PROTEIN ADSORPTION ON EX VIVO CATHETERS AND POLYMERS EXPOSED TO PERITONEAL DIALYSIS EFFLUENT

Naoko Yanagisawa, Dai-Qing Li, and Åsa Ljungh

Medical Microbiology, Dermatology and Infection, Lund University, Lund, Sweden

Objective: Adsorption of proteins on catheters exposed both ex vivo and in vitro to dialysate of patients on peritoneal dialysis (PD) was studied.

Methods: Peritoneal dialysis effluent was collected from 5 patients with end-stage renal disease on continuous ambulatory PD. Tenckhoff catheters were obtained from 16 patients. Deposition of proteins on excised Tenckhoff catheters and tubing of different materials exposed to PD effluent in vitro was studied using 125I-iodine-labeled antibodies. Adhesion of Staphylococcus aureus and Staphylococcus epidermidis strains was quantified on tubing exposed to PD effluent in vitro.

Results: The presence of albumin, transferrin, immunoglobulin G, fibrinogen, fibronectin, von Willebrand factor, vitronectin, and thrombospondin was determined at various concentrations in PD effluent. All proteins analyzed were detected on PD catheters removed from patients. The extent of protein deposition on Tenckhoff catheters exposed to PD effluent, in vitro, rapidly reached a plateau and remained constant, as it did on polyvinyl chloride and polyethylene tubing. Adhesion of staphylococci was enhanced on Tenckhoff catheters exposed to PD effluent compared to unused PD solution.

Conclusions: The data identify surface exposed proteins that may serve as adhesion sites for microbes on peritoneal catheters indwelled in patients undergoing PD.


KEY WORDS: Biomaterial; protein adsorption; medical grade silicone; polyethylene; polyvinyl chloride; Staphylococcus epidermidis; vitronectin; von Willebrand factor.

Background: Deposition of proteins on surfaces of medical devices has been recognized to putatively relate to the process of regulation of biomaterial-associated complications by attachment of fibrin clots, eukaryotic cells, and microbes. The molecules adsorb to a varying extent, depending not only on the physicochemical properties of the biomaterial, but also on the composition of the host fluid.

Objective: Adsorption of proteins on catheters exposed both ex vivo and in vitro to dialysate of patients on peritoneal dialysis (PD) was studied.

Methods: Peritoneal dialysis effluent was collected from 5 patients with end-stage renal disease on continuous ambulatory PD. Tenckhoff catheters were obtained from 16 patients. Deposition of proteins on excised Tenckhoff catheters and tubing of different materials exposed to PD effluent in vitro was studied using 125I-iodine-labeled antibodies. Adhesion of Staphylococcus aureus and Staphylococcus epidermidis strains was quantified on tubing exposed to PD effluent in vitro.

Results: The presence of albumin, transferrin, immunoglobulin G, fibrinogen, fibronectin, von Willebrand factor, vitronectin, and thrombospondin was determined at various concentrations in PD effluent. All proteins analyzed were detected on PD catheters removed from patients. The extent of protein deposition on Tenckhoff catheters exposed to PD effluent, in vitro, rapidly reached a plateau and remained constant, as it did on polyvinyl chloride and polyethylene tubing. Adhesion of staphylococci was enhanced on Tenckhoff catheters exposed to PD effluent compared to unused PD solution.

Conclusions: The data identify surface exposed proteins that may serve as adhesion sites for microbes on peritoneal catheters indwelled in patients undergoing PD.


KEY WORDS: Biomaterial; protein adsorption; medical grade silicone; polyethylene; polyvinyl chloride; Staphylococcus epidermidis; vitronectin; von Willebrand factor.

Background: Deposition of proteins on surfaces of medical devices has been recognized to putatively relate to the process of regulation of biomaterial-associated complications by attachment of fibrin clots, eukaryotic cells, and microbes. The molecules adsorb to a varying extent, depending not only on the physicochemical properties of the biomaterial, but also on the composition of the host fluid.

Objective: Adsorption of proteins on catheters exposed both ex vivo and in vitro to dialysate of patients on peritoneal dialysis (PD) was studied.

Methods: Peritoneal dialysis effluent was collected from 5 patients with end-stage renal disease on continuous ambulatory PD. Tenckhoff catheters were obtained from 16 patients. Deposition of proteins on excised Tenckhoff catheters and tubing of different materials exposed to PD effluent in vitro was studied using 125I-iodine-labeled antibodies. Adhesion of Staphylococcus aureus and Staphylococcus epidermidis strains was quantified on tubing exposed to PD effluent in vitro.

Results: The presence of albumin, transferrin, immunoglobulin G, fibrinogen, fibronectin, von Willebrand factor, vitronectin, and thrombospondin was determined at various concentrations in PD effluent. All proteins analyzed were detected on PD catheters removed from patients. The extent of protein deposition on Tenckhoff catheters exposed to PD effluent, in vitro, rapidly reached a plateau and remained constant, as it did on polyvinyl chloride and polyethylene tubing. Adhesion of staphylococci was enhanced on Tenckhoff catheters exposed to PD effluent compared to unused PD solution.

Conclusions: The data identify surface exposed proteins that may serve as adhesion sites for microbes on peritoneal catheters indwelled in patients undergoing PD.


KEY WORDS: Biomaterial; protein adsorption; medical grade silicone; polyethylene; polyvinyl chloride; Staphylococcus epidermidis; vitronectin; von Willebrand factor.

Peritoneal dialysis (PD) is an established form of therapy in the management of end-stage renal disease and entails a permanently indwelled catheter placed percutaneously into the peritoneal cavity. Morbidity due to catheter-related complications, including luminal obstruction (1), exit-site/tunnel infection (2), and peritonitis (3), has limited the more widespread use of the technique. Staphylococcus aureus accounts for the majority of episodes of exit-site/tunnel infection. Coagulase-negative staphylococci, predominantly S. epidermidis, are the most frequently isolated organisms in peritonitis associated with PD, followed by S. aureus (2). Nasal carriage of S. aureus has been recognized as a risk factor for endogenous infections associated with PD (4). Staphylococcus epidermidis strains of skin flora have been shown to spread in a contiguous route, by which microbes pass subcutaneously along the catheter surface and traverse the peritoneum (3).

Soon after implantation, the surface of foreign body materials becomes coated with host serum proteins. Staphylococcal infection associated with indwelling medical devices initiates when microbes attach to and colonize the surface. Once primary attachment of planktonic cells has occurred, microbes generate developmental signals through activation of a number of genes, such as the intracellular adhesion locus and the sigma factor in the genus Staphylococcus, and transform to metabolically distinct sessile cells, which thereafter generate extracellular factors that assist in the formation of biofilm (5). Formation of biofilm by staphylococci is a major concern in catheter-related infections in PD because it protects microorganisms from opsonophagocytosis and antibiotics (3). For the purpose of attachment to host proteins, staphylococci carry several surface receptors with the ability to bind plasma and extracellular matrix proteins, which include the microbial surface components-recognizing adhesive matrix molecules (MSCRAMMs) anchored to the cell wall by the LPXTG motif, such as protein A (Spa) (6), clumping factors (Clf A and B) (7,8), fibronectin-binding proteins (Fnbp A and B) (9), and the cell wall-associated, but not covalently linked,
extracellular proteins such as coagulase, extracellular fibrinogen-binding protein (Efb) (10,11), extracellular adherence protein (Eap) (12), and autolysin (13). Binding of receptors to host proteins that rapidly coat indwelling medical devices after implantation not only promotes adherence of microbes to the surfaces of biopolymers, but also prevents activation of the complement system (14).

Protein adsorption depends on the competitive processes among the constituents, and has been shown to be different in a complex- than in a single-protein solution (15). The conformation of protein layers on medical devices varies with the protein composition of the fluid (16) as well as with the physicochemical properties of the biomaterial (17). Protein adsorption from blood plasma and serum to surfaces of medical devices has been extensively studied (18,19). Profiles of proteins on the surfaces of catheters exposed to peritoneal dialysate, however, have not been investigated. The purpose of this study was to demonstrate the presence of host proteins that are prone to possessing biological functions on the surface of PD catheters. This report is the first attempt to demonstrate adsorption of individual proteins from PD effluents on Tenckhoff catheters and other polymer tubing. The amount of proteins in PD effluent that are prone to become associated with catheter-related complications was quantified. The results suggest that catheter surfaces are coated with host proteins immediately after implantation, which may mediate bacteria to bind to the surface.

MATERIALS AND METHODS

CHEMICALS

Fresh human plasma was purchased from the blood bank of Lund University Hospital. Fibronectin (Fn) and vitronectin (Vn) were purified from human plasma as previously described (20,21). Human thrombospondin (TSP) and recombinant von Willebrand factor (vWF) were kind gifts from Professor J. Lawler, Boston, USA, and Professor F. Dorner, Vienna, Austria, respectively. Rabbit antibodies to human Vn and TSP were raised from serum as previously described (22). Human albumin (Alb), immunoglobulin G (IgG), and transferrin (Tf) were from Sigma Chemical Co., St. Louis, Missouri, USA. Human fibrinogen (Fg) was from Imco AB, Stockholm, Sweden. Rabbit antibodies to human Alb, IgG, Fg, Fn, Tf, and vWF, and swine anti-rabbit antibody were purchased from Dakopatts Immunoglobulins A/S, Copenhagen, Denmark. The NaI was from Amersham Plc., Little Chalfont, Buckinghamshire, UK. Iodo Beads were from Pierce Chemicals, Rockford, Illinois, USA. Polyvinylidene difluoride (PVDF) membranes were from Micron Separations Inc., Westborough, Massachusetts, USA. All common chemicals were purchased from Kebo, Spånga, Sweden.

EX VIVO PD CATHETERS

Excised Tenckhoff catheters of medical grade silicone were from 16 patients. The patients’ profiles are listed in Table 1. Tubes were cut transversely into 5-mm-long pieces and rinsed three times for 10 minutes each in 0.07 mol/L phosphate-buffered saline, pH 7.2 (PBS) prior to measurement of protein deposition.

PERITONEAL DIALYSIS EFFLUENT

Peritoneal dialysis effluent was obtained from 5 patients with chronic renal failure on continuous ambulatory peritoneal dialysis (CAPD). Dialysate from patients with signs of infection or subjected to antibiotic treatment was discarded. Effluent from each patient was filtered using Millex-GV 0.22-µm filters (Millipore, Stockholm, Sweden) to omit any bacterial contamination, and was immediately stored at –70°C until use, at the longest for 5 months. Bacterial contamination in the effluent was simultaneously confirmed by negative detection of colonies after cultivation in both aerobic and anaerobic conditions.

POLYMER TUBING

For in vitro studies, medical grade silicone Tenckhoff catheters (516/5019651; Fresenius Medical Care Deutschland GmbH, Bad Homburg, Germany), polyvinyl chloride (PVC) tubes (Portex 800/000/180; Hemex Medical, Sollentuna, Sweden) (23), each with an outer diameter of 5 mm and an inner diameter of 3 mm, and polyethylene (PE) tubes (CP6407-020; AB Kemila-Preparat, Sollentuna, Sweden) with an outer diameter of 6 mm and an inner diameter of 3 mm, were used. All tube materials were cut transversely into 5-mm-long pieces and were subjected to exposure to PD fluids.

EXPOSURE OF POLYMER TUBING TO POOLED PD EFFLUENT

Peritoneal dialysis effluent collected from 5 patients was thawed from –70°C and was pooled together to incubate with polymer tubing materials. We used the incubation model of shear rate flow developed by Ortega–Vinuesa et al. to adsorb total serum proteins to silicone materials (24), with minor modifications. In brief, incubation of each piece of tubing placed in a cylinder with a diameter of 10 mm was performed on a horizontal gyratory shaker (MST4;
IKA-Werke, Staufen, Germany) under 60 rpm at 37°C with 1-mL volume of PD effluent. The exposure time of the polymer tubing was 1, 4, 24, and 48 hours, adapted from the procedure for various polymeric materials to adsorb proteins from human plasma by Falkenback et al. (19) and cerebrospinal fluid by Lundberg et al. (25).

DETECTION OF DEPOSITED PROTEINS

The extent of adsorbed proteins was determined both on ex vivo Tenckhoff catheters and on tubing exposed to PD effluent in vitro. Catheter pieces (5 mm long) were incubated with rabbit antibodies against each protein, diluted 1:100 in PBS with 1% bovine serum albumin (BSA) in a total volume of 0.5 mL for 1 hour at 22°C. The pieces of tubing were rinsed three times for 10 minutes each, blocked with 1% BSA-PBS for 1 hour at 37°C, and rinsed again. Swine anti-rabbit Ig was labeled with Na125I according to a modified chloramine-T method using Iodo Beads (26). The specific activity was 1.2 × 10^6 cpm/µg Ig. Radiolabeled antibody was diluted 1:100 in 1% BSA-PBS in a total volume of 0.5 mL and incubated for 1 hour at 22°C. The pieces were rinsed and the amount of bound radioactivity corresponding to the extent of deposition of adsorbed proteins was measured on a gamma counter (LKB-Wallac 2772; Wallac, Turku, Finland) (25). The extent of deposition was expressed as the percentage of total added radioactivity per surface area of each catheter piece.

PROTEIN CONTENT OF PD EFFLUENT

Albumin and IgG were included for investigation of their presence in the peritoneal dialysate because of their high quantity in blood, from which dialysate is ultrafiltered. Predominant pathogens of infections associated with PD, Staphylococcus species, have been shown to express binding ability of physiological fluid proteins, such as Fg (7,10,11,27,28), Fn (8,9), Vn (23,26), Tf (29), vWF (30), and TSP (31). Therefore, these proteins were chosen for investigation. Peritoneal dialysis effluents collected from patients were thawed from –70°C. The protein content of PD effluent from each of the 5 patients was determined separately with a GS-710 Calibrated Imaging Densitometer (BioRad Laboratories GmbH, Munich, Germany) on PVDF membranes using purified proteins as standards. The concentration of each protein in each patient effluent sample was measured in triplicate; the mean value of 5 patient samples was calculated.

BACTERIAL STRAINS AND CULTURE CONDITIONS

Twelve staphylococci strains were used in the experiments. Four S. aureus strains (C1932, C5192, C1734, and C4302) and five S. epidermidis strains (C4226, C5436, C1274, C4136, and C3672) were isolated from PD fluid obtained from patients with CAPD-associated peritonitis (Lund University Hospital, Lund, Sweden). Staphylococcus aureus Newman

**TABLE 1**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Duration of PD (months)</th>
<th>Duration of implantation (months)</th>
<th>PD regimen</th>
<th>Reason for excision</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>M</td>
<td>5</td>
<td>5</td>
<td>NIPD</td>
<td>Renal transplant</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>F</td>
<td>51</td>
<td>51</td>
<td>CAPD</td>
<td>Intracerebral hematoma</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>F</td>
<td>121</td>
<td>25^a</td>
<td>CAPD</td>
<td>Ultrafiltration failure</td>
</tr>
<tr>
<td>4</td>
<td>68</td>
<td>F</td>
<td>64</td>
<td>83</td>
<td>CAPD</td>
<td>Ultrafiltration failure</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>F</td>
<td>19</td>
<td>19</td>
<td>NIPD</td>
<td>Renal transplant</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>M</td>
<td>23</td>
<td>23</td>
<td>CAPD</td>
<td>Renal transplant</td>
</tr>
<tr>
<td>7</td>
<td>61</td>
<td>M</td>
<td>50</td>
<td>50</td>
<td>CAPD</td>
<td>Exit-site infection</td>
</tr>
<tr>
<td>8</td>
<td>36</td>
<td>F</td>
<td>4</td>
<td>4</td>
<td>CAPD/APD</td>
<td>Noncompliance</td>
</tr>
<tr>
<td>9</td>
<td>3 weeks</td>
<td>M</td>
<td>5 days</td>
<td>5 days</td>
<td>CAPD</td>
<td>Maple syrup urine disease</td>
</tr>
<tr>
<td>10</td>
<td>39</td>
<td>F</td>
<td>14</td>
<td>15</td>
<td>CAPD/APD</td>
<td>Exit-site infection/peritonitis</td>
</tr>
<tr>
<td>11</td>
<td>76</td>
<td>M</td>
<td>2</td>
<td>2</td>
<td>CAPD</td>
<td>Peritonitis</td>
</tr>
<tr>
<td>12</td>
<td>62</td>
<td>M</td>
<td>5</td>
<td>5</td>
<td>CAPD</td>
<td>Peritonitis</td>
</tr>
<tr>
<td>13</td>
<td>96</td>
<td>M</td>
<td>19</td>
<td>22</td>
<td>CAPD/NIPD</td>
<td>Ultrafiltration failure</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>M</td>
<td>24 days</td>
<td>24 days</td>
<td>CAPD</td>
<td>Colon perforation</td>
</tr>
<tr>
<td>15</td>
<td>56</td>
<td>M</td>
<td>4</td>
<td>6</td>
<td>CAPD</td>
<td>Peritonitis</td>
</tr>
<tr>
<td>16</td>
<td>56</td>
<td>M</td>
<td>11</td>
<td>11</td>
<td>CAPD</td>
<td>Exit-site infection</td>
</tr>
</tbody>
</table>

NIPD = nightly intermittent peritoneal dialysis; CAPD = continuous ambulatory peritoneal dialysis; APD = automated peritoneal dialysis.

a Tenckhoff catheter was re-implanted subsequent to an episode of tunnel infection.
was used as a reference strain as it expresses protein A (Spa) (6) and binds Fg (7,10) and Fn (9). Staphylococcus aureus Cowan 1, which binds Fg (11), Fn (8), Vn (26), VWF (30), and TSP (31), and a slime non-producing, Fg-binding S. epidermidis 19 isolated from peritonitis (27) were also included as reference strains. Bacterial cells were grown in Todd–Hewitt broth at 37°C for 22 hours on a gyratory shaker with vigorous agitation, and were washed twice in PBS. The bacterial concentration was adjusted to 1 × 10^8 cfu/mL by calculation from a standard curve of optical density at 540 nm, and immediately used for the binding assays.

**BACTERIAL ADHESION ASSAYS**

Peritoneal dialysis effluent collected from 5 patients was thawed from −70°C, pooled together, and used immediately to incubate tubing materials prior to incubation with bacteria in the same volume and flow rate as was used in the detection of adsorbed proteins. Control tubing pieces were incubated with an equal volume of unused PD solution (Gambrosol trio 10, 2.5% glucose; Gambro Lundia AB, Lund, Sweden). To examine the extent of bacterial binding to Tenckhoff catheters, the incubation time with PD effluent was chosen to adsorb the maximal amount of proteins on the catheter surface. Protein adsorption on medical grade silicone Tenckhoff catheters exposed to PD effluent was investigated by in vitro incubation time of 1 hour was chosen for exposure of PD fluids to catheters prior to bacterial inoculation. After incubation with PD effluent or unused solution, materials were washed with PBS and blocked with 1% BSA-PBS for 1 hour at 37°C. Bacteria were resuspended in 1% BSA-PBS at a concentration of 10^7 cells/mL. Initial binding of bacteria to the PD effluent-exposed catheter surface was investigated by incubating each tubing in 1 mL of the bacterial suspension for 1 hour at 37°C, according to Yu et al. (32). Subsequent to initial attachment of planktonic bacteria, irreversible adhesion of sessile bacteria occurs in sequential steps involving secretion of extracellular polysaccharides embedding the cells in the biofilm. Bacterial colony counts resembling those in the later phase of attachment were analyzed 48 hours after bacterial inoculation, according to Dasgupta et al. (33).

**BACTERIAL ADHESION ASSAY BY BIOLUMINESCENCE**

Bacterial adherence was quantified using a modified bioluminescence assay (14,30). After incubation with bacteria, catheter pieces were washed three times in 0.1 mol/L Tris-acetate, 2 mmol/L EDTA, pH 7.75 (TAE), and placed in sterile glass tubes. Adenosine triphosphate (ATP) was extracted from adhered bacteria by addition of 1 mL 2.5% trichloroacetic acid (TCA) to the catheter pieces. Extraction fluid (100 µL) was immediately transferred to 700 µL TAE in a clean polystyrene cuvette (Clinicon AB, Bromma, Sweden). Light emission corresponding to the amount of sample ATP (Ismp) was recorded in a luminometer (LKB Wallac 1250 Luminoimeter, Wallac) with automated addition of 200 µL ATP monitoring reagent (BioThema AB, Haninge, Sweden) to each cuvette. Light emission (Ismp) of sample plus 10 µL of ATP standard (BioThema AB) was recorded after each sample measurement. A blank containing 700 µL TAE and 100 µL 2.5% TCA was measured simultaneously and was calibrated. The amount of ATP in each sample was calculated according to, ATP (Ismp) = 10^7 × Ismp / (Ismp + std – Ismp). The number of bacterial cells that had attached to the polymeric piece per surface area was calculated from a standard curve of ATP concentration versus viable staphylococcal cells (correlation coefficient 0.98).

**STATISTICS**

Mann-Whitney U test was performed to investigate the significance of the amount of each protein, or bacterial colony counts, adsorbed on catheters. Kruskal–Wallis ANOVA was performed to determine significant differences in the amount of each adsorbed protein between exposure times or materials. A p value < 0.05 was considered significant.

**RESULTS**

**LEVEL OF PROTEINS IN PD EFFLUENT**

The amount of each protein was quantified in PD effluent samples from each of the 5 patients separately in triplicate, and the mean concentration value (SEM) of all 5 patient samples was calculated. Albumin, Tf, IgG, Fg, Fn, Vn, VWF, and TSP were present in the peritoneal dialysate at various levels, as shown in Table 2.

**DETECTION OF ADSORBED PROTEINS ON TENCKHOF CATHETERS EX VIVO**

Tenckhoff catheters removed from patients revealed significant values of adsorbed proteins IgG, Alb, Fg, Fn, Tf, Vn, VWF, and TSP (Figure 1). The studied Tenckhoff catheters had been removed from a heterogeneous group including 8 patients with history of infection and variable duration of implantation. In order to clarify
whether these variables have an impact on the quantity and quality of proteins detected on the catheter surface, an attempt was made to statistically compare positive and negative histories of infection. However, a history of infection did not influence the amount of each protein on the catheter surface (IgG, Alb, Fg, Fn, Tf, Vn, vWF, and TSP, respectively; Patients 3, 7, 10 – 12, 14 – 16 vs Patients 1, 2, 4 – 6, 8, 9, 13; \( p > 0.05 \) Mann–Whitney U test, data not shown). A statistical approach was also made for comparison between durations of implantation. However, the dwell time of catheters in the range of samples included in the study did not influence the amount of each protein on the catheter surface [IgG, Alb, Fg, Fn, Tf, Vn, vWF, and TSP, respectively; 5 days – 5 months (\( n = 6 \)) vs 6 – 83 months (\( n = 10 \)), \( p > 0.05 \) Mann–Whitney U test, data not shown].

### PROTEIN ADSORPTION ON POLYMER TUBING EXPOSED TO PD EFFLUENT IN VITRO

Since microbes commonly gain access to the device shortly after insertion (3), protein profiles during the first 48 hours were investigated in more detail (Figure 2). Medical grade, silicone Tenckhoff catheters are of the most frequently used material for peritoneal catheters in PD, thus it was used to analyze protein deposition in vitro. The amount of proteins on the surfaces reached maximum level after 1 hour of exposure to peritoneal dialysate, and the levels remained constant. Protein deposition from PD effluent was investigated on different surfaces. PVC and PE were chosen as clinically relevant materials. Binding of each protein at each time point was compared between three materials. All three materials bound each protein to equivalent extents at each time point (medical grade silicone vs PVC vs PE at exposure intervals: 1 hour, \( p > 0.05 \); 4 hours, \( p > 0.05 \); 24 hours, \( p > 0.05 \); 48 hours, \( p > 0.05 \); Kruskal–Wallis ANOVA).

### BACTERIAL ADHESION TO TUBING EXPOSED TO PD EFFLUENT IN VITRO

Since the amount of adsorbed protein from PD effluent reached maximum level beyond 1 hour of in-

---

**Table 2**

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Dialysate protein contenta (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>3382±375</td>
</tr>
<tr>
<td>Transferrin</td>
<td>290±35</td>
</tr>
<tr>
<td>IgG</td>
<td>258±56</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>229±47</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>72±7</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>26±3</td>
</tr>
<tr>
<td>Vitronektin</td>
<td>4.6±2.6</td>
</tr>
<tr>
<td>Thrombospondin</td>
<td>0.002±0</td>
</tr>
</tbody>
</table>

\( ^a \) Concentration of each protein in each patient PD effluent was measured in triplicate. Data presented are mean value ± SEM of protein amount of 5 patients' samples.

---

**Figure 1** — Detection of proteins on excised Tenckhoff catheters. Percentages bound of total added radioactivity (cpm) per surface area (cm²) of ex vivo Tenckhoff catheters. Data presented are mean values of catheter pieces from 16 patients, tested in duplicate. Mean value of two exposed samples incubated without primary antibodies was subtracted from each datum of the same patient. Horizontal bars denote median values of each bound protein. Alb = albumin; Fg = fibrinogen; Fn = fibronectin; Tf = transferrin; Vn = vitronectin; vWF = von Willebrand factor; TSP = thrombospondin.
Cubation, tubing materials were incubated with PD effluent for 1 hour at 37°C prior to addition of bacteria. One hour after inoculation with bacteria, all S. aureus [Figure 3(a)] and five S. epidermidis strains [Figure 3(b)] were bound to Tenckhoff catheter pieces exposed to used PD effluent at significantly greater extents compared to those exposed to unused PD solution. Adhered bacterial counts of strain Newman, as well as three clinical isolates of S. aureus and all strains of S. epidermidis, increased after 48 hours compared to 1 hour of bacterial incubation (p < 0.05 Mann–Whitney U test, data not shown). At 48 hours, viable cell counts of all S. aureus and three S. epidermidis strains were significantly greater on catheters exposed to PD effluent compared to those exposed to unused solution (Figure 3).

**DISCUSSION**

A considerable variety of proteins representing several functional classes were found in the PD effluent. The concentrations of Alb (34), IgG (35), Fg (34), Fn (36), and Tf (34) in the PD fluid were similar to those presented elsewhere. There has been no previous report on the presence of Vn, vWF, and TSP in peritoneal dialysate. Correlation between the amount of proteins lost in the peritoneal dialysate and their molecular weights was observed, indicating diffusion of proteins from adjacent blood vessels. However, vWF was found in the PD effluent at extremely high levels relative to the assumed concentration of each protein in normal blood. This may indicate local production of the protein in the peritoneal cavity (37).

Tenckhoff catheter surfaces have been assessed in high resolution detail by means such as scanning electron microscopy (38,39) and confocal laser scanning microscopy (39) where the topography of the catheter surface biofilm, consisting of a multilayered conformation of host and bacterial cells, was evaluated. The extent of individual proteins on different samples can be compared using radiolabeled antibodies, as shown. Previous studies of protein adsorption to various types of clinically relevant materials have obtained results similar to our study after perfusion with diluted serum, where quantified protein amounts were greatest for Alb, IgG, and Fg. Thrombospondin and vWF adsorbed to the least extents (18,19). Cerebrospinal fluid shunts have been shown ex vivo to adsorb Vn, Fn, and Fg, as well as TSP despite the low concentration of TSP in cerebrospinal fluid (25). However, our data on ex vivo Tenckhoff catheters are not compatible with the finding on hemodialysis membranes, where Vn and TSP were lacking (28). The variation in the adsorbed proteins may explain the different surface events on different medical devices, since the adsorbed proteins constituting the outermost...
layer may be prone to expose biologically active domains.

To reproduce deposition of proteins on patient-derived catheters, in vitro exposure of PD effluent was attempted. Fluid flow in the peritoneal cavity is achieved by continuous ultrafiltration and is influenced by several factors, such as routine exchanges of dialysis fluid and intraperitoneal hydrostatic pre-
sure (40). Shear stress influences the amount and configuration of the proteins that adsorb to biomaterial surfaces (16,24). To mimic the flow condition to which the Tenckhoff catheter is exposed in the peritoneal cavity, incubation was performed under agitation to maintain fluid movement surrounding the surface (24). Comparison of the adsorbed proteins was made between the excised Tenckhoff catheters and the tubing exposed to PD effluent in vitro. In vitro exposure of PD effluent, revealing Alb, Fg, Fn, Tf, and Vn on the surfaces of Tenckhoff catheters, was equivalent in both quality and quantity to catheters exposed in patients (48 hours’ incubation vs ex vivo catheters: Fg, Fn, and Vn, respectively, p > 0.05; Alb and Tf respectively, p > 0.01; Mann Whitney U test). These results may suggest that the procedure used in the present study is able to deliver a sufficient number of molecules to the surface, and is relevant for studies regarding surface-bound proteins in PD for longer periods of exposure. Three hydrophobic materials of medical grade silicone, PVC, and PE bound each protein to similar extents at all time points. This may indicate that the hydrophobicity of a material’s surface may play an important role in rapidly adsorbing proteins, a role more profound than the underlying physicochemical properties (16).

Colonies of six S. aureus strains, including strains Newman and Cowan 1, were greater on catheter surfaces exposed to PD effluent compared to that on surfaces exposed to unused solution, beyond 1 hour to 48 hours of bacterial incubation. The numbers of adhered cells of all six S. epidermidis strains on catheters exposed to PD effluent were greater than on those exposed to unused solution after 48 hours, although the difference was not significant for three S. epidermidis strains, including strain 19, despite the significant enhancement of cell adhesion on effluent-exposed catheters at 1 hour of bacterial incubation. This may be due to the postadsorption changes in configuration in the immobilized proteins in dynamic conditions after 48 hours, resulting in increased exposure of domains recognized by S. aureus, such as the α- and γ-chains of Fg bound by Efb (10,11) and ClfA (7,8) respectively, contrary to the β-chain of Fg by Fg-binding protein (Fbe) of S. epidermidis strain 19 (27).

Colonies of adhered bacteria on catheter pieces exposed to solution without proteins increased over time, up to 48 hours. This may be due to binding of bacteria to the surface by hydrophobicity or electrostatic charge. However, at both early and late phases of exposure to bacteria, the extent of adhered viable cells of all S. aureus and S. epidermidis strains was greater on catheters exposed to used PD effluent compared those exposed to solution without protein content. This may indicate that the extent of staphylococcal adhesion may be enhanced through a higher affinity, mediated through ligand and receptor domain interactions by protein content in the PD fluid adsorbed on the polymeric surface. Although bacteria with high Vn-binding capacity bound to shunts perfused with cerebrospinal fluid, bacterial binding was only partially blocked by anti-Vn antibody (23). Whether one or several of the surface-exposed proteins correspond to the adhesion of bacteria to Tenckhoff catheters is to be further investigated.

The wide variety of proteins bound on peritoneal catheters may also contribute to the complexity of adhesin-targeted vaccine design toward catheter-related bacterial infections in PD (41). Specific integrins providing receptors for ligands, including Fg, Fn, Vn, vWF, and TSP, have been shown to be expressed on macrophages while undergoing differentiation in the peritoneal cavity (42). The presence of surface-exposed proteins may predispose to infection by exposing a broad capacity of binding of microbes as well as of eukaryotic cells to form biofilms on PD catheters.

Acknowledgments

This study was supported by Scandinavia-Japan Sasakawa Foundation, Swedish Medical Research Council (6×11229), and Faculty of Medicine, University of Lund. Dr. O. Sørensen, Department of Nephrology, Lund University Hospital, Lund, is appreciated for the supply of excised Tenckhoff catheters and allowing us to use their supply of peritoneal dialysis effluent. Dr. K. Tanda, Hakodate-Chuo Hospital, Hokkaido, and Drs. T. Chikaraishi, S. Owada, and T. Yasuda, St. Marianna University Hospital, Kanagawa, are appreciated for the supply of excised Tenckhoff catheters.

References

6. Palmqvist N, Foster T, Tarkowski A, Josefsson E. ACKNOWLEDGMENTS

This study was supported by Scandinavia-Japan Sasakawa Foundation, Swedish Medical Research Council (6×11229), and Faculty of Medicine, University of Lund.

Dr. O. Sørensen, Department of Nephrology, Lund University Hospital, Lund, is appreciated for the supply of excised Tenckhoff catheters and allowing us to use their supply of peritoneal dialysis effluent. Dr. K. Tanda, Hakodate-Chuo Hospital, Hokkaido, and Drs. T. Chikaraishi, S. Owada, and T. Yasuda, St. Marianna University Hospital, Kanagawa, are appreciated for the supply of excised Tenckhoff catheters.
Protein A is a virulence factor in Staphylococcus aureus arthritis and septic death. Microb Pathog 2002; 33: 239–49.


