. The microcirculation of the parietal peritoneum was imaged with IVM and the number of (A) rolling and (B) adherent leukocytes in the peritoneal venules as well as the number of (C) extravascular leukocytes were quantified. Mice that had catheter implants, regardless of the presence of the dialysis solution, had significantly increased numbers of rolling and extravascular leukocytes. Data recorded as mean $\pm$ SEM and analyzed with ANOVA with Bonferroni correction, 4 – 6 venules averaged per count, $n = 4 - 6$ mice, [^] $p < 0.05$ and ^{**} $p < 0.001$ compared with naïve controls. IVM = intravital microscopy; SEM = standard error of the mean.

exposure to conventional dialysis solutions induced increased numbers of rolling leukocytes compared with the buffer control and this was not attributed to the catheter implant (10,11). Together, these studies indicate that exposure to PD fluids increases baseline rolling in mesenteric venules, and, during inflammation, PD fluids can lead to an increase or a decrease in leukocyte-endothelial cell interactions depending on the pro-inflammatory stimulus. Also, the peritoneal catheter appeared to have little effect on the inflammatory response of the visceral peritoneum microcirculation in these animal studies.

The microcirculation of the parietal peritoneum was chosen for examination in our study due to its major contributions to mass transport in dialysis (16,17) and the observation that the abdominal wall exhibits the greatest morphological changes in biopsies from PD patients (21). In particular, we were interested in this surface because the catheter implant runs directly through this tissue. In the mouse parietal peritoneum microcirculation, leukocyte rolling is mediated by P-selectin and β_2 integrin/CD18 (19). In the current report, all catheterized animals had an increased number of rolling leukocytes, and 6-week exposure to dialysis solution did not alter leukocyte-endothelial cell interactions in an additive fashion to the effects of the catheter. This is in contrast to the 2 studies which found that the venules of the visceral peritoneum had significantly increased numbers of rolling leukocytes after subacute exposure to conventional PD fluids but the catheterized control groups did not have altered baseline rolling compared with the naïve animals (10,11). Together, these findings highlight an important difference in the inflammatory responses of the visceral and parietal peritoneum microvascular beds to the PD fluids and the catheter implant.

Inflammation involves neutrophil mobilization from the bone marrow into the blood (22). The total blood leukocyte counts were elevated in the catheterized groups but a significant increase was only observed in the catheter control group compared with naïve mice. Interestingly, this group also had a significant increase in systemic monocytes. Monocyte recruitment to implants as a host response to foreign bodies is well described, where monocytes are recruited and macrophages adhere and fuse to form foreign body giant cells (23). No

TABLE 1
Systemic Leukocyte Counts

Group	Total cells $\times 10^9/L$	Neutrophils	Lymphocytes	Monocytes
Baseline	4.08 $\pm$ 0.67	0.99 $\pm$ 0.27	3.06 $\pm$ 0.42	0.037 $\pm$ 0.010
Naïve	5.74 $\pm$ 0.83	1.43 $\pm$ 0.37	4.17 $\pm$ 0.56	0.10 $\pm$ 0.02
Catheter	10.87 $\pm$ 2.31 ^{^*}	3.17 $\pm$ 0.79	5.13 $\pm$ 2.06	0.64 $\pm$ 0.23 ^{^*}
Catheter + dialysis	8.87 $\pm$ 1.27 [^]	2.47 $\pm$ 1.14	6.01 $\pm$ 0.90	0.37 $\pm$ 0.09

IVM = intravital microscopy; SEM = standard error of the mean.

After the completion of surgery and IVM observations, blood was collected via cardiac puncture and total systemic leukocyte counts and differential leukocyte counts were performed. Catheterized animals without dialysis had significantly increased numbers of systemic leukocytes and systemic monocytes compared with naïve controls. Data recorded as mean $\pm$ SEM and analyzed with ANOVA with Bonferroni correction, $n = 4 - 6$ mice.

[^] $p < 0.05$ compared with baseline.

^{*} $p < 0.05$ compared with naïve controls.

Figure 3 — Catheterized control mice and dialyzed mice had similar histologic alterations in the parietal peritoneum. (A) H&E-stained sections of the left anterior abdominal wall, collected after completion of IVM, show a thin peritoneal layer in the naïve mice while the catheter control and dialyzed groups both had peritoneal thickening, hypercellularity and increased vascularization after 8-week exposure to the catheter implant. The second row shows increased vascular density in the submesothelial zone. (B) Picro-sirius red-stained sections of the abdominal wall show that the thickened submesothelial extracellular matrix was rich in collagen, characteristic of fibrotic tissues. Representative images, $n = 4 - 6$ mice, scale bar = 100 μm . H&E = hematoxylin and eosin; IVM = intravital microscopy.

significant differences in the number of systemic neutrophils were observed between any of the groups. Although surgical preparation and IVM imaging are expected to contribute to the inflammation, the systemic leukocyte counts and differential white blood cell counts were not significantly different in the naïve mice used for IVM compared with baseline controls that were not subjected to intravital imaging. This indicates that the effects of the procedure were minimal in our study.

Part of the damage to the peritoneal membrane observed in chronic animal models of PD was shown to be caused by the presence of the catheter, where injection of a sterile 4% glucose solution through a catheter resulted in increased thickness and vascular density of the submesothelial compact zone in the parietal peritoneum, and increased growth factor

expression compared with solution-treated rats with an IP needle injection (12). Furthermore, animals with silicone catheters tunnelled into the peritoneal cavity that were not exposed to dialysis solutions exhibited changes in the peritoneum at 4 weeks after catheter implantation (13). Interestingly, these animals had more striking histopathologic alterations of the peritoneum than animals treated with a 4% glucose solution through a catheter for 20 weeks. Animals that did not have a catheter implanted but received daily IP injections of the dialysis solution, on the other hand, exhibited relatively less alterations in the peritoneum (13). In the current study, we observed significantly increased levels of fibrosis, manifested by increased peritoneal thickness, and increased vascular density after 8-week exposure to the peritoneal catheter. Collagen

Figure 4 — The peritoneal catheter induced severe fibrosis and angiogenesis in the peritoneal layer. The (A) thickness and (B) vascular density of the peritoneal layer were quantified from the H&E-stained sections. The catheter control group exhibited similar levels of fibrosis and vascularization in the peritoneal layer of the upper and lower abdominal wall as the dialyzed mice. Data recorded as mean $\pm$ SEM and analyzed with ANOVA with Bonferroni correction, $n = 4 - 6$ mice, [^] $p < 0.05$ and ^{*} $p < 0.01$ compared with naïve controls. H&E = hematoxylin and eosin; SEM = standard error of the mean.

deposition, an indicator of peritoneal fibrosis (24–27), was detected by picro-sirius red, which demonstrated that the thickened submesothelial matrix is rich in collagen fibers in catheterized animals. Also, in the IVM images, the submesothelial connective tissue appeared to have less organized collagen bundles in the catheterized animals compared with naïve mice. The pathological alterations were similar between catheterized animals with and without dialysis fluid, indicating that the catheter was mainly responsible for these fibrotic changes.

In transforming growth factor beta 1 (TGF- β 1)-induced fibrosis of the parietal peritoneum, the basement membrane was displaced from its position directly beneath the mesothelium to several cell layers beneath the mesothelial layer due to cellular expansion above and below the basement membrane, while a new basement membrane formed near the mesothelium (28). In the normal human visceral and parietal peritoneum, a network of elastic fibers was observed immediately beneath the mesothelial basement membrane (29). We also observed a layer rich in elastic fibers running along the mesothelial layer in the naïve animals. Similarly to what was observed in

the basement membrane changes in fibrosis (28), the elastic layer spanned the thickened submesothelial matrix closer to its base (near the musculature) in the catheterized control and dialysis solution-treated mice in our study. Taken together, these observations indicate that 8-week exposure to the catheter caused peritoneal fibrosis, which included collagen deposition and dislocation of the elastic fibers from directly beneath the mesothelium to several cell layers beneath the mesothelial surface.

Peritoneal dialysis promotes new vessel formation (11) and it is easier for neutrophils to transmigrate because there is less resistance in the maturing vessel wall with underdeveloped basal lamina that are less abundant in pericytes. We observed a significant increase in the number of extravascular leukocytes in both groups of catheterized mice compared with naïve animals. However, again, there was no difference with the addition of PD solution, indicating that the increased leukocyte extravasation was a response to the catheter rather than the solution. Examination of the leukocyte-endothelial cell interactions with *in vivo* models of PD is important as shown by a double-layer transwell model of the peritoneal membrane with an endothelial and a mesothelial layer (30). This model showed that the endothelial cells are the rate determinants of neutrophil migration across this double layer while the mesothelial barrier did not have an additive effect (30). Thus, the ability of neutrophils to interact with and migrate across the endothelium impacts neutrophil accumulation in the peritoneal tissue and cavity, which is important in bacterial peritonitis where clearance of bacteria is correlated with neutrophil influx in the peritoneal cavity (30). We observed an approximately 5-fold increase in IP leukocytes in the dialyzed mice compared with baseline values, although the increase was not significant as determined by a Student's *t*-test. This correlates with observations in PD patients, where leukocytosis is detected in spent dialysate in the absence of infection in the first 2 weeks after catheter implantation (31).

The animal model in our study does not precisely simulate PD, and so its clinical relevance has limitations. Peritoneal dialysis patients experience some degree of kidney failure and develop uremia, while the mice in our study were nonuremic in an effort to avoid complications of surgery with nephrectomy and development of uremia. However, uremic animal models indicate that there are differences in peritoneal permeability to solutes (32), and uremia impacts inflammatory responses (33). Furthermore, our model involved injections of solutions through silicone catheters which were not drained, and 1 injection was made per day, unlike the 4 – 6 fluid exchanges performed in continuous ambulatory PD per day. Lastly, the catheter to peritoneal cavity ratio was greater in our mouse model than in PD patients, which can promote more local inflammatory responses in the peritoneum. Nevertheless, our model utilized a conventional dialysis solution and silicone catheter material commonly used in PD and responses were assessed by cutting-edge *in vivo* imaging. Thus, these results still offer valuable insight into the inflammatory microvascular responses to the peritoneal catheter.

Figure 5 — (A) Elastic tissue (arrows) shown in resorcin-fuchsin-stained sections of the anterior abdominal wall counterstained in van Gieson's solution and Weigert's iron hematoxylin. (B) The network of elastic fibers lined the mesothelium in the naïve mice and was found at the base of the thickened submesothelial extracellular matrix in the catheterized control mice and dialyzed mice (arrows). (C) Elastic fibers did not appear near the peritoneal surface in the catheterized control and dialyzed groups. Representative images, $n = 4 - 6$ mice, scale bar = 100 μm .

Our *in vivo* data showed that increased leukocyte recruitment and histopathologic alterations in the parietal peritoneum and the underlying microcirculation resulted from the presence of the peritoneal catheter and not the PD solution. The pro-inflammatory effects of the catheter are significant because inflammation was found to be associated with fibrosis and functional deterioration of the peritoneum (34). Furthermore, these results stress the importance of distinction between the effects of the catheter and PD fluid with animal models. As well, our report on the responses to a foreign body in the parietal peritoneum microcirculation is a novel addition to IVM models of chronic inflammation.

DISCLOSURE

The authors have no financial conflicts of interest to declare.

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