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Biofilm formation and changes in bacterial cell surface hydrophobicity during growth in a CAPD model system

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Abstract

Peritonitis is a frequent complication of continuous ambulatory peritoneal dialysis (CAPD), with patients suffering recurrent attacks. The microorganisms most frequently implicated in the infection are the skin microflora, in particular, the coagulase-negative staphylococci such as *Staphylococcus epidermidis*. These microorganisms gain access to the peritoneal cavity via the in-dwelling silicone rubber catheter in the abdominal wall and often persist as biofilms on the surface of the catheter. The surface characteristics of *S. epidermidis* were monitored during growth in a CAPD in-vitro model together with their ability to adhere to silicone rubber substrata. Fresh dialysis fluid exerted an injurious effect on the cells leading to a decrease in cell numbers but during the simulated dialysis period the cells adapted to the applied stresses. Over a 96-h period in the model both a clinical isolate and a skin isolate of *S. epidermidis* adopted a more hydrophobic phenotype. The data presented here show that the bacteria grown in this in-vivo reflective CAPD model continually adapt to their environment and become more tolerant to the stresses imposed. The adapted cells were seen to colonise silicone rubber substrata.

Introduction

Continuous ambulatory peritoneal dialysis (CAPD) is an alternative treatment to haemodialysis for patients with end-stage renal failure who are awaiting transplantation. While it provides a number of advantages to the patient in terms of convenience and increased mobility, it carries with it the inherent risk of peritonitis. Infective peritonitis causes significant morbidity and mortality and recurrent episodes are associated with fibrosis of the peritoneal membrane, leading to reduction in dialysis efficiency and failure of the technique (Gokal & Mallick 1999). The primary cause of CAPD peritonitis is infection by coagulase-negative staphylococci derived from the skin microflora (Bint et al 1987; Chew et al 1997). To precipitate and sustain an infection, these commensals must adapt to the hostile environment and nutrient limitations of the dialysed peritoneum (Wilcox et al 1990). CAPD patients have an in-dwelling silicone rubber catheter inserted through the abdominal wall and this subsequently enters the peritoneal cavity. This catheter acts as a primary route for the coagulase-negative staphylococci to access the peritoneal cavity and cause peritonitis. In particular, their ability to adhere to the catheter provides a protective niche. Microflora such as *Staphylococcus epidermidis* thus are opportunist pathogens because they persist in biofilms within which they are protected from the host immune system and antibiotic attack (Stewart & Costerton 2001). To understand the complex processes of pathogenicity, it is necessary to have an appreciation of the interplay between growth rate, composition of nutrients and inhibitors and the effect of these parameters on cell surface characteristics. Brant et al (1998) have developed an in-vitro model of peritonitis in CAPD, which integrates the parameters of environmental gaseous tension, the changing biochemical profile of the dialysate and the periodic challenge of fresh fluid. Dwell times of 4 h during the day and 10 h at night were used to reflect the in-vivo situation. Biochemical analysis of dialysate within the model showed that the profiles for creatinine, glucose and protein approximated those found in patients. *S. epidermidis* growing planktonically in synthetic dialysis effluent (SDE) or pooled

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human dialysate (PHD) were seen rapidly to adapt to the environment after an initial lag phase with a tendency to aggregate together over the 50-h period of operation. Such aggregation is indicative of increasing hydrophobicity and propensity to adhere.

Microbial colonisation of medical implants, such as CAPD catheters, is a major problem and leads to a high incidence of biofilm-associated bacterial infections, such as peritonitis (Allison & Gilbert 1992).

The rate and strength of bacterial attachment is influenced by environmental conditions, such as electrolyte concentration, pH, temperature and nutrient flux. In addition, the physiochemical characteristics of the substratum, such as its electrostatic charge, surface free energy, hydrophobicity and roughness, can all influence the strength and rate of bacterial attachment (Allison & Gilbert 1992; Fletcher 1996). Values for most of these parameters are derived from the measurement of contact angles (θ) of liquids on those surfaces.

Bacterial attachment has also been related to cell surface hydrophobicity (Hogt et al 1983), which can be directly measured by techniques such as the hydrophobic interaction assay and the salt aggregation test (Denyer et al 1993; Fletcher 1996). Hydrophobic interaction chromatography of bacterial cells is a procedure based on the hydrophobic interaction between non-polar residues on a gel bed, usually octyl or phenyl sepharose, and non-polar regions on the bacterial surface. This has been adapted to form the basis of the hydrophobic interaction assay (HIA; Denyer et al 1993) in which a bacterial suspension is mixed with a gel suspension and the extent of bacterial adhesion to the gel is measured by optical density change. The salt aggregation test (SAT) is based on the precipitation of cells from suspension by the addition of increasing concentrations of ammonium sulfate to cause bacterial aggregation. Aggregation is thought to arise through the reduction of repulsive charges and the subsequent dominance of hydrophobic interactions at the outer surface layer of the bacteria.

This paper investigates the formation of biofilms on silicone rubber substrates within the CAPD model and discusses this in relation to changes in cell surface hydrophobicity as a result of cellular adaptation.

Materials and Methods

Collection and pooling of used human dialysate

Dialysis fluid was collected on the day of drainage from patients established on CAPD and pooled according to the methods described in Brant et al (1998). This fluid was subsequently known as pooled human dialysate (PHD).

Preparation of fresh dialysis fluid and synthetic dialysis effluent (SDE)

Fresh dialysis fluid was prepared as follows ($g\,L^{-1}$): sodium lactate 5, NaCl 5.6, $CaCl_2$ 0.26, $MgCl_2.6H_2O$ 0.15, sodium metabisulfite 0.05, glucose either 13.6 or 38.6, distilled water to required volume. The solution of lactate and sodium chloride was autoclaved at 121°C for 15 min. A stock glucose solution was autoclaved separately (115°C, 10 min) before adding aseptically. $CaCl_2$, $MgCl_2$ and sodium metabisulfite stock solutions were filter-sterilized before aseptic addition. This composition was formulated to reflect that of commercially available fluid (Dianeal, Travenol).

Synthetic dialysis effluent (SDE) was designed to mimic human dialysate and consisted of the following ($g\,L^{-1}$): nutrient broth no. 2 dried powder 1, NaCl 8, KH_2PO_4 0.12, KCl 0.197, $CaCl_2$ 0.133, $MgCl_2$ 0.81, urea 1.32, creatinine 0.083, glucose 3.37, distilled water to required volume. The solution, but without $CaCl_2$, $MgCl_2$, urea, creatinine and glucose, was autoclaved at 121°C for 15 min. A stock glucose solution was autoclaved separately (115°C, 10 min) and added aseptically. $CaCl_2$, $MgCl_2$, urea and creatinine stock solutions were also sterilized separately by filtering before aseptic addition. For certain experiments 1% v/v fetal bovine serum (Life Technologies Ltd, Paisley, Scotland) was added to SDE to allow for protein pre-conditioning of the biomaterial before bacterial adhesion (Holmes et al 1989).

Microbial cultures

A clinical isolate of *S. epidermidis* (designated strain 901) was isolated from infected dialysis fluid obtained from a CAPD patient suffering peritonitis (attending the Renal Unit, The City Hospital, Nottingham, UK). In addition, swabs were taken from the skin (hands and abdomen) of healthy subjects and bacterial isolates purified and identified. Cultures of *S. epidermidis* were identified by API Staph and analysed using the salt aggregation test (SAT; see later) to determine their surface hydrophobicity. The most hydrophilic strain (SAT value = 3.8) was selected for further work.

Stocks of all bacterial cells were kept at −70°C in nutrient broth (no. 2; Oxoid, Basingstoke, UK) supplemented with 10% v/v glycerol. Before use, frozen stocks were thawed and cultured in 100 mL of nutrient broth and their identity confirmed using API Staph and Gram staining. Surface characteristics were routinely monitored using contact-angle determination to detect any significant changes. All working cultures were maintained on nutrient agar according to the procedures described by Brant et al (1998). Suspensions of cells were created in phosphate-buffered saline (PBS), adjusted to a concentration of 2×10^8 colony-forming units (CFU) mL^{-1} and 0.2 mL used to inoculate 10 mL of medium contained in a universal jar (either SDE or PHD). These were then incubated statically for 16 h at 37°C in 5% CO_2–95% air to provide a starter culture for the in-vitro CAPD model.

Viable counts

Viability was assessed by preparing ten-fold serial dilutions in sterile PBS (containing 1% Tween 80) and spreading 0.2 mL of the appropriate dilution, in triplicate, on to over-dried nutrient agar plates and incubating at 37°C for 24 h.

To determine the number of bacteria adhering to the silicone rubber substrates (see below), any adherent bacteria were transferred into 10 mL PBS in a sterile Petri dish by scraping the surfaces of the rubber with a glass spreader. The suspension was transferred to a sterile boiling tube that was vortex-mixed for 15 s, after which 0.2 mL volumes of serial dilutions of the suspension were plated in triplicate on to over-dried agar. Examination of scraped and washed surfaces by fluorescence microscopy indicated that very few residual bacteria remained attached.

In-vitro CAPD model design and operation

The CAPD model used was a modification of that described by Brant et al (1998) adapted to accommodate silicone rubber substrates for the growth of biofilms.

The modified glass model is shown in Figure 1, and the complete apparatus was sterilized by dry heat at 175°C for 1 h. The model was assembled at least 12 h before use to allow the gaseous environment inside the model to equilibrate with that of the incubator. To initiate an experiment, a starter culture of *S. epidermidis* strain 901 or skin isolate was used to inoculate 100 mL pre-warmed SDE or PHD equilibrated at 5% CO_2–95% air, which was added to the model. The model was run on a cyclical basis to reflect the dwell times of 4 h during the day and 10 h at night interspersed with fluid changes observed clinically. This has been described in detail by Brant et al (1998).

During the running of the model, SDE or PHD were continuously added and so it was necessary to apply a correction factor to compensate for the effect of this dilution process on cell numbers (Brant et al 1998). The rate of dilution was calculated to be $10^{-0.44}$ and this was applied to the analysis of growth curves, allowing plots to be derived which describe the population size that would arise if there were no dilution; the true growth rate at any stage can be thus be determined.

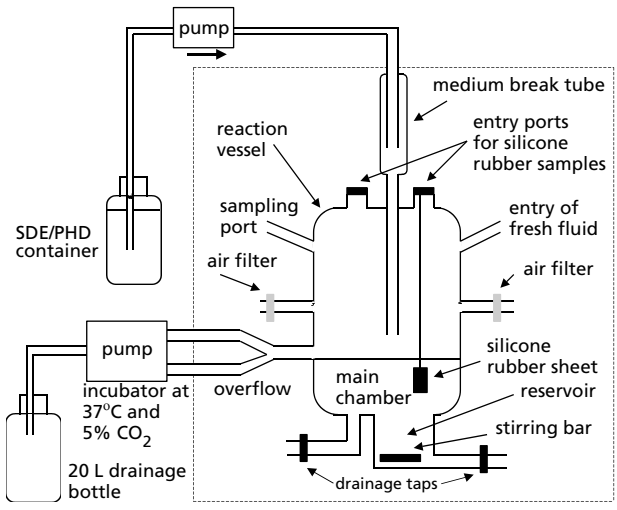

Figure 1 Diagram of in-vitro continuous ambulatory peritoneal dialysis model system.

Silicone rubber substrates

The catheters employed in CAPD are constructed from silicone rubber and so sheets of this material were used as substrata to investigate bacterial biofilm formation.

Standard silicone rubber sheets (Fisher Scientific, Leicestershire, UK) were cut into rectangular sections (2 × 1 cm), rinsed in distilled water to remove any residues present and sterilised by exposure to dry heat at 175°C for 1 h. Medical grade silicone rubber sheets of the same type used in CAPD catheters were provided by Hy-Med Ltd Medical Products (Fordingbridge, UK). These sheets had been sterilised using the same sterilisation process (ethylene oxide) employed for CAPD catheters. The sheets were cut into 2 × 1 cm sections under aseptic conditions before use.

Dynamic contact angle (DCA) measurements of silicone rubber surfaces

The wettability of the silicone rubber substrata was determined using a Cahn DCA analyser. The wetting medium employed was high-performance liquid chromatography (HPLC)-grade water, the surface tension of which was determined by measuring its contact angle onto a flamed glass cover-slip. This value was then used to determine the dynamic contact angle at the water–silicone rubber interface. The standard and medical-grade silicone rubber (both sterile) were placed on the DCA analyser and their advancing and receding angles were determined.

Adherence of bacteria to silicone rubber substrates

S. epidermidis strain 901 and the skin isolate were grown in SDE re-suspended to give cell concentrations of 10^6, 10^7 and 10^8 CFU mL^{-1} and dispensed (10 mL) into universal containers containing both types of silicone rubber. After 4 h, the silicone rubber was rinsed three times in sterile distilled water and viable counts were performed. The experiment was repeated with the addition of fetal bovine serum (1% v/v) to the SDE. In addition, *S. epidermidis* strain 901 and the skin isolate at a concentration of 10^8 CFU mL^{-1} in SDE were dispensed (10 mL) into universal containers containing both types of silicone rubber, which were incubated for 1, 2, 3, 4 and 5 h. After each time interval, the silicone rubber was rinsed three times in sterile distilled water and viable counts were performed. Again, the experiment was repeated with addition of fetal bovine serum (1% v/v) to the SDE. *S. epidermidis* strain 901 and the skin isolate were each grown in the CAPD in-vitro model for a total of 96 h and the number of bacteria adhering to each silicone rubber sample was determined after 24, 48, 72 and 96 h.

Bacterial surface characterisation

Hydrophobic interaction assay (HIA)

The CAPD model was set up as described above and inoculated with a starter culture of *S. epidermidis* strain

901 in SDE. Samples were taken at various time intervals and evaluated for viable count and surface hydrophobicity of the cells using HIA. The model was sampled by collecting 50 mL of suspension from the overflow half way through a dwell period and by collecting all of the suspension (190 mL) at each change-over point. The number of cells obtained from 50 mL was only sufficient to perform one HIA control and one HIA test, meaning that hydrophobicity was only determined with any surety at the end of each dwell. Therefore, the model was run for increasingly longer times so that it could be stopped and drained mid-dwell, to allow several replicate measurements to be made.

Samples of *S. epidermidis* 901 and skin isolate were collected from the model and centrifuged (5000 *g*, 15 min). The cells were washed three times in PBSS (PBS buffer (pH 7.2) containing 2 M NaCl) (5000 *g*, 10 min), before being re-suspended in PBSS to a concentration of 10^8 CFU mL^{-1}.

Phenyl sepharose gel CL-4B (Sigma) was washed in PBSS and the swollen gel suspended to a concentration of 30% w/v in PBSS. Gel suspension (1 mL) was added to 1 mL of each test cell suspension in glass test tubes. Controls were prepared by adding 0.7 mL of PBSS (equivalent to the volume of suspending fluid in the 30% gel suspension) to 1 mL of cells. All the tubes were vortex-mixed for 30 s and then allowed to stand at room temperature to allow the gel to settle (or centrifuged at 100 *g* for 2 min) before the absorbance (420 nm) of the supernatant liquids of the test and control suspensions was measured. The results were expressed as percentage adhesion calculated as a percentage decrease in absorbance of test compared with the absorbance of the control.

Salt aggregation test (SAT)

Samples of *S. epidermidis* 901 and skin isolate were removed from the model and centrifuged (5000 *g*, 15 min). The cells were washed three times in phosphate solution (0.002 M Na_2HPO_4; pH 7.2) (5000 *g*, 10 min) and then re-suspended in phosphate solution to a final concentration of 10^9 CFU mL^{-1}.

A series of ammonium sulfate solutions were prepared (0.2–4.0 M, in 0.2-M increments) in phosphate solution. Equal volumes (50 μL) of cell suspension and ammonium sulfate solution were placed in turn on a glass slide, mixed and gently rocked for 2 min. The SAT value was recorded as the lowest concentration of ammonium sulfate that caused aggregation of the cells in 2 min when the solution was viewed against a darkened background.

Results

Growth, hydrophobicity and salt tolerance of planktonic cells in CAPD model

The CAPD in-vitro model was established as described above and run for progressively longer times up to 77 h (Figure 2). Viable counts were made periodically, with one immediately before and after each addition of fresh dialysis fluid and at 5, 10 14 and 24 h. Cell numbers decreased during the first 5 h, but by 14 h cell numbers had increased to approximately the initial inoculum size and by 24 h (after the long simulated night-time dwell) this had been exceeded by 100 times to approximately 10^7 CFU mL^{-1}. During the subsequent 24-h cycles, cell numbers followed a similar cyclical pattern but did not experience the dramatic drop observed during the first 10 h of the first cycle.

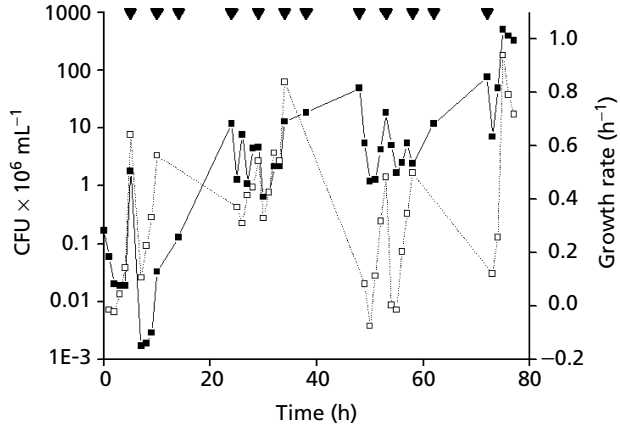

Figure 2 Cell count (■) and growth rate (□) of *S. epidermidis* strain 901 over 77 h (triangles indicate fluid change).

By correcting for the reduction in cell numbers caused by the addition of fresh dialysis fluid to the model, the true growth rate may be calculated. Growth rates can be compared with cell numbers (Figure 2) enabling an assessment of growth phase to be made. Upon the addition of fresh dialysis fluid to the model, a brief period of cell death occurred (not exceeding 2 h) followed by cell growth. Cell growth rate achieved a maximum (0.5–0.7 h^{-1}) just before the dialysis fluid was changed and then fell with the introduction of fresh dialysis fluid, on occasions to zero (indicative of entry into stationary phase). Maximum growth rate was achieved on the 4th day (0.95 h^{-1}). The relationship between viable count and associated cell surface hydrophobicity (as measured using HIA) is shown in Figure 3. Before its addition to the model, the adsorption of the inoculum was 32%; this value represents the hydrophobicity of the cells during stationary phase grown in batch PHD. There was little change in hydrophobicity ($P > 0.05$; analysis of variance) until 4 h when adsorption decreased to 15% ($P < 0.002$); this coincided with the bacteria beginning to grow. As growth slowed at 8 h, so hydrophobicity increased only to decrease again during growth until the cells entered into stationary phase at the end of the overnight dwell and adsorption returned to 34%. Clearly, the cells were adapting to the changing conditions within the CAPD in-vitro model.

Adsorption of *S. epidermidis* strain 901 and skin isolate to standard and medical-grade silicone rubber at different cell concentrations

The wettability of standard and medical-grade silicone rubber was determined using DCA measurements. Standard silicone rubber gave a mean advancing angle of

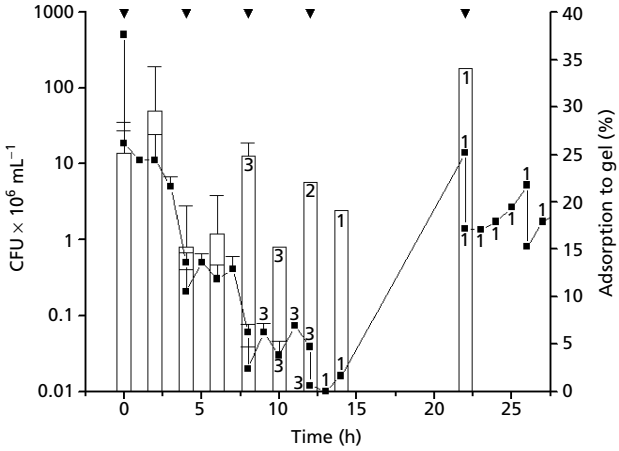

Figure 3 Cell count (■) and hydrophobicity (measured by HIA; open bars) of *S. epidermidis* strain 901 cultured in SDE in the CAPD in-vitro model. Triangles indicate fluid change; the numbers adjacent to the symbols and bars indicate the number of replicates, unless otherwise stated, n = 5, mean ± s.d.

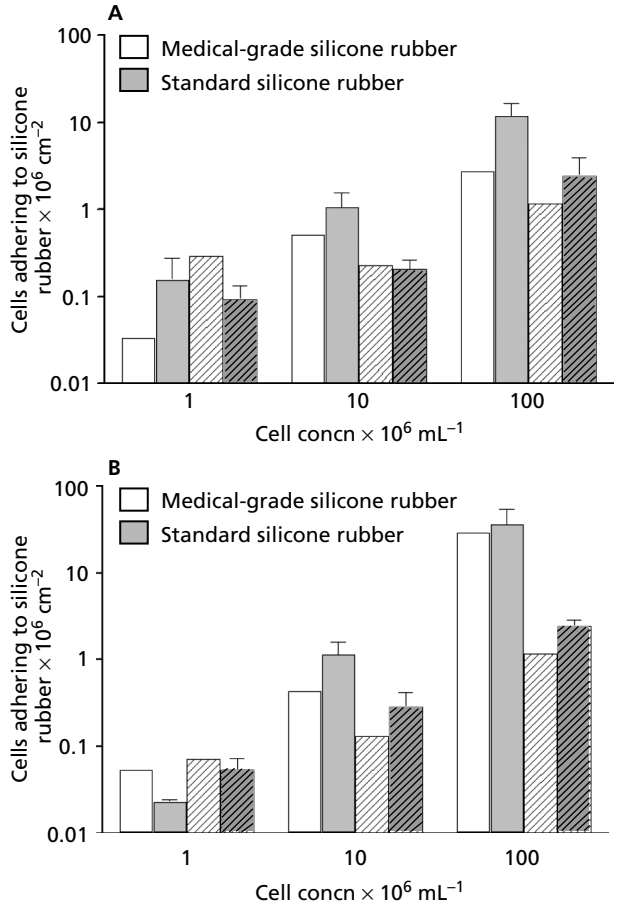

Figure 4 Adsorption isotherms of *S. epidermidis* strain 901 (A) and *S. epidermidis* skin isolate (B) to medical-grade and standard silicone rubber without (unpatterned bars) and with (patterned bars) the addition of serum to the SDE after 4 h contact (n = 6, mean ± s.d., standard silicone rubber).

129.00 ± 1.10° and a mean receding angle of 81.67 ± 1.03°. Medical-grade silicone rubber had advancing and receding angles of 106.17 ± 2.56° and 71.33 ± 4.68°, respectively. Advancing angles for both types of silicone rubber indicate that they are hydrophobic but the standard silicone rubber is more hydrophobic than the medical-grade silicone rubber.

The adsorption of *S. epidermidis* strain 901 to standard and medical-grade silicone rubber at various cell concentrations is shown in Figure 4A. As the cell concentration increased, so too did adsorption to the rubber substrates ($P < 0.01$ for standard silicone rubber, analysis of variance). The cells attached less readily to medical-grade silicone rubber than to standard silicone rubber. The addition of serum to the SDE generally reduced the number of bacteria adhering to the substrates ($P < 0.05$ for standard silicone rubber, analysis of variance) (patterned bars). Similar results were seen for the adsorption of *S. epidermidis* skin isolate (Figure 4B). Increasing the cell concentration increased the number of cells adhering to the silicone rubber substrates ($P < 0.01$ for standard silicone rubber, analysis of variance) and, for a given concentration of cells, the number of cells adhering to the substrate was reduced by the presence of serum ($P < 0.1$ for standard silicone rubber, analysis of variance).

Adsorption kinetics of *S. epidermidis* strain 901 and skin isolate to standard and medical-grade silicone rubber at different time intervals

In the absence of serum, bacterial adherence to standard silicone rubber was greater than to medical-grade silicone rubber for both strains of *S. epidermidis* at all time points (Figure 5A, B, square symbols). The effect was more marked for strain 901 (Figure 5A). Addition of serum to the SDE significantly reduced the adherence of strain 901 to standard silicone rubber ($P < 0.001$, analysis of variance) (Figure 5A, closed symbols). At 5 h this reduction was from a mean of 12 million cells/cm^2 to a mean of 1.4 million cells/cm^2. However, the adherence of strain 901 to medical-grade silicone rubber was largely unaffected by the presence of serum, with an adherence of 0.7 million cells/cm^2 in the absence of serum at 5 h and an adherence of 1.4 million cells/cm^2 in the presence of serum at 5 h (Figure 5A). Addition of serum to the SDE significantly reduced the adherence of the skin isolate to standard silicone rubber ($P < 0.001$, analysis of variance) (Figure 5B, closed symbols). The number of cells from the skin isolate adhering to both types of silicone rubber decreased from 10.0 million cells/cm^2 and 6.2 million cells/cm^2 to 1.6 million cells/cm^2 and 1.9 million cells/cm^2 at 5 h for standard silicone rubber and medical-grade silicone rubber, respectively (Figure 5B). The variability observed in this experiment is probably a reflection of cellular aggregation as epifluoresence microscopy examination of washed surfaces revealed few remaining bacteria. Adherence in larger numbers to standard

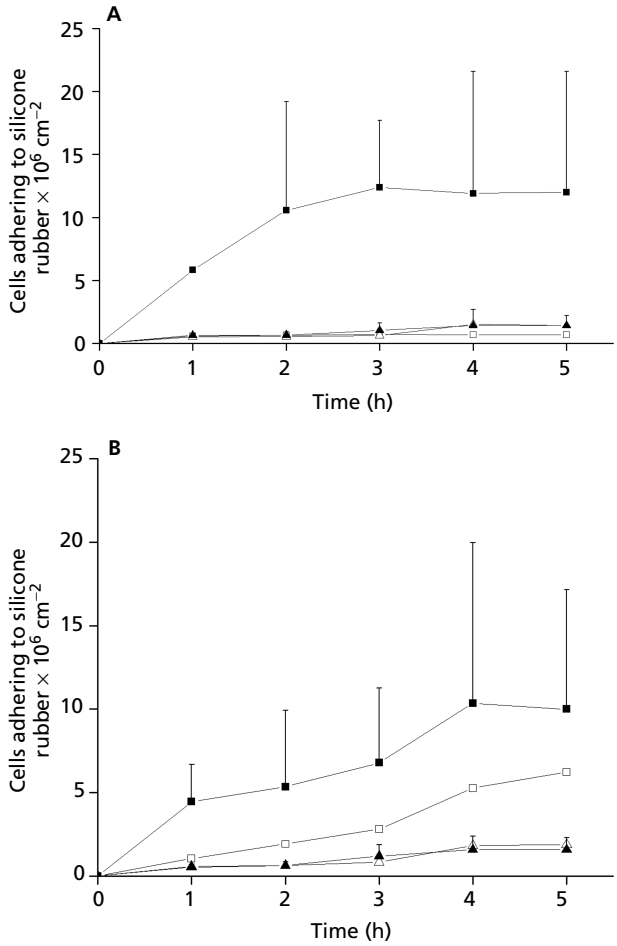

Figure 5 Adsorption kinetics of *S. epidermidis* (A) strain 901 and skin isolate (B) to medical-grade (open symbols) and standard silicone rubber (closed symbols) at different time intervals with (triangles) and without (squares) the addition of serum to the SDE (n = 6, mean ± s.d., standard silicone rubber).

silicone rubber may have resulted in cellular aggregation, which was more difficult to break up.

Viable count and hydrophobicity determination of cells adhering to standard and medical-grade silicone rubber

S. epidermidis strain 901 and the skin isolate were each grown in SDE in the CAPD in-vitro model for a total of 96 h and the numbers of bacteria adhering to each silicone rubber sample were determined after 24, 48, 72 and 96 h. Results show that strain 901 adhered well to the standard silicone rubber in comparison with the skin isolate where adherence to medical-grade silicone rubber appeared to be slightly better (Figure 6). The inoculum cultures of strain 901 and the skin isolate gave SAT values of 0.8 and 3.8, respectively. For both strains, the SAT value after 96 h was 0.2. When each strain was sub-cultured and grown in nutrient broth or SDE their SAT values reverted back to their initial values.

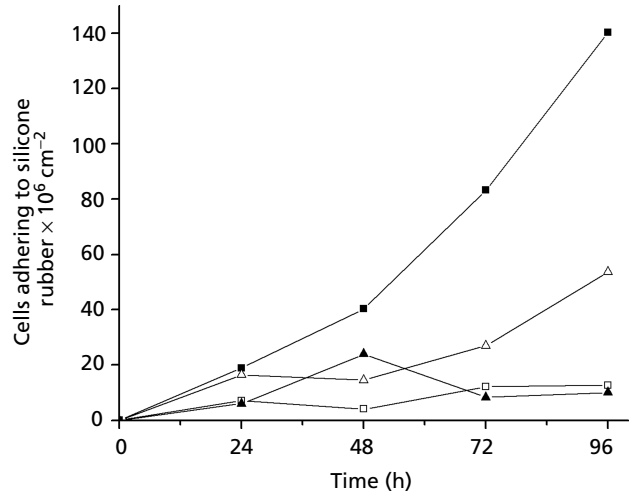

Figure 6 *S. epidermidis* strain 901 (squares) and skin isolate (triangles) adhering to medical-grade (open symbols) and standard silicone rubber (closed symbols) after culturing in SDE in the CAPD in-vitro model for different periods of time (n = 1).

Discussion

The growth of planktonic *S. epidermidis* (both 901 and the skin isolate (data not shown)) exhibited the characteristic adaptation pattern reported previously by Brant et al (1998). On initial addition of fresh fluid the culture growth rate was very slow, such that the doubling time of the cells did not exceed the washout rate (Figure 2). As a result, culture density fell. By the time of the second addition of fresh fluid, the cells had adapted somewhat and the growth rate increased slightly. However, it was not until the first overnight dwell period that the cells became fully adapted and grew back to their original cell density. Thereafter, the cells grew quickly after each new addition of fresh fluid. Examination of planktonic cells within the model showed that their hydrophobicity varied depending upon their stage of growth. Generally, the cells were more hydrophilic in nature while in exponential growth but became more hydrophobic as the growth rate fell (Figure 3). By the end of the overnight dwell time the cells were at their maximal hydrophobicity. This observation is in agreement with Brant et al (1998) who found that after a few days growth in the model, the cells had clumped visibly to form large aggregates that were unable to stay in suspension.

It is generally accepted that three important elements — substrata, suspending fluids and bacteria — are involved in determining the initial adhesion process to biomaterials (Tunney et al 1996). Upon implantation in the body, medical devices are usually surrounded by biological fluids, such as urine, blood, saliva and synovial fluids, which contain proteins such as fibronectin, laminin, fibrin, collagen and immunoglobulins that are almost immediately coated on to the biomaterial surface to form a conditioning film. The conditioning film may act to alter the substratum by decreasing its hydrophobicity, or lowering the surface

energy of a clean, high-energy surface. Generally, conditioning films have little effect on low energy surfaces (Baier 1980).

A range of techniques has been applied to measure bacterial cell surface hydrophobicity, including HIA, contact angle, SAT and bacterial adherence to hydrocarbons (BATH) (Denyer et al 1993). Although a number of studies have reported reasonable correlation between these methods, there inevitably will be discrepancies since the methodologies used probe different components of the cell surface.

Bacteria attach more readily to hydrophobic (low-energy) surfaces than to hydrophilic (high-energy) surfaces (Fletcher & Marshall 1982; Pringle & Fletcher 1983). The standard silicone rubber sheet was found to be more hydrophobic than the medical-grade silicone rubber and it was shown that the cells adhered to the former in greater numbers (Figures 4 and 5). Merritt et al (1997) found that adherence of organisms to materials can differ from one strain to another, probably due to variations in their physicochemical cell surface properties (van der Mei et al 1997). In general, the more hydrophilic skin isolate did not adhere in such great numbers as strain 901. Addition of serum to the SDE led to a reduction in cell adhesion (Figures 4 and 5). This effect was more marked for the standard silicone rubber sheet than the less hydrophobic medical-grade silicone rubber sheet. It was presumed that the serum caused the formation of a protein-conditioning film, which reduced the hydrophobicity of the surface of the silicone rubber.

Cells growing in the CAPD model readily formed biofilms on the surface of the silicone rubber sheets (Figure 6). As before, *S. epidermidis* strain 901 produced a denser biofilm on standard silicone rubber than on the medical-grade silicone rubber, but the skin isolate colonised the medical-grade silicone rubber to a greater extent from the 72 h time point. Strain 901 was a hydrophobic microorganism and remained so after growth in the model. However, when the skin isolate was removed from the surface and assessed for its hydrophobicity it was found to have altered to a hydrophobic phenotype. The data presented in this study show that bacteria grown in the CAPD model continually adapt to their environment. They become tolerant to the stresses imposed by the fresh dialysis fluid and alter their hydrophobicity to allow adherence at surface–liquid interfaces.

Conclusions

This paper reports findings on the adaptability of skin microflora in a model system simulating microbial contamination of CAPD. *S. epidermidis* was able to overcome the initial toxicity of fresh dialysis fluid and the stresses imposed by PHD and SDE and adapt to the cyclical changes within the environment. After about four cycles of fluid change within the model system the cells began to grow well and ultimately adopted a more hydrophobic phenotype. This adaptation facilitated adherence to the silicone rubber of the catheter material and promoted biofilm formation. This protective environment leads to the persistence of *S. epidermidis* in the repeated episodes of peritonitis typical of long-term CAPD treatment.

The model described in this paper has allowed a detailed study of the adaptability of contaminating microorganisms under conditions of cyclical stress imposed by CAPD. In the future, the same model could permit the study of the effectiveness of many of the proposed new therapies for combating biofilm formation and reducing catheter-biofilm-related infections in patients undergoing CAPD.

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