Urol Res (1989) 17:349–352

Urological Research

Original articles

Infection of catheterised patients: bacterial colonisation of encrusted Foley catheters shown by scanning electron microscopy

A. J. Cox[1], D. W. L. Hukins[1], and T. M. Sutton[2]

[1] Department of Medical Biophysics, University of Manchester, Manchester, and
[2] Bard Ltd., Clacton-on-Sea, Essex, United Kingdom

Accepted: February 1, 1989

Summary. The surfaces of 32 encrusted urinary catheters were examined by scanning electron microscopy to investigate the association of bacteria with the encrusting deposits. Deposits consisted of struvite crystals surrounded by aggregates of very small crystallites of hydroxyapatite. Underneath these minerals there was a layer of closely packed bacteria. Impressions of bacteria were also observed in hydroxyapatite. Crystals were often engulfed by the bacterial layer, which thus appeared to bind the crystals to each other and to the catheter surface. This thick layer of bacteria associated with crystals may protect both the bacteria from antibiotics and the crystals from acidic bladder washout solutions intended to dissolve them. Furthermore, the existence of this sessile population explains why urease-producing bacteria are not invariably detected in the urine of patients with encrusted catheters. The observation of this bacterial layer (or "biofilm") by scanning electron microscopy provided direct evidence for infection being implicated in catheter encrustation.

Key words: Urinary infection – Urinary catheters – Encrustation – Bacterial colonisation

Introduction

Scanning electron microscopy has been used to show that encrusting deposits on urinary catheters are associated with bacteria which colonise the catheter surface. This observation provides evidence for infection as the precursor to encrustation and explains why infections of the urinary tract are so difficult to eradicate in catheterised patients.

Encrusting deposits consist of a mixture of struvite (ammonium magnesium phosphate hexahydrate) and a poorly crystalline form of hydroxyapatite, containing some carbonate [5, 8, 13]. These minerals precipitate from urine when it becomes alkaline, as a result of infection by bacteria (especially *Proteus sp.*) which secrete urease [11]. Therefore, it has been proposed that the aetiology of encrustation is closely similar to that of "infection stone" formation [14,20]. The evidence for this proposal is conflicting. On the one hand, published scanning electron micrographs of four encrusted Foley catheters have revealed the presence of bacteria [2, 18]; on the other hand, *Proteus* cannot be detected in the urine of all patients with encrusted catheters [1, 4, 20]. Examination of a very much larger sample of catheters by scanning electron microscopy confirms the importance of bacteria in encrustation and resolves the apparent conflict in the experimental evidence.

Materials and methods

Over 50 used catheters were obtained from long-stay geriatric patients from 4 British hospitals; 32 of these were selected for detailed examination, for the reasons given below. Six of the catheters had been stored in phosphate buffered saline, containing 25% ethanol. All catheters were sterilised by ^{60}Co irradiation and air-dried. No other fixing or dehydration procedures were used, in order to reduce the opportunities for the introduction of artefacts. This precaution was important because the specimens consisted of crystals and biological structures on top of bulky, robust polymers (latex and/or silicone), which are poor electrical and thermal conductors. Such inhomogeneous samples are susceptible to damage from treatments where large temperature gradients might occur (e.g. freeze-drying and sputtering) and flaking-off of surface deposits could be expected.

Catheters were cut longitudinally in order to assess the degree of encrustation on both inner and outer surfaces. Most deposits were found to occur in the lumen immediately below the catheter eye. Initially catheters were selected that appeared encrusted to the naked eye; however, when these were viewed under the scanning electron microscope, their luminal surfaces were found to be completely obscured by crystals. It was then impossible to obtain any information about the formative stages of the encrustation process. Therefore, catheters were subsequently selected that appeared to be barely encrusted, and which were of all-silicone or silicone elastomer-

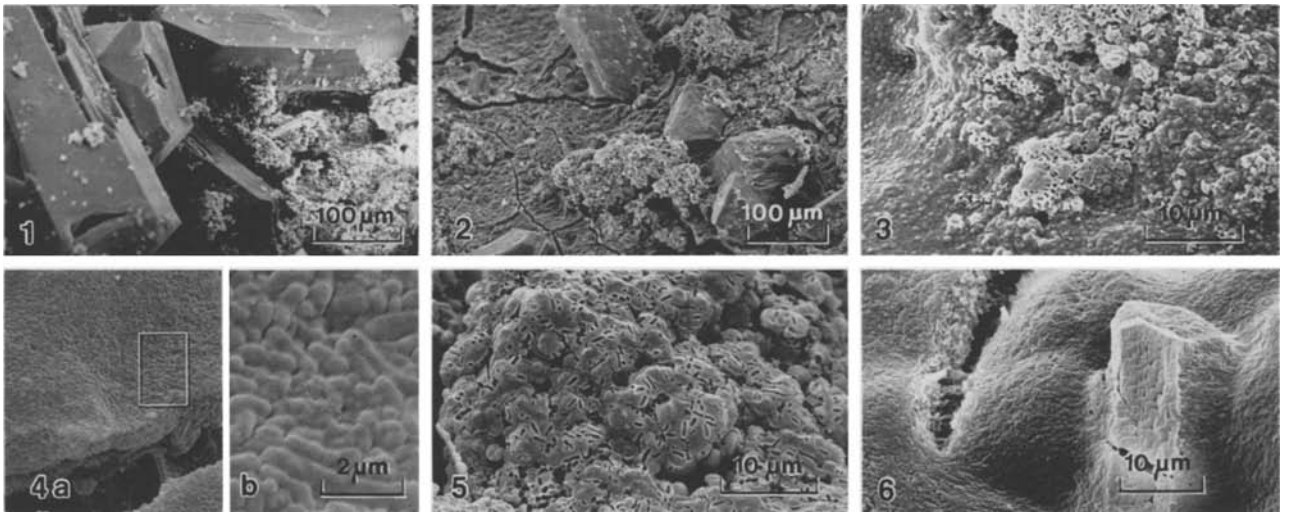

Fig. 1. Scanning electron micrograph showing the deposit on an encrusted catheter. Two crystal species can be identified: struvite crystals are relatively massive and have characteristic Y-shaped cracks on their faces, whereas hydroxyapatite presents a powdery appearance, since its individual crystallites are approximately one thousand times smaller

Fig. 2. Scanning electron micrograph of the luminal surface of a lightly encrusted catheter. Struvite and hydroxyapatite can be identified, but here a surface film can be distinguished underneath

Fig. 3. Scanning electron micrograph in which the surface film can be seen to consist of bacteria, on top of which a crust of hydroxyapatite is forming. This presents a fibrous appearance due to the impressions of cocci left in it

Fig. 4a, b. Scanning electron micrograph of a surface layer of bacteria which is several micrometers thick. The zoom picture on the right hand side (at five times the magnification of the left-hand side picture) resolves individual bacteria and shows that they are very closely packed

Fig. 5. Scanning electron micrograph showing impressions of rod-shaped bacteria in the hydroxyapatite

Fig. 6. Scanning electron micrograph of a struvite crystal engulfed by the bacterial layer

coated latex construction. These materials have relatively smooth surfaces on which features such as crystals and bacteria could be readily distinguished, whereas latex surfaces are usually very rough and irregular [6]. The features observed on the outer surfaces were similar to those on the inner surfaces, but they are not considered here because the outer surfaces would have been subjected to friction during removal of the catheter. In contrast the luminal environment would remain undisturbed.

Pieces approximately 35 mm × 5 mm were cut from the catheters from a region 10 cm distal to the eye. They were mounted on specimen stubs with the luminal surface uppermost. Silver paint or Leit-C Plast (a conductive plasticine) was applied to ensure good electrical and thermal contract between the upper surface of the specimen (a fairly thick insulator) and the stub; both materials were obtained from Agar Aids Ltd. (Stanstead, Essex, U.K.). Gold sputtering was carried out using an Edwards S150 sputterer, at a low sputtering current with specimen cooling. Both of these precautions prevented overheating, which produced spurious surface cracks. Specimens were examined in either of two Cambridge Instruments Stereoscan SEMs (Models 150 and 360) at an accelerating voltage of 20 kV and a 45°C specimen tilt.

Results

Figure 1 shows a scanning electron micrograph of a heavily encrusted luminal surface on which struvite and hydroxyapatite crystals can be readily identified by their characteristic appearance [7]. Relatively large struvite crystals are surrounded by very much smaller (about 0.2 µm) crystallites of hydroxyapatite, which present a powdery appearance. A three-dimensional network of the two mineral phases extends into the lumen. Further precipitation of these minerals would eventually be expected to block the lumen and prevent the free flow of urine.

Scanning electron micrographs of lightly encrusted regions invariably revealed a film of bacteria underlying the mineral deposits. An example is shown in Fig. 2, where the two mineral components and bacteria may be clearly seen. Again, blocky struvite crystals are surrounded by aggregates of much smaller hydroxyapatite crystallites. Underlying these is a flaking film, which is composed of multitudes of densely packed bacteria. At first sight this film could be mistaken for delamination of the catheter surface. However, at higher magnifications individual bacteria may be resolved, as in Figs. 3 and 4.

Furthermore, scanning electron micrographs also reveal an intimate association between bacteria and hydroxyapatite deposits. In Fig. 3 the hydroxyapatite crust has a somewhat fibrous appearance because of the round holes in it. These holes can be seen to be impressions (rather like footprints) left by cocci that

had existed within the crust. The association between bacteria and hydroxyapatite deposition is clearly shown in Fig. 5, where "footprints" of rod-shaped bacteria are apparent. Because the hydroxyapatite particles are so small, it gives the appearance of a continuous medium in which bacterial impressions are cast – just as the fine particles of plaster of Paris enable it to be used to produce detailed castings of larger features.

Bacteria may also engulf crystals once they have formed. Figure 6 shows the film of bacteria covering a struvite crystal. This process would be expected to bind the crystals of both minerals together, and to help bind a growing deposit of encrusting minerals to the catheter surface. Indeed, such layers containing closely packed bacteria have frequently been observed to grow to a thickness of several micrometers; Fig. 4 provides an example.

Discussion

It was clear that bacteria had colonised the surfaces of nearly all the catheters examined; this observation provides direct evidence to substantiate the view that the aetiology of encrustation is similar to that of the formation of infection stones [14, 20]. The sequence of events would then be: (i) bacteria colonise the catheter, (ii) bacteria secrete urease, (iii) urease hydrolyses urea in the urine to form ammonia, (iv) the urine becomes alkaline, and (v) struvite and hydroxyapatite are deposited [11]. It is established that struvite and hydroxyapatite are precipitated from alkaline urine [10, 16], and that catheters can be encrusted in vitro by the addition of urease to artificial urine [9, 17]. Nevertheless, previous evidence for this sequence has tended to be incomplete or indirect; it has included comparisons of pH [20] and bacterial counts [21] for urine from recently inserted and used catheters, culturing bacteria from the outer surfaces of used catheters [15], and analysing the chemical composition of the encrusting deposits [5, 8, 13]. Scanning electron microscopy provides direct evidence for the association of bacteria with the encrusting deposits.

The thick layer of bacteria which colonises the catheter surface appears not only to precede mineral deposition but also to bind the crystals together as encrustation proceeds. Thus the bacteria could stabilise the growing layer of encrusting deposits, leading to eventual blockage of the catheter. A similar layer was observed previously in an examination of a single used catheter, where it was described as a "bacterial biofilm" [18].

The formation of a thick layer of bacteria also indicates that the luminal surface of the catheter offers a suitable substrate for colonisation, as well as a relatively protected environment for further growth. The difficulty in eradicating bacterial infecitons of the urinary tract in catheterised patients [14] is, therefore, not surprising. An alternative explanation, that residual urine is responsible for the persistence of infection [22], appears less important – given the thickness of the bacterial layer on the catheter itself. Indeed, it has been shown that systemic antibiotics have little effect on the bacteria present on the catheter surface [15]. It has been suggested previously that bacteria are implicated in forming a matrix which external agents have difficulty penetrating [18]. Changing the catheter would appear to be an important part of the treatment of persistent infection.

However, there have been various reports that urease-producing bacteria are not invariably cultured from urine samples obtained from catheterised patients [1, 4, 20]. One explanation for this anomalous result is that the urease production is due to organisms such as *Ureaplasma urealyticum* [12] or *Corynebacterium sp.* [23], which are not usually detected by conventional bacterial culture methods. Another is that, since the population distribution of bacterial strains present in urine is known to fluctuate with time [3], the urease-producing organisms responsible for encrustation are no longer present at the time of sampling. Our results suggest that a more likely reason is that the bacteria remain associated with the catheter surface, in a thick layer, and so may not be detected in the urine. Localised urease production from within such a layer has been demonstrated in the formation of experimentally induced bladder stones in rats infected with *Proteus sp.* [19].

In conclusion, the existence of thick layers of bacteria on the luminal surfaces of the catheters provides direct evidence for infection being involved in the encrustation process. The existence of this sessile population offers an explanation as to why bacteria are not invariably found in the urine of patients with encrusted catheters. It also shows how the constituent bacteria may be protected from the effects of antibiotics, not only because they lie deep within this film but also because they may be incorporated within the hydroxyapatite. Conversely, the bacterial layer may protect the mineral components from acidic bladder washouts which are intended to dissolve them. The affinity of bacteria for polymer surfaces is therefore the primary reason for catheter encrustation.

References

1. Alling B, Brandberg A, Seeberg S, Svanborg A (1973) Aerobic and anaerobic microbial flora in the urinary tract of geriatric patients during long-term care. J Infect Dis 127:34–39

2. Axelsson H, Schonebeck J, Winblad B (1977) Surface structure of used and unused catheters. Scand J Urol Nephrol 11:283–287
3. Breitenbucher R (1984) Bacterial changes in the urine samples of patients with long-term indwelling catheters. Arch Intern Med 144:1585–1588
4. Brocklehurst JC, Brocklehurst S (1978) The management of indwelling catheters. Br J Urol 50:102–105
5. Bruce AW, Sira SS, Clark AF, Awad AF (1974) The problem of catheter encrustation. Can Med Assoc J 111:238–241
6. Cox AJ (in press) Comparison of catheter surface morphologies. Br J Urol
7. Cox AJ, Hukins DWL (in press) Morphology of mineral deposits on encrusted urinary catheters investigated by scanning electron microscopy. J Urol
8. Cox AJ, Harries JE, Hukins DWL, Kennedy AP, Sutton TM (1987) Calcium phosphate in catheter encrustation. Br J Urol 59:159–163
9. Cox AJ, Hukins DWL, Davies KE, Irlam JC, Sutton TM (1987) An automated technique for in vitro assessment of the susceptibility of urinary catheter materials to encrustation. Eng Med 6:37–41
10. Elliot JS, Quaide WL, Sharp RF, Lewis L (1958) Mineralogical studies of urine: the relationship of apatite, brushite and struvite to urinary pH. J Urol 80:269–271
11. Griffith DP, Musher DM, Itin C (1976) Urease: the primary cause of infection-induced urinary stones. Invest Urol 13:346–350
12. Hedelin H, Brorson J-E, Grenabo L, Pettersson S (1984) *Ureaplasma urealyticum* and upper urinary tract stones. Br J Urol 56:244–249
13. Hukins DWL, Hickey DS, Kennedy AP (1983) Catheter encrustation by struvite. Br J Urol 55:304–305
14. Kunin CM (1987) Care of the urinary catheters 5. In: Detection, prevention and management of urinary tract infections, 4th ed chapt 5. Lea and Febiger, Philadelphia, pp 245–297
15. Kunin CM, Steele C (1985) Culture of the surfaces of urinary catheters to sample urethral flora and study the effect of antimicrobial therapy. J Clin Microbiol 21:902–908
16. Linder PW, Little JC (1986) Prediction by computer modelling of the precipitation of stone forming solids from urine. Inorg Chim Acta 123:137–145
17. Miller JM, Opher AW (1974) An enzymatic method to make struvite crystals. Invest Urol 11:402–403
18. Nickel JC, Gristina AG, Costerton JW (1985) Electron microscopic study of an infected Foley catheter. Can J Surg 28:50–52
19. Nickel JC, Olson M, McLean RJC, Grant SK, Costerton JW (1987) An encological study of infected urinary stone genesis in an animal model. Br J Urol 59:21–30
20. Norberg A, Norberg B, Lundbeck K, Parkhede U (1980) Urinary pH and the indwelling catheter. Upsala J Med Sci 85:143–150
21. Rubin M, Berger SA, Zodda FN, Gruenwald R (1980) Effect of catheter replacement on bacterial counts in urine aspirated from indwelling catheters. J Infect Dis 142:291
22. Rubino SM, Scialabra MA (1983) A clinical evaluation of a modified Foley catheter. Am J Obstet Gynecol 146:103–104
23. Soriano F, Ponte C, Santamaria M, Aguado JM, Wilhelmi I, Vela R, Cifuentes L (1985) *Corynebacterium* group D2 as a cause of alkaline-encrusted cystitis: report of four cases and characterization of the organisms. J Clin Microbiol 20:788–792

Dr. D. W. L. Hukins
Department of Medical Biophysics
University of Manchester
Manchester M13 9PT
United Kingdom