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THE STRUCTURE OF URINARY CATHETER ENCRUSTING BACTERIAL BIOFILMS

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Abstract

A major complication of long-term urethral catheterization is catheter blockage by encrustation. We have examined 20 encrusted catheters and in each case crystal formation was associated with the presence of bacterial biofilms on the luminal surfaces. Scanning electron microscopy and X-ray microanalysis indicated the presence of struvite and hydroxyapatite in the biofilms. Urease producing bacteria were colonizing 16 of the catheters. Proteus mirabilis was the commonest species being recovered from ten of the catheters. These results support the hypothesis that catheter encrustation has a similar etiology to that of infection-induced urinary stones and confirm that the important target for any attempt to control catheter encrustations is Pr. mirabilis.

Key Words: Urethral catheters, urinary tract infection, bacterial biofilms, catheter encrustation, calcification.

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Introduction

Indwelling urethral catheters are used in enormous numbers to deal with the problems of urinary retention and incontinence in elderly and chronically debilitated patients. Despite many ingenious attempts to improve the design of these devices and their urine drainage systems, they remain the major source of the infections that are acquired in health care facilities [15]. Even with careful aseptic management and maintenance of the closed drainage system, bacteriuria will occur in about half of the patients within 10-14 days and all of those undergoing long-term catheterization will be bacteruric by about day 28. Polymicrobial populations of drug resistant organisms colonize the bladder urine and are extremely difficult to eliminate with antibacterial agents while the catheter is in place [2].

Although these infections are generally asymptomatic, the patients are at risk from a range of complications which make them more vulnerable than non-catheterized individuals. A major problem with long-term catheterization is the formation of encrustations on the surfaces of the catheter [5,8]. These encrustations can cover the surface of the balloon, obstruct the eyelet and the lumen of the catheter, thus blocking the drainage of urine from the bladder. The hard crystalline deposits can cause trauma to the bladder mucosa and to the urethra on withdrawal. The blockage results in incontinence due to urine by-passing the catheter, or urine retention in the bladder with the associated acute pain and distress. Bacteriuria in the presence of blockage may culminate in episodes of fever, sepsis and shock which if not immediately lethal may require prolonged treatment with antibiotics [11]. Unnoticed catheter blockage can thus have serious consequences. In patients in their own homes where professional assistance is not immediately available, blockage can be particularly distressing. In some populations of elderly patients, up to half have been reported to suffer from repeated catheter blockage [12,13].

In this paper, we present our observations on the composition of the bacterial communities colonizing 20 encrusted catheters removed from patients undergoing long-term bladder management. Scanning electron microscopy and X-ray microanalysis were also used to examine the structure of the encrustations. The results provide some support for the mechanism of catheter encrustation proposed by Cox et al. [4].
Materials and Methods

Catheters
Encrusted silicone or silicone coated Foley catheters freshly removed from patients undergoing long-term catheterization in local hospitals, nursing homes and in the community were transported to the laboratory for examination.

Characterization of the biofilm communities
To characterize the bacterial communities colonizing the lumen of the catheters, sections (1 cm long) were cut from the region of the catheter within the retention balloon. The sections were rinsed once gently in 10 ml of buffer (Hanks-Hepes pH 7.4) and then placed in 10 ml of nutrient broth (Oxoid). Disruption of the luminal biofilm was then achieved by sonication for 5 min (Transonic Water Bath, Camlab, UK.) followed by vortex mixing for 2 min. Samples of the broth suspension were then plated out on CLED agar (Oxoid) and incubated for 24 h at 37°C. The resulting isolates were characterized using the appropriate identification kits (API, UK.)

Scanning electron microscopy
Sections of catheters (1 cm in length) were taken from the region adjacent to the retention balloon on the side away from the catheter tip. These were rapidly plunged into liquid nitrogen cooled propane and transferred to liquid nitrogen. Cross sections were then produced by freeze fracturing samples in a specially designed copper block which held the catheter and a blade in position and facilitated the production of reproducible cross sections. The samples were then freeze-dried for 24 h at -80°C, mounted fractured surface uppermost onto aluminium stubs, sputtered with gold and examined in a JEOL JSMS200 scanning electron microscope.

To examine the nature of the crystalline formations in the biofilms, further sections of catheter were plunged into liquid nitrogen, freeze dried, mounted and carbon coated for X-ray microanalysis in the scanning electron microscope.

To observe the nature of the surfaces of the biofilms, longitudinal sections of each catheter were prepared from the region adjacent to the retention balloon. The sections were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h and were then washed overnight in the phosphate buffer before being post-fixed in Millonig’s phosphate buffered osmium tetroxide (1.0%) for 1 h. The samples were dehydrated in a graded series of aqueous ethanol solutions (30-100%). Following dehydration, the samples were critically point dried using liquid CO₂. Finally, the samples were mounted on aluminium stubs, sputtered with gold and examined in the scanning electron microscope.

Results
Bacteriological analysis on the catheters revealed that all 20 were colonized by at least one organism. Eight catheters were colonized by single species biofilms, six by Proteus mirabilis and one by Pseudomonas aeruginosa. Mixed community biofilms containing up to four species were present on the other 12. Urease producing organisms Morganella morganii, Pseudomonas aeruginosa, P. mirabilis and Klebsiella pneumoniae were present on 10 of these. 16 of the catheters were thus colonized by urease producers. The other organisms isolated were Acinetobacter calcoaceticus (in combination with Enterococcus faecalis) and Enterobacter aerogenes (in

Figure 1. Freeze-dried preparations showing freeze fractured cross-sections of catheters colonized by pure cultures of Proteus mirabilis (Figs. 1A, 1B) and by a mixed community biofilm composed of Morganella morganii, Enterococcus faecalis, Escherichia coli and Pseudomonas aeruginosa (Fig. 1C).
Encrusting catheter biofilms

Figure 2. Freeze dried preparations showing the surfaces of encrusting biofilms. Fig. 2A is a *Proteus mirabilis* biofilm. Figs. 2B and 2C are from catheters colonized by *Proteus mirabilis* and *Enterococcus faecalis*, and Fig. 2D is a mixed community composed of *Providencia stuartii*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. In each case, large crystals typical of struvite (a) and small amorphous particles characteristic of hydroxyapatite (b) are visible.

Figure 3. X-ray microanalytical spectra of crystals present in the biofilms. The spectrum typical of those produced by the large crystals (Fig. 3A) confirms the presence of magnesium and (Fig. 3B) is a spectrum of the amorphous material indicating the presence of calcium.
combination with *Pr. mirabilis*). The freeze-fractured cross sections of the catheters (Figure 1) clearly show the partial or complete occlusion of the lumen by encrustation. The structure of the crystals found in four typical catheter encrustations is shown in Figure 2. X-ray microanalytical spectra of the large "coffin" shaped crystals and the small amorphous particles are shown in Figures 3A and 3B. Surface views of encrusted catheters prepared by fixing, washing, post-fixing and critical point drying are shown in Figure 4. In each case, the preparation of these specimens has removed the crystalline material and revealed the underlying bacterial biofilms.

**Discussion**

On the basis of a scanning electron microscopy study of encrusted catheters, Cox *et al.* [4] proposed a mechanism for catheter encrustation. While they did not identify the bacteria that were growing on the catheters, they suggested that encrustation is initiated by colonization with urease producing bacteria. The urease in the biofilm then hydrolyses urea to produce ammonia and the resulting alkaline environment facilitates the crystallization of struvite and hydroxyapatite. The biofilm matrix serves to bind the crystals together, stabilizing the growing encrustation that eventually blocks the catheter. The results of bacteriological analysis of the biofilm communities on the 20 catheters obtained in our survey largely substantiate this hypothesis.

Nine different bacterial species were recovered from the encrusted catheters. *Pr. mirabilis* was the most commonly isolated species being present on ten of the catheters (six as single species biofilms and four in mixed communities). Scanning microscopy on freeze fractured cross sections of the catheters revealed that they all had lumen occluding encrustations such as those shown in Figure 1. It also appeared that the catheters with the most extensive encrustation (Figure 1B) were colonized by pure cultures of the urease producer *Pr. mirabilis*.

Previous studies [3, 7, 9] have shown that the encrustations consist of a mixture of struvite (ammonium magnesium phosphate hexahydrate) and a poorly crystalline form of calcium phosphate (hydroxyapatite). The scanning micrographs (Figure 2) of the surfaces of the encrustations reveal the presence of the large coffin shaped crystals (a) typical of struvite and the small amorphous particles (b) characteristic of hydroxyapatite. The X-ray microanalysis of these structures (Figures 3A and 3B) confirmed the presence of magnesium and phosphorus in the struvite shaped crystals and of calcium and phosphorus in the amorphous particles.

In Figure 4, it is apparent that the preparation of the specimens by fixing, washing, dehydrating and critical point drying, has removed much of the encrustation from the catheter surface and revealed the underlying layers of bacteria.

The control of catheter encrustation is commonly attempted by the instillation of acidic solutions but the rational basis of this treatment is not clear [14]. A recent clinical trial in elderly female patients showed that acidic bladder washouts performed twice weekly for three weeks had no demonstrable effect in preventing catheter encrustation [10]. It has also been suggested [6] that long-term acidification of urine might provide an answer to the problem. A recent *in vitro* study [1] however, clearly demonstrated that acidification of urine in the presence of urease, produced only a temporary drop in pH. Attempts to prevent the rise in pH simply causes more urea to be
converted into ammonia. These authors concluded that acidification of the urine without elimination of bacteria which produce urease is not feasible as a method for preventing encrustation of indwelling urinary catheters.

Our results support that conclusion and confirm that the important target for any attempt to control catheter encrustations is *P. mirabilis*. We wish to suggest that replacement of a blocked catheter should be accompanied by antibiotic therapy targeted at *P. mirabilis* to ensure that the replacement catheter does not become immediately colonized by this organism.

References


Discussions with Reviewers

W. Schmitz: Catheterization is a common clinical practice with a lot of clinical indications. What criteria were used to select the twenty catheters for your investigation?

Authors: Over a three month period, we asked nursing staff in a spinal injuries unit and a geriatric ward at two hospitals and local district nurses responsible for bladder management in nursing homes and domiciliary care, to notify us when they removed a blocked catheter. The twenty catheters we collected came from elderly and chronically debilitated patients undergoing long-term indwelling catheterization to deal with problems of urinary incontinence or retention. The catheters were thus obtained from a range of situations where encrustation and blockage presents as a clinical problem.

H. Hedelin: Was there any difference in the amount of encrustations between silicone or silicone coated latex catheters?

W. Schmitz: Could you find out some differences in the kind or amount of encrustations between different kinds of catheters or different kind of colonized bacteria on the catheters?

Authors: Encrustations were found on both silicone and silicone coated latex catheters. While we had data on the length of time each catheter had been in place, it was not possible to be sure about how long each of the catheters had been colonized by an encrusting biofilm. We therefore did not attempt an analysis of encrustation related to the catheter type or the organisms colonizing the catheter. Perhaps these questions could be answered by a laboratory study in model systems under controlled conditions.

R.J.C. McLean: Based on visual observations, were crystals visible in catheter lumens and were they coated in slime i.e. biofilm material?

Authors: In some cases, crystalline material was visible to the naked eye. In general however, the appearance was one of thick layers of material embedded in a mucoid matrix.

R.J.C. McLean: What was the pH of the urine in these patients? Normally it is alkaline if struvite is present, however biofilms can create alkaline microenvironments which allow struvite to form in urine with neutral or acid pH (McLean et al., J. Urol. 146: 1138-1142, 1991).

Authors: We did not measure the pH of urine samples from the patients as it had become clear to us, from work with model systems, that the pH of urine and that of an encrusting biofilm can be very different.

R.J.C. McLean: In figure 2, regions marked "b" are interpreted as amorphous carbonateapatite. Could they in fact be calcified biofilms?

Authors: The removal of this material by the sample preparation procedures of fixing, washing, dehydrating and critical point drying, revealed the presence of underlying bacterial cells. So our interpretation is that the bacterial biofilm becomes calcified by deposition of hydroxyapatite in the matrix. We considered that the material labelled "b" was probably hydroxyapatite because of its amorphous nature and because X-ray microanalysis detected the presence of calcium and phosphorus (Figure 3B).

W. Schmitz: Instillation of acidic solutions cannot prevent catheter encrustation. However, as we could show in our investigation (Cells and Materials 1993 (1) pp 1-10) that existing encrustations can be removed or dissolved with these solutions. Positive effects of this treatment (in
approximately two day terms) are a) prevention of catheter blockage b) less trauma on catheter withdrawal and c) less remaining encrustation material in the bladder and decreased risk of reinfection. What do you think about it?

Authors: The study referred to here is most interesting. It shows that in an in vitro model system, the acid solutions Suby G and Solution R, were capable of dissolving catheter encrustations produced by *Pr. mirabilis*. In contrast, the recent clinical trial of these same two solutions in elderly female patients undergoing long-term catheterization, reported by Kennedy *et al.* (1992) (ref10) indicated that twice weekly bladder washouts had no demonstrable effect in preventing crystal formation or in the amount of encrustation found on the catheters. They also found evidence of adverse effects on the bladder epithelium. It may well be that the enrusting biofilms produced by *Pr. mirabilis* on catheters in your model, in artificial urine over 30h, are more susceptible to dissolution than those formed on catheters in vivo over much longer periods. We have some reservations about the treatment you suggest, as the frequent, regular use of acidic washouts might also lead to bladder irritation.

H. Hedelin: You claim that the target of attempts to control catheter encrustations is *Pr. mirabilis* but how should this be done in long-term indwelling catheters? If eradicated by antibiotics, the infection relapses when the treatment is terminated.

Authors: In our experience, the current practice of simply replacing blocked catheters, leads to their rapid recolonization, repeated blockage and results in patients being designated as "blockers". We are suggesting that it would be worthwhile investigating the effect of targeting *Pr. mirabilis* with antibiotics at the time of catheter change. In this way it might be possible to prevent or delay recolonization of the catheter with the urease producer that is mainly responsible for encrustation. It is certainly true that patients who are treated with antibiotics for catheter associated urinary tract infections, rapidly become reinfected when treatment is completed. It is possible that a major source of reinfection is the biofilm on the catheter, as bacteria in this mode of growth are extremely resistant to antibiotics. We are not aware of a study that has examined the combined effects on infection, of antibiotic treatment and catheter change. Perhaps we should also explain that our intention was to suggest that any attempt to control encrustation, whether by antibiotic treatment, bladder washouts, or by seeking to devise catheter surfaces that resist colonization, should consider *Pr. mirabilis* as the prime target.

D.W.L. Hukins: What antibiotics would the authors suggest are most suitable for targeting *Proteus mirabilis* and at what concentrations should they be present in the urine if they are to be effective?

Authors: The antibiotic sensitivity of each particular catheter colonizing strain would need to be considered of course but commonly *Pr. mirabilis* is sensitive to trimethoprim (2mg/l), cefuroxime (0.25mg/l), nalidixic acid (4mg/l) and norfloxacin (0.03mg/ml). The concentrations of these drugs attained in urine from normal dosages are trimethoprim 100mg/l, cefuroxime 500mg/l, nalidixic acid 250mg/l and norfloxacin 250mg/ml. It is not certain what concentrations of these antibiotics would be required to eliminate organisms from the catheter biofilms. If however, catheters were changed at the time of treatment, normal dosages should clear bacteria from the bladder and hopefully delay recolonization of the new catheter.

D.W.L. Hukins: Is there a danger, in targeting *Proteus mirabilis*, that other urease producing bacteria will colonize the catheter?

Authors: We feel that the control of catheter blockage and encrustation is not going to be easy but a successful strategy would produce a major advance in the care of patients undergoing bladder management by long-term indwelling catheterization. If it proves to be the case that eradication of *Pr. mirabilis* by antibacterial agents merely allows other urease producers to colonize and encrust catheters, then alternative strategies will have to be sought. Perhaps it would be worthwhile exploring the use of urease inhibitors.

K.A. Getliffe: Catheter encrustation occurred in the absence of urease producers in some catheters. Is it likely that urease producers colonizing these catheters were not detected or that catheter encrustation can occur under natural physiological conditions?

Authors: It is of course possible that in some cases, urease producers colonizing catheters were not detected by our isolation techniques, particularly if they were present as minority populations in the biofilm. Catheter encrustation by calcium and magnesium salts might also be possible in urine that was alkaline because of dietary or physiological factors. It seems clear however, that in the majority of cases, the problem of encrustation is generated by biofilms with urease activity.

K.A. Getliffe: Is it necessary to target *Proteus mirabilis* with a specific antibiotic or would a broad spectrum agent be effective against the range of urease producers identified?

Authors: A broad spectrum antibiotic such as ciprofloxacin, could well be effective against the range of urease producers that we found on the catheters. On the other hand, because of the important need to limit the development of resistance to such drugs, it would in our opinion, be preferable to use a drug with a narrower spectrum of activity, specifically aimed at the source of the urease.

K.A. Getliffe: It seems to me that 17/20 catheters were colonised by urease producers and not 16/20 as stated in the paper: 6 catheters colonised by *Proteus mirabilis* only, 1 catheter were colonised by *Providencia stuartii* only, and 10 catheters colonised by mixed cultures including urease producers.

Authors: Strains of *Providencia stuartii* vary in their ability to produce urease and this particular organism was urease negative. So, as we stated, 16/20 catheters were colonised by urease producers.