Attachment of staphylococci to different plastic tubes *in vitro*

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Summary. Adherence of 18 staphylococcal strains to 13 types of uncoated plastic tubes made from 10 different plastic materials were investigated by binding of radiolabelled bacteria in phosphate-buffered saline for 2 h at 37°C. The different materials could be divided into five groups based on their ability to bind staphylococci. Lowest adhesion was found for plasticised polyvinylchloride. Simple assays for the relative binding of peroxidase-labelled human IgG or fibrinogen did not predict the result of adhesion studies. Neither bacterial surface hydrophobicity measured in a two-phase partitioning assay, nor hydrophobicity of materials (wettability) as measured by their contact angles in water correlated with bacterial adhesion. Adhesion of staphylococci to certain plastic materials was greatly influenced by the method used for sterilisation of the material.

Introduction

Infections associated with foreign bodies, such as intravenous catheters, are most often caused by staphylococci, especially *Staphylococcus epidermidis* and *S. aureus*.^{1–3} It must be assumed that the initial binding of the bacteria to the foreign body plays a role in the pathogenesis of these infections.⁴

Only a limited number of studies have been reported in which the attachment of bacteria to different plastic materials has been compared.^{5–7} In the present study we investigated the adhesion of staphylococcal strains to plastic tubes manufactured from different materials, and correlated this with the surface hydrophobicity of the bacteria, the hydrophobicity (wettability) of the materials, and with protein binding to these materials.

Materials and methods

Bacteria

Eighteen staphylococcal strains were used. Five *S. aureus* strains were isolated from blood, including strains E 2371 and the H strain.^{8,9} Thirteen *S. epidermidis* strains were isolated from foreign body infections: six of these were from patients with intravenous line infection, four from peritonitis in patients on peritoneal dialysis and three were blood culture isolates from patients with infections of

prosthetic heart valves. All strains were stored at –80°C.

Radioactive labelling of bacteria

Staphylococci were labelled with thymidine-methyl-³H (specific activity 5 Ci/mmol; Amersham, Bucks). For each strain, several colonies were inoculated into 5 ml of Mueller-Hinton broth containing 0.02 mCi thymidine-methyl-³H. After incubation for 18 h at 37°C, the bacteria were washed three times by centrifugation at 2000 *g* for 10 min in phosphate-buffered saline (PBS), pH 7.2, and adjusted to an absorbance (E_{650}) of 0.4 (*c.* 5×10^8 cfu/ml). The actual cfu/ml was determined by viable counts. The adhesion of bacteria after growth in Mueller-Hinton broth has previously been found to be similar to that of bacteria grown in tryptic soy broth.¹⁰

Bacterial surface hydrophobicity

Hydrophobicity of bacteria was investigated by a modified two-phase partitioning assay.¹¹ Briefly, stock solutions (25% w/w) of dextran T500 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) and polyethylene glycol (PEG; Union Carbide, New York, USA) were prepared in 0.03 M Tris buffer, pH 7.0. PEH 163 g and dextran 230 g were mixed with 350 ml of 0.03 M Tris buffer, pH 7.0, and incubated overnight. Then 2 ml of the top phase (PEG-rich) and 2 ml of the bottom phase (dextran-rich) were mixed in acid-

Table I. Component materials of plastic tubes

Code	Material
PVC (normal)	Polyvinylchloride (plasticised)
PVC (sateen)	Polyvinylchloride (plasticised)
PE _H (cold)	Polyethylene (melt index 0.7)
PE _H (normal)	Polyethylene (melt index 0.7)
PE _L (cold)	Polyethylene (melt index 0.3)
PE _L (normal)	Polyethylene (melt index 0.3)
EVA	Ethylenevinylacetate
EBA	Ethylenebutylacrylate
TPU	Thermoplastic polyurethane
PP	Polypropylene
PEBA	Polyether-block-amide
PP-EPDM	Polypropylene ethylene propylene diene monomer
SEBS	Styrene-ethylene-butylene-styrene copolymer

washed glass vials. Radiolabelled bacteria (100 µl) were added and mixed by gentle inversion 20 times. After incubation at 4°C for 2 h, radioactivity of the upper 0.5 ml of the top phase (hydrophilic) and the lower 0.5 ml of the bottom phase (hydrophobic) were determined by liquid scintillation counting. A hydrophobicity index—cpm (bottom phase) divided by cpm (top phase)—was calculated.

The analytical variation was determined by investigating the 18 strains twice on different days. The variation was determined by the formula: $SD = (\Sigma d^2/2n)$, where SD is the standard deviation, Σd^2 is the sum of squared differences of double determinations, and n is the number of observations. The inter-day and day-to-day variation was $13\% \pm 23\%$ (99% confidence limit) and $12\% \pm 18\%$, respectively.

Fibrinogen and IgG

Human fibrinogen was obtained from IMCO, Stockholm, Sweden and 0.5 g was added to 20 ml gelatine-sepharose to remove contaminating fibronectin.¹² Purified human IgG was a gift from Kabi, Stockholm, Sweden. Human fibrinogen and IgG were coupled to horseradish peroxidase according to the method described by Mathiesen *et al.*¹³

Preparation of plastic tubes

Silicone tubes with an internal diameter of 3 mm and an external diameter of 5 mm were obtained from Dow Corning Corporation (Midland, MI, USA) and used as a reference material in almost all experiments performed. Silicone was selected as a reference material as we have previously used this material extensively to characterise the adhesion of staphylococci to these catheters.¹⁰ Tubes with identical dimensions to the silicone tubes were manufactured in different plastic materials (table I). The manufacturing of the tubes took place under "clean room facilities" and proper conditions recommended by the raw material

suppliers. Documentation on the biocompatibility of the selected materials was obtained from the raw material supplier, either as documentation of passing USP "Biological tests for plastics", or as an FDA or BGA approval (food contacting materials). All materials selected, shown in table I, are at present used in different applications for medical devices or implants.

The two different surfaces, sateen or cold (normal), are the types of surfaces used normally when manufacturing plasticised PVC tubes (table I). To obtain similar differences in the surface appearance for polyethylene, the same production conditions were applied when manufacturing PE_H (melt index 0.7) and PE_L (melt index 0.3). These tubes are then referred to as cold (sateen) or normal (table I).

Sterilisation of tubes

All tubes were sterilised by two sterilisation methods frequently used for medical devices— β -irradiation and ethylene oxide sterilisation—under appropriate conditions for medical devices, as described in the Pharmacopoeias, the "Code of practice for radiation sterilization of medical products", and in the Danish "Announcement of medical utensils".

Surface hydrophobicity of plastic tubes

Surface hydrophobicity (wettability) of the different plastic tubes was analysed by measuring wetting angles with water, using the dynamic contact angle measurement method measuring both the advancing and receding contact angles.¹⁴ The advancing contact angle is deduced from immersing the tube into water, and the receding contact angle is deduced from emersion. This technique has the advantage that the measuring can take place at the tube itself. The tubes were cut into 3-cm long segments before assay, and all experiments were performed in triplicate. The analytical variation was determined for 14 tubes by performing the assay on different days. The variation was $0.3\% \pm 1.0\%$ (99% confidence limit) and $11\% \pm 35\%$ for advancing and receding angles, respectively.

Binding of human IgG and fibrinogen to polymer tubes

The relative binding of these proteins was measured by incubation of 1.0-cm segments of each tube for 30 min at 20°C in 1 ml of peroxidase-labelled fibrinogen or IgG, diluted 1 in 2000 or 1 in 10000, respectively, in PBS. These experimental conditions were chosen after experiments with different dilutions of proteins and different incubation periods. After incubation, unbound protein was removed by washing the segments five times in PBS, and the segments were then incubated in 1 ml of sodium acetate buffer (10 mM, pH 5.2) containing 5 mM H₂O₂, 0.76 M N,N-dimethylformamide (Sigma) and 3 mM 3-amino-9-

ethyl-carbazole (Sigma). The enzyme reaction was stopped by the addition of 0.4 ml of 1 M H₂SO₄, and the colour-reaction was quantified as optical density (OD) at 490 nm in a spectrophotometer after removal of the tube.

The binding relative to the binding to silicone tubes was calculated by dividing the mean OD value of three measurements by the mean OD (triplicates) obtained with silicone segments.

The analytical variation was determined for 13 tubes by performing the assay in duplicate on different days. The variation on the same day and on different days was $19\% \pm 33\%$ (99% confidence limit) and $22\% \pm 41\%$, respectively, for IgG and $14\% \pm 29\%$ and $17\% \pm 32\%$, respectively, for fibrinogen.

Adherence of bacteria to plastic tubes

The adherence assay has been described recently in detail.¹⁰ Briefly, three catheter tube segments of 1.5 cm were incubated in 3 ml of solution containing radioactively labelled bacteria for 120 min at 37°C and, after washing five times in saline, tube-bound radioactivity was determined by liquid scintillation counting. The number of adherent bacteria can be calculated from the following three values: the catheter-related radioactivity, the radioactivity of labelled bacteria/ml, and the cfu/ml. All assays were performed in triplicate and adherence was expressed as a percentage of that to silicone tubes. Binding of a given strain to different tubes was performed on the same day with the same bacterial suspension giving an analytical variation of $7 \pm 14\%$ (99% confidence limit).¹⁰

Statistical methods

The Mann-Whitney U test and Spearman rank correlation test were applied; *p* values < 0.05 were considered to be significant.

Table II. Adhesion of 18 staphylococcal strains to different plastic tubes as a percentage of adhesion to silicone tubes

Material	Percentage adhesion* to tubes sterilised by	
	β -irradiation	ethyleneoxide
PVC (normal)	4 (1–81)	11 (3–44)
PVC (sateen)	6 (1–50)	17 (1–79)
TPU	53 (22–209)	55 (26–107)
PP	73 (33–211)	76 (37–132)
SEBS	85 (35–118)	98 (26–208)
PE _H (cold)	89 (21–198)	105 (50–474)
EVA	91 (60–265)	105 (75–375)
PE _H (normal)	94 (48–232)	108 (66–602)
EBA	102 (69–226)	147 (98–496)
PE _L (normal)	107 (70–239)	130 (83–493)
PE _L (cold)	115 (57–271)	119 (61–315)
PP-EPDM	174 (65–785)	256 (82–1134)
PEBA	319 (8–2899)	198 (7–1157)

* Median (range).

Results

Adhesion of staphylococci to different materials

Table II shows the adhesion of staphylococci to the different plastic tubes. For most materials, the adhesion was reasonably similar irrespective of the sterilisation procedure used (table II). The median adhesion of β -irradiated tubes correlated to the median adhesion of tubes sterilised by ethylene oxide ($Rho = 0.8$; $p < 0.001$).

Based on the data in table II, five groups of material could be identified. Group I (lowest adhesion) included the two PVC types; group II included TPU and PP, group III SEBS, PE_H (cold), EVA, and PE_H (normal); group IV EBA, PE_L (normal) and PE_L (cold); and group V (highest adhesion) included PP-EPDM and PEBA. Nearly all materials in each of these arbitrary groups showed significantly different binding compared to materials from other groups.

Some materials (PP-EPDM and PEBA) showed great variation in the binding of individual bacterial strains. However, if the strains were ranked according to their binding ability for each material, nearly the same ranking was obtained for all materials, indicating that the same strains showed high or low adhesion to all materials (data not shown).

Binding of human IgG and fibrinogen to plastic tubes

The relative binding of human IgG and fibrinogen to the different plastic tubes is shown in table III. Most materials, except for PEBA, showed greater binding of both IgG and fibrinogen when compared with silicone (100%). Fibrinogen bound at about the same level to materials sterilised by the two different methods, but IgG bound better to some materials (PVC, EVA, EBA and TPU) when ethylene oxide had been used (table III).

The binding of proteins to the tubes did not correlate with the median adhesion of bacteria. In the case of IgG, Rho was -0.07 ($p = 0.59$) and -0.31 ($p = 0.31$) for β -irradiation and ethylene oxide, respectively, and for fibrinogen Rho was -0.01 ($p = 0.97$) and 0.10 ($p = 0.75$), respectively.

Bacterial surface hydrophobicity

The median hydrophobicity index for the 18 staphylococcal strains investigated was 1.8 (range: 0.1–9.3). When this index was correlated with adhesion to individual plastic tubes, no significant correlation coefficients were found (table IV).

Surface hydrophobicity of the different plastic tubes

Surface hydrophobicity, expressed as advancing and receding contact angle, for the different materials is shown in table V. The different sterilisation

Table III. Binding of human IgG and fibrinogen to different plastic tubes as a percentage of binding to silicone tubes

Material	Percentage binding of IgG to tubes sterilised by		Percentage binding of fibrinogen to tubes sterilised by	
	β -irradiation	ethylene oxide	β -irradiation	ethylene oxide
PVC (normal)	365	853	164	211
PVC (sateen)	318	807	155	219
PE _H (cold)	310	301	221	286
PE _H (normal)	261	351	228	279
PE _L (cold)	380	360	222	298
PE _L (normal)	296	300	221	296
EVA	486	709	343	363
EBA	564	926	238	391
TPU	62	132	112	181
PP	270	262	240	289
PEBA	36	49	66	71
PP-EPDM	175	176	141	145
SEBS	221	355	201	266

Table IV. Correlation (Spearman rank correlation) between hydrophobicity and adhesion for 18 bacterial strains

Material	Rho (p value) for bacteria hydrophobicity and adhesion to tubes sterilised by	
	β -irradiation	ethylene oxide
PVC (normal)	0.18 (0.48)	-0.01 (0.98)
PVC (sateen)	0.17 (0.49)	0.03 (0.92)
PH _H (cold)	0.27 (0.27)	-0.13 (0.61)
PH _H (normal)	0.22 (0.38)	-0.33 (0.19)
PE _L (cold)	0.34 (0.16)	-0.48 (0.06)
PE _L (normal)	0.48 (0.06)	-0.16 (0.51)
EVA	-0.03 (1.00)	-0.10 (0.70)
EBA	0.17 (0.51)	-0.23 (0.35)
TPU	0.37 (0.14)	0.39 (0.11)
PP	0.28 (0.26)	0.16 (0.51)
PEBA	0.40 (0.10)	0.32 (0.19)
PP-EPDM	0.05 (0.84)	0.09 (0.73)
SEBS	0.05 (0.84)	0.32 (0.20)

methods did not markedly influence the contact angles. For both types of sterilisation, the advancing and receding angles showed a high degree of correlation (Rho = 0.74, $p < 0.01$ for β -irradiation, and Rho = 0.68, $p < 0.01$ for ethylene oxide). However, there was no correlation between median bacterial adhesion to the different materials and their contact angles (β -irradiation: Rho = -0.03, $p = 0.91$ for advancing and -0.36, $p = 0.23$ for receding angle; ethylene oxide: Rho = 0.08, $p = 0.79$ for advancing and 0.36, $p = 0.23$ for receding angle).

There was a weak positive correlation between the advancing contact angle of different β -irradiated materials and their binding of fibrinogen (Rho = 0.56, $p = 0.04$), but not between the receding angle and fibrinogen binding (Rho = -0.46, $p = 0.11$). The binding of fibrinogen to ethylene oxide-sterilised materials and the binding of IgG to both types of

Table V. Wettability (advancing or receding contact angle in water as a percentage of that of silicone) of different plastic tubes

Material	Percentage adhesion (contact angle, degrees) to tubes sterilised by			
	β -radiation		ethylene oxide	
	Advancing	Receding	Advancing	Receding
PVC (normal)	95 (88)	96 (67)	86 (85)	87 (64)
PVC (sateen)	97 (90)	87 (62)	97 (96)	111 (81)
PE _H (cold)	90 (83)	77 (54)	82 (81)	80 (59)
PE _H (normal)	91 (84)	73 (51)	81 (81)	90 (66)
PE _L (cold)	95 (88)	83 (59)	88 (86)	91 (67)
PE _L (normal)	95 (88)	74 (52)	96 (94)	96 (70)
EVA	91 (84)	70 (49)	86 (85)	70 (52)
EBA	94 (87)	85 (60)	92 (91)	92 (67)
TPU	95 (88)	92 (65)	96 (95)	58 (43)
PP	92 (85)	73 (51)	90 (89)	89 (65)
PEBA	94 (87)	61 (43)	82 (81)	54 (40)
PP-EPDM	107 (99)	109 (77)	100 (99)	109 (79)
SEBS	100 (92)	87 (61)	96 (95)	93 (92)

* Percentage adhesion of adhesion to silicone tubes (degrees contact angle).

materials did not correlate with contact angle measurements (data not shown).

Influence of sterilisation procedure

The adhesion of four strains (two *S. epidermidis* and two *S. aureus* strains, which showed the highest binding to most polymers) to seven sterilised materials was compared with adhesion to unsterilised materials (table VI). Both types of PVC gave highly increased adhesion when β -irradiation was used compared with ethylene oxide treatment (table VI). Also, SEBS showed higher binding of bacteria to β -irradiated material, whereas for TPU and PP-EPDM, ethylene oxide sterilisation gave the highest adhesion (table VI).

Table VI. Adhesion (as a percentage of adhesion to non-sterilised tubes) of four staphylococcal strains to seven plastic materials

Material	Percentage adhesion to tubes sterilised by	
	β -Irradiation	Ethylene oxide
PVC (normal)	349 (131–682)*†	113 (97–201)
PVC (sateen)	286 (183–335)†	130 (120–269)
TPU	88 (80–105)	121 (110–145)‡
PP	88 (86–95)	99 (84–113)
PEBA	120 (102–180)	115 (104–142)
PP-EPDM	86 (75–94)	106 (91–133)‡
SEBS	97 (79–123)	73 (58–81)

* Median (range).

† Increased adhesion ($p < 0.01$) compared to ethylene oxide.‡ Increased adhesion ($p < 0.01$) compared to β -irradiation.

In the case of PP and PEBA, the adhesion seemed similar irrespective of the method of sterilisation.

Discussion

The pathogenesis of most foreign body infections has not been clarified, and the number of infections occurring either during or after implantation is unknown. Therefore, the choice of plastic surfaces used for experimental purposes is not well defined and could be either the native surface or a surface covered with host-derived proteins, cell or tissue debris, or both. Furthermore, the composition of the coating substances on the plastic material, when exposed *in vivo*, is almost unknown, and clinically relevant coating conditions have not been established for experimental studies. In the present study, we investigated the binding of bacteria in PBS as a representation of the adhesion that may occur during implantation of a new foreign body. Others have performed assays in the presence of albumin 0.5% to reduce "unspecific adhesion",¹⁵ and such an assay may simulate an infection that occurs after the foreign body has been inserted. We believe that during the insertion of a catheter the binding of bacteria (contamination during the handling of the material) will occur directly on the catheter without any plasma proteins pre-adsorbed to the material, and the assay used in this study is a more accurate reflection of this.

Although the individual staphylococcal strains showed great variation in adhesion to certain materials, it was possible to distinguish plastic with high and low adhesion capacity (table II). PVC showed the lowest adhesion capacity and there was no major difference in adhesion for the two different surfaces of this material (table II). TPU and PP also showed a relatively low degree of adhesion, while the highest degree of adhesion was found with PP-EPDM and PEBA (table II). Few authors have studied adhesion of staphylococci to different plastics *in vitro*. Ludwicka *et al.*⁷ investigated the adhesion of five *S. epidermidis*

strains to seven plastics including TPU, PP and PE. They observed that hydrophobic strains bound less to TPU than to the two other materials but binding of the hydrophilic strains to TPU was greater.⁷ Kristinsson⁶ found no difference between the binding of staphylococci to silicone or TPU. The reason for the low binding to PVC cannot be explained, but the main differences between PVC and the other materials are the chloride content and the high content (up to c. 40% w/w) of plasticiser.

When a plastic tube is suspended in a solution containing proteins the surface is rapidly coated with proteins. We have used an assay that only arbitrarily measures the relative amount of either human IgG or human fibrinogen that binds in a given period. Our aim was to investigate whether such a simple assay could predict the ability of the material to bind bacteria. However, there was no correlation between protein binding and bacterial adhesion. Indeed, in the case of IgG, binding was higher to PVC than that to PEBA. It seemed that both fibrinogen and IgG bound least to PEBA, TPU and the silicone tube used as control (table III). Also, the binding of IgG was more influenced by the sterilisation method than that of fibrinogen (table III). Most proteins, both surface proteins from staphylococci and human plasma proteins, inhibit the adhesion of staphylococci to silicone catheters,¹⁰ and the binding of proteins other than the two tested here may predict bacterial adhesion better.

Hydrophobic interactions have been suggested as important factors in the early steps of microbial adhesion to polymers.^{7,16,17} However, the experimental background for this hypothesis is not clear. Increasing hydrophobicity of materials, as measured by their contact angles in water, gave increasing adhesion of marine pseudomonas strains.¹⁶ Similar findings were found for a few *S. epidermidis* strains by Hogt *et al.*⁵ and Ludwicka *et al.*,⁷ but Hogt *et al.*,¹⁷ in a subsequent study, concluded that adhesion was unrelated to bacterial hydrophobicity. Also, Dankert *et al.*¹⁸ found that neither surface charge nor hydrophobicity of the bacteria correlated directly with binding to hydrophobic materials. Bacterial hydrophobicity measured as adherence to xylene in an aqueous phase was found to be significantly related to adhesion to teflon catheters by Pascual *et al.*¹⁹ However, in that study, the bacteria were grown in Mueller-Hinton broth for the adhesion test and in tryptone soy broth for the hydrophobicity test and it has been found that growth of bacteria in different media may change their hydrophobicity substantially.^{20,21} Recently, Kristinsson⁶ showed no correlation between binding of staphylococci to xylene in an aqueous phase and adhesion to silicone catheters, when bacteria for both assays were grown in the same medium.

Our findings in this study strongly challenge the belief that hydrophobic interactions are major determinants of staphylococcal adhesion to polymers. There was a complete lack of correlation between

bacterial surface hydrophobicity and adhesion of the bacteria to a wide range of polymer tubes (table IV), and a lack of correlation between surface hydrophobicity of the plastic materials and bacterial adhesion. However, the hydrophobicity of the various plastic materials showed only minor differences (table V). The lack of variation in surface properties of these highly different materials may be due to their additive content. These additives (e.g., stabilisers and lubricants), which are necessary for the manufacturing of the plastic tubes, are often low mol. wt substances, which may migrate to the surface and there influence the binding. Whether bacterial surface hydrophobicity plays a role in binding to plastic materials coated with host-derived proteins also needs to be investigated.

The clinical significance of the data obtained in the present study is difficult to evaluate. We do not know how well the adhesion of bacteria to plastic materials *in vitro* correlates with the rate of clinical infections related to different foreign bodies. A range of other factors may affect the risk of infection, including the design and size of the foreign body, the degree of tissue damage, and the ability of the foreign body to activate the complement system and the coagulation system. At present there is a need for reliable in-vitro methods

for predicting the in-vivo performance. Only limited clinical data are available on the differences in infection and contamination rates of foreign bodies of different materials. Sheth *et al.*²² demonstrated a significantly increased number of bacteraemias related to the use of PVC as compared to polytetrafluoroethylene as materials in central venous catheters; and Karlan *et al.*²³ showed that myringotomy tubes made of polytetrafluoroethylene gave fewer ear infections than myringotomy tubes made of silicone. Clinical studies like these, or perhaps involving experimental animal models, in which different polymers can be compared, may determine, in the future, which type of material is most appropriate.

In conclusion, when the binding takes place in buffer, different plastic tubes bind staphylococci very differently, with the lowest binding to PVC. No correlation was found between hydrophobicity of either the plastic material or the bacteria and the adhesion of staphylococci, but the sterilisation method used for the plastics may greatly influence adhesion. Simple binding assays for IgG and fibrinogen did not predict the extent of bacterial adhesion.

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