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Effect of plastic catheter material on bacterial adherence and viability

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Summary. The kinetics of adherence of single isolates of *Staphylococcus aureus*, *S. epidermidis*, *Pseudomonas aeruginosa* and *Escherichia coli* to catheters made of polyvinyl chloride (PVC), Teflon®, siliconised latex, polyurethane and Vialon® was evaluated by a radiometric assay. Radiolabelled bacteria (10^8 cfu/ml) were incubated in vials containing 1-cm lengths of catheter for up to 3 days. The peak of maximal adherence to each biomaterial was reached after 24 h for *P. aeruginosa* and after 72 h for the other strains. Bacterial adherence to PVC and siliconised latex was significantly higher (2–6 times; $p<0{\cdot}05$) than to the other biomaterials for all the strains. The lowest values of adherence were observed with polyurethane and Vialon® for the staphylococci but with Teflon® for *E. coli* and *P. aeruginosa*. Bacterial viability and growth was evaluated in eluates obtained from incubation of segments of each catheter in buffer for 24 h. None of the eluates affected the viability of the staphylococci. However, all of them, significantly increased the growth of *E. coli* and *P. aeruginosa* with the exception of the eluate from siliconised latex, in which the inoculum count was reduced to an undetectable level for *E. coli*. We conclude that bacterial adherence to catheters may depend in part on the nature of the biomaterial and that certain substances eluted from the catheters may affect the viability and growth of different micro-organisms.

Introduction

Intravascular and urinary catheters are frequently used in hospitals. Several studies have demonstrated significant infection rates associated with local skin conditions, methods used in the placement of such devices and the duration of catheter usage.[1,2] Catheter-associated infections are a significant source of nosocomial morbidity and mortality.[3] The mechanisms by which infections develop are not fully understood but the phenomenon of adherence to the biomaterials appears to be a critical factor in initiating colonisation and subsequent infection.[4]

The nature and chemical composition of the biomaterials commonly used to make medical devices differ, and many of them contain different additives and plasticisers to improve their physico-chemical properties and biocompatibility.[5]

The adherence of different micro-organisms to, and their survival in, catheters is promoted not only by bacterial factors but additional bacterium-device interactions could participate in this phenomenon.[6] It has been proposed that some micro-organisms, e.g., coagulase-negative staphylococci, could metabolise some of the components of plastic catheters in the absence of other nutrients and use them to sustain growth on the surface of biomaterials.[7]

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We have developed an experimental in-vitro system for evaluating quantitatively the adherence of bacteria over a period of 3 days to five intravascular and urinary catheters composed of polyvinyl chloride (PVC), Teflon®, siliconised latex, polyurethane and Vialon®. The effect of eluates of these biomaterials on the growth of different micro-organisms in the absence of other nutrient sources has also been assessed.

Materials and methods

Bacterial strains and radioactive labelling

One strain each of *Escherichia coli* (HUS 25), *Pseudomonas aeruginosa* (HUS 36), *Staphylococcus aureus* (HUS 41) and *S. epidermidis* (HUS 59) were used. The strains were isolated from blood cultures of patients with catheter-associated infections. Strains were identified by standard methods. Heavy suspensions of each strain in Tryptone Soy Broth (Oxoid) with glycerol 10% were stored in small volumes at −70°C.

For adherence assays, several colonies from a nutrient-agar plate were inoculated into 5 ml of Mueller-Hinton broth containing ($^{2-3}$H) adenine (specific activity 24 Ci/mmol; Amersham) 10 µl.[8] Before use, bacteria were washed three times with phosphate-buffered saline (PBS) and resuspended to obtain a final concentration of 10^8 cfu/ml.

Catheters

Catheters made of five different biomaterials were used: (a) polyvinyl chloride (PVC) (Drum-Cartridge® catheter, Abbott Laboratories, Eire); (b) Teflon® (Abbocath R–T, Abbott Laboratories); (c) latex (siliconised) two-way paediatric Foley catheter (Inmed®; Inmed USA, Malaysia); (d) polyurethane (Cavafix Certo®; B. Braun Melsugen AG, Germany); and (e) Vialon® (Viacath®, Becton Dickinson, USA). For adherence assays, catheters were cut under sterile conditions into 1·0-cm segments and pre-incubated in 10ml of sterile isotonic PBS at 37°C for 1 h.[9]

Eluates of the catheters were prepared by incubating 30 segments of each in 10 ml of PBS at 37°C for 24 h under sterile conditions.

Bacterial adherence to urinary catheters

For the adherence assays, 10 ml of radioactive bacterial suspension (10^8 cfu/ml) were incubated with 30 segments of each catheter type in 25-ml screw-capped glass vials at 37°C. After 5 min, 1, 6, 24 and 72 h, two catheter segments were removed, washed five times in cold PBS to remove all non-adherent bacteria and deposited in polypropylene vials. (Biovial®; Beckman Instruments, USA) with 2·5 ml of scintillation fluid (Aqua Luma Plus, Lumac/3M, The Netherlands). Catheter-bound radioactivity was determined in a scintillation counter (1701 LS Beckman Instrument, Inc). From this value, the number of bacteria adherent per cm^2 of catheter was calculated by dividing the number of bacteria adherent to a catheter piece by the total surface area of the piece. The relationship between radioactivity and number of bacteria was obtained by counting in a biovial 100 μl of the initial inoculum.

Bacterial growth in catheter eluates

Each strain was inoculated into catheter eluates at a final concentration of 10^3 cfu/ml. At timed intervals, 10-μl samples were diluted in ice-cold PBS and pour plates made in Mueller-Hinton Agar (Oxoid); colonies were counted after incubation for 48 h at 37°C.

Statistical methods

Results were expressed as mean and SD. Differences amongst groups were compared by analysis of variance and the Bonferroni method[10] was used to assess statistical significance at $p \leqslant 0{\cdot}05$.

Results

Bacterial adherence to catheters

The kinetics of adherence of *S. epidermidis* and *E. coli* to the plastic catheters up to 72 h of incubation are shown in figs. 1 and 2. After incubation for 24 h,

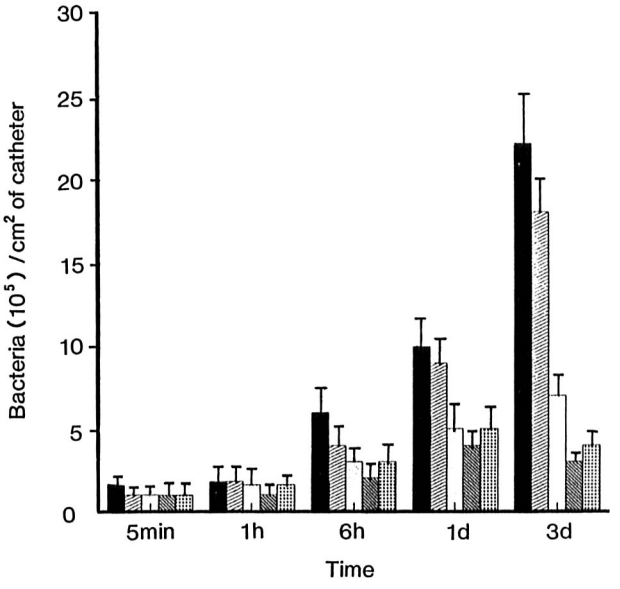

Fig. 1. Mean adherence of *S. epidermidis* to catheters of different materials (n=3; bar=SD), ■, PVC; ▨, siliconised latex; □, Teflon®; ▧, polyurethane; ⊡, Vialon®.

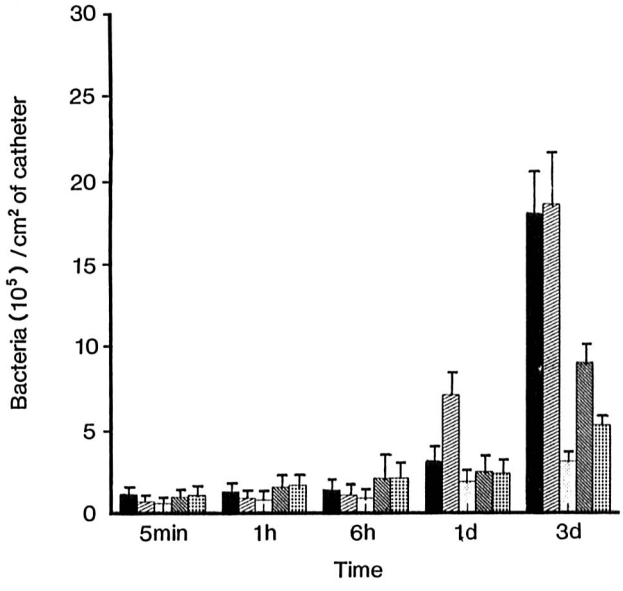

Fig. 2. Mean adherence of *E. coli* to catheters of different material (n=3; bar=SD): ■, PVC; ▨, siliconised latex; □, Teflon®, ▧, polyurethane; ⊡, Vialon®.

adherence of both micro-organisms was significantly higher to PVC and siliconised latex than to Teflon®, polyurethane or Vialon®. Similar behaviour was observed with *S. aureus* and *P. aeruginosa*. The peak of bacterial adherence was reached between 24 and 72 h for *E. coli* and staphylococci (table I). However, *P. aeruginosa* adhered to the biomaterial more rapidly, reaching maximal adherence between 6 h for PVC and 24 h for the other biomaterials.

Maximal bacterial adherence was observed with PVC followed by siliconised latex for *P. aeruginosa* and staphylococci (table I). The adherence of *E. coli* to both biomaterials was similar but was significantly greater than that observed with Teflon®, polyurethane and Vialon®. The lowest adherence values were

Table I. Maximum levels of bacterial adherence to five types of plastic catheter during incubation for 72 h at 37°C

Catheter material	Mean* (SD) number of adherent bacteria (10^5/cm^2)			
	S. aureus	*S. epidermidis*	*E. coli*	*P. aeruginosa*
Siliconised latex	26·6 (3·4)	17·9 (1·7)	18·6 (1·8)	24·4 (3·0)†
PVC	42·0 (4·6)	22·9 (2·8)	18·5 (1·8)	40·8 (3·8)‡
Teflon®	11·4 (1·2)	6·8 (0·4)	2·9 (0·1)	9·8 (1·0)†
Polyurethane	15·1 (1·4)	3·9 (0·6)†	9·3 (0·5)	13·5 (1·2)†
Vialon®	7·5 (0·9)	5·1 (0·3)†	4·5 (0·2)	11·5 (1·2)†

*Mean of three experiments.
†Maximum adherence measured after incubation for 24 h; ‡after 6 h.

obtained with Teflon® for gram-negative bacteria, polyurethane for *S. epidermidis* and Vialon® for *S. aureus*.

Bacterial growth in catheter eluates

The growth rates of bacteria in eluates obtained from the incubation of catheter segments in PBS for 24 h at 37°C are shown in tables II and III. After incubation for 24 h, numbers of *E. coli* were significantly higher in PVC, Teflon®, polyurethane and Vialon® eluates than in the controls. However, in siliconised latex eluates the number of viable organisms was reduced to undetectable levels (< 10 cfu/ml). All the eluates stimulated the growth of *P. aeruginosa* (table II) after incubation for 24 h.

Different results were observed with staphylococci (table III). None of the catheter eluates significantly increased the growth of either *S. aureus* or *S. epidermidis*; only the Teflon® eluate significantly decreased the viable number of *S. epidermidis* after incubation for 24 h.

Discussion

Bacterial adherence to prosthetic materials can be measured by quantitative culture,[11] microscopy,[12] chemiluminescence[13] or the use of radiolabelled bacteria.[6] The latter method has provided a simple and reproducible method for quantifying the bacterial biomass on the surface of biomaterials.[6] This method is particularly useful for measuring the initial interactions between bacteria and polymers, which are mainly mediated by attracting and repelling forces such as hydrophobic interactions between the two surfaces.[6]

In our study, adherence was measured over a longer period of time than is usual in such studies. Very little difference in adherence was shown at 1 h or 6 h, but marked differences developed after 24 h, depending on the strain and the catheter material involved. The increased adherence measured may have been due to microcolony formation and growth may be required for this adherence to occur. However, lack of correlation between longer term adherence and growth in catheter eluates suggests that factors other than growth due to soluble catheter components is involved. Further studies are needed with scanning electron-microscopy to compare the quantitative data with morphological studies. Observations (unpublished) with different strains of *P. aeruginosa* showed that, at 24 h, bacteria on a PVC catheter are immersed in an amorphous material which is not observed with polyurethane, whereas adherence in this study was similar for both types of catheter material.

Our results indicate that catheter material type may be important in adherence of bacteria to catheter

Table II. Growth of *E. coli* and *P. aeruginosa* in eluates from different catheters

Catheter material	Mean (SD) number of cfu (10^4/ml)			
	E. coli		*P. aeruginosa*	
	6 h	24 h	6 h	24 h
None (control)	0·8 (0·1)	1·5 (0·6)	5·5 (1·5)	600 (31·1)
Siliconised latex	0	0	7·5 (1·8)	1150 (111)†
PVC	1·1 (0·2)	11·0 (2·2)†	6·5 (1·7)	3150 (320)†
Teflon®	1·5 (0·2)†	160·0 (10·3)†	7·8 (2·2)	2980 (262)†
Polyurethane	2·0 (0·4)†	250·0 (19·8)†	7·0 (2·1)	3250 (342)†
Vialon®	0·6 (0·2)	60·0 (5·8)†	6·9 (2·2)	3506 (298)†

0 = < 10 cfu/ml.
*Mean of three experiments; initial inoculum 10^3 cfu/ml.
†$p < 0·05$ compared to the controls.

Table III. Growth of *S. epidermidis* and *S. aureus* in eluates from different catheter materials

Catheter material	Mean (SD) number of cfu (10^3/ml)			
	S. epidermidis		*S. aureus*	
	6 h	24 h	6 h	24 h
None (control)	1·0 (0·3)	0·6 (0·3)	0·6 (0·3)	0·1 (0·04)
Siliconised latex	1·0 (0·4)	0·5 (0·2)	0·6 (0·2)	0·2 (0·09)
PVC	0·7 (0·3)	0·2 (0·1)	0·3 (0·1)	0·2 (0·08)
Teflon®	0·4 (0·3)	0·1 (0·06)†	0·7 (0·3)	0·2 (0·08)
Polyurethane	0·7 (0·3)	0·6 (0·2)	0·5 (0·2)	0·2 (0·1)
Vialon®	0·6 (0·2)	0·4 (0·2)	0·5 (0·2)	0·2 (0·09)

See footnotes to table II.

surfaces. In our assays, polyurethane and Vialon® offered the greatest resistance to adherence of staphylococci and Teflon® for *E. coli* and *P. aeruginosa*. Previous studies[11] with a blood-agar-roll technique, which measures viable bacteria, have shown that the initial adherence of coagulase-negative staphylococci to PVC catheters was also greater than that observed with Teflon® catheters. The results obtained with single isolates cannot be extrapolated to the whole species because it is known that there is variation between strains of a species both in adherence and in other surface properties.

The measurement of bacterial adherence to clean catheters is a simplification of the events that occur *in vivo* because the catheters are rapidly coated with different proteins, fluids and cells, but the initial adherence may depend mainly on the catheter biomaterial.

The survival *in vitro* of coagulase-negative staphylococci adherent to intravascular catheters in the absence of conventional nutrients has been described.[7] It has been proposed that these micro-organisms could use some of the components of the catheters as nutrients for growth.[4] It may also be possible that adherence to the surface of foreign objects may allow organisms to become metabolically dormant.[7] To consider this hypothesis, we tested the effect of catheter components on bacterial growth. We used eluates obtained from the incubation of catheter segments in PBS as growth media. Growth of *E. coli* and *P. aeruginosa* in eluates from PVC, Teflon® and both polyurethanes was significantly greater than that observed in the controls. However, siliconised latex eluate was toxic for *E. coli* but increased the growth of *P. aeruginosa*. None of the eluates tested increased the growth of either *S. aureus* or *S. epidermidis*. Staphylococci have complex nutritional requirements and it seems less likely that they would grow in a catheter eluate in PBS. Many biomaterials, e.g., PVC, contain several additives to make them flexible enough to be used in catheters. Others, e.g., siliconised latex, come from different tropical countries; in the manufacturing process, several chemicals are used and the exact formulae are unknown. Some of these substances could be eluted from the catheter into the medium and could be used by different micro-organisms.

Although these findings require further characterisation by more extensive in-vitro and clinical investigation to define the mechanisms involved in the pathogenesis of catheter-related infections, these preliminary data suggest that the effect of new biomaterials on bacterial adherence and viability should be evaluated before clinical trials to obtain catheters that could prevent bacterial adherence and colonisation.

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References

1. Cleri DJ, Corrado ML, Seligman SJ. Quantitative culture of intravenous catheters and other intravascular inserts. *J Infect Dis* 1980; 141: 781–787.
2. Maki DG, Goldman DA, Rhame FS. Infection control in intravenous therapy. *Ann Intern Med* 1973; **79**: 867–887.
3. Maki DG. Nosocomial bacteriemia: an epidemiologic overview. *Am J Med* 1981; **70**: 719–732.
4. Peters G, Locci R, Pulverer G. Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters. *J Infect Dis* 1982; **146**: 479–482.
5. Welch GW, McKeel DW, Silverstein P, Walker HL. The role of catheter composition in the development of thrombophlebitis. *Surg Gyn Obstet* 1974; **138**: 421–424.
6. Pascual A, Fleer A, Westerdaal NAC, Verhoef J. Modulation of adherence of coagulase-negative staphylococci to Teflon catheters *in vitro*. *Eur J Clin Microbiol* 1986; **5**: 518–522.
7. Franson TR, Sheth NK, Menon L, Sohnle PG. Persistent in vitro survival of coagulase-negative staphylococci adherent to intravascular catheters in the absence of conventional nutrients. *J Clin Microbiol* 1986; **24**: 559–561.
8. Verhoef J, Peterson PK, Quie PG. Kinetics of staphylococci opsonization, attachment, ingestion and killing by human polymorphonuclear leukocytes: a quantitative assay using

(^{3}H)-thymidine labelled bacteria. *J Immunol Methods* 1977; **14**: 303–310.
9. Ashkenazi S, Mirelman D. Adherence of bacteria to pediatric intravenous catheters and needles and its relation to phlebitis in animals. *Pediatr Res* 1984; **18**: 1361–1366.
10. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods used in circulation research. *Circ Res* 1980; **47**: 1–9.
11. Sheth NK, Rose HD, Franson TR, Buckmire FLA, Sohnle PG. *In vitro* quantitative adherence of bacteria to intravascular catheters. *J Surg Res* 1983; **34**: 213–218.
12. Hogt AH, Dankert J, Hulstaert CE, Feijen J. Cell surface characteristics of coagulase-negative staphylococci and their adherence to fluorinated poly (ethylenepropylene). *Infect Immun* 1986; **51**: 294–301.
13. Kristinsson KG. Adherence of staphylococci to intravascular catheters. *J Med Microbiol* 1989; **28**: 249–257.