Adhesion of Coagulase-negative Staphylococci to Biomaterials

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The adhesion of two *Staphylococcus epidermidis* strains and one *Staphylococcus saprophyticus* strain on to poly(tetrafluoroethylene-co-hexafluoropropylene) (FEP)-fluorocarbon and cellulose acetate was studied *in vitro*. Both *S. epidermidis* strains showed a more hydrophobic character than the encapsulated *S. saprophyticus* as determined by the bacterial affinity towards xylene. *Staphylococcus epidermidis* showed a significantly higher adhesion on to the hydrophobic FEP than *S. saprophyticus*. The adhesion of staphylococci on to the more hydrophilic cellulose acetate was always low. Treatment of *S. epidermidis* with pepsin or extraction with aqueous phenol yielded cells with a decreased hydrophobicity, which resulted in a decreased adhesion on to FEP. Cells with a decreased hydrophobicity showed a lower rate of reaggregation in suspension. The hydrophobicity and the adhesion on to FEP of *S. epidermidis* were not affected by exposure to a subminimal inhibitory concentration of penicillin. The strong interaction between *S. epidermidis* and FEP, which appeared not to be influenced by the age or the metabolic stage of the bacteria, is mainly caused by hydrophobic bonding.

INTRODUCTION

The adhesion of bacteria on to mammalian tissue surfaces is recognized as an important initial step in the pathogenesis of an infectious process (Ofek & Beachey, 1980). Infections associated with prosthetic implants and medical devices might also be preceded by the adhesion of bacteria on these devices. Despite the serious consequences of implant- and device-associated infections, few reports on bacterial adhesion on to biomaterials are available (Holt, 1970; Moore *et al.*, 1980; Locci *et al.*, 1981; Christensen *et al.*, 1982). The micro-organisms most often involved in such infections are coagulase-negative staphylococci (Garvey, 1980). Locci *et al.* (1981) studied the adhesion of *S. epidermidis* on to various intravenous catheters and indicated that the initial adhesion occurred in irregularities along the otherwise smooth catheter surface from which micro- and macrocolonies developed. Slime-producing staphylococci adhering to such catheters obtained from patients with intravascular catheter-associated infections were cemented in a matrix of unidentified amorphous material, whereas non-slime-producing staphylococci adhered as individual cells not encased in an adhesive layer (Christensen *et al.*, 1982).

We have studied *in vitro* the adhesion of three strains of coagulase-negative staphylococci on to two different biomaterials. The materials tested were poly(tetrafluoroethylene-co-hexafluoropropylene) (FEP), a hydrophobic material used in vascular grafts, intravenous catheters and trachea prostheses, and cellulose acetate (CA), a more hydrophilic material used for the coating of sorbents in haemoperfusion systems.

Abbreviations: CA, cellulose acetate; DPCC, diphenylcarbonyl chloride; FEP, poly(tetrafluoroethylene-co-hexafluoropropylene); LTA, lipoteichoic acid; MIC, minimum inhibitory concentration; TLCK, *N*- α -tosyl-L-lysine chloromethyl ketone; TSB, trypticase soy broth.

METHODS

Bacterial strains and growth conditions. Two strains of *Staphylococcus epidermidis* and one strain of *Staphylococcus saprophyticus* were used. *Staphylococcus epidermidis* strain 1 was cultured from blood samples collected from a child with bacteraemia and the other *S. epidermidis* strain 2 was isolated from a bottle containing an intravenous fluid. *Staphylococcus saprophyticus* was isolated from the bed of a surgical patient nursed in the Intensive Care Unit. Micro-organisms were Gram-positive cocci in clusters and were coagulase-negative when tested by the slide and the tube test using rabbit plasma (Sylvana, Grand Island, N.Y., U.S.A.). Each strain was further characterized using the API Staph gallery (API System S.A., Montalieu Vercieu, France). Strains 1 and 2, coded as 6706113 were identified as *S. epidermidis*. Strain 3, coded as 6630112 was identified as *S. saprophyticus*. Both *S. epidermidis* strains were sensitive and *S. saprophyticus* was moderately sensitive to lysostaphin (Sigma) (Kloos & Schleifer, 1975). All three staphylococcal strains were oxidase-negative (Faller & Schleifer, 1981). The *S. saprophyticus* strain was encapsulated, as demonstrated by the India ink wet-film method (Duguid, 1951) and by the Muir method (Cowan & Steel, 1974), whereas neither *S. epidermidis* strain was encapsulated or produced slime when tested according to the method of Christensen *et al.* (1982).

Bacteria were grown for 18 h at 37 °C in 50 ml Trypticase Soy Broth (TSB; BBL) in a rotary shaker (New Brunswick Scientific Co.) at 120 r.p.m. Late-exponential phase cells were obtained by culturing 2 ml of the overnight culture in 100 ml fresh TSB at 37 °C for 4.5 h; stationary phase cells were obtained by incubating for 18 h. Cells were harvested by centrifugation (Beckman Instruments Inc., model J2-21) at 20 000 *g* for 10 min at 4 °C, washed three times with 60 ml amounts of NaCl/phosphate buffer (0.1 M-NaCl and 13 mM-phosphate buffer, pH 7.2) and resuspended in 60 ml of the same buffer. Clusters of bacteria were dispersed by aspirating and expelling each suspension twice through a sterile 25 gauge steel needle attached to a syringe (B-D Plastipak, Dublin, Ireland). Then the suspension was filtered through a membrane filter (type SC, pore size 8 µm; Millipore). Microscopic examinations on wet mounts of the filtered suspensions showed that the suspensions primarily contained single cells. The optical density of the suspensions was measured at 540 nm (model 24 spectrophotometer, Beckman Instruments Inc.). The number of viable colony forming units was determined by spreading 0.1 ml portions from serial tenfold dilutions of bacterial suspensions in TSB on to blood agar plates (Oxoid). After incubation at 37 °C for 18 h, the numbers of colonies were counted. Bacterial suspensions in NaCl/phosphate buffer were adjusted to an OD of 1.0 and contained approximately 10^9 c.f.u. ml⁻¹.

In order to measure the reaggregation of bacteria, 3 ml of the bacterial suspensions in NaCl/phosphate buffer were sealed in spectrophotometer tubes (45 × 10 × 10 mm, Thovadec Hospidex, Nieuwkoop, the Netherlands) and incubated in a rotary shaker incubator (120 r.p.m., 37 °C). Optical densities of the suspensions were measured at 540 nm at various time intervals up to 2.5 h.

Bacterial cell surface hydrophobicity. The cell surface hydrophobicity of the bacteria was measured using the method described by Rosenberg *et al.* (1980). To round-bottom test tubes (10 mm inner diameter) containing washed cells suspended in 3 ml NaCl/phosphate buffer and adjusted to an OD of approximately 1.0, various volumes of *p*-xylene (Brocades, Maarssen, the Netherlands) ranging from 0 to 0.5 ml were added. The mixtures were vigorously agitated for 60 s using a Whirlimixer (Cenco, Breda, the Netherlands). After 20 min, the aqueous phase was removed with a Pasteur pipette and transferred to a 4.5 ml cuvette. The OD was measured at 540 nm. The fractional decrease in OD of the bacterial suspensions was determined as a function of the xylene volume added.

Polymer films. Films of FEP and CA were used for determining the bacterial interaction with a hydrophobic surface and a non-hydrophobic surface, respectively. Cellulose acetate, type 'TV 20' was purchased from Fabelta S.A., Tubize, Belgium; the degree of substitution was 2.49 (40.1% acetyl), the number average molecular weight ($\bar{M}_n$) was 53 000. Films of CA were prepared as follows: CA (10 g) was dissolved in acetone (350 ml), the solution was membrane filtered (Type LS, pore size 5 µm; Millipore) and concentrated under reduced pressure to a residual weight of approximately 100 g. The solution was cast on a glass plate (70 × 17 cm) and subsequently dried by exposure to a low flow of nitrogen gas for 18 h at room temperature. The film was detached from the glass surface by immersion in water and then dried at room temperature. Circular specimens (34 mm diameter) were cut from the films with a dinting punch, rinsed with distilled water and dried at room temperature. After sterilization by γ -irradiation (2.5 Mrad, Gammaster, Ede, the Netherlands) contact angle measurements were made using a sessile drop (water) technique (Adamson, 1976). After a contact time of 30 s, the water contact angle on CA was $63^\circ \pm 5^\circ$ (mean value $\pm$ S.D. of ten measurements). Differential scanning calorimetry (DSC-2, Perkin Elmer, Norwalk, Conn., U.S.A.) studies revealed no crystallinity in the CA films.

Circular specimens of FEP were obtained from a sheet of FEP-fluorocarbon, type '500 A' (Du Pont de Nemours, Geneva, Switzerland). The specimens were ultrasonically cleaned (50 KHz, Branson 221, Branson B.V., Soest, the Netherlands) in a 1% (v/v) detergent solution (RBS 25, Hicol, Rotterdam, the Netherlands) for 30 min, followed by extensive rinsing with distilled water, ethanol and diethyl ether. The FEP specimens were sterilized by dry heat for 3 h at 175 °C. The FEP water contact angle was determined to be $108^\circ \pm 2^\circ$ (mean value $\pm$ S.D. of ten measurements).

In order to determine that the two polymer films were not contaminated with bacterial lipopolysaccharide, specimens (10 mm²) obtained before sterilization were incubated at 37 °C in 0.1 ml pyrogen-free physiological saline with 0.1 ml Limulus lysate preparation (Limulus amoebocyte lysate, Mallinckrodt, Inc., St Louis, Mo., U.S.A.). No gelation reaction was observed after 1 h. As a positive control the test was performed on specimens contaminated with 0.1 ml of a standard *Escherichia coli* endotoxin preparation (50 pg ml⁻¹, Mallinckrodt).

Bacterial adhesion and desorption. Circular specimens of the two polymer films were placed into the wells of polystyrene culture dishes (six-well, 35 mm inner diameter, Costar, Cambridge, Mass., U.S.A.), fixed to the bottoms with glass rings (30 mm inner diameter) and rinsed with NaCl/phosphate buffer (3 ml). After the addition of 3 ml bacterial suspension (NaCl/phosphate buffer), films were incubated at 37 °C in a rotary shaker (120 r.p.m.) for 2.5 h. The films were then rinsed eight times with 3 ml amounts of NaCl/phosphate buffer and treated with 4% (w/v) glutaraldehyde in NaCl/phosphate buffer to fix adhering bacteria. The films were finally rinsed with distilled water and dried at room temperature. Adhering bacteria were counted by bright-field microscopy (Olympus BHB/PM-10M, Olympus Optical Co. Ltd, Tokyo, Japan). The number of adhering bacteria per mm² of the films was determined by examining six 0.05 mm² areas on each film. When aggregates of bacteria were observed, each aggregate was considered to be one unit. All experiments were performed in duplicate.

To study the influence of pH on the bacterial adhesion, bacteria were suspended in either NaCl/citrate buffer (0.1 M-NaCl and 0.1 M-citrate, pH 3.0), NaCl/acetate buffer (0.1 M-NaCl and 0.1 M-acetate, pH 5.0), NaCl/phosphate buffer (0.1 M-NaCl and 0.1 M-phosphate, pH 7.2), or NaCl/carbonate buffer (0.1 M-NaCl and 0.1 M-carbonate, pH 9.4). In some experiments, 20 mM-EDTA (disodium salt, Merck) was added to the bacterial suspension.

To study the desorption of adhering bacteria, polymer films were exposed for 2.5 h to suspensions containing either *S. epidermidis* strain 1 or *S. saprophyticus* with approximately 5×10^8 cells ml⁻¹, followed by one, eight or sixteen rinses with 3 ml amounts of NaCl/phosphate buffer. The effect of proteolytic enzymes or a chelating agent on desorption was studied as follows. FEP films, exposed for 2.5 h to a *S. epidermidis* strain 1 suspension containing 1×10^9 cells ml⁻¹, were rinsed eight times with NaCl/phosphate buffer and were subsequently incubated for 1.5 h at 37 °C in NaCl/phosphate buffer (0.1 M-NaCl and 0.1 M-phosphate, pH 8.0) containing 0.1% (w/v) trypsin (type XI, DPCC-treated; Sigma) or 0.1% (w/v) α -chymotrypsin (type VII, TLCK-treated; Sigma), or in NaCl/citrate buffer (pH 3.0) containing 0.1% (w/v) pepsin (Sigma), or in NaCl/phosphate buffer containing 20 mM-EDTA. After the treatments, the films were washed three times with NaCl/phosphate buffer. The adhering bacteria were fixed with glutaraldehyde and counted as described above.

Pretreatment of bacteria. The following methods were used to determine the effect of pH, bacterial viability, proteolytic enzymes, a low concentration of penicillin and extraction with aqueous phenol on bacterial reaggregation, cell surface hydrophobicity and adhesion.

To study the influence of pH, bacteria were suspended in buffer solutions as described above.

To obtain non-viable bacteria, bacterial suspensions in NaCl/phosphate buffer containing 10^9 to 10^{10} cells ml⁻¹ were placed in a waterbath at 60 °C for 1 h or incubated in 2% (v/v) formaldehyde at room temperature for 1 h. The bacteria were then sedimented by centrifugation and washed three times with NaCl/phosphate buffer. Samples cultured from either the heat- or formaldehyde-treated bacterial suspensions showed no growth after 48 h.

Exponential phase bacterial cells were incubated for 1.5 h at 37 °C in suspensions containing trypsin, chymotrypsin or pepsin as described above. The suspensions were then chilled rapidly, centrifuged (10000 g, 4 °C, 10 min) and the bacteria were washed three times with ice-cold NaCl/phosphate buffer (pH 7.2). In some experiments 20 mM-EDTA was added to the bacterial suspension.

Lipoteichoic acid (LTA)-deficient bacteria were obtained using two methods. Firstly, the minimal inhibitory concentration (MIC) of penicillin for both *S. epidermidis* strains was determined by the standard serial twofold dilution technique. The MIC of strain 1 and strain 2 were 7.5 U ml⁻¹ and 0.02 U ml⁻¹, respectively. Bacteria were then cultured in TSB containing a subminimal inhibitory concentration of penicillin (0.25 MIC) to stimulate the release of LTA (Alkan & Beachey, 1978). Bacteria were harvested after 4.5 h incubation at 37 °C and washed as usual. For the second method, bacteria grown in TSB overnight, centrifuged (10000 g, 4 °C, 10 min) and washed with distilled water, were extracted using the hot (65–68 °C) aqueous phenol method (Westphal *et al.*, 1952; Wicken *et al.*, 1973) modified according to Kessler & Shockman (1979) by the addition of 10 mM-MgCl₂ to the aqueous phase. Cells harvested by centrifugation (10000 g, 4 °C, 10 min) were finally washed three times with NaCl/phosphate buffer.

In order to obtain decapsulated *S. saprophyticus*, cells were either grown in TSB at 20 °C or 42 °C for 48 h, or were mechanically agitated in NaCl/phosphate buffer at 40 °C for 5 min (Stinson & Van Oss, 1971).

Preparation of crude lipoteichoic acid. A crude LTA preparation, obtained using the hot aqueous phenol extraction method (Westphal *et al.*, 1952; Wicken *et al.*, 1973) modified according to Kessler & Shockman (1979) was dialysed against demineralized water at 4 °C for 48 h. Desoxyribonuclease (type I, Merck), and ribonuclease (Merck) were added to digest polynucleotides prior to further dialysis at 4 °C for 15 h. The crude LTA was

Fig. 1. Optical densities of bacterial suspensions as a function of time. *Staphylococcus epidermidis* strain 1 (○) and *S. saprophyticus* (●) suspensions were prepared from bacteria grown in TSB at 37 °C for 4.5 h. Bacteria were washed, and suspended in NaCl/phosphate buffer, pH 7.2, and filtered through a membrane filter (pore size 8 µm). *Staphylococcus epidermidis* strain 1 was pretreated with pepsin (□) and strain 2 with hot aqueous phenol (▽). *Staphylococcus epidermidis* strain 1 was also suspended in 20 mM-EDTA (△).

concentrated using a rotary evaporator (model RV 4, Bühler, Tübingen, Germany), lyophilized (Edwards, Crawley, Sussex) and stored at 4 °C.

The crude LTA was added to suspensions of bacteria in NaCl/phosphate buffer to a final concentration of 0.5% (w/v). Films of FEP were treated with solutions of crude LTA in NaCl/phosphate buffer (1 mg ml⁻¹) for 1 h at room temperature. The films were then rinsed carefully with buffer for 15 min avoiding air contact with the polymer surfaces. Contact angles for these specimens were 104° ± 2° (mean ± S.D. of ten measurements).

Statistical methods. For calculations of *P* values the Student's *t*-test of significance between two sample means was used (Fisher & Yates, 1957).

RESULTS

Reaggregation of bacteria

Optical density values of *S. epidermidis* suspensions decreased at a higher rate than those of *S. saprophyticus* (Fig. 1). Plots of the decrease in OD of *S. epidermidis* strain 1 suspensions were similar for pH values ranging from 5.0 to 9.4. However, in NaCl/citrate buffer, pH 3.0, *S. epidermidis* rapidly reaggregated to macroscopic clusters. Suspensions prepared from *S. epidermidis* cells extracted with aqueous phenol or treated with pepsin showed only a minimal decrease in OD (Fig. 1). The OD plots of suspensions containing bacteria treated with trypsin or chymotrypsin, or grown in TSB containing 0.25 MIC of penicillin were similar to those of non-treated bacteria grown in TSB. An initial decrease in OD which levelled out after approximately 1.5 h was observed when EDTA was added to the bacterial suspension (Fig. 1).

Bacterial cell surface hydrophobicity

The affinities of the three staphylococcal strains towards xylene are presented in Fig. 2. Both *S. epidermidis* strains, but not *S. saprophyticus*, exhibited high affinity towards xylene (Fig. 2*a*). Treatment of both *S. epidermidis* strains with pepsin resulted in a substantially decreased xylene affinity, whereas trypsin or chymotrypsin had no effect (Fig. 2*b*). The cell surface hydrophobicity of bacteria suspended in pepsin-free NaCl/phosphate buffer, pH 3.0, and subsequently resuspended in NaCl/phosphate buffer, pH 7.2, was unchanged. Extraction with aqueous phenol was performed using *S. epidermidis* strain 2. The extracted cells showed a decreased affinity towards xylene. Adding 0.5% (w/v) crude LTA to a *S. epidermidis* suspension also decreased the xylene affinity of the bacteria (Fig. 2*c*). Bacteria grown in TSB containing 0.25 MIC of penicillin showed the same affinity as non-treated bacteria.

Fig. 2. Fractional decrease of optical density of bacterial suspensions as a function of xylene volume added according to Rosenberg *et al.* (1980). Optical density was measured spectrophotometrically at 540 nm using NaCl/phosphate buffer suspensions of: (a) *S. epidermidis* strain 1 (○), strain 2 (△), and *S. saprophyticus* (●); (b) *S. epidermidis* strain 1 pretreated with trypsin (○), chymotrypsin (△) or pepsin (□); and (c) *S. epidermidis* strain 2 grown in TSB with a subminimal inhibitory concentration of penicillin (○), extracted with hot aqueous phenol according to Westphal *et al.* (1952) and Wicken *et al.* (1973) (△), or *S. epidermidis* strain 2 containing 0.5% (w/v) crude lipoteichoic acid obtained from an aqueous phenol extract of *S. epidermidis* strain 2 (□).

Fig. 3. Numbers of bacteria adhering on to FEP-fluorocarbon as a function of exposure time. Specimens of the films were incubated with suspensions of *S. epidermidis* strain 1 in NaCl/phosphate buffer containing 1×10^7 (○), 5×10^7 (△), or 1×10^8 (□) cells ml⁻¹ at 37 °C for various time intervals up to 3 h. The polymer films were then rinsed eight times with 3 ml amounts of buffer (see Methods).

Adhesion of bacteria

Higher numbers of *S. epidermidis* strain 1 adhered on to FEP when exposure times were prolonged and when the initial cell concentration was increased (Fig. 3). Saturation coverage of *S. epidermidis* was always reached after 2 h exposure time with bacterial cell concentrations of 10^7 cells ml⁻¹ or more. In all further experiments an exposure time of 2.5 h was used. Exponential phase bacteria adhered in the same number as stationary phase bacteria. In further experiments exponential phase bacteria were used. Similar numbers of *S. epidermidis* adhered on to FEP at pH values of 5.0, 7.2 and 9.4. Adhesion of *S. epidermidis* strain 1 on to CA was much less than on to FEP (Fig. 4a). The numbers of this micro-organism adhering on to polymer films appeared to be dependent on the initial bacterial cell concentrations, in contrast to the numbers of adhering *S. saprophyticus* (Fig. 4b).

Fig. 4. Numbers of adhering bacteria found on FEP-fluorcarbon and CA films. Specimens of the films were incubated with various concentrations of bacteria suspended in NaCl/phosphate buffer for 2.5 h at 37 °C. The polymer films were then rinsed eight times with 3 ml amounts of buffer (see Methods). Each point in the graphs represents a mean value obtained from duplicate experiments. (a) *Staphylococcus epidermidis* strain 1 on FEP (○) and on CA (▽); (b) *S. saprophyticus* on FEP (●) and on CA (▼).

Table 1. Effect of pretreatment of *S. epidermidis* (strain 1) and *S. saprophyticus* on adhesion on to FEP-fluorcarbon

Specimens of the polymer films were incubated with bacterial suspensions in NaCl/phosphate buffer, containing 5×10^8 or 1×10^9 cells ml^{-1} for 2.5 h at 37 °C and rinsed eight times with 3 ml amounts of buffer. Further details on pretreatment of bacteria are given in Methods.

$10^{-9} \times$ Initial concn of bacteria incubated with film (ml^{-1})	Pretreatment of bacteria	$10^{-3} \times$ No. of adhering bacteria (mm FEP film) $^{-2} \pm$ s.d.		
		<i>S. epidermidis</i> strain 1	<i>S. epidermidis</i> strain 2	<i>S. saprophyticus</i>
0.5	None	16 ± 1	ND	0.3 ± 0.1
0.5	Heat (60 °C)	14 ± 7	ND	0.4 ± 0.2
0.5	Formaldehyde (2%, w/v)	17 ± 2	ND	0.3 ± 0.1
1.0	None	52 ± 17	46 ± 8	0.3 ± 0.1
1.0	Trypsin (0.1%, w/v)	68 ± 8	42 ± 4	0.2 ± 0.1
1.0	Chymotrypsin (0.1%, w/v)	47 ± 6	45 ± 6	0.3 ± 0.2
1.0	Pepsin (0.1%, w/v)	$1.4 \pm 0.3^*$	$11 \pm 7^*$	0.6 ± 0.6

ND, Not determined.

* Significant ($P < 0.001$) difference between control and test. Other differences were not significant ($P > 0.1$).

Effect of pretreatment of bacteria on adhesion

Heat-killed or formaldehyde-fixed *S. epidermidis* strain 1 and *S. saprophyticus* adhered on to both polymer films to the same extent as the viable bacteria. No significant differences in the adhesion of the *S. epidermidis* strains and *S. saprophyticus* on to FEP were observed between trypsin- or chymotrypsin-treated cells and non-treated cells. Pepsin-pretreatment of *S. epidermidis* strongly reduced their adhesion on to FEP as compared to non-treated bacteria (Table 1). The presence of EDTA in the suspension during the adhesion test did not affect bacterial adhesion on to FEP (result not shown). *Staphylococcus saprophyticus* cells, either mechanically agitated in warm saline (Stinson & Van Oss, 1970), or grown at 20 °C or 42 °C in

Table 2. *Lipoteichoic acid-related effects on the adhesion of S. epidermidis (strain 2) on to FEP-fluorocarbon and CA*

The polymer films were incubated for 2.5 h at 37 °C with NaCl/phosphate suspensions containing 1×10^9 cells of *S. epidermidis* strain 2 ml⁻¹, which were either non-treated, exposed to 0.25 MIC penicillin, or treated with hot aqueous phenol. Crude LTA was added to the bacterial suspensions in a final concentration of 0.5% (w/v). The polymer films were preincubated with crude LTA in NaCl/phosphate buffer (1 mg ml⁻¹) for 1 h at room temperature. Further details are given in Methods.

Experimental conditions	10 ⁻³ × No. of adhering bacteria (mm film) ⁻² ± S.D.	
	FEP	CA
Control	24 ± 6	0.6 ± 0.6
Bacteria exposed to 0.25 MIC of penicillin	23 ± 6	1.5 ± 0.8
Bacteria treated with hot aqueous phenol	12 ± 1*	ND
Bacterial suspension with added crude LTA (final concn 0.5%, w/v)	13 ± 4*	0.2 ± 0.2
FEP films preincubated with crude LTA	18 ± 4*	ND

ND, Not determined.

* Significant ($P < 0.001$) difference between control and test. Other differences were not significant ($P > 0.2$).

Table 3. *Desorption of bacteria from FEP-fluorocarbon or CA*

The polymer films were incubated with bacterial suspensions in NaCl/phosphate buffer containing 5×10^8 cells ml⁻¹ for 2.5 h at 37 °C and rinsed once, eight or sixteen times with 3 ml amounts of buffer. FEP films were also incubated with bacterial suspensions containing 1×10^9 cells ml⁻¹, washed eight times with 3 ml of NaCl/phosphate buffer and further incubated in enzyme-containing solutions or in a solution of EDTA for 1.5 h at 37 °C and then rinsed three times with NaCl/phosphate buffer. Further details are given in Methods.

10 ⁻⁹ × Initial concn of bacteria incubated with film (ml ⁻¹)	Bacterial species	Treatment	10 ⁻³ × No. of adhering bacteria (mm film) ⁻² ± S.D.	
			FEP	CA
0.5	<i>S. epidermidis</i> strain 1	Rinsed once	15 ± 3	2.7 ± 1.4
		Rinsed eight times	14 ± 2	0.3 ± 0.3*
		Rinsed sixteen times	13 ± 2	0.1 ± 0.1*
0.5	<i>S. saprophyticus</i>	Rinsed once	0.5 ± 0.2	0.2 ± 0.1
		Rinsed eight times	0.3 ± 0.2	0.2 ± 0.1
		Rinsed sixteen times	0.4 ± 0.2	0.2 ± 0.2
1.0	<i>S. epidermidis</i> strain 1	Enzyme-free buffer	52 ± 17†	0.6 ± 0.5
		Trypsin (0.1%, w/v)	60 ± 10	ND
		Chymotrypsin (0.1%, w/v)	61 ± 11	ND
		Pepsin (0.1%, w/v)	0.9 ± 0.2*	ND
		EDTA (20 mM)	46 ± 9	ND

ND, Not determined.

* Significant ($P < 0.001$) difference between control (film washed once, or treatment with enzyme-free buffer) and test reactions. Other differences were not significant ($P > 0.2$).

† Data obtained by treatment of films with enzyme or EDTA-free NaCl/phosphate buffer or NaCl/citrate buffer were used as control and were not significantly different from data obtained with non-treated films.

order to obtain decapsulated bacteria, showed no significantly different adhesion on to either FEP or CA as compared to non-treated cells. In India ink wet-film preparations no decapsulated cells were seen.

Lipoteichoic acid related effect on adhesion

Staphylococcus epidermidis strain 2 exposed to 0.25 MIC penicillin to stimulate LTA release, adhered to the same extent on to either FEP or CA as bacteria not exposed to penicillin (Table 2). However, *S. epidermidis* strain 2 obtained after hot aqueous phenol treatment to extract LTA, adhered in lower numbers on to FEP than non-extracted bacteria. Addition of crude LTA to the *S. epidermidis* (strain 2) suspension decreased the bacterial adhesion on to FEP. Crude LTA-preincubated FEP films had a lower water contact angle ($104^\circ \pm 2^\circ$) than non-treated films ($108^\circ \pm 2^\circ$). Adhesion of *S. epidermidis* strain 2 on to such LTA-treated films was less than on to untreated FEP films.

Desorption of adhering bacteria

After frequent rinses, the numbers of *S. epidermidis* strain 1 adhering on to CA decreased significantly, but the numbers of bacteria adhering on to FEP were not reduced. The numbers of *S. saprophyticus* adhering on to either of the polymer films were not lowered after repeated rinses (Table 3).

Pepsin treatment of FEP films with adhering *S. epidermidis* strain 1 resulted in a significant desorption of bacterial cells, whereas trypsin, chymotrypsin or EDTA treatment did not reduce the number of adhering bacteria (Table 3).

DISCUSSION

The results demonstrated, that the encapsulated strain of *Staphylococcus saprophyticus* showed a hydrophilic character, as determined by the method of Rosenberg *et al.* (1980). These cells reaggregated slightly and adhered much less to FEP-fluorocarbon films than those of the two other hydrophobic *Staphylococcus epidermidis* strains. *Staphylococcus epidermidis*, rendered less hydrophobic by exposure to pepsin, showed reaggregation patterns and adhesion on to FEP similar to *S. saprophyticus*. These findings suggest that the occurrence of spontaneous reaggregation of staphylococci and their ability to adhere on to the hydrophobic polymer film was related to the presence of hydrophobic cell surface constituents. The mechanism involved in both phenomena might be hydrophobic bonding (Magnussen, 1982; Doyle *et al.*, 1982), although the bacterial reaggregation can also be affected by differences in the bacterial cell surface charge. Adhesion of staphylococci on to the more hydrophilic CA films was always low and not directly related to the bacterial cell surface hydrophobicity.

The cell surface constituents of the staphylococci involved in the adhesion on to the polymers are unknown. Recently, slime produced by *S. epidermidis* was suggested to play a major role in the adhesion of this species on to biomaterial surfaces (Christensen *et al.*, 1982). However, we used non-slime-producing staphylococcal strains. The interaction of *Staphylococcus aureus* with epithelial cells is reported to be mediated by teichoic acid (Aly *et al.*, 1980). Lipoteichoic acid is thought to be involved in the adhesion of group A streptococci to epithelial cells (Ofek *et al.*, 1975). Staphylococci, deficient in LTA after extraction with hot aqueous phenol showed a decreased adhesion on to FEP suggesting the involvement of LTA in the adhesion process. However, extraction of a staphylococcus and a streptococcus strain with hot aqueous phenol removed only 10% of their total LTA content (Huff, 1982), whereas constituents other than LTA are also extracted (Silvestri *et al.*, 1978). Therefore, the decrease in hydrophobicity and the reduced adhesion of the extracted cells might be due to the removal of constituents other than LTA or to conformational changes of surface molecules.

Gram-positive bacteria exposed to subinhibitory concentrations of cell-wall active antibiotics are depleted in their LTA or teichoic acid contents (Alkan & Beachey, 1978; Ramírez-Ronda *et al.*, 1981). In our study, staphylococci exposed to a subinhibitory concentration of penicillin showed no change in their hydrophobicity and adhered on to FEP as did cells not exposed to penicillin, confirming the results obtained by Wadström *et al.* (1981), who showed that release of LTA did not change the hydrophobicity of staphylococci. Our results indicate that LTA is not a mediator in the adhesion of staphylococci on to FEP, although the adhesion of *S. epidermidis* on

to FEP films which has been preincubated with crude LTA extracted from staphylococci was reduced. However, this reduction can be explained by a decreased hydrophobicity of the polymer film as determined by contact angle measurements. Because pepsin treatment of *S. epidermidis* decreased adhesion and caused desorption of adhering staphylococci, it is likely that protein-containing constituents are involved in the interaction of staphylococci with FEP.

The adhesion of a Gram-negative marine *Pseudomonas* species on to various polymer surfaces was correlated with the polymer surface hydrophobicity (Fletcher & Loeb, 1979). The number of adhering bacteria increased with increasing hydrophobicity of the polymers tested. Absolom *et al.* (1979) reported that surface hydrophobicity (i.e. contact angle) was inadequate as a parameter for the prediction of cellular adhesion on to solid surfaces. They proposed instead that the change in Helmholtz free energy for the overall cellular adhesion process is the appropriate parameter. These authors found that the cell adhesion on to different substrates was also dependent on the surface tension of the liquid medium in which the cells were suspended and the surface tension of the cells. The surface tension of the hydrophobic *S. epidermidis* strain is less than that of the suspending liquid (NaCl/phosphate buffer). Therefore, our finding that adhesion of *S. epidermidis* on to FEP was greater than to the less hydrophobic CA was in agreement with their results. The surface tension of the more hydrophilic *S. saprophyticus* and of the pepsin-treated or aqueous phenol-extracted *S. epidermidis* might be close to that of the suspending liquid, which could explain the low adhesion of these micro-organisms on to both polymer materials (Hogt *et al.*, 1982).

The adhesion of *S. epidermidis* to FEP showed plateau values related to exposure time and cell concentration, suggesting an equilibrium Langmuir-type adhesion. However, such an equilibrium is only reached if the adhesion of *S. epidermidis* is reversible, which is uncertain. Srinivasan & Ruckenstein (1981), proposing a kinetic model for the adhesion of cells on to solid surfaces, suggested that the rate of adhesion will be governed by the existing potential energy barriers, provided that the total free energy change for adhesion is negative. Cells interact with a surface via Van der Waals attractive forces and forces associated with electrostatic double layers. The latter are usually repulsive and might generate a substantial energy barrier for adhesion (D.L.V.O.-theory). The plateau values observed in the adhesion of *S. epidermidis* to FEP might be kinetically controlled by the increasing potential energy barrier due to the coverage-induced heterogeneity occurring during the adhesion process. Differences in the rate of reaggregation of the test strains in suspension can also be described using the D.L.V.O.-theory (Curtis, 1969). The presence of the hydrophilic capsule in *S. saprophyticus* or the changes in *S. epidermidis* surface constituents due to pepsin treatment or due to extraction with aqueous phenol, might increase the electrostatic repulsion between micro-organisms, thereby limiting the amount of effective collisions and the rate of reaggregation.

Apart from electrostatic and dispersion forces, additional interactions are possible for bacteria in close contact with surfaces (Marshall *et al.*, 1971). Our findings suggest that the adhesion of *S. epidermidis* on to FEP was probably caused by hydrophobic bonding which is not or only slightly affected by the age and the metabolic stage of the bacteria. More studies of this sort with encapsulated and unencapsulated organisms are required before generalizations can be made.

Because after insertion of implants or use of medical devices, most artificial surfaces are rapidly covered by a protein layer (Feijen *et al.*, 1979), the adhesion of staphylococci on to protein-coated polymers is currently being investigated.

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