Adherence of staphylococci to intravascular catheters

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Summary. Adherence of seven strains of Staphylococcus epidermidis and three strains of S. aureus to three types of intravascular catheters was assessed by ATP bioluminescence, by culture after ultrasonication and by scanning electron microscopy. The catheter materials studied were silicone elastomer, thermoplastic polyurethane and polyurethane coated with Hydromer®, a coating which absorbs water and provides a hydrophilic sheath around the catheter. The adherence assays were performed in phosphate-buffered saline on a rotary shaker at 37°C, with the catheters precoated with serum and uncoated, and the results were correlated with bacterial hydrophobicity. There was wide strain-to-strain variation in bacterial adherence; S. aureus and slime-producing S. epidermidis strains adhered better than did non-slime-producing strains. Overall, there was less bacterial adherence to Hydromer®-coated catheters than to polyurethane and silicone catheters but it was unrelated to bacterial hydrophobicity. Serum coating of catheters resulted in marked reduction of bacterial adherence.

Introduction

Intravascular catheters are essential in the management of acutely ill patients. Long intravenous lines are often left in situ for long periods to maintain infusions of hyperosmolar fluids and drugs and for haemodynamic monitoring. Silicone catheters (Hickman® and Broviac®) are widely used to maintain long-term intravenous access in patients with haematological malignancy and in patients on total parenteral nutrition. The major complication of prolonged intravascular catheterisation is infection, leading to bacteraemia that may be life-threatening (Sheretz et al., 1983). Press et al. (1984) quoted an average infection rate of 0.14/100 catheter days.

The intravascular portion of a catheter is encased by fibrin within 5–7 days (Hoshal et al., 1971) and catheter thrombosis is a major risk factor in the development of catheter infection (Press et al., 1984). Thrombogenicity is associated with the stiffness (Stenqvist et al., 1983) and the nature (Borow and Crowley, 1985) of the catheter material. Catheter manufacturers have concentrated mainly on making biocompatible catheters with a low thrombogenic potential, rather than catheters with antibacterial properties. However, bacterial adherence is important in the initial stages of infections of prosthetic devices (Peters et al., 1982) and coagulase-negative staphylococci (CNS) and Staphylococcus aureus are the commonest organisms causing such infections (Press et al., 1984; Sattler et al., 1984; Harvey et al., 1986). The aim of this study was to investigate the initial adherence of CNS and S. aureus to two commonly used catheter materials, silicone and polyurethane, and to compare these with the adherence to polyurethane coated with Hydromer®, which makes surfaces more hydrophilic and reduces thrombogenicity (Borow and Crowley, 1985; Seifert and Greer, 1985).

Materials and methods

Bacteria

Three S. aureus strains (C710, C1583, C1662) and four S. epidermidis strains (C1276, C1543, A1085, A2057) were isolated from infected long intravenous catheters in the Bacteriology Department, Royal Hallamshire Hospital, Sheffield. S. epidermidis strain KH11 was from Professor G. Peters, Hygiene Institute, Cologne, West Germany. Two S. epidermidis strains (C362, C988) isolated as skin commensals were also included. Four of the S. epidermidis strains produced extracellular slime as detected by the method described by Christensen et al. (1982). All strains were stored at −20°C in glycerol 10% v/v broth. They were cultivated overnight at 37°C in Tryptone Soy Broth (TSB, Oxoid) when required.
Strains were harvested by centrifugation, washed twice in 10-ml volumes of phosphate-buffered saline (PBS, pH 7.4), and resuspended in PBS by vortex mixing. The bacterial suspension was filtered through membrane filters (type NC 14-0 μm, Millipore Co., MA, USA) to remove clumps and adjusted to a density of (0.5–1) × 10^9 cfu/ml by optical-density measurement at 540 nm (OD_{540}) and calibrated by viable counts. The slime-producing strains of *S. epidermidis* were treated by ultrasonication (12 m peak-to-peak, for 1 min; MSE ultrasonic disintegrator) before filtering.

**Measurement of hydrophobicity**

Bacterial hydrophobicity was assessed by measuring adherence to xylene in an aqueous phase (Rosenberg, 1984). Analytical grade p-xylene (0.25 ml) was added to 3 ml of the bacterial suspension that had been adjusted to an OD_{540} of 1.0 (c. 10^9 cfu/ml). The suspension was vortex-mixed for 60 s and left for phase separation for 30 min. The aqueous phase was removed and its OD_{540} measured. Percentage hydrophobicity was expressed as

\[
\frac{\text{OD}_{540\text{ after phase separation}}}{\text{OD}_{540\text{ of original suspension}}} \times 100.
\]

**Intravenous catheters**

All three types of catheters used were supplied by British Viggo: silicone elastomer, gauge 14, with barium sulphate 12% (SIL); thermoplastic polyurethane (Pellethane® 2363 90A), gauge 16 (PUR); and polyurethane coated with Hydromer®, gauge 14 (HYD). Hydromer coating is a hydrogel material made by the interaction of polyvinylpyrrolidone with an isocyanate prepolymer. It is chemically bonded to the polyurethane catheter material. The Hydromer coating acts like a sponge absorbing water from the blood to provide a hydrophilic sheath around the catheter. The catheters were provided sterile and stored in plastic bags. To provide easy access to the bacteria to the whole catheter surface, the catheters were split longitudinally with a sterile scalpel before coating with Hydromer; this avoided damage to the Hydromer coat and its subsequent loss.

**Serum treatment**

Serum was collected from four healthy volunteers and pooled. Catheter pieces were immersed in whole undiluted serum for 30 min at room temperature. Excess serum was shaken off the catheters before use in the adherence assays.

**Initial adherence**

Catheters were placed in 7-ml glass screw-capped bottles containing 2 ml of bacterial suspension (0.5–1) × 10^9 cfu/ml; these were placed on a rotary shaker in a 37°C water bath for 1 h. Care was taken to ensure total submersion of the catheters without air bubbles. Bacterial suspensions without catheters were included as standards for the ATP bioluminescence assays. After the bacteria had been allowed to adhere, catheters were removed from the bottles and placed in glass 28-ml containers. After washing gently three times with 10-ml volumes of PBS, excess buffer was shaken off and the catheters assessed for bacterial attachment. The adherence experiments were done in duplicate for each strain on at least two occasions.

**Assessment of bacterial attachment**

Bacterial attachment was assessed quantitatively by ATP bioluminescence and by culture after ultrasonication. Scanning electronmicroscopy (SEM) was used for qualitative assessment of adherence.

**ATP bioluminescence.** The method used was a modification of that of Ludwicka et al. (1985). Catheter pieces were placed in small plastic capules and 200 μl of trichloroacetic acid 2-5% w/v was added; this released bacterial ATP and inactivated ATP-degrading enzymes. A blank control was prepared at the same time with 50 μl of PBS instead of the catheters. After mixing, a 50-μl sample was transferred to a plastic cuvette and 1 ml of ATP-monitoring reagent (LKB-Wallac 1243–200, 1 vial reconstituted with 50 ml of Tris-EDTA buffer, pH 7.75) was added. The light generated was assayed by a LKB 1250 Luminometer. To calculate the amount of ATP in each sample, 20 μl of ATP standard (LKB-Wallac 1243–201; 1 vial reconstituted with 10 ml of Tris-EDTA buffer) was then added to each sample, and the increase in light emission measured. The amount of ATP was calculated by dividing the light generated by the bacterial ATP (mV_{bact}) by that generated by the bacterial ATP in the standard (20-μl standard contains 0.2 nmol). The result obtained with the blank (calculated in the same way) was subtracted from this to obtain the true value.

\[
\text{ATP (nmoles)} = \left( \frac{mV_{bact}}{mV_{bact + \text{stand}}} \right) \times 0.02 - \text{blank}
\]

The ATP content of a 50-μl sample of the blank control suspension (without catheters) and two ten-fold dilutions was measured for each strain. From a standard curve of ATP concentration (nmoles) against viable count it was possible to calculate the number of bacterial cells adherent to each catheter.

**Culture.** The catheter pieces were placed in 10 ml of PBS in a glass 28-ml container and sonicated at 12 m (peak-to-peak) for 3 min, in an ultrasonic disintegrator (MSE); 1 ml of each of three ten-fold dilutions was used to make pour-plates with nutrient agar. After incubation for 18 h at 37°C, colonies were counted to estimate the number of cfu/ml and the cfu/cm² of catheter was then calculated.
Scanning electronmicroscopy. Immediately after washing, the catheter pieces were fixed in gluteraldehyde 3% v/v in sodium cacodylate buffer (300 mOsm, pH 7.4) for at least 24 h at room temperature. The catheters were then washed in the same buffer, dehydrated through increasing ethanol concentrations and critical-point dried in a Polaron E-3000 drier with liquid CO₂. After drying, the catheters were fastened with double-sided sellotape and coated with gold for 3 min in a sputter coater (Edwards Sl50) with a gold-plated cathode at 50 mA in an atmosphere of argon for examination in a scanning electronmicroscope (Philips 50) at 15 kV with 45° tilt. The catheter surfaces were examined for adherent bacteria and surface irregularities.

Statistics

Results were compared by the Student's t test and, where appropriate, by paired t-tests.

Results

Comparison of the bioluminescence assay and culture

There was good agreement between the ATP bioluminescence assay and culture after ultrasonication (correlation coefficient, r = 0.62). The correlation between the two assay systems is shown in fig. 1. The results of culture were slightly lower than those of the ATP assays, especially for polyurethane catheters.

Adherence to different catheter materials

Conventional catheters. There was great strain-to-strain variation and, although the bacteria appeared to adhere better to polyurethane than to other catheters when measured by ATP bioluminescence, the difference was not statistically significant (fig. 2). With some of the Hydromer-coated catheters, the damaged lining on the inside of the catheters flaked off while on the rotary shaker. This was confirmed by electronmicroscopy. The results obtained by culture after ultrasonication were slightly lower than those obtained by the ATP bioluminescence assays, especially with the polyurethane catheters.

Serum-treated catheters. Exposure of catheters to serum resulted in a significant reduction in bacterial adherence to all types of catheter, when adherence was measured by culture after sonication (mean adherence 4 × 10⁶ cfu versus 1 × 10⁵ cfu, p < 0.001; fig. 3). When, however, adherence was measured by ATP bioluminescence (data not shown in figs.), the reduction in bacterial adherence (significance analysed by paired t-tests) was significant for polyurethane catheters (1.1 × 10⁷ cfu versus 3.2 × 10⁶ cfu, 0.05 < p < 0.1) but not for silicone (5 × 10⁶ cfu versus 1.9 × 10⁶ cfu, 0.05 < p < 0.1) or Hydromer-coated catheters (6.3 × 10⁶ cfu versus 1 × 10⁷ cfu, 0.2 < p < 0.25).

Because of this inconsistency the effects of serum
on ATP bioluminescence were assessed. Incubation of staphylococci with serum for $\geq 30$ min increased their ATP content slightly; it then remained stable (fig. 4) rather than decreasing, as it did when serum was not present.

**Precut completely Hydromer-coated catheters.** The Hydromer coat did not flake off these catheters and the bacteria adhered significantly less well to be Hydromer-coated catheters ($t=2.6$, $DF=6$, $p<0.05$), as measured by ATP bioluminescence (fig. 5), despite the surface area being increased by the fluid expansion of the Hydromer coating.

**Adherence by different bacterial strains**

When assessed by ATP bioluminescence, *S. aureus* and slime-producing strains of *S. epidermidis* adhered significantly better to the three types of catheters than did non-slime-producing *S. epidermidis* strains ($p<0.01$) (fig. 6). The results of culture after ultrasonication (not shown) were similar.

**Hydrophobicity**

All strains of *S. epidermidis* were very hydrophilic (i.e., hydrophobicity 0–6.5%) whereas the three *S. aureus* strains were relatively more hydrophobic (i.e., hydrophobicity 20–23%). The ability to produce slime did not appear to influence the hydrophobicity of *S. epidermidis* (table).

**Correlation of hydrophobicity with adherence**

Overall, there was no correlation between bacterial hydrophobicity and adherence. The relatively
ADHERENCE OF STAPHYLOCOCCI

Fig. 5. Comparison of adherence (measured by ATP bioluminescence) of staphylococci on to plain and Hydromer-coated polyurethane catheters.

Hydrophobic strains (C710, C1583 and C1662) adhered very well to all catheters; however, some very hydrophilic strains (e.g., C362 and C1543, both producing slime) also adhered extremely well.

Scanning electronmicroscopy

When catheter surfaces were examined by SEM there were marked differences in appearance. Polyurethane catheters were smooth and even with very few surface irregularities (fig. 7); silicone catheters had a rough surface and holes were common (figs. 8 and 9). Catheter surfaces coated with Hydromer had a wavy, crater-like appearance (fig. 10). The Hydromer was examined only in this dry state after it had been soaked with liquid and then dried. It was difficult to find bacteria on intact Hydromer-coated surfaces, but they were abundant where the Hydromer coating had flaked away. The S. aureus and slime-producing S. epidermidis strains tested adhered preferentially in sheets or clusters (fig. 7); the non-slime-producing strains adhered as single cells. Almost all holes and irregularities were filled with bacterial cells (fig. 9). Some of the cells

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain no.</th>
<th>Slime production</th>
<th>Hydrophobicity (%)</th>
<th>Adherence (cfu x 10⁶/cm²) to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PUR*</td>
</tr>
<tr>
<td>S. aureus</td>
<td>C710</td>
<td>. . . .</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>S. aureus</td>
<td>C1583</td>
<td>. . . .</td>
<td>23</td>
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<td>C1662</td>
<td>. . . .</td>
<td>22</td>
<td>40</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>C988</td>
<td>−</td>
<td>3</td>
<td>1.1</td>
</tr>
<tr>
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<td>C1276</td>
<td>−</td>
<td>6</td>
<td>3</td>
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<td>A2057</td>
<td>−</td>
<td>6</td>
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</tr>
<tr>
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<td>+</td>
<td>6</td>
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<td>+</td>
<td>0</td>
<td>2</td>
</tr>
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<td>C1543</td>
<td>+</td>
<td>0</td>
<td>112</td>
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<tr>
<td>S. epidermidis</td>
<td>KH11</td>
<td>+</td>
<td>6</td>
<td>28</td>
</tr>
</tbody>
</table>

* Catheters were of polyurethane (PUR), silicone (SIL) or Hydromer-coated polyurethane (HYD) (see Materials and methods).
. . . . = Not relevant.

Table: Slime production, hydrophobicity and adherence (measured by ATP bioluminescence) of the staphylococcal strains investigated
Fig. 7. *S. aureus* strain C1583 adhering to surface of polyurethane catheter (bars = 10 μm).

Fig. 8. *S. aureus* strain C1583 adhering to surface of silicone catheter (bars = 10 μm).

appeared to attach themselves by means of projections from the cells to the catheter surface (fig. 11).

Discussion

Bacterial biomass adherent to prosthetic materials can be measured by direct microscopy (Hogt *et al.*, 1986), by quantitative culture (Sheth *et al.*, 1983; Mackenzie and Rivera-Calderon, 1985), by use of radiolabelled bacteria (Pascual *et al.*, 1986) or by assay of ATP by bioluminescence (Harber *et al.*, 1983). Each method has some disadvantages, but ATP bioluminescence provides a rapid and simple quantitative assay of bacterial biomass.
attached to synthetic polymer surfaces (Ludwicka et al., 1985). The latter method was modified to measure the number of bacteria attached to commercially produced catheter tubing, and the results correlated well with results obtained by culture after ultrasonication. Culture gave slightly lower results than the ATP assay, particularly with polyurethane catheters to which bacteria may have adhered more avidly so that the degree of ultrasonication was insufficient to remove all adherent cells from the catheter surface; alternatively, adherent cells may have slightly higher ATP content than cells in suspension. The discrepancy between methods was much more marked with serum-treated catheters; this was found to be because serum increased the ATP content of the bacterial
cells. The ATP content remained almost constant up to 90 min whereas that of the cells in buffer without serum declined significantly. Therefore, measurement of cells adherent to serum-treated catheters by ATP bioluminescence produces spuriously high results.

SEM showed that fewer organisms adhered to the Hydromer coating than to other surfaces, a finding that contradicted the results of the initial quantitative assays. However, in some of the initial assays the damaged Hydromer coating on the inside of the catheters flaked off leaving exposed areas of polyurethane and there were more bacteria adherent to the exposed surfaces than to Hydromer coating. Therefore, catheters coated with Hydromer after they had been cut were obtained from the manufacturer and compared with uncoated catheters. This time ATP bioluminescence showed significantly less adherence to the Hydromer-coated catheters, despite the increase in surface area caused by the fluid soaking of the Hydromer coat. The mean adherence of all strains to silicone, polyurethane and “damaged” Hydromer catheters was not statistically different.

There was wide strain-to-strain variation in bacterial adherence. *S. aureus* and slime-producing *S. epidermidis* strains adhered significantly better to all catheters than did the non-slime-producing *S. epidermidis* strains. This is in agreement with the findings of Christensen *et al.* (1985) who found a good correlation between slime production and adherence. Conversely, Pascual *et al.* (1986) found that slime-producing CNS adhered less quickly and less strongly than other CNS, but they incubated their catheters without shaking. The stress or challenge of shaking demonstrates differences in adherence between slime-producing and non-producing strains (Kristinsson *et al.*, 1987).

Bacterial adherence to prosthetic biomaterials or plastics is poorly understood. It depends upon attracting and repelling forces between the two surfaces and it might be thought that hydrophobic bacteria would be rejected from aqueous fluids and attracted towards non-aqueous phases, e.g., solid surfaces (Marshall, 1985). All strains tested were hydrophilic (0–23%) and it is likely that growth in TSB enhances production of hydrophilic sites on the surface of staphylococci. There was no correlation between hydrophobicity and adherence, but this narrow hydrophobicity range makes correlation more difficult. Reifsteck *et al.* (1987) investigated the hydrophobicity-hydrophilicity of staphylococci but included only one strain of *S. epidermidis*, and found only slight correlation between hydrophobicity and adherence to silicone catheters. Pascual *et al.* (1986) found a significant positive correlation between hydrophobicity and adherence and concluded that hydrophobicity played a powerful role, but Hogt *et al.* (1985) concluded that adherence was not related to hydrophobicity. However, each study used different catheter materials and different methods. Furthermore growth of bacteria in different media changed their hydrophobicity (Ljungh *et al.*, 1985); there-
fore, to relate hydrophobicity and adherence, cells must be grown in the same medium and conditions for both tests. Pascual et al. (1986) used cells grown in Mueller-Hinton broth for adherence assays but in TSB for the hydrophobicity tests; information is lacking from Hogt et al. (1985).

Measuring bacterial adherence to clean catheters is an over-simplification of events in vivo. Much of the catheter surface is coated within seconds with body fluids and proteins, followed by platelets and fibrin. Once coated, the catheter material makes little difference to bacterial adherence, but initial adherence may depend on the catheter material. Coating the catheters with serum, intended to imitate those conditions, led to a significant decrease in bacterial adherence to all catheters, confirming the results of Hogt et al. (1985) and Pascual et al. (1986).

Assay of bacterial biomass by ATP bioluminescence makes it feasible to compare bacterial adherence to different catheters, but the present method is cumbersome for testing large numbers of strains under different conditions. More rapid methods, based on a microtitration format now being developed, will enable in-vitro evaluation of bacterial adherence to new catheter materials before clinical trials.

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REFERENCES


