Conditioning fluid influences on the surface properties of silicone and polyurethane peritoneal catheters: implications for infection

S. P. GORMAN, D. S. JONES, W. M. MAWHINNEY, J. G. McGOVERN, C. G. ADAIR
School of Pharmacy, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK

Catheter-related infection remains a considerable problem in continuous ambulatory peritoneal dialysis (CAPD). This study examined the adherence of clinical isolates of *Staphylococcus epidermidis* to commercially available polyurethane and silicone peritoneal catheters in the presence and absence of a proteinaceous conditioning film. In addition, the effects of the conditioning film on the surface properties (advancing and receding contact angles, and surface rugosity) of these biomaterials were investigated. Bacterial adherence to polyurethane and silicone catheters, pre-treated with phosphate-buffered saline (PBS) or artificial spent peritoneal dialysate (ASD) for 1 h at 37 °C, was examined using a radiometric (2-^{3}H-adenine) adherence assay. The advancing and receding contact angles and the surface rugosity of ASD- and PBS-treated biomaterials were examined using a dynamic contact angle analyser and an atomic force microscope, respectively. The bacterial isolates were selected to represent high and low cell surface hydrophobicity. The hydrophobic isolate exhibited both a significantly greater rate and a significantly greater extent of adherence than the hydrophilic isolate to both catheter materials, independent of pre-treatment. In general, pre-treatment of the catheter materials with ASD significantly decreased the subsequent adherence of both isolates owing to the deposition of a conditioning film on the surface of the biomaterial. ASD treatment also decreased both the advancing and receding contact angles and the surface rugosity of both catheter materials. This study highlights the influence of both bacterial cell surface hydrophobicity and biomaterial surface conditioning films on bacterial adherence to CAPD catheters. In addition, it is recommended that the effects of proteinaceous conditioning films on biomaterial surface properties should be considered when assessing materials for medical devices and products.

1. Introduction

The initial event in medical-device-related infection is generally accepted as being adherence of the micro-organism to the device surface. This allows the micro-organism to overcome, in the first instance, the flushing mechanisms of body secretions [1]. Following adherence, micro-organisms may form colonies and exude exopolymeric substances to form a microbial biofilm. In this environment, micro-organisms are protected from host-defence mechanisms, antibiotics and biocides and are, therefore, frequently difficult and costly to eradicate [2].

In patients with end-stage renal disease, continuous ambulatory peritoneal dialysis (CAPD) has become a widely accepted form of dialysis. However, peritonitis presents as a frequent complication in patients undergoing CAPD. The coagulase-negative staphylococci, *Staphylococcus epidermidis*, in particular, are the most frequent cause of infection [3]. The presence of microbial biofilm on the surface of in-dwelling peritoneal catheters has been confirmed by several workers [4, 5] and has been suggested as a cause of relapsing peritonitis [6]. In the light of this, elucidation of the mechanisms of interaction of micro-organisms with biomaterials and the development of novel biomaterials that will reduce or inhibit microbial adherence would provide considerable benefit [7]. Factors reported to be of importance in microbial adherence to inert materials include microbial cell surface hydrophobicity [8], microbial cell surface charge [9], biomaterial hydrophobicity [10, 11] and microrugosity [12, 13].

The majority of commercially available peritoneal catheters are composed of polyurethane or silicone. The cell surface characteristics of *S. epidermidis* isolated from microbial biofilm on the surface of retrieved peritoneal catheters have been previously reported by us [14]. Typically, the isolates demonstrated wide ranges of cell surface hydrophobicity and surface charge. The present study compares the adherence of two of these clinical isolates chosen to represent the extremes of cell surface hydrophobicity

0957–4530

presenting clinically in catheter-related peritonitis. In addition, the influence of a proteinaceous conditioning film on the surface properties of polyurethane and silicone catheters was investigated in relation to bacterial adherence to the biomaterials.

2. Materials and methods

2.1. Chemicals

Potassium dihydrogen orthophosphate, disodium hydrogen orthophosphate, sodium chloride and xylene were purchased from BDH (Poole, Dorset, UK). 2-^{3}H-Adenine was purchased from Amersham (Buckinghamshire, UK). Scintillation cocktail (Optiphase Hi-Safe) was obtained from LKB Scintillation Products (UK) and octyl-sepharose from Pharmacia (Uppsala, Sweden). Sterile polyurethane CAPD catheters (batch number 068-299) and sterile silicone catheters (batch number 404772-90032) were supplied by Gambro (UK). All other chemicals were of AnalaR, or equivalent, quality and were purchased from Sigma Chemical Co. (St Louis, USA).

2.2. Bacterial isolates

S. epidermidis strains were isolated from biofilms formed on peritoneal catheter surfaces *in vivo* and maintained in the cryoprotective fluid of a Protect Bacterial Preserve System (Technical Consultants Ltd, UK) at $-20\,^{\circ}C$ as previously reported by us [5].

2.3. Growth conditions

Growth and radiolabelling of *S. epidermidis* was performed as previously reported by us [13] but with the additional presence of 5% CO_2 as suggested by Denyer *et al.* [15]. Briefly, one colony of each isolate was inoculated into Mueller–Hinton broth (100 ml) containing (2-^{3}H)-adenine (37 MBq ml^{-1}) and incubated for 18 h at 37 °C in an orbital shaker. The microbial suspension was then centrifuged (5000*g*; 10 min; 4 °C), washed three times and finally resuspended in cold phosphate-buffered saline (PBS) (pH 7.4; 0.01 M) to approximately 10^7 colony-forming units (cfu) ml^{-1} prior to inclusion in the adherence assay. Scintillation cocktail (5 ml) was vortex mixed with 1 ml of the radiolabelled bacterial suspension for 1 min and its radioactivity determined in a liquid scintillation counter. All counts per minute (CPMs) were converted into decays per minute (DPMs) through the use of appropriate quench correction curves. Four replicates were examined and the DPMs per viable bacterium calculated.

2.4. Adherence of *Staphylococcus epidermidis* to polyurethane and silicone catheters

Catheter sections (0.5 cm long) were soaked in artificial spent dialysate (ASD) containing protein to simulate the *in-vivo* situation [16] or in PBS at 37 °C for 1 h. Following three rinses in cold PBS the sections were incubated at 37 °C in radiolabelled bacterial suspension (20 ml; about 10^7 cfu ml^{-1}). At pre-determined intervals up to 120 h, sections were removed and gently washed five times in sterile cold PBS to remove non-adherent bacteria. The final wash fluid was shown not to contain CPMs above background levels. Each catheter section was then mixed with scintillation fluid (5 ml; 1 min) and the associated radioactivity determined as before. The DPMs of each sample and the number of colony-forming units per square centimetre of catheter surface was calculated [13].

2.5. Measurement of biomaterial advancing and receding contact angles

Advancing and receding contact angles of PBS-treated and ASD-treated silicone and polyurethane peritoneal catheters were determined in quadruplicate using a dynamic contact angle analyser (DCA 312, Cahn Instruments) at 25 °C. The wetting medium used was reagent grade 1 water from a Milli-Q system (Millipore UK Ltd.).

2.6. Evaluation of biomaterial surface rugosity

The surface rugosity of PBS-treated and ASD-treated catheters was determined using atomic force microscopy (AFM) with a Burleigh personal atomic force microscope (Burleigh Instruments NY, USA). Surface rugosity was calculated as the Z_{RMS} value, i.e., the root mean square of Z, the vertical dimension of the surface. At least 50 measurements of this surface parameter were performed for each biomaterial and the mean ($\pm$standard deviation (SD)) calculated.

2.7. Statistical analysis

All adherence experiments were performed in quadruplicate, and the mean and standard deviation of each group calculated and presented in all figures. Differences in mean groups were calculated using an unpaired two-tailed t test. Statistical evaluation of conditioning film effects on biomaterial advancing and receding contact angles and surface rugosity were performed using a two-way analysis of variance (ANOVA). *Post-hoc* analysis of differences between individual means were examined using Fisher's least-significant-difference test. In all statistical tests, $p < 0.05$ was accepted to denote significance.

3. Results

Adherence of the hydrophilic and hydrophobic isolates of *S. epidermidis* to polyurethane catheters is influenced by catheter pre-treatment with either PBS (Fig. 1) or ASD (Fig. 2). Maximum adherence of the hydrophobic isolate to PBS-treated and ASD-treated polyurethane occurred following 24–48 h and 48 h contact, respectively. In contrast, maximum adherence of the hydrophilic isolate to PBS-treated and ASD-treated polyurethane occurred following 120 h and 48–72 h contact, respectively. The hydrophobic

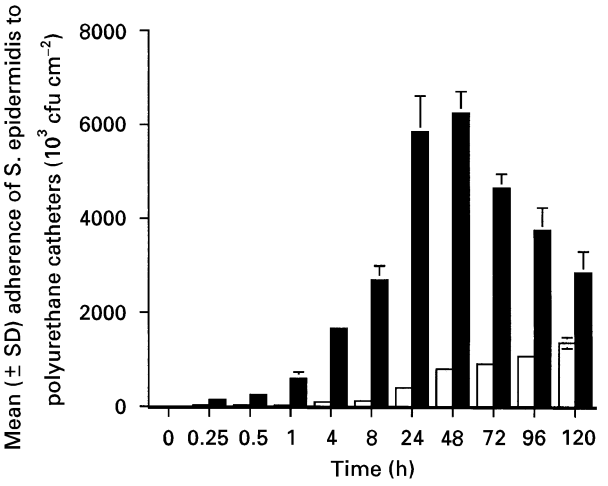

Figure 1 Adherence of hydrophobic (■) and hydrophilic (□) isolates of *S. epidermidis* to polyurethane peritoneal catheters pre-treated with PBS.

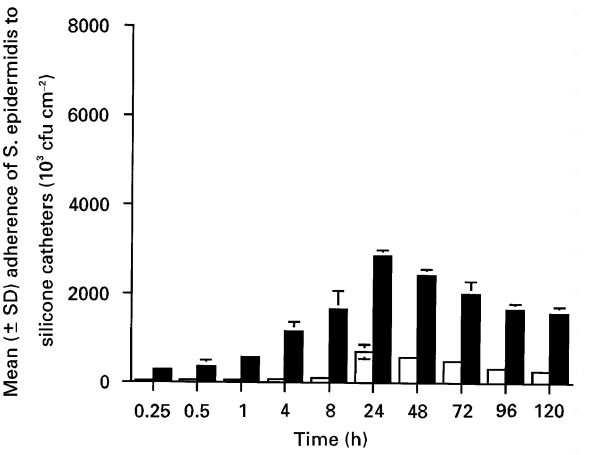

Figure 3 Adherence of hydrophobic (■) and hydrophilic (□) isolates of *S. epidermidis* to silicone peritoneal catheters pre-treated with PBS.

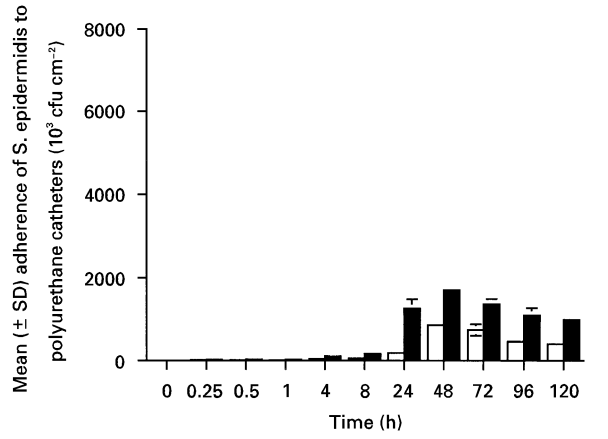

Figure 2 Adherence of hydrophobic (■) and hydrophilic (□) isolates of *S. epidermidis* to polyurethane peritoneal catheters pre-treated with ASD.

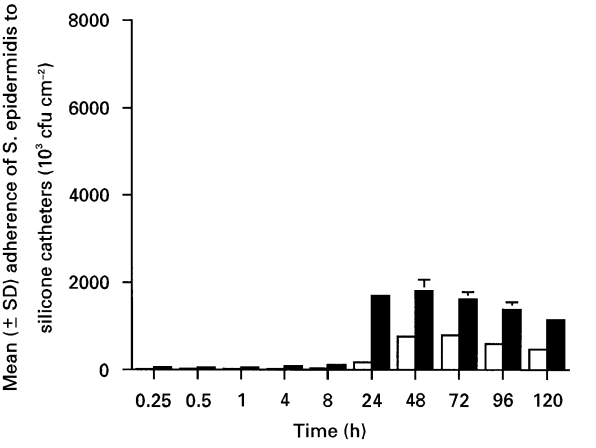

Figure 4 Adherence of hydrophobic (■) and hydrophilic (□) isolates of *S. epidermidis* to silicone peritoneal catheters pre-treated with ASD.

isolate of *S. epidermidis* demonstrated a significantly greater rate and extent of adherence to both PBS- and ASD-treated polyurethane than did the hydrophilic isolate. In addition, both isolates exhibited significantly reduced adherence to the ASD-treated polyurethane at each sampling period in comparison with PBS-treated polyurethane.

The time of contact between the hydrophilic and hydrophobic isolates and silicone catheters, pretreated with either PBS or ASD, affected adherence. The maximum adherence of the hydrophobic and hydrophilic isolates to PBS-treated silicone occurred following 24–48 h contact (Fig. 3). Treatment of silicone with ASD resulted in maximum adherence of the hydrophilic isolate after 48–72 h whereas maximum adherence of the hydrophobic isolate occurred following 24–72 h contact (Fig. 4). The hydrophobic isolate of *S. epidermidis* again demonstrated significantly greater adherence to both PBS and ASD-treated silicone than did the hydrophilic isolate. In addition, ASD treatment of silicone significantly reduced the adherence of the hydrophobic isolate in comparison with PBS treatment. However, adherence of the hydrophilic isolate of *S. epidermidis* to ASD-treated silicone was significantly less following 24 h contact, and yet significantly greater after 48 h contact than adherence to the PBS-treated silicone.

At all sampling periods, adherence of the hydrophobic and hydrophilic isolates was greater to PBS-treated polyurethane than to PBS-treated silicone. In general, no significant differences were apparent between the adherence of *S. epidermidis* isolate to either ASD-treated silicone or polyurethane.

Table I shows the effects of a conditioning film, derived from ASD, on the advancing and receding contact angles and surface rugosity of silicone and polyurethane catheters. The advancing and receding contact angles of PBS-treated silicone were observed to be statistically greater than for PBS-treated polyurethane. Both biomaterials in the presence of an ASD-induced conditioning film exhibited decreased advancing and receding contact angles in comparison with their PBS-treated counterparts. However, the advancing and receding contact angles of ASD-treated silicone were statistically lower than for polyurethane. In addition, significant differences were observed between biomaterial (silicone or polyurethane) and pre-treatment (ASD or PBS) with

TABLE I The effects of an ASD conditioning film on the surface properties of silicone and polyurethane peritoneal catheters

Biomaterial	Conditioning film	Mean ($\pm$SD) biomaterial surface properties		
		Advancing contact angle (deg)	Receding contact angle (deg)	Surface rugosity (nm)
Silicone	PBS	97.95 ± 0.74	64.39 ± 1.89	57.15 ± 4.25
Silicone	ASD	67.00 ± 0.49	44.69 ± 0.36	45.8 ± 3.40
Polyurethane	PBS	91.03 ± 0.19	60.18 ± 0.43	50.43 ± 5.35
Polyurethane	ASD	84.79 ± 0.54	51.67 ± 1.03	33.43 ± 2.29

respect to both advancing and receding contact angles. Finally, there was no significant difference between the surface rugosity of PBS-treated silicone and PBS-treated polyurethane. The presence of a conditioning film significantly reduced the surface rugosity of both biomaterial types. The smoothest surface under examination was ASD-treated polyurethane.

4. Discussion

This study demonstrates the ready adherence of both highly hydrophobic and hydrophilic clinical isolates of *S. epidermidis* to two commercially available peritoneal catheter materials. The isolates of *S. epidermidis* were selected according to their disparate cell surface hydrophobicities and represent the expected range of bacterial cell surface hydrophobicities (CSHs) encountered clinically in microbial biofilm on peritoneal catheters [14]. Bacterial adherence increased with time, reaching a peak at about 48 h and then gradually decreased. Such a trend has been reported previously for adherence of *Pseudomonas spp.* [17]. The rate and extent of adherence of the hydrophobic *S. epidermidis* isolate to both biomaterials, regardless of pre-treatment, was significantly greater than that of the hydrophilic isolate, indicating the importance of a hydrophobic microbial surface to the adherence process with this type of biomaterial. Previously, Ashkenazi *et al.* [8] reported that hydrophobic isolates of *Staphylococcus aureus*, *Escherichia coli* and *Serratia marcescens* adhered better to Teflon and polyethylene than their more hydrophilic counterparts. Similarly, Stenstrom [18] related bacterial adhesion to mineral particles to the high CSH exhibited, and Gilbert *et al.* [19] correlated increased CSH and adherence of the micro-organisms *S. epidermidis* and *E. coli* to glass surfaces. The present study illustrates the importance of microbial CSH in microbial adherence to inert surfaces which have been modified by protinaceous conditioning films.

It is appreciated that the physical properties of a biomaterial will influence microbial adherence. Initial reports suggested that the extent of bacterial adhesion to inert surfaces was greatest on surfaces of lowest wettability, i.e., greatest hydrophobicity [10]. However, this study has shown greater adherence of *S. epidermidis* to the more hydrophilic (PBS-treated) polyurethane catheters than to silicone catheters. Similarly, Wilkins *et al.* [12] reported a lack of correlation between the critical surface tension (a measure of hydrophobicity) of polymeric monofilaments and adherence of both *S. aureus* and *E. coli*. In addition, it has been reported that the adherence of *Streptococcus sanguis* 12 was greater to glass (hydrophilic) than to the more hydrophobic polymer, fluorethylene propylene [11]. These workers concluded that the greater adherence to glass was due to the more favourable thermodynamics of this surface. Indeed, within the present study, this factor may contribute to the greater adherence of both isolates of *S. epidermidis* to the more hydrophilic polyurethane catheters. However, it is important to remember that microbial adherence to inert surfaces involves several complex processes [13] and consequently the differences between the adherences of the two *S. epidermidis* isolates to the different catheter materials may not be adequately explained by alterations in any single physical parameter. Adherence to biomaterials is also affected by the growth conditions of the bacteria, as reported by Denyer *et al.* [15]. In recognition of this we have employed a growth atmosphere of 5% CO_2 representing the physiological conditions within the peritoneal cavity. Recently, Wilcox and Schumacher-Perdreau [20] also recommended the use of human body fluids as suitable media for the growth of coagulase-negative staphylococci because of their effects on subsequent adherence to biomaterials. The use of such fluids rather than artificial media may, therefore, have a further influence on adherence of our isolates to silicone and polyurethane.

Inert surfaces and most bacteria in contact with body fluids are rapidly coated with proteins to form a conditioning film which may alter non-specific and specific adherence by altering surface properties [21]. In this study, ASD-containing serum was employed to simulate *in-vivo* conditions within the peritoneum. The modification of *S. epidermidis* adherence to polyurethane and, in most cases, silicone following pretreatment with ASD highlights the effects of adsorbed constituents, notably proteins, on the adherence process. Similarly, adherence of coagulase-negative staphylococci in the presence of protein has been reported to be significantly reduced to fluorinated polyethylene–polypropylene films [22] and to polyethylene, nylon and poly(vinyl chloride) catheters [23]. The reduced adherence following deposition of the conditioning film may be explained, in part, by the observed decrease in both the advancing and the receding contact angles (and hence increased surface free energies) which will alter the thermodynamics of

adhesion [11]. The reduced surface rugosity, a parameter which has been previously shown by us to affect bacterial adherence to biomaterials [13], may also play a role in this regard as it is possible that nanometre differences in the biomaterial topography may affect their interaction with microbial adhesins. The dissimilarities between the surface properties of ASD-treated silicone and polyurethane (Table I) suggest that the consistency or uniformity of the conditioning film deposited on each biomaterial was somewhat dependent on the surface properties of the biomaterial. Indeed these differences are highlighted by the statistical interaction term with respect to biomaterial contact angles in the ANOVA. In this, the reductions in both the advancing and the receding contact angles for silicone following treatment with ASD were greater than those observed for ASD-treated polyurethane. Consequently, it is inappropriate to assume that the similarities in adherence of *S. epidermidis* to ASD-treated silicone and polyurethane were directly due to a uniformity induced in the material surface properties following adsorption of ASD. However, it may be that ASD conditioning of surfaces provides similar proteinaceous binding sites for adherence and this is responsible for the observed similarities in adherence. In practice, the conditioning film acts as a preferential interface for microbial adherence. Consequently, the contribution of conditioning films to the surface properties of biomaterials is of considerable significance in the infectious process. It is worth noting that in the clinical situation micro-organisms within the peritoneum will be coated with a conditioning film. Previously we have shown that the CSH of micro-organisms coated with conditioning films from body fluids is decreased [24]. This will, therefore, further affect adherence to peritoneal catheters. In general, however, the majority of micro-organisms, whether devoid of or coated with a conditioning film, will demonstrate a CSH within the range selected in this study. Interestingly, Harkes *et al.* [25] have suggested that strategies to reduce catheter-associated bacteriuria should involve inhibition of bacterial growth on the surface of the catheter rather than by modification of the physicochemical properties of the catheter surface. Importantly, this study has shown that physicochemical modification of peritoneal catheters associated with the deposition of a surface conditioning film significantly reduced subsequent microbial adherence. Elucidation of the molecular basis of the anti-adherent effects of this conditioning film may assist future developments for biomaterials designed to reduce the incidence of catheter-associated infections.

Acknowledgement

We are grateful for the financial support of the Northern Ireland Kidney Research Fund.

References

1. S. P. GORMAN, in "Mechanism of action of chemical biocides", Blackwell Publications Technical Services No. 27, edited by S. P. Denyer and W. B. Hugo (Blackwell, Oxford, 1991) 271.
2. W. M. MAWHINNEY, C. G. ADAIR and S. P. GORMAN, *Int. J. Pharm. Pract.* **1** (1992) 136.
3. W. M. MAWHINNEY, C. G. ADAIR and S. P. GORMAN, *Int. J. Pharm. Pract.* **1** (1991) 1.
4. W. P. REED, P. D. LIGHT and K. A. NEWMAN, in "Frontiers in peritoneal dialysis", edited by J. F. Maher (Field, Rich and Associates, New York, 1986) p. 176.
5. S. P. GORMAN, C. G. ADAIR and W. M. MAWHINNEY, *Epidem. Infect.* **112** (1994) 551.
6. R. C. EVANS and C. J. HOLMES, *Antimicrob. Agents Chemother.* **31** (1987) 889.
7. S. P. DENYER, G. W. HANLON, M. C. DAVIES and S. P. GORMAN, in "Microbial biofilms: formation and control", Blackwell Publications Technical Services No. 30, edited by S. P. Denyer, S. P. Gorman and M. Sussman (Blackwell, Oxford, 1993) 147.
8. S. ASHKENAZI, E. WEISS and M. M. DRUCKER, *J. Lab. Clin. Med.* **107** (1986) 136.
9. J. A. ONAOLAPO, M. I. A. EL-HAFFAR, D. TOWNLEY and R. M. M. KLEMPERER, *J. Med. Microbiol.* **24** (1987) 227.
10. M. FLETCHER and G. I. LOEB, *Appl. Environ. Microbiol.* **37** (1979) 67.
11. H. J. BUSSCHER, J. SJOLLEMA and H. C. VAN DER MEI, in "Microbial cell surface hydrophobicity", edited by R. J. Doyle and M. Rosenberg (American Society for Microbiology, Washington, DC, 1990) p. 335.
12. K. M. WILKINS, G. P. MARTIN, G. W. HANLON and C. MARRIOTT, *Int. J. Pharm.* **57** (1989) 1.
13. S. P. GORMAN, W. M. MAWHINNEY, C. G. ADAIR and M. ISSOUCKIS, *J. Med. Microbiol.* **38** (1993) 411.
14. D. S. JONES, C. G. ADAIR, W. M. MAWHINNEY and S. P. GORMAN, *Int. J. Pharm.* **131** (1996) 83.
15. S. P. DENYER, M. C. DAVIES, J. A. EVANS, R. G. FINCH, D. G. E. SMITH, M. H. WILCOX and P. WILLIAMS, *J. Clin. Microbiol.* **28** (1990) 1813.
16. C. J. HOLMES, R. E. EVANS and E. VONESH, *Biomaterials* **10** (1989) 625.
17. L. MARTINEZ-MARTINEZ, A. PASCUAL and E. J. PEREA, *J. Med. Microbiol.* **34** (1991) 7.
18. T. A. STENSTROM, *Appl. Environ. Microbiol.* **67** (1989) 53.
19. P. GILBERT, D. J. EVANS, E. EVANS, I. G. DUGUID and M. R. W. BROWN, *J. Appl. Bacteriol.* **71** (1991) 72.
20. M. H. WILCOX and F. SCHUMACHER-PERDREAU, *J. Hosp. Infect.* **26** (1994) 239.
21. G. REID, R. DAVIDSON and J. D. DENSTEDT, *Surf. Interface Anal.* **21** (1994) 581.
22. A. H. HOGT, J. DANKERT and J. FEIJEN, *J. Gen. Microbiol.* **13** (1985) 2485.
23. J. CARBELLO, C. M. FERREIROS and M. T. CRIADO, *Med. Microbiol. Immunol.* **180** (1991) 139.
24. J. G. McGOVERN, D. S. JONES, A. D. WOOLFSON and S. P. GORMAN, *J. Pharm. Pharmacol.* **47** (12B) (1995) 1063.
25. G. HARKES, J. DANKERT and J. FEIJEN, *Appl. Environ. Microbiol.* **58** (1992) 1500.

Received 29 August
and accepted 9 September 1996