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The role of adhesion in the migration of bacteria along intrauterine contraceptive device polymer monofilaments

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Summary

Using an in vitro model simulating the female genital tract, it was demonstrated that bacterial adhesion to intrauterine contraceptive device (IUD) tails is of importance in the transmission of bacteria along such surfaces. The process of bacterial adhesion to polymer monofilaments was subsequently investigated. Organisms were seen to adhere reversibly at first and then to produce polymeric material to achieve stronger, irreversible adhesion. Using scanning electron microscopy, the extent of bacterial adhesion was shown to be dependent upon several factors including the bacterial species and the microrugosity of the threads. A greater number of bacteria were found adhering to the monofilaments when there was no agitation of the washing or dehydrating solutions compared to the standard preparatory techniques.

Introduction

Women using intrauterine contraceptive devices (IUDs) have been assessed as having a 3–5-fold increased risk of developing pelvic inflammatory disease (PID) (Edelman et al., 1982). The role of the IUD in the pathogenesis of PID may be to allow colonisation of bacteria on its surface, hence reducing the effectiveness of normal defence mechanisms. Various workers have consequently examined the surfaces of IUDs removed from patients using both light and electron microscopy. IUDs removed from uteri after hysterectomy provide the most reliable samples, since the withdrawal of an IUD through the cervix and vagina will undoubtedly lead to the presence of contaminant material on the device, although with care it is possible to distinguish between material that was in close association in situ and contaminants arising during the removal process.

Potts and Pearson (1967) found that after removal IUDs were covered with a layer of cells including polymorphonuclear leucocytes, uterine epithelial cells and probably degenerate remains of macrophages. In addition, extracellular material was present which consisted of small bundles of collagen fibres, PAS-positive material and 'supposed calcium salts'. On 2 out of 55 IUDs investigated, unidentified rod-shaped bacteria were visible.

Both transmission and scanning electron microscopy (SEM) coupled with X-ray micro-analy-

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sis were used by Sheppard and Bonnar (1980) to investigate inert and medicated IUDs removed from uteri immediately after hysterectomy. The cellular material identified on the inert IUDs consisted mainly of macrophages, with some polymorphonuclear leucocytes, erythrocytes and a few platelets and fibrin fibres. Copper devices were covered principally with polymorphonuclear leucocytes and the inert arms of a Progestasert ® IUD were found to be coated more extensively than the progesterone releasing stem. All devices had calcium deposits, with an increase in deposition with time.

Marrie and Costerton (1983) observed IUDs removed from patients using scanning and transmission electron microscopy and found that the IUD tails had considerable amounts of material on their surfaces. Bacteria were observed either totally embedded in this material or adhering via apparent 'pseudopodia' of amorphous material. Transmission electron microscopy of the adherent material revealed both Gram-positive and Gram-negative bacteria embedded in a fibrous matrix, along with human leucocytes and cellular debris. Spornitz et al. (1984) also observed the tails from removed IUDs and showed that coating material consisted of cellular debris, bacteria and mucus, the exact characteristics of which varied according to the area of the genital tract to which the tail had been exposed. The thickness of the material was related to the duration of use of the IUD and the thicker the adsorbed coating, the more likely that bacteria were to be found on the intrauterine section of the tail.

It is therefore apparent that the IUD surface and polymer tail in situ are modified markedly by the adhesion of a wide range of biological materials, including bacteria. The IUD tail may act as an initial substrate for bacterial adhesion and once attached, the bacteria may multiply and grow along the IUD surface, hence gaining entry into the otherwise sterile uterus.

In a previous study, an in vitro model was utilised to investigate the progression of bacteria through gels along polymer monofilament threads (Wilkins et al., 1989a). A range of bacterial species were demonstrated to progress along all materials tested but in the absence of solid substrate no migration was observed. In addition, the extent of bacterial transmission appeared to be determined primarily by the motility of the organism, since motile species (*Escherichia coli* and *Serratia marcescens*) progressed to a greater extent than non-motile types (*Branhamella catarrhalis* and *Staphylococcus aureus*). The purpose of this study was to investigate the role of adhesion in the transmission of microorganisms along monofilament threads and to assess quantitatively and qualitatively the progress of adherence to such surfaces.

Materials and Methods

Polymer threads

Polyvinylidene chloride (PVDC) monofilament was obtained from Nymofil Ltd. (Poulton-le-Fylde, U.K.). Nylon monofilament from the Progestasert IUD was obtained from Alza Corp. (Palo Alto, CA). Polyethylene monofilament from the Lippes loop IUD was obtained from Ortho-Cilag Pharmaceuticals Ltd., (High Wycombe, U.K.). Polypropylene monofilament from the Multiload Cu250 IUD was obtained from Organon Laboratories Ltd. (Morden, U.K.). Before use all threads were placed under a bactericidal ultraviolet unit (Hanovia Lamps, Slough, U.K.) for 1 h.

Media

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

Organisms

The bacterial species used were obtained from the National Collection of Industrial Bacteria (NCIB), Aberdeen. The bacteria selected were *E. coli* NCIB 8196, *S. aureus* NCIB 10788 and *Serratia marcescens* NCIB 2302. The bacterial cells were maintained on nutrient agar slopes at 4°C and subcultured at approx. 3-month intervals.

Scanning electron microscopy of thread sections from the in vitro model

In vitro models containing a polypropylene or polyethylene monofilament were set up as de-

scribed previously (Wilkins et al., 1989a). *S. aureus* or *Se. marcescens* at a concentration of approx. 2×10^6 colony forming units (cfu) ml^{-1} in nutrient broth were used as the bacterial inoculum. After 96 h incubation at 37°C the tubing was sectioned using a sterile scalpel blade, and placed into 10-ml volumes of sterile 1/4 strength Ringer to allow the sodium carboxymethylcellulose (SCMC) gel to disperse. The threads were removed and fixed initially in 0.5% glutaraldehyde/phosphate-buffered saline (PBS), pH 7.2, for 15 min and then 5% glutaraldehyde/PBS, pH 7.2, for 1 h. The threads were washed in PBS for 15 min, employing constant agitation. This washing procedure was repeated a further four times. Subsequently, threads were dehydrated through a graded ethanol series made up with distilled water of 10, 20, 30, 50, 70, 90, 95, 100, 100 and 100%. The threads were agitated with each solution for 15 min. Finally, the threads were critical point dried using carbon dioxide, mounted on metal stubs, sputter gold coated and observed under a scanning electron microscope (SEM) (model ISI-100A, International Scientific Instruments, Manchester).

The use of scanning electron microscopy to measure the extent of bacterial adhesion to thread surfaces

Cultures of *S. aureus* and *E. coli* were obtained by inoculating 100 ml nutrient broth with a single bacterial colony and incubation without shaking overnight at 37°C. Cells were collected by centrifugation ($3000 \times g$ for 20 min), washed once in 1/4 strength Ringer solution and resuspended in 1/4 strength Ringer solution, the concentration being adjusted photometrically to $0.8–1.9 \times 10^8$ cfu ml^{-1} by reference to previously constructed calibration curves. Thread sections (1 cm lengths) were incubated with the bacterial suspension for different time intervals (15 min to 24 h) at 37°C in a shaking water bath (114 throws min^{-1}). After incubation the threads were removed and prepared for observation with the SEM as described above. Photographs were taken randomly of the thread surfaces for all incubation times. For those threads incubated for 2 h, however, photographs were taken at five points on each sample and the number of bacteria counted in that defined area. The experiments were repeated on 2 or 3 occasions. The photographs in each series were taken at the same magnification (either × 4000 or ×2000; as reproduced herein, ×3200 or ×1600, respectively). The initial area was selected randomly and then the subsequent photographs were taken using a spacing interval of 1 mm. Threads investigated were polyethylene, polypropylene, nylon and PVDC.

Experiments investigating the adhesion of *S. aureus* and *E. coli* to all thread types were repeated employing minimum agitation of wash or dehydrating solutions.

The adhesion of *S. aureus* and *E. coli* to all thread types was also examined when cells were resuspended in PBS. In these cases PBS replaced 1/4 strength Ringer solution throughout.

Differences in the numbe of bacteria adhering to each monofilament were analysed for statistical significance using the two-tailed Mann-Whitney U-test (Siegal, 1956).

Determination of the strength of bacterial adhesion to threads

Polyethylene threads (2-cm lengths) were incubated with bacterial suspensions of *E. coli* for 1, 5, 15, 30, 60, 120, 180 or 240 min at 37°C. The threads were removed and each placed in 2.5 ml of 1/4 strength Ringer solution and agitated in a water bath at 66 throws min^{-1} for 1 min. The threads were then each placed in another 2.5 ml of 1/4 strength Ringer solution and agitated in a water bath at 160 throws min^{-1} for 10 min. Finally, the threads were each transferred to 2.5 ml of 1/4 strength Ringer solution and sonicated in a sonic bath for 1 min (this low level of sonication had previously been shown not to have a detrimental effect on bacterial cells). 0.2-ml portions of each 1/4 strength Ringer solution sample were placed onto overdried nutrient agar plates, which were subsequently incubated at 37°C for 48 h.

Results

The incubation of *S. aureus* with the 4 polymer monofilaments resulted in the adhesion of large numbers of cells. Table 1 illustrates the adhesion

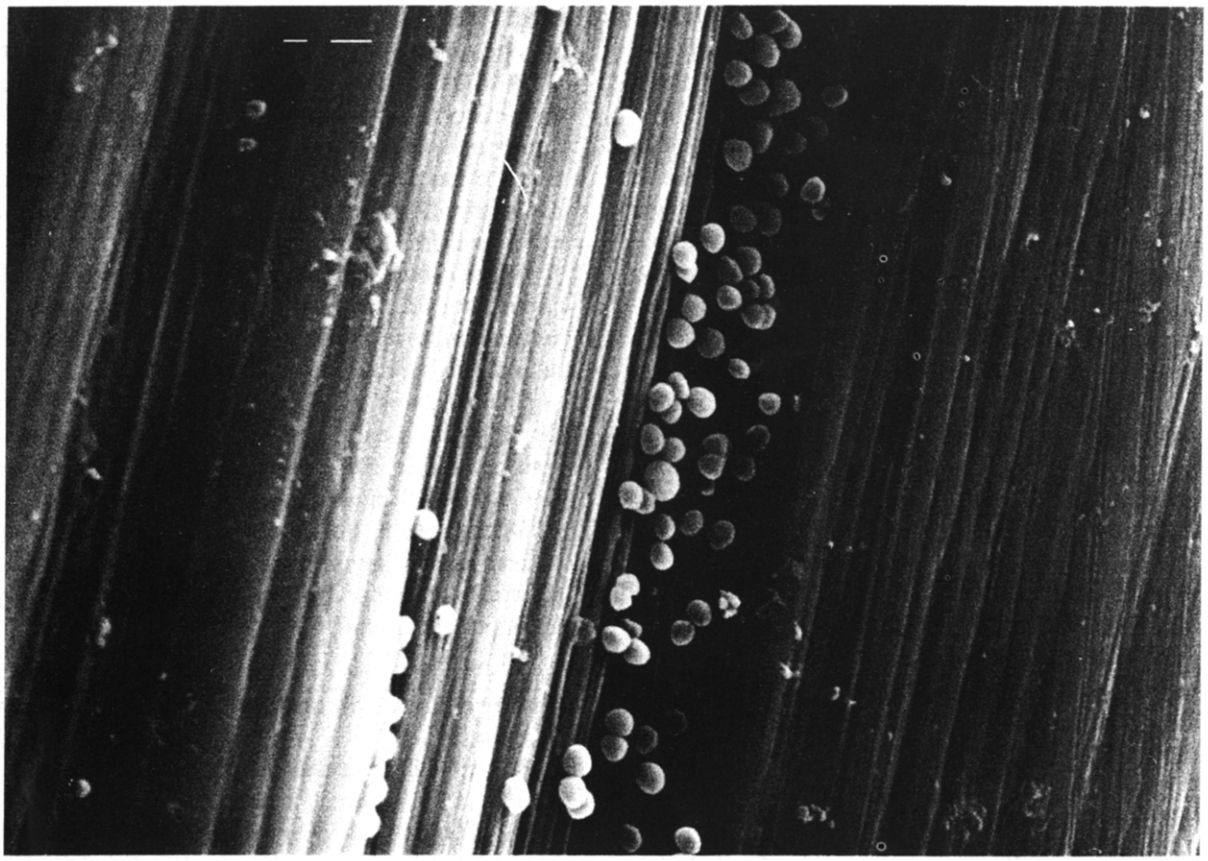

Fig. 1. *S. aureus* adhered to IUD monofilament after 2 h incubation. The cells have no obvious attachment mechanism and are found principally in the deep grooves on the surface.

of *S. aureus* to the 4 thread types with either 1/4 strength Ringer solution or PBS as the suspending fluid. In each of these conditions the adhesion to nylon and polyethylene was significantly greater than adhesion to polypropylene ($p < 0.05$). Adhesion to any given polymer was not significantly different when the two suspending fluids were compared.

TABLE 1

Mean number of S. aureus cells adherent to polymeric monofilaments (cm^{-2})

Thread type	Suspending media		
	1/4 strength Ringer solution	1/4 strength Ringer solution (low agitation)	PBS
PVDC	8.58×10^4	2.50×10^6	1.53×10^4
Nylon	2.86×10^5	1.75×10^6	2.31×10^5
Polyethylene	3.37×10^5	1.56×10^6	1.50×10^5
Polypropylene	6.13×10^4	3.86×10^5	1.02×10^4

TABLE 2

Mean number of E. coli cells adherent to polymeric monofilaments (cm^{-2})

Thread type	Suspending media		
	1/4 strength Ringer solution	1/4 strength Ringer solution (low agitation)	PBS
PVDC	2.14×10^4	4.60×10^5	5.96×10^5
Nylon	1.22×10^4	1.26×10^5	2.94×10^5
Polyethylene	9.50×10^4	3.31×10^5	5.96×10^5
Polypropylene	1.53×10^4	0.92×10^5	2.53×10^5

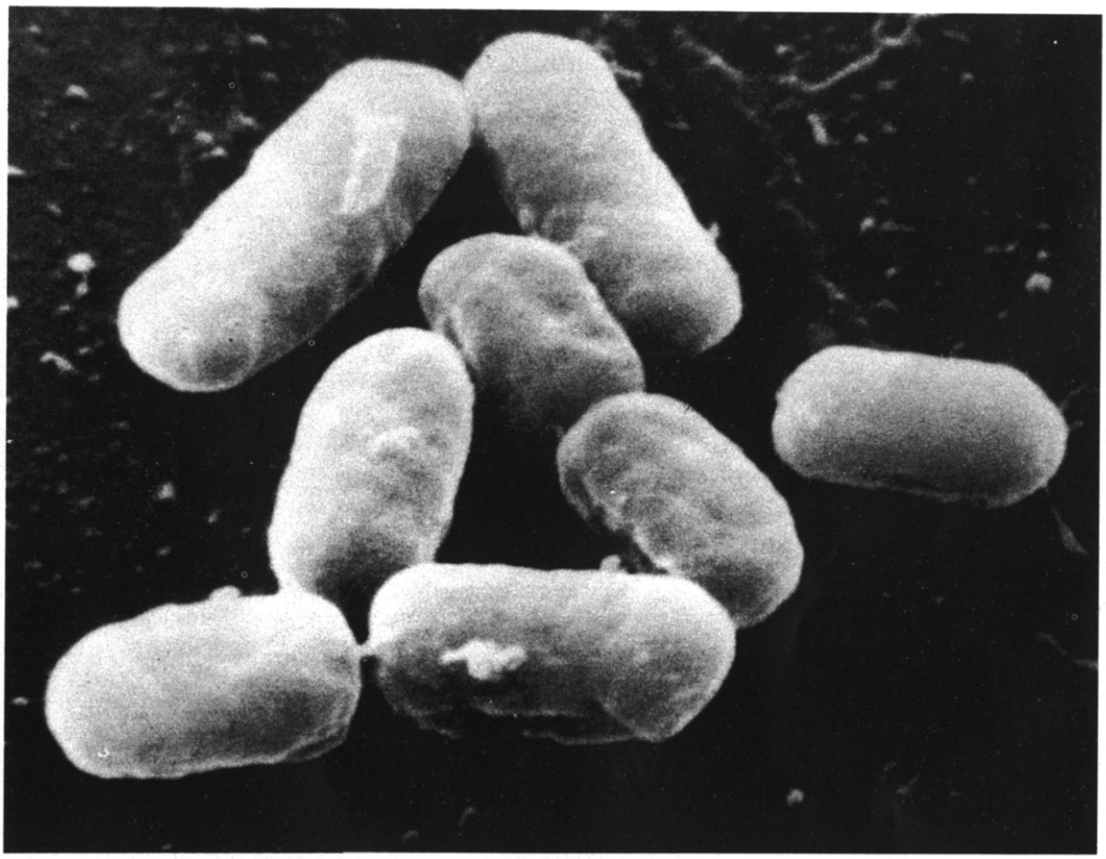

Fig. 2. *E. coli* adhered to IUD monofilament after 2 h incubation. The cells have no obvious attachment mechanism.

When the experiments in 1/4 strength Ringer solution were repeated with minimal agitation of washing or dehydration solutions the number of cells adhering was significantly greater ($p < 0.05$) for each polymer type (Table 1). Most pronounced was the adhesion to PVDC when agitation of fluids in contact with the threads was kept to a minimum, since the adhesion was almost 30-times greater than when the standard preparative technique was used.

Examination of electron micrographs showing adhesion of *S. aureus* to IUD polymer monofilaments indicates that after 2 h incubation the cells adhere to the surface with no obvious attachment mechanism. The cells shown in Fig. 1 are typical of those normally seen in that the major sites of adhesion are within the longitudinal grooves made in the surface during manufacture. The 3 threads taken from IUDs all exhibited this scoring effect and similar patterns of adhesion were noted. PVDC was unusual in that half of the surface was scored while the other half was smooth. Adhesion of all bacterial types occurred predominantly in the roughened areas.

The quantitative data for the attachment of *E. coli* to the polymer substrata are given in Table 2. When PBS was used as the suspending fluid no significant difference was observed in adhesion to the different polymer types. However, when 1/4 strength Ringer solution was used adhesion to polyethylene was significantly greater than adhesion to polypropylene, nylon and PVDC ($p < 0.05$). There was no significant effect of suspending fluid on numbers of bacteria adhered to the other polymer types.

Preparation of samples in the absence of high agitation resulted in a significant increase in adhesion for all polymers used ($p < 0.05$). This in-

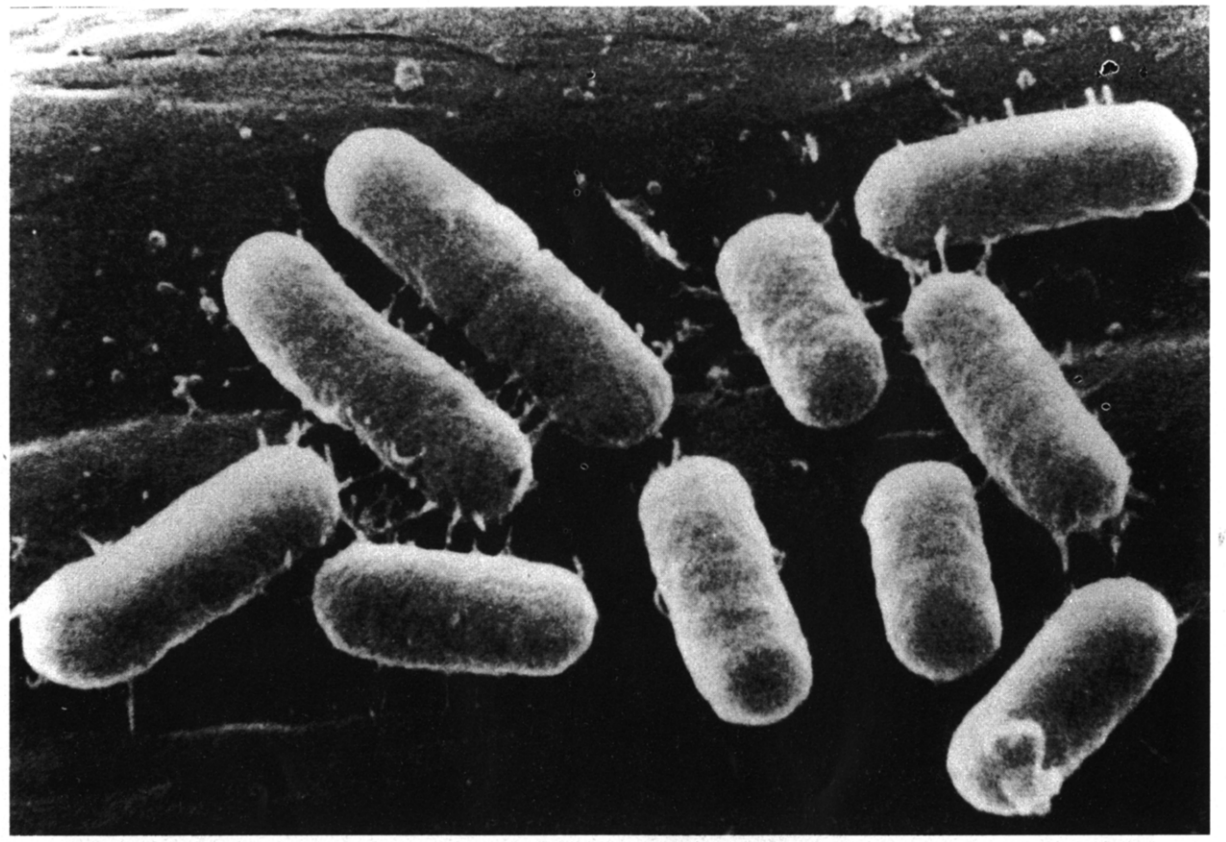

Fig. 3. *E. coli* adhered to IUD monofilament after 20 h incubation. An extracellular biopolymeric material appears to be anchoring the cells to the surface.

crease was over 20-fold in the case of PVDC and over 30-fold for nylon.

Electron micrographs of *E. coli* attached to polymer surfaces after 2 h show no obvious attachment mechanism (Fig. 2) but after 20 h incubation distinct extracellular biopolymeric material was observed attaching the cells to the surface (Fig. 3). The phenomenon was also observed for *S. aureus* on prolonged incubation.

Results obtained from studies investigating the strength of *E. coli* adhesion to polyethylene thread are listed in Table 3. It can be seen that an incubation time of 30 min or less enabled bacteria to adhere to the thread but that this adhesion was of a very loose nature and all bacteria could be removed by shaking the thread in 1/4 strength Ringer solution at a slow speed, hence there was

TABLE 3

The influence of incubation time on the strength of adhesion of E. coli to polyethylene monofilament thread

*, bacterial growth; −, absence of bacterial growth

Incubation time of *E. coli* with polyethylene thread (min)	Bacteria present in 1/4 strength Ringer solution after		
	Slow shake	Fast shake	Soni-cation
0	−	−	−
1	*	−	−
5	*	−	−
15	*	−	−
30	*	−	−
60	*	*	−
120	*	*	−
180	*	*	*
240	*	*	*

no bacterial growth evident after fast shake and sonication. Incubation times of 1–4 h led to a gradual increase in the strength of bacterial adhesion. After 1 h incubation, all bacteria could be removed from the IUD thread by shaking in 1/4 strength Ringer solution at a fast speed, but after 3 h incubation, sonication was needed to remove some bacteria.

The micrographs taken of thread sections from the in vitro model utilising *Se. marcescens* show that adhesion may play a role in the progression of motile species along IUD threads. Sections of threads taken from near the inoculum show a dense accumulation of cells (Fig. 4) but there are discrete clusters of adherent cells visible further along the threads (Fig. 5).

Discussion

The scanning electron micrographs of thread sections from the in vitro model clearly show the importance of adhesion in the transmission of bacteria along monofilament materials. The presence of discrete clusters of *Se. marcescens* cells (Fig. 5) adherent to upper portions, compared to an even distribution lower down (Fig. 4), suggests that motile bacteria may initially adhere to the thread, divide, break free, move along the thread surface, and then re-adhere and begin the process of cell division again.

E. coli and *S. aureus* cells were initially seen adhering to the surfaces of the monofilaments with no obvious attachment mechanism; after a 20

Fig. 4. *Se. marcescens* adhered to polyethylene thread removed from in vitro model. Section is close to the inoculum reservoir and shows high density of adherent cells.

Fig. 5. *Se. marcescens* adhered to polyethylene thread removed from in vitro model. Section shown is a part of the thread distant from the inoculum reservoir. The cells adhere in discrete clusters.

h incubation period, however, distinct polymeric material was observed attaching both cell types to the polymer monofilaments. A similar pattern of bacterial adhesion was observed by Peters et al. (1982) whilst investigating the colonisation of catheter surfaces by coagulase-negative staphylococci. The staphylococcal cells usually possessed a clean surface during the early stages of adhesion but with lengthening incubation periods many cells became covered with a 'slimy material', the amount of which clearly increased with time.

As the time of incubation increased so the force necessary to remove bacteria increased. After 1 h incubation, all bacteria could be removed from the IUD thread by shaking in 1/4 strength Ringer solution at a fast speed but after 3 h incubation, sonication was needed to remove those which were firmly adhering. This increase in strength of bacterial adhesion is likely to correlate with an increase in linkages between the bacterial extracellular biopolymeric material and the IUD thread. Both these results and those from the SEM studies support the proposal that bacterial adhesion is a two-stage process. Organisms may adhere to surfaces reversibly at first and then subsequently synthesise polymeric material to achieve irreversible adhesion (Rutter, 1980), the increase in strength of adhesion of bacteria with time being the result of a continuing extracellular polymer production with the consequent creation of additional bonds with the surface (Costerton and Irvin, 1981).

SEM studies demonstrated that bacterial adherence to monofilaments is dependent upon many factors including material type, surface microrugosity, bacterial species and composition of the

cell suspending liquid. In particular, such studies highlighted the extreme care that must be taken when utilising SEM as a means of investigating bacterial adhesion to surfaces. For example, *S. aureus* adhesion was shown to be greatest to polyethylene, with only 25% of this number adherent to PVDC monofilament (Table 1). When the SEM experiments were repeated, however, with no agitation of washing or dehydrating solutions, far more *S. aureus* were seen adhering to all thread types (Table 1) indicating that standard SEM preparatory techniques had led to the removal of many cells in earlier experiments. In addition, when low agitation was employed the *S. aureus* cells were seen adhering in largest numbers to PVDC monofilament. These results together suggest that although *S. aureus* cells may adhere preferentially to PVDC monofilament, this adherence is of a weak nature compared to adhesion to other thread types and consequently cells are far more readily removed.

Similarly, the pattern of *E. coli* adherence to monofilaments appeared to be method dependent. *E. coli* adhesion was greatest to polyethylene with only 20% of this number adherent to PVDC (Table 2). When SEM experiments were repeated with no agitation of washing or dehydrating solutions, far more *E. coli* cells were seen adhering to all thread types with the highest numbers of *E. coli* cells being visible on PVDC monofilaments (Table 2). Thus, although *E. coli* cells may preferentially adhere to PVDC, as with *S. aureus*, this adhesion is apparently weaker than the adhesion to other thread types.

Interestingly, the scanning electron micrographs showed bacterial cells preferentially adhering to the rougher grooved sections of monofilament surfaces rather than to the smoother areas. This was particularly apparent for microbial adsorption to PVDC. Possible explanations for this include increased surface area available for contact, increased charge density and shielding from shearing forces.

The results from this study concur with a number of previous investigations. Baker (1984) showed that roughening the surfaces of substrata submerged in river water led to a greatly increased rate of bacterial colonisation. Similarly, adherent bacteria were found to be concentrated in the crevices of detrital material from river water (Geesey and Costerton, 1979). In addition, cocci were found to adhere initially in the pits and surface irregularities of human dental enamel and cementum (Nyvad and Fejerskov, 1980) and in the surface irregularities of catheter materials (Peters et al., 1982; Franson et al., 1984). Microrugosity was also found to be a contributory factor in determining the extent of bacterial adhesion to polymer monofilaments (Wilkins et al., 1989b).

The strength of bacterial attachment to surfaces therefore appears to increase with time and during the initial reversible stages measurement of numbers adhering can be greatly influenced by the method of assessment. When this is standardised the extent of adhesion is shown to be dependent upon the bacterial species and the nature of the surface. The results suggest that adhesion may play an important part in movement of bacteria along solid substrates and as such may facilitate the ascent of microorganisms along IUD marker tails from the vagina into the otherwise microbiologically sterile uterus.

References

Baker, J.H., Factors affecting the bacterial colonisation of various surfaces in a river. *Can. J. Microbiol.*, 50 (1984) 511–515.

Costerton, J.W. and Irvin, R.T., The bacterial glycocalyx in nature and disease. *Annu. Rev. Microbiol.*, 35 (1981) 299–324.

Edelman, D.A., Berger, G.S. and Keith, L., The use of IUDs and their relationship to pelvic inflammatory disease: A review of epidemiologic and clinical studies. *Curr. Probl. Obstet. Gynaecol.*, 6 (1982) 33–34.

Franson, T.R., Sheth, N.K., Rose, H.D. and Sohnle, P.G., Scanning electron microscopy of bacteria adherent to intravascular catheters. *J. Clin. Microbiol.*, 20 (1984) 500–505.

Geesey, G.G. and Costerton, J.W., Microbiology of a northern river: bacterial distribution and relationship to suspended sediment and organic carbon. *Can. J. Microbiol.*, 25 (1979) 1058–1062.

Marrie, T.J. and Costerton, J.W., A scanning and transmission electron microscopic study of the surfaces of intrauterine contraceptive devices. *Am. J. Obstet. Gynecol.*, 146 (1983) 384–394.

Nyvad, B. and Fejerskov, O., Early microbial colonisation of human tooth surfaces: A SEM study. *J. Ultrastruct. Res.*, 73 (1980) 131.

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.

Potts, M. and Pearson, R.M., A light and scanning electron microscope study of cells in contact with intrauterine contraceptive devices. *J. Obstet. Gynaecol. Br. Commonw.*, 74 (1967) 129–136.

Rutter, P.R., The physical chemistry of the adhesion of bacteria and other cells. In Curtis. A.S.G. and Pitts, J.D. (Eds.) *Cell Adhesion and Motility*, Cambridge University Press, Cambridge, 1980, pp. 103–134.

Sheppard, B.L. and Bonnar, J., Scanning and transmission electron microscopy of material adherent to intrauterine contraceptive devices. *Br. J. Obstet. Gynaecol.*, 87 (1980) 155–162.

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

Spornitz, U.M., Makabe, S., Hafez, E.S.E. and Van Os, W.A.A., Scanning electron microscopy of the intrauterine device. *Contraceptive Delivery Syst.*, 5 (1984) 29–45.

Wilkins, K., Hanlon, G.W., Martin, G.P. and Marriott, C., The migration of bacteria through gels in the presence of IUCD monofilament tails. *Contraception*, 39 (1989a) 205–215.

Wilkins, K., 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 (1989b) 1–7.