International Journal of Pharmaceutics, 99 (1993) 293–301

IJP 03260

The effects of polyHEMA coating on the adhesion of bacteria to polymer monofilaments

J. Bilbruck [1], G.W. Hanlon and G.P. Martin [1]

Pharmaceutical Sciences Research Group, Department of Pharmacy, University of Brighton, Brighton (UK)

(Received 28 January 1993)
(Accepted 26 March 1993)

Key words: Bacterial adhesion; PolyHEMA; *Escherichia coli*; Polyester; Polyvinylidene chloride; Nylon; Work of adhesion

Summary

Bacterial adhesion to implants is the initial stage in the pathogenesis of device-related infections, the extent of attachment being dependent on the surface properties of both the bacteria and the substrate. One approach to preventing infection involves incorporating antiadhesive agents onto the surface of indwelling devices. Hydrogels of poly(2-hydroxyethylmethacrylate) (polyHEMA) homopolymer have been shown previously not to support the attachment and growth of mammalian cells but their interaction with bacterial cells has not been extensively investigated. The purpose of this study was to modify the surfaces of polymeric monofilaments by coating them with polyHEMA, and then test their ability to support bacterial attachment before and after coating. Monofilaments composed of polyester, polyvinylidene chloride (PVDC) and nylon were dip-coated in a 5% (w/v) polyHEMA solution in 95% (v/v) ethanol. Work of adhesion (W_A) was determined from determination of the contact angle between the monofilaments and water. Adhesion of *E. coli* was assessed using a bioluminescent method based on adenosine triphosphate determination and scanning electron microscopy was used to investigate the surface morphologies of the monofilaments. Coating the monofilament fibres with polyHEMA increased the value of W_A from 100.6, 94.6 and 91.2 mJ m^{-2} for PVDC, polyester and nylon, respectively, to a common value of 113.7 mJ m^{-2}, indicating that the monofilaments were more hydrophilic after coating. Adhesion of *E. coli* cells to all monofilaments occurred rapidly and was almost complete after 2 h incubation. The coating of the monofilaments with polyHEMA reduced the surface rugosity of the monofilaments and significantly reduced the numbers of adherent bacteria. The modification of the surface in this manner is likely to have implications for situations where bacterial adhesion is a factor in predisposing infection.

Introduction

The potential medical uses of indwelling prosthetic devices and implants such as sutures, various types of catheters, intrauterine contraceptive devices (IUCDs), contact lenses, prosthetic heart valves and artificial joints has increased dramatically over recent years. As applications have become more extensive a wide variety of biomaterial components has been created to meet the specific functional requirements of systems and organs. The materials from which they are manufactured can be metals, ceramics and sometimes modified natural materials, although the majority of implants tend to be made of synthetic polymers such as polyethylene, polypropylene and

Correspondence to (present address): G.P. Martin, Department of Pharmacy, King's College London, Manresa Road, London, SW3 6LX, U.K.

[1] *Present address:* Department of Pharmacy, King's College London, Manresa Road, London, SW3 6LX, U.K.

polytetrafluoroethylene (PTFE) (Jansen et al., 1988).

The two major impediments to the extended use of implanted biomaterials are the lack of successful tissue integration and the possibility of device-related infection. These two phenomena are interrelated and based on similar molecular mechanisms. On insertion an indwelling device represents a ready surface for colonisation. Macromolecules, tissue cells and bacteria will compete for the vacant binding sites at this surface and if the first colonising cells are tissue, then subsequent arrivals will be confronted by a layer of living, integrated cells. This integrated surface will be resistant to bacterial colonisation due to its viability, intact cell membrane, glycocalyx and host defence mechanisms (Gristina, 1987). Conversely, if initial adhesion and colonisation is by bacteria, infection is likely to result and tissue integration will be prevented. This relationship between bacteria and tissue cells has been conceptualised as a 'race for the surface' of the implant material, with the outcome being either integration or infection (Gristina et al., 1988). Infection is therefore a frequent complication of the presence of implants and can occur in both transient and permanent devices. Bacterial adhesion to the implant is the initial stage in the pathogenesis of device-related infections, and the extent of this attachment is dependent on the surface properties of both the bacteria and the substrate.

The use of IUCDs is associated with an increased risk of PID compared to women using no method of contraception (Grimes, 1987). Many studies have investigated the role of the monofilament marker tail, attached to such devices, in the development of infection of the female genital tract (Purrier et al., 1979; Sparks et al., 1981; Skangalis et al., 1982). These workers concluded that the tail of the IUCD can facilitate the ascent of bacteria from the vagina into the uterine cavity, and therefore may be implicated in the aetiology of PID. Support for this hypothesis has come from in vitro studies which investigated the progression of bacteria, through gels, along various polymer monofilament threads (Wilkins et al., 1989). A range of bacterial species were demonstrated to progress along all materials tested, but in the absence of a solid substrate no migration was observed. Further studies showed that bacterial adhesion to the marker tail is important in the transmission of organisms along such surfaces (Wilkins et al., 1990). The extent of this adhesion was shown to be dependent upon the bacterial species and the nature of the surface. The IUCD tail therefore acts as an initial substrate for bacterial adhesion, and once attachment has occured the bacteria can multiply and grow along the surface. Little work, however, has been carried out regarding the prevention of bacterial adhesion to the surface of these monofilament tails, and the influence this may have on uterine contamination.

One possible approach to preventing infection involves the incorporation of antiadhesive agents onto the surface of indwelling devices. Anionic polyelectrolytes, such as heparin have been shown to duplicate the antiadhesive activity of the luminal mucin layer in the bladder (Ruggieri et al., 1987). By binding heparin to the surface of latex catheter material these workers demonstrated a reduction in bacterial attachment to less than 10% of control, untreated latex, and proposed that urethral catheters, treated in this way, may delay the acquisition of associated urinary tract infection. Siliconisation has also been found to reduce the transmission of *E. coli* along IUCD monofilaments (Hanlon et al., 1982).

Synthetic hydrogels have been shown to exhibit an outstanding tolerance in biological systems, and for this reason they have great potential for use in a wide range of biomedical applications (Ratner and Hoffman, 1976). Hydrogels of poly(2-hydroxyethyl methacrylate) (polyHEMA) homopolymer do not normally support the attachment and growth of mammalian cells (Lydon, 1986; McAuslan and Johnson, 1987). In contrast to mammalian cell adhesion, however, bacterial attachment to hydrogels is a field which, as yet, has received little attention. If it is the case that the adhesion of bacteria is also not supported on polyHEMA hydrogels, this could be advantageous in circumstances where bacterial attachment and subsequent colonisation has been shown to be detrimental e.g. the development of infec-

tion associated with implanted medical devices. The purpose of the present study was therefore to modify the surfaces of polymeric monofilaments by coating them with polyHEMA, and then test their ability to support bacterial attachment before and after coating. Bacterial adhesion was assessed using a bioluminescent method based on adenosine triphosphate (ATP) determination which provided a convenient, rapid and sensitive technique for determining bacterial numbers.

Materials and Methods

Polymer monofilaments

Polyester and polyvinylidene chloride (PVDC) monofilaments were obtained from Nymofil Ltd, Poulton-le-Fylde, Lancashire. Nylon monofilament (as attached to the Progestasert IUCD) was obtained from Alza Pharmaceuticals, Palo Alto, CA, U.S.A.

Media and chemicals

Nutrient broth (C.M. 1) and nutrient agar (C.M. 3) were obtained from Oxoid Ltd, Basingstoke, Hampshire. Both were prepared according to the manufacturer's instructions and sterilised by autoclaving at 121°C for 15 min.

Poly(2-hydroxyethyl methacrylate) (polyHEMA) was obtained from Aldrich Chemical Co., Gillingham, Dorset, U.K.

Lyophilised ATP monitoring reagent and ATP standard were obtained from LKB Wallac, Wallac Oy, Turku, Finland. One vial of lyophilised ATP monitoring reagent was reconstituted by adding 10 ml of double distilled, deionised water, and mixing gently, according to manufacturer's instructions. One vial of ATP standard was also reconstituted with 10 ml double distilled, deionised water in a similar manner. This gave a final stock solution of 10 μM ATP.

Organisms

Escherichia coli NCIB 8196 was obtained from the National Collection of Industrial Bacteria (NCIB), Aberdeen, and was maintained on nutrient agar at 4°C and subcultured at approx. 3 month intervals.

Coating of monofilaments

Prior to coating, PVDC, polyester and nylon monofilaments were washed in a 1% (v/v) Decon 90 (BDH Chemicals Ltd, Poole, Dorset) solution for 2 h. They were then rinsed thoroughly in distilled water, and finally soaked in distilled water (frequently changed) for at least 24 h. Monofilaments were coated by dip-coating in a 5% (w/v) polyHEMA solution in 95% (v/v) ethanol, and then drying at room temperature for 1 h. The coated monofilaments were stored in distilled water (frequently changed) for at least 24 h prior to use.

Determination of work of adhesion

The work of adhesion (W_A) values for uncoated and polyHEMA coated monofilaments were calculated by determining the contact angle (θ) of a single liquid (in this case water) placed on the polymer surface. A length of monofilament (approx. 4 cm) was placed on a platform sited between the condensor and lens of a projector. Contact angles were determined by a sessile drop technique. A small drop of water (approx. 0.5 μl) was placed onto the monofilament surface using a 25 μl Hamilton microlitre syringe, and its magnified image was projected onto a screen situated approx. 1.5 m away. The equilibrium contact angle for the liquid on the polymer was measured from the image, the value of the angle being dependent upon the properties of both liquid and solid. For each polymer five contact angles were determined along the length of the monofilament, for three batches of monofilament. Recorded values, therefore, represented the mean of 15 readings. The lamp in the projector was switched off after each angle determination to prevent undue heating of the sample, and hence reduce evaporation of the water. All measurements were made at 21°C. The liquid surface tension of water was determined at 21°C using a Du Noüy tensiometer (Cambridge Scientific Instruments Ltd, Cambridge).

Scanning electron microscopy

The monofilament under investigation (1 cm length) was mounted on a metal stub, sputter gold coated to eliminate surface charging and

observed under a scanning electron microscope (Model ISI-100A, International Scientific Instruments, Manchester).

Assessment of bacterial adhesion

Polyester, nylon and PVDC monofilaments were cut into 8 cm lengths, cleaned and rinsed, and then placed under a bactericidal ultraviolet light unit at a distance of 20 cm for 1 h (rotating occasionally). Coated monofilaments were prepared by dip-coating in a 5% (w/v) polyHEMA solution as described before. After air-drying, the coated threads were exposed to ultraviolet radiation, as before, and equilibrated in sterile water for at least 24 h prior to use. A culture of *E. coli* was obtained by inoculating 100 ml nutrient broth with a single colony from a nutrient agar plate and incubating overnight, without shaking, at 37°C. The cells were harvested by centrifugation ($4300 \times g$, 20 min, 20°C), washed once in phosphate buffered saline (PBS) and finally resuspended in PBS. The cell concentration was assessed photometrically by reference to the previously constructed calibration curve, and the suspension adjusted accordingly to give a concentration of 2×10^9 cells ml^{-1}. A sample (6 ml) of this suspension was added to 18 ml PBS in a sterile 25 ml universal bottle to give a final working concentration of 5×10^8 cells ml^{-1}. Lengths of monofilament (50×8 cm) were then incubated in this *E. coli* suspension at 37°C. On immersion of the monofilaments care was taken that the total surface area of each thread was exposed to the *E. coli* suspension. The suspension was gently agitated with a shaker, to prevent settling of the cells and bunching of the threads. Monofilaments were incubated in the bacterial suspension for various periods of time (2, 4, 6 and 24 h), and at the appropriate time in each experiment all monofilaments were removed and washed three times in 10 ml aliquots of PBS. The monofilaments were then transferred to 10 ml of a 0.05% (w/v) trichloroacetic acid (TCA) solution containing 2 mM EDTA, held in a polypropylene centrifuge tube. This was vortex mixed vigorously for 10 s and then left for 10 min. After extraction, 400 μl of the acid extract was added to 600 μl of 0.1 M Tris-acetate 2 mM EDTA buffer. The light emitted by the firefly assay of ATP was measured at 20°C using a Lumac Biocounter (Model 2010, Lumac bv, 6370 AC Landgraaf, The Netherlands). The assay of ATP was conducted by first adding 100 μl of ATP monitoring agent to a plastic cuvette and measuring the background light emission (B) in the luminometer. To this was added 300 μl of the diluted acid extract, and the constant level of emission (S) was recorded. Finally, 100 μl of a 1 in 10 dilution of the ATP standard stock solution was added and the increase in light emission (I) measured. In each series of assays a blank, using PBS instead of bacterial sample, was assayed in the same manner, as a control. The amount of ATP in each sample was calculated using the equation:

$$[\text{ATP}]\ (\mu\text{mol}) = [(S - B)/I] \times \text{amount of ATP standard}\ (\mu\text{mol})$$

The blank (calculated in the same way) was subtracted from this to obtain the final ATP concentration. The gradually decreasing ATP level/cell with incubation time during the time course of the experiments was compensated for by the preparation of a standard curve, at the same times as the measurements of attached bacteria. This value was converted to a bacterial cell count by reference to a previously prepared calibration plot. The number of attached cells cm^{-2} could then be calculated by knowing the monofilament surface area. All data obtained from both surface characterisation and adhesion experiments were analysed using the two-tailed Mann-Whitney U-test for unpaired non-parametric data (Siegal, 1956).

Results

Determination of work of adhesion

Very small drops (approx. 0.5 μl) of the organic liquids were used in the measurement of contact angles on the monofilament surfaces, in order to eliminate gravitational distortion of

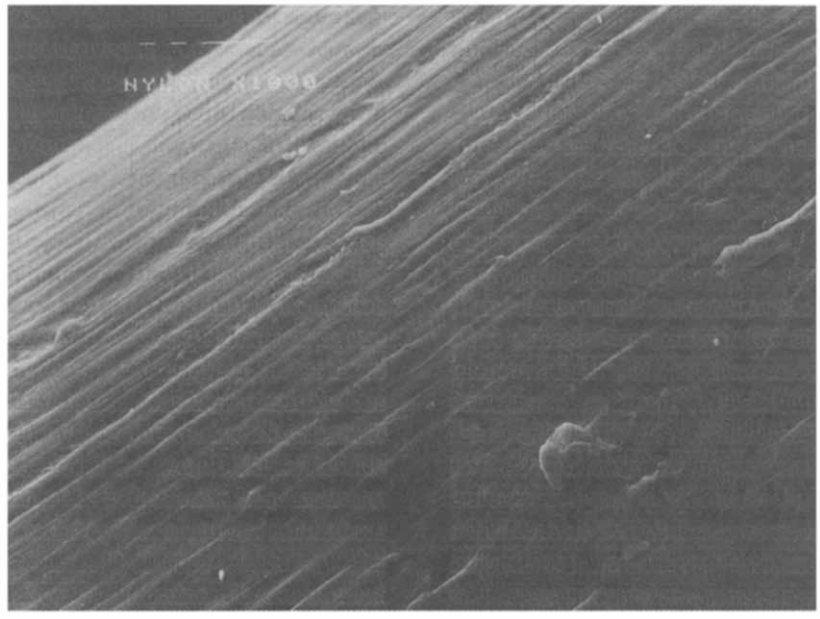

Fig. 1. SEM of the surface of an uncoated nylon monofilament. Right-hand marker bar = 1 μm.

shape. The determined contact angle was therefore independent of the liquid drop volume and temperature variations were not considered to influence results unduly. In this way any experimental error associated with this technique was minimised. The contact angles produced by drops

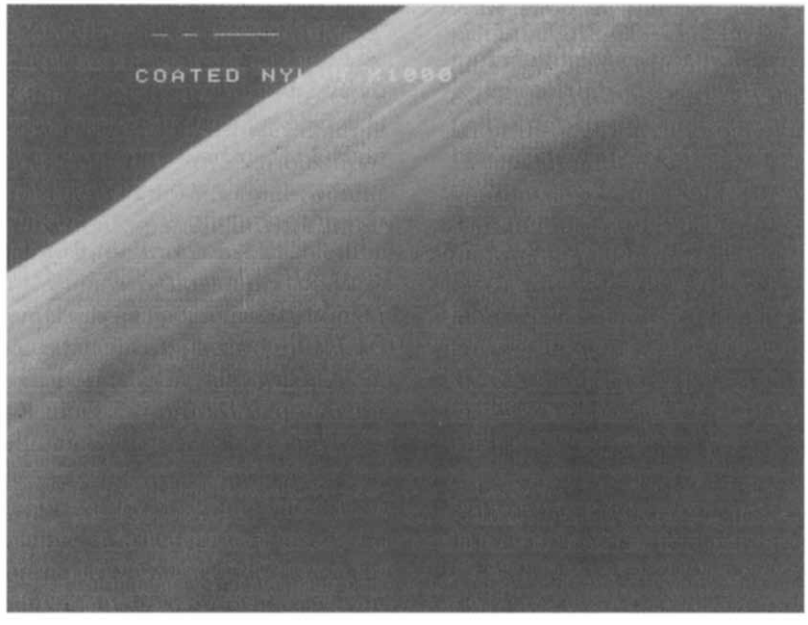

Fig. 2. SEM of the surface of a polyHEMA coated nylon monofilament. Right-hand marker bar = 1 μm.

TABLE 1

W_A (mJ m^{-2}) values determined for dip-coated and uncoated monofilaments (n = 6)

Thread type	$W_A \pm$SD	
	Uncoated	Dip-coated
PVDC	100.6±5.0	113.5±2.5
Polyester	94.6±5.7	114.0±4.5
Nylon	91.2±5.1	113.7±3.4

of water on the substrate surfaces were used to determine W_A values for the monofilaments tested, using the equation:

$$W_A = \gamma_{LV}(1 + \cos\theta) + \pi$$

where W_A is the work of adhesion (mJ m^{-2}), θ denotes the contact angle of water on substratum (°), γ_{LV} is the liquid surface tension of water (mN m^{-1}) and π represents the spreading pressure of adsorbed vapours on solid. The value of γ_{LV} determined for water was 72.2 ± 0.4 mN m^{-1}, and for the purpose of calculations it was assumed that π (the spreading pressure of adsorbed vapours on solid) was negligible, which is a valid assumption for surfaces where $\theta > 0°$. W_A values for both uncoated and polyHEMA coated monofilaments are given in Table 1. Of the uncoated monofilaments PVDC has the highest W_A value and nylon the lowest. For all monofilament types dip-coating with polyHEMA significantly increases W_A to the same value ($p < 0.05$).

Scanning electron microscopy

Scanning electron microscopy was used to investigate the surface morphologies of the monofilaments before and after coating with polyHEMA. Typical results from these examinations are shown in the scanning electron micrographs (SEMs) of uncoated and polyHEMA coated nylon monofilament given in Figs 1 and 2. The surfaces of all the uncoated monofilaments showed a high degree of microrugosity, i.e., surface roughness. The three types of monofilament all exhibited longitudinal grooves, probably made on the surface during manufacture. On coating with polyHEMA the monofilament surfaces appeared much smoother and more homogeneous.

Bacterial adhesion

The time course of adhesion of *E. coli* to polyester, nylon and PVDC monofilaments over a 24 h period is shown in Fig. 3. The adhesion profile is the same in all cases, in that the majority of attachment occurs during the first 2 h, after which there is a general flattening of the curve with a slight increase up to 24 h incubation.

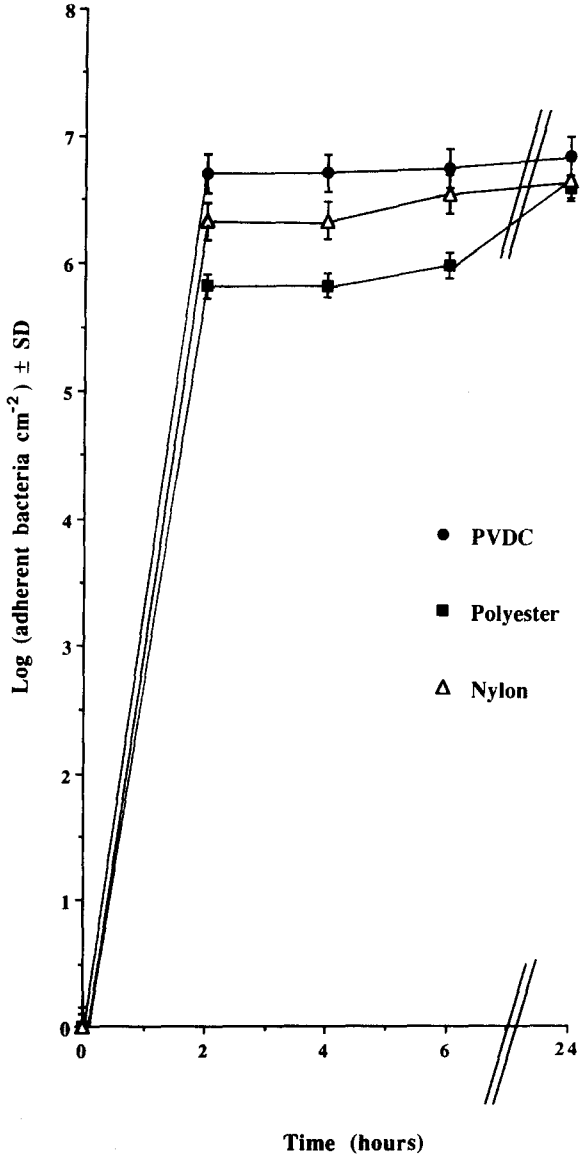

Fig. 3. Time course of adhesion to various monofilaments ($n = 6$).

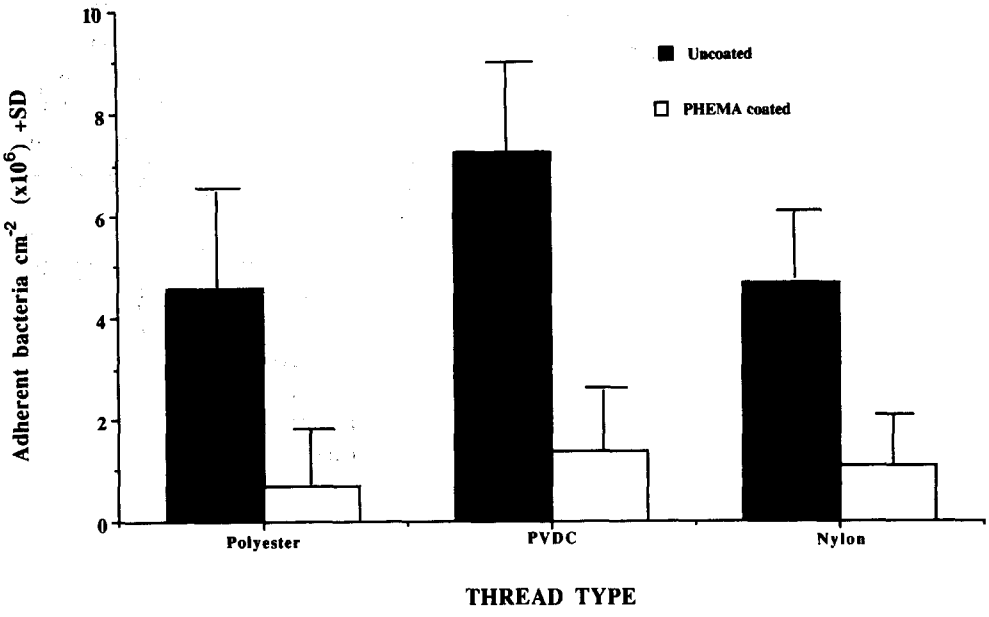

Fig. 4. Adhesion of bacteria to uncoated and polyHEMA coated monofilaments after 24 h ($n = 6$).

Attachment of the bacteria seems to be greatest to the PVDC monofilaments, and this is more clearly shown in Fig. 4, which also shows the effect on adhesion of coating the various monofilaments with polyHEMA. Fig. 4 indicates that after 24 h incubation *E. coli* adhered in significantly greater numbers to PVDC monofilaments than to polyester or nylon monofilaments ($p < 0.05$). More striking, however, is that in all cases coating with polyHEMA significantly reduced the adhesion of *E. coli* to the surface of the monofilaments ($p < 0.05$). Bacterial adhesion to the polyHEMA layer was independent of the monofilament type to which the polyHEMA was coated ($p < 0.05$).

Discussion

The work of adhesion can be used as a measure of the wettability of a substrate. These values therefore provide a means for ranking materials in terms of their relative surface energies. An increase in W_A represents an increase in surface energy, and is indicative of a more hydrophilic surface. Dip-coating with polyHEMA changed significantly the W_A values of the different monofilaments ($p < 0.05$) to the same value (Table 1). This suggests that when monofilaments are coated with polyHEMA they acquire the surface characteristics of the hydrogel layer, and these properties are independent of the monofilament type. This increase in W_A values indicated that the monofilaments had become more hydrophilic on coating. A previous study has reported the contact angle of water (in air) on polyHEMA to be $59.5 \pm 2.3°$, which equates to a W_A value of 108.8 ± 2.5 mJ m^{-2} (Holly and Refojo, 1976) and is therefore in relatively good agreement with the values obtained in this present study. W_A, in this case, describes the thermodynamic energy of separation at the interface between the monofilament surface and water. It has been suggested that W_A is a realistic parameter of substrate wettability with bacterial attachment, because it is at this solid-aqueous medium interface that attachment occurs (Pringle and Fletcher, 1983). Maximum bacterial attachment has been shown to occur within a substratum W_A range of 75–105 mJ m^{-2}. All of the uncoated monofilaments tested had W_A values within this range, but coating with polyHEMA increased this

value beyond the upper limit. By this criterion it might be expected that the monofilaments would be less likely to support bacterial adhesion if coated with polyHEMA.

The results given in Fig. 3 show that the majority of the adhesion of *E. coli* cells occurs during the first 2 h of incubation. This concurs with other studies using sutures and related materials, where a significant increase in bacterial attachment during the first 1–2 h was followed by a flattening of the adhesion profile (Ashkenazi and Mirelman, 1984; Shuhaiber et al., 1989). These earlier studies, however, report no further increase in adhesion after the first 2 h, unlike the present study, which shows a continued but small increase in adhesion between 6 and 24 h. ($p < 0.05$). Wilkins et al. (1990) showed that *E. coli* cells initially adhered to the surface of polymeric monofilaments with no obvious attachment mechanism. After 20 h incubation, however, distinct polymeric material was observed attaching the bacteria to the monofilaments. Similar observations were reported by Peters et al. (1982), whilst investigating the colonisation of catheter surfaces by staphylococci. The staphylococcal cells generally possessed clean surfaces during the early stages of adhesion, but with lengthening incubation periods many cells became covered with a 'slimy material', the amount of which increased with time. It is possible that the presence of this extracellular polymeric material after long incubation periods may increase the strength of adhesion, and also facilitate the attachment of other cells, thereby increasing the number of bacteria attached to the surface of the monofilament.

The degree of bacterial adhesion observed in this study (10^5–10^6 adherent bacteria cm^{-2}) was in good agreement with some reports (Shuhaiber et al., 1989; Wilkins et al., 1990), but lower than in other investigations where adhesion was shown to be of the order of 10^6–10^7 bacteria cm^{-2} (Sugarman and Musher, 1981; Ashkenazi and Mirelman, 1984). Bacterial adhesion in these latter studies was investigated using clinical isolates; this may explain the increased adhesion compared to the present study which employed a laboratory maintained bacterial strain. However, it is not appropriate to make direct comparisons between studies, due to differences in the bacterial species and substrates investigated, and the concentrations of the bacterial suspensions used. Different centrifugation and resuspension processes may also modify the cell surface components of organisms and therefore influence their adhesive properties.

The results given in Fig. 4 show that *E. coli* adhered in greater numbers to the PVDC monofilaments and significantly less to the nylon and polyester monofilaments ($p < 0.05$). There was no significant difference in adhesion between the latter two types of monofilament. These results concur with previous work which showed, using three different methods, that the number of *E. coli* cells adherent to PVDC monofilaments was greater than that adherent to nylon monofilaments (Wilkins et al., 1990).

In all cases coating of the monofilaments with polyHEMA was shown to significantly reduce bacterial adhesion to the thread surface ($p < 0.05$) (Fig. 4). The attachment of bacteria to the polyHEMA layer was independent of the monofilament type on which the polyHEMA was coated ($p < 0.05$). This suggests that, when coated, the monofilaments adopt the surface characteristics of the polyHEMA layer. In this way the W_A value of the monofilament surface is increased and it becomes more hydrophilic. These results therefore seem to support other studies where bacteria have been found to attach in higher numbers to low energy surfaces, and less to more hydrophilic surfaces (Fletcher and Loeb, 1979; Gerson and Scheer, 1980; Pringle and Fletcher, 1983). This increase in surface energy is not the only parameter to influence bacterial adhesion, and other factors such as changes in the surface charge and microrugosity, i.e., surface roughness of the monofilament may also contribute to the overall effect. The SEMs shown in Figs 1 and 2 are typical of the broad differences in the surface morphologies of uncoated and polyHEMA coated monofilaments. The uncoated threads exhibited a high degree of microrugosity, and previous studies have shown that bacteria preferentially adhere to rougher sections of monofilament surface, as opposed to smoother areas (Wilkins et al., 1990). There are several possible explanations for this

phenomenon: (i) An increase in microrugosity creates a greater available substratum surface area for bacterial attachment. (ii) The bacterial glycocalyx may adhere better to rough areas than to smooth areas. (iii) There is an increase in charge density on rough surfaces. (iv) Detachment of bacteria is likely to be reduced as the cells will be protected from shear forces imposed by the bulk liquid flow. When coated with polyHEMA, the surface of the monofilament became visually much smoother (Fig. 2). It would therefore seem that a reduction in the surface microrugosity, by coating with polyHEMA, may also be a contributory factor in rendering the monofilament less likely to support bacterial attachment.

In conclusion, therefore, it has been found that the modification of monofilament surfaces by coating with polyHEMA significantly reduces bacterial adhesion. This is possibly attributable to an increase in surface energy and a decrease in microrugosity upon coating with polyHEMA. The modification of the surface of substrates in this manner may therefore have implications to situations where bacterial adhesion has been shown to predispose to infection.

References

Ashkenazi, S. and Mirelman, D., Adherence of bacteria to pediatric intravenous catheters and needles and its relation to phlebitis in animals. *Pediatr. Res.*, 18 (1984) 1361–1366.

Fletcher, M. and Loeb, G.I., Influence of substratum characteristics on the attachment of a marine pseudomonad to solid surfaces. *Appl. Environ. Microbiol.*, 37 (1979) 67–72.

Gerson, D.F. and Scheer, D., Cell surface energy, contact angles and phase partition: III. Adhesion of bacterial cells to hydrophobic surfaces. *Biochim. Biophys. Acta*, 602 (1980) 506–510.

Grimes, D.A., Intrauterine devices and pelvic inflammatory disease: recent developments. *Contraception*, 36 (1987) 97–109.

Gristina, A.G., Biomaterial-centered infection: Microbial adhesion versus tissue integration. *Science*, 237 (1987) 1588–1595.

Gristina, A.G., Naylor, P. and Myrvik, Q., Infections from biomaterials and implants: a race for the surface. *Med. Prog. Technol.*, 14 (1988) 205–224.

Hanlon, G.W., Patel, J. and Marriott, C., Modification of IUCDs as a possible means of preventing uterine infections. *J. Pharm. Pharmacol.*, 34 (1982) 31P.

Holly, F.J. and Refojo, M.F., Water wettability of hydrogels. In Andrade, J.D. (Ed.), *Hydrogels for Medical and Related Applications*, ACS Symposium Series, Washington: ACS, 1976, Vol. 31, pp. 252–266.

Jansen, B., Peters, G. and Pulverer, G., Mechanisms and clinical relevance of bacterial adhesion to polymers. *J. Biomat. Appl.*, 2 (1988) 520–543.

Lydon, M.J., Synthetic hydrogels as substrata for cell adhesion studies. *Br. Polym. J.*, 18 (1986) 22–27.

McAuslan, B.R. and Johnson, G., Cell responses to biomaterials: I. Adhesion and growth of vascular endothelial cells on poly(hydroxyethyl methacrylate) following surface modification by hydrolytic etching. *J. Biomed. Mater. Res.*, 21 (1987) 921–935.

Peters, G., Locci, R. and Pulverer, G., Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters. *J. Infect. Dis.*, 146 (1982) 479–482.

Pringle, J.H. and Fletcher, M., Influence of substratum wettability on attachment of freshwater bacteria to solid surfaces. *Appl. Environ. Microbiol.*, 45 (1983) 811–817.

Purrier, B.G., Sparks, R.A., Watt, P.J. and Elstein, M., In vitro study of the possible role of the intrauterine contraceptive device tail in ascending infection of the genital tract. *Br. J. Obstet. Gynaecol.*, 86 (1979) 374–378.

Ratner, B.D. and Hoffman, A.S., Synthetic hydrogels for biomedical applications. In Andrade, J.D. (Ed.), *Hydrogels for Medical and Related Applications*, ACS Symposium Series, Washington: ACS, 1976, Vol. 31, pp. 1–36.

Ruggieri, M.R., Hanno, P.M. and Levin, R.M., Reduction of bacterial adherence to catheter surface with heparin. *J. Urol.*, 138 (1987) 423–426.

Shuhaiber, H., Chugh, T. and Burns, G., In vitro adherence of bacteria to sutures in cardiac surgery. *J. Cardiovasc. Surg.*, 30 (1989) 749–753.

Siegal, S., *Nonparametric Statistics for the Behavioural Sciences*, McGraw Hill, London, 1956.

Skangalis, M., Mahoney, C.J. and O' Leary, W.M., Microbial presence in the uterine cavity as affected by varieties of intrauterine contraceptive devices. *Fertil. Steril.*, 37 (1982) 263–269.

Sparks, R.A., Purrier, B.G.A., Watt, P.J. and Elstein, M., Bacteriological colonisation of uterine cavity: role of tailed intrauterine contraceptive device. *Br. Med. J.*, 282 (1981) 1189–1191.

Sugarman, B. and Musher, D., Adherence of bacteria to suture materials. *Proc. Soc. Exp. Biol. Med.*, 167 (1981) 156–160.

Wilkins, K.M., Hanlon, G.W., Martin, G.P. and Marriott, C., The influence of critical surface tension and microrugosity on the adhesion of bacteria to polymer monofilaments. *Int. J. Pharm.*, 57 (1989) 1–7.

Wilkins, K.M., Hanlon, G.W., Martin, G.P. and Marriott, C., The role of adhesion in the migration of bacteria along intrauterine contraceptive device polymer monofilaments. *Int. J. Pharm.*, 58 (1990) 175–184.