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Microbial colonization of implanted silicone and polyurethane catheters

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Catheters explanted from nephropathic children were tested for microbial colonization, biofilm formation and surface defects chargeable to the implantation into the organism. Infection symptoms were detected in 13.6% of cases, versus 16% of colonization detected in the absence of clinical signs of infection. PU catheters showed slightly higher colonization/infection rates, perhaps due to the implant location. Biofilm was observed on both silicone and PU catheters, independently of the duration of catheterization; a lower amount of organic deposits was observed on the external catheter surfaces. Surface morphology of the catheters seemed to affect biofilm deposition, cavities and scratches present on both unused and explanted catheters providing preferential sites of deposit formation. Surface characteristics as well as biofilm possibly affected bacterial attachment in an *in vitro* adherence test. The presence of antibiotic molecules trapped in the biofilm was hypothesized to explain partial inhibition of *S. epidermidis* and *S. aureus* adhesion to catheter implanted in patients who underwent antibiotic therapy.

1. Introduction

Indwelling medical devices are routinely used as therapeutic tools in medicine. They have become increasingly useful, particularly in the management of certain classes of patients such as infants and patients with drug- or disease-induced immunospuppression. However, long-term use of such devices is often hampered by the development of foreign-body associated infections [1].

Soon after implant, devices are coated by a film (biofilm) composed of molecules present in the body fluids, in a process known as "surface conditioning" [2]. The composition of this biofilm is influenced by the chemical nature and design of the implanted device. The presence of "conditioned" indwelling foreign bodies provides a suitable support for microbial colonization, enabling even non-pathogenic bacteria to cause infections. Implanted biomaterials also allow for the unusual persistence of pathogens at the site of infection. As a consequence, the removal of infected prosthesis is often the only way to eradicate the infection, with all the risks and costs this implies. In some cases, bacterial colonization may also be present without giving clinical signs of infection, and still presents problems for the functionality of the device [3].

All these factors, i.e. biomaterial surface morphology [4, 5], the chemical nature of the implant [1] and the biofilm covering foreign bodies [6], have been considered as favouring the development of infection foci on devices.

We investigated the microbial colonization, associated either with overt of subclinical infections, of 44 polyurethane (PU) and silicone catheters explanted from nephrophatic paediatric patients. The microscopical features of these devices, in comparison to the unused ones, were also examined.

2. Materials and methods

2.1. Catheters

Thirteen PU and 31 silicone catheters, of either intravascular (IV) or peritoneal (IP) location (Table I), were explanted from children or young adults, ages ranging from 4 days to 15 years, after a total of 3185 days of usage (range 5–1000 days).

2.2. Microbiology

Soon after removal, all catheters were processed for microbiological and ultrastructural analysis. Catheters were cut under sterile conditions and segments of the tip, the intermediate and the emergence tract were separately cultured on blood agar, mannitol-salt plates, McConkey agar, and nutrient broth. Incubation at 37 °C followed, for up to two weeks. When turbidity of the broth culture or colonies were observed on agar, microorganisms were identified according to routine laboratory procedures.

Definition of catheter-associated infection was according to Maki [7]; colonization was defined as the

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isolation of the same microorganism from at least two of the three segments of the catheter or even from the tip only in patients with a previous history of sepsis or peritonitis and, possibly, confirmed with the observation of bacterial forms by scanning electron microscopy (SEM). Fever, higher level of protein C reactive, and high leucocyte count were taken as symptoms of infection.

2.3. Scanning electron microscopy

2 mm segments of each catheter tract were cut open to show the internal surface, as well as the external one, and prefixed with 0.1 M Na-cacodylate buffered-glutaraldehyde for a minimum of 2 h. Postfixation with 1% OsO_4 was followed by critical point drying (Balzers CPD 010), gold coating (Balzers SCD 040) and observation by a Cambridge 360 SEM at 15 kV with a 50 nm probe.

2.4. Bacterial strains

Five *Staphylococcus epidermidis* and 2 *Staphylococcus aureus* strains from biomaterial-associated infections were used for the adherence test. All strains were characterized (Table II) for their antibiotic sensitivity and hydrophobicity [8]. Slime production was evaluated through a procedure modified [9] from the original one first described by Christensen [10].

2.5. Adherence test

Segments of non-colonized catheters were stored for no more than 2 h in phosphate-buffered saline (PBS) and challenged with bacterial suspension in PBS (OD 0.01) for 18 h at 37 °C in a shaking incubator. Samples were subsequently processed for SEM as described above. For each sample bacterial cells were counted (50 fields at 8000X magnification) and the adhesion rate expressed as bacteria/mm^2.

TABLE I Characteristics of catheters considered in the study

Catheter type	Location	Material	Number
Tenckoff	I.P.	Silicone	13
Curled	I.P.	Silicone	5
Quinton	I.V.	Silicone	13
Unicath	I.V.	Polyurethane	13
Total			44

3. Results

3.1. Microbiology

Microbiological analysis provided evidence of colonization in 14 cases (31.8%), with a slightly higher relative risk of colonization for PU catheters (5 of 13 PU catheters versus 9 of 31 silicone catheters). Among PU catheters, only in one case (2.2%) the infection was symptomatic and directly related to the catheter. In one more case, signs of infection were present albeit the catheter was removed for restored renal functions. Four patients with implanted silicone catheters showed infection symptoms. Colonized PU catheters had a medium implantation time of 11.8 versus 20.3 days for the non-colonized ones; colonized and non-colonized silicone catheters were implanted for 130 and 77 days, respectively. Several different microorganisms were isolated (Table III) including *S. aureus*, coagulase-negative staphylococci (CNS), *Micrococcus kristinae*, *Candida spp.*, *Enterococcus faecium*, *Klebsiella pneumoniae*; mixed infections were also observed. There was no preferential colonization of a single species to a specific material.

3.2. Scanning electron microscopy

SEM studies revealed the presence of heavy organic deposits on both luminal and, to a lower extent, external catheter surfaces (Fig. 1a, b). Fibrillar material was often observed to obstruct exit holes on the catheter tip (Fig. 1c). Deposits, as well as bacterial cells, were detectable particularly along surface irregularities; scratches, wave-like patterns and cavities were observed on the surface of both explanted and new catheters analysed for comparison (Fig. 1d). In a few cases, a pseudo-colonization by epithelial-like cells, on peritoneal catheters only, was also detected (Fig. 1e).

3.3. Adherence test

To evaluate the influence of the molecular biofilm on bacterial colonization, an *in vitro* adherence test with clinical isolates of *S. aureus* and *S. epidermidis*, whose characteristics are listed in Table II, was performed on new and explanted non-colonized catheters (Table IV). Adherence rates to four different unused catheters were comparable for each strain considered. On the other hand, explanted catheters showed different reactivity towards microbial colonization; in particular, low adhesion rates were observed when catheters

TABLE II Characteristics of coagulase-negative and -positive staphylococcal strains utilized in the *in vitro* adherence test

Strain	Source	Antibiotic resistance	Hydrophobicity (%)	Slime production
S. epidermidis 3561	Blood	Caz, Ox, Cro, Amp	28	S.P.
S. epidermidis 948	Blood	Caz, Ox, Cro, Amp	22	S.P.
S. epidermidis 930	Blood	Amp	42	S.P.
S. epidermidis 465	Catheter tip	–	15	S.P
S. epidermidis 462	Catheter tip	Amp, Ak	36	W.P.
S. aureus 377	Peritonitis	Amp, Ak	44	W.P.
S. aureus 399	Catheter tip	Amp, Ak	47	N.P.

Caz = ceftazidime (30 μg/μl); Ox = oxacillin (5 μg/μl); Cro = ceftriaxone (30 μg/μl); Amp = ampicillin (10 μg/μl); Ak = amikacin (30 μg/μl)
S.P. = strong producer; W.P. = weak producer; N.P. = non-producer

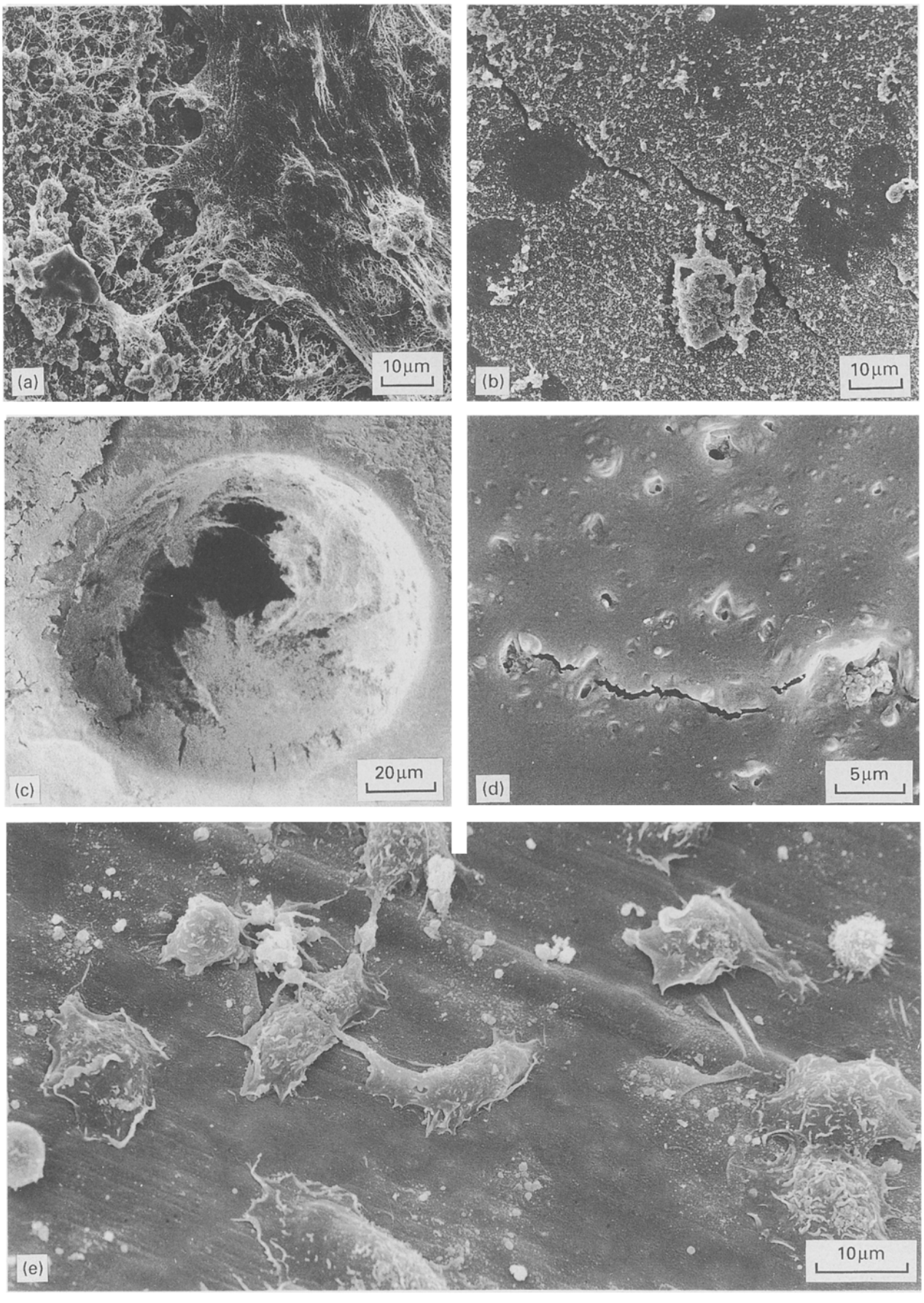

Figure 1 (a) Internal and (b) external surface of a silicone peritoneal catheter. The biofilm deposit is thicker and more abundant on the luminal surface than the external one. (c) Organic material is also heavily deposited on a giugular catheter tip almost obstructing the exit hole. (d) A long scratch and cavities are visible on the surface of a polyurethane catheter. (e) Fibroblast-like cells are seen to colonize the inner surface of a silicone peritoneal catheter.

explanted from patients under antibiotic therapy were challenged with strains sensitive to the given antibiotics. Note the adhesion rates of *S. epidermidis* 948 and 3561 strains (both resistant to ceftazidime and sensitive to amikacin) to catheter 5 and 6 (Table IV), which had been implanted in patients treated with ceftazidime and amikacin, respectively.

4. Discussion

Infection is one of the most common causes of catheter failure, as well as the most difficult to manage [11], most often requiring catheter removal [12].

While a variety of microorganisms have been found to cause such infections, CNS, specifically *S. epidermidis*, represents the leading cause of both intra- [13] and extravascular [14] catheter-associated infections. In our study, microbiological analysis confirmed that *Staphylococcus spp.* is commonly isolated from implanted biomaterials, followed by fungal species. Colonization and overt infection rates were comparable (16% versus 13.6%), in agreement with Haslett *et al.* [13] who reported 9% colonization among 502 IVC implanted in an adult intensive care unit (48% by *S. epidermidis*), and an 11.8% rate of infection.

Data suggested that longer catheterization periods were associated with a higher colonization rate among silicone catheters; this type of association was expected, because of the higher probability of exposure to possible contaminating microorganisms. On the other hand, PU-colonized or infected catheters had a medium implant time shorter than the non-colonized ones; since these devices were all implanted in the femoral vein, this location (near the inguinal, highly contaminated, skin region) may have accounted for the higher colonization rates. A higher risk of penetration of the infecting organism at the moment of insertion rather than during catheter handling may also be considered.

After just 5 days (the minimum implantation period in our study) a heavy deposit on catheters was observed; biofilm thickness was, however, not dependent on the length of catheterization. Surface irregularities possibly provided the starting point for biofilm formation, and should be considered a major point of interest by the medical devices manufacturing companies. The relatively lower biofilm formation on the external catheter surface we observed, was recently described by Raad *et al.* [15], who gave as explanation the mechanical stripping of the external biofilm during catheter removal through the subcutaneum and skin. Biofilm quality, as well as quantity, might also have accounted for the differential behaviour of materials towards colonization. Indwelling prosthesis have been shown to become covered by organic biofilm soon after implantation [2]. Vaudaux *et al.* [17] observed a significantly higher level of fibronectin deposition on Hickman and polyvinylchloride compared to PU catheters. The slightly higher colonization rates for PU as against silicone catheters may then reflect a differential chemical composition of the biofilm. Fibronectin has been reported to have a supporting action in the adherence of *S. aureus* and, less frequently, of *S. epidermidis*, to catheters [16, 17]; in contrast, Muller *et al.* [18] showed that plasma molecules covering cannulae inhibited the adhesion process. We did not analyse the biofilm composition in our specific cases; however, results of the *in vitro* adherence test suggested that antibiotic molecules also may become trapped in the biofilm, thus affecting the microbial viability, and adherence ability, during the initial stage of colonization. As far as the role played by the bacterial factors was concerned, it seemed that slime production rather than hydrophobicity was effective in supporting colonization. This is a quite

TABLE III Bacterial and fungal species contaminating or infecting PU and silicone catheters explanted from nephropathic patients

Strain	Catheter		Number and (%)
	Silicone	PU	
S. epidermidis	1	2	3 (21.5)
S. aureus	3	0	3 (21.5)
S. warneri	1	0	1 (7.1)
M. kristinae	0	1	1 (7.1)
C. tropicalis	0	1	1 (7.1)
C. parapsilosis	2	0	2 (14.4)
S. epidermidis + *K. pneumoniae*	1	0	1 (7.1)
S. epidermidis + *S. aureus*	1	0	1 (7.1)
S. haemolyticus + *E. faecium*	0	1	1 (7.1)
Total	9	5	14 (100)

TABLE IV *In vitro* adherence rates of staphylococcal strains to explanted catheters (number of bacteria $\times 10^5$ per mm^2)

Strain	Adhesion to explanted non-colonized catheter							
	1 (PU) (Amp, cro)[a]	2 (silicone) –	3 (PU) –	4 (silicone) (Caz)[a]	5 (silicone) (Caz)[a]	6 (PU) (Ak)[a]	7 (silicone) (Ak)[a]	8 (PU) (Caz)[a]
S. epidermidis 3561	90	0	3	10	94	0	64	61
S. epidermidis 948	11, 2	0, 6	12, 5	–	11, 2	0, 5	0, 2	2, 2
S. epidermidis 930	–	0	1, 6	6	1, 6	–	0, 8	1, 6
S. epidermidis 465	4, 5	–	0, 4	–	–	–	0	0, 9
S. epidermidis 462	0, 5	0	1, 6	–	1, 1	0, 6	2, 8	–
S. aureus 377	1, 1	1	–	0	0, 6	0, 1	0, 1	0, 3
S. aureus 399	–	2, 8	0	2, 7	3, 7	–	1, 6	0, 1

[a] antibiotic therapy, if any, administered to the patient during catheter implant (abbreviations as in Table II)
– biofilm thickness prevented bacterial counts

controversial point since a number of papers suggested a role for hydrophobicity in the adhesion process while others underlined the importance of slime [19–22]. We observed considerable variation among the adhesion rates of the different strains to each catheter; these rates probably reflected a balance of factors intervening in bacteria–catheter interactions, such as a differential biofilm composition, including antibiotic molecules trapped in it, and bacterial adherence factors.

In conclusion, our data confirmed staphylococci as the commonest etiologic agents of catheter infections. Colonization was present in indwelling catheters, even in the absence of clinically evident infection, highlightening the importance of a thorough catheter handling during care and/or surgical procedures. A slight preponderance of colonization among PU catheters might have, in our cases, been due to the anatomical location of the implant; the real importance of the role of the material type will need the analysis of a larger number of samples.

An interesting point is represented by the possible trapping of antibiotic molecules in the biofilm; were this to be confirmed, it will be necessary to consider such an effect when planning the profilactic protocol routinely used in catheterized patients.

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