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ORIGINAL PAPER

A study of the structure of the crystalline bacterial biofilms that can encrust and block silver Foley catheters

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Abstract The aim of this study was to examine the structure of the crystalline bacterial biofilms that encrust and block silver/hydrogel-coated latex catheters. Scanning electron microscopy was used to examine the crystalline deposits that were found encrusting catheters obtained from six patients undergoing long-term catheterization in a community setting. Large populations of bacilli and cocci were seen on all catheters developing on a basal foundation layer of crystalline material. These observations show that in patients prone to catheter encrustation, crystalline material formed in the urine can cover the surfaces of silver catheters. Extensive bacterial biofilms then develop on the crystals, shielded from the underlying silver. It is suggested that if antimicrobials are to be incorporated into catheters to prevent encrustation, they must diffuse out from the catheter surface and reduce the viable cell populations of the urease producing bacteria that elevate the urinary pH and trigger crystal formation.

Keywords Silver catheters · Bacterial biofilms · Urolithiasis · Catheter encrustation · Urinary tract infections

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Introduction

The Foley catheter marketed by Bard (Crawley, UK) as their IC catheter, has metallic silver chemically anchored in a coating of gold and palladium to a latex base. An external hydrogel layer gives the catheter its lubricity. Silver ions are apparently released slowly over long-periods and exert their antibacterial activity in the urine at both the internal and external surfaces. It has been claimed that these coatings endow the catheters with the ability to resist bacterial colonization and biofilm development [1, 2]. The biofilms formed on catheters by urease producing bacteria particularly *Proteus mirabilis,* are responsible for the most troublesome complications in the care of patients undergoing long-term catheterization [3]. These organisms generate alkaline urine, producing conditions in which calcium and magnesium phosphates come out of solution and form crystalline biofilms that encrust and eventually block the catheters [4]. Evidence from laboratory tests in models of the catheterized bladder indicates that the Bard IC catheters are just as vulnerable to encrustation and blockage by *P. mirabilis* biofilms as hydrogel-coated latex catheters [5, 6]. Encouraged by the comments in the literature that the silver catheters were resistant to biofilm formation, however, community nursing staff in the Bristol area tried the IC catheters for the management of patients who were recurrently blocking their catheters. In the event the IC catheters blocked and were found to be extensively encrusted. The availability of the silver catheters from these patients however, has allowed us to perform a scanning electron microscopic study of the structure of the crystalline biofilms that formed in vivo on these catheters. The observations suggest a mechanism that enables bacteria to colonize silver catheters and have important implications for the development of novel encrustation-resistant catheters.

Patients and methods

The catheters

Bard IC catheters were collected from six patients undergoing long-term catheterization in their own homes in the Bristol area. They were stored at 4°C until they could be prepared for electron microscopy. Sections (1 cm in length) were cut from the catheters at the eye-hole and immediately below the retention balloon.

Scanning electron microscopy

They were prepared for electron microscopy by fixing in 2.5% glutaraldehyde in 0.1M phosphate buffer pH 8 (4 h at room temperature or overnight at 4°C). They were then washed in the buffer for 15 min before post-fixing using a 1:1 solution of 0.05M buffer, 1% osmium tetroxide for 1 h. A further 15-min wash in distilled water was carried out before samples were dehydrated in an ascending ethanol series. Sublimation dehydration was then performed using hexamethyldisilizane (HMDS), i.e. 70%, 90% 100%, 100% ethanol, 100% ethanol:HMDS (1:1). Finally they were washed twice in 100% HMDS (15 min each step) and left to dry in air. The samples were gold sputter-coated (Edwards S150P sputter-coater), and visualised using a Philips XL-20 scanning electron microscope accelerating voltage 15–25kv.

The bladder model

The laboratory models used to determine the concentration of silver eluting into urine as it flows through the IC catheter have been described previously [7]. They consist of a glass chamber maintained at 37°C by a water jacket. Each model was sterilized by autoclaving and then a 14 Fr silver/hydrogel-coated latex IC catheter (Bard) was inserted into the chamber through an outlet at the base. The catheter retention balloons were inflated in the usual way with 10 ml of water, sealing the outlet from the bladder. Sterile pooled human urine was supplied to the chamber at 0.5 ml/min, so that residual volumes are collected below the catheter eye-holes before flowing through the drainage tubes into the collecting bags.

Assay of silver in urine

The silver content of samples of urine that had drained through IC catheters from the models was determined by atomic absorbance spectroscopy (AAS) using an aa/ae spectrophotometer 457 (Thermo Electron Corporation, Waltham, Massachusetts, US). The spectrophotometer was calibrated to produce a standard curve using silver standards purchased from Spectosol (VWR International Ltd., Poole, Dorset, UK) Samples were vapourized in an air/acetylene flame and the silver content determined at λ 328.1 nm. The lower limit of silver detection was determined at 0.05 μg Ag/ml.

Results

Visual examination revealed that the catheters from all six patients were encrusted. Figure 1 shows an electron micrograph of a lumenal section of a catheter that had blocked after 11 days in situ. The catheter was clearly occluded by extensive deposits of crystalline material composed of amorphous aggregates typical of apatite and large crystals characteristic of struvite [8]. Micrographs at higher magnification revealed the presence of large numbers of bacilli on the surface of crystalline material (Figs. 2, 3). Encrustation was also visible around the eye-holes of the catheters. Figure 4 shows crystalline deposits on the outer surface and the eye-hole rim of a catheter that was removed from a patient after just 5 days. Examination of this material revealed that it was composed of bacterial biofilm that had formed on a crystalline foundation layer (Fig. 5). The microcrystalline structure of this foundation layer can be seen clearly on the eye-hole (Figs. 6, 7) and lumenal surfaces (Fig. 8). Both cocci and bacilli were found on most catheters indicating the presence of mixed species biofilms typical of those found on catheters from patients undergoing long-term catheterization [9, 10]. In all six cases the encrusting deposits proved to be crystalline bacterial biofilm.

In the experiments to determine the extent of silver released from catheters, models were assembled and supplied with human urine at 0.5 ml/min continuously for 24 h.

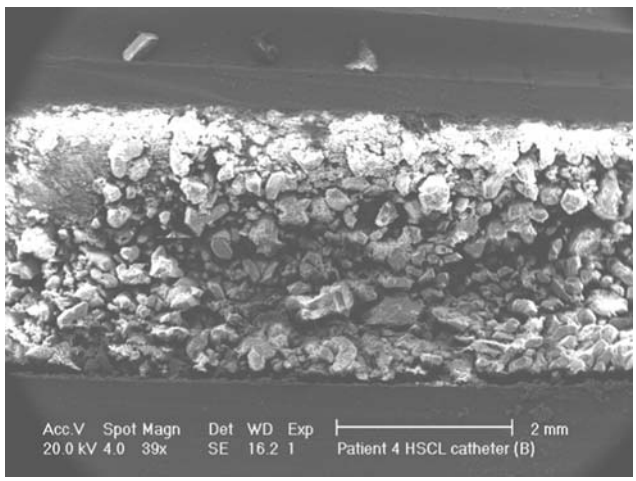

Fig. 1 A longitudinal section of an IC catheter removed from patient 4 after 11 days in situ. Extensive crystalline material can be seen occluding the catheter lumen

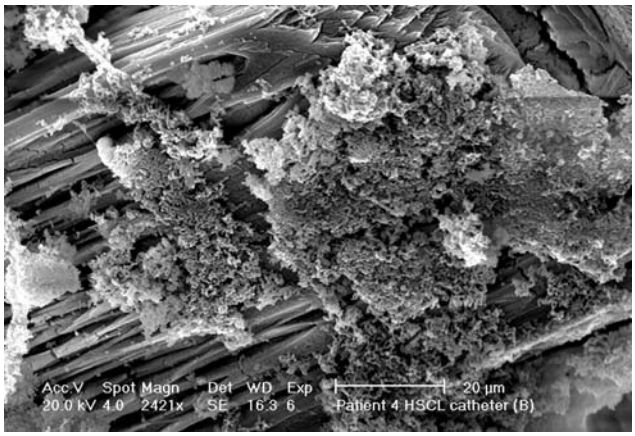

Fig. 2 The micrograph illustrates the bacterial colonization of plates of crystals in the material occluding the lumen of the catheter from patient 4

Fig. 3 The nature of the encrustation on the lumenal section of the catheter removed from patient 2 after 14 days. Large numbers of bacilli can be seen colonizing crystals

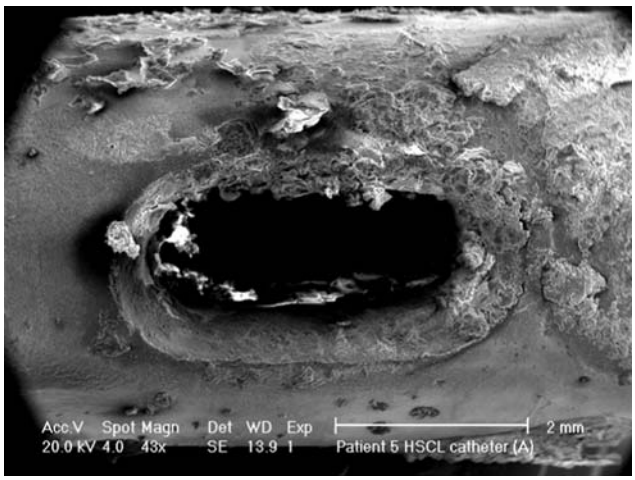

Fig. 4 The encrustation around the eye-hole of the catheter removed from patient 5 after just 5 days

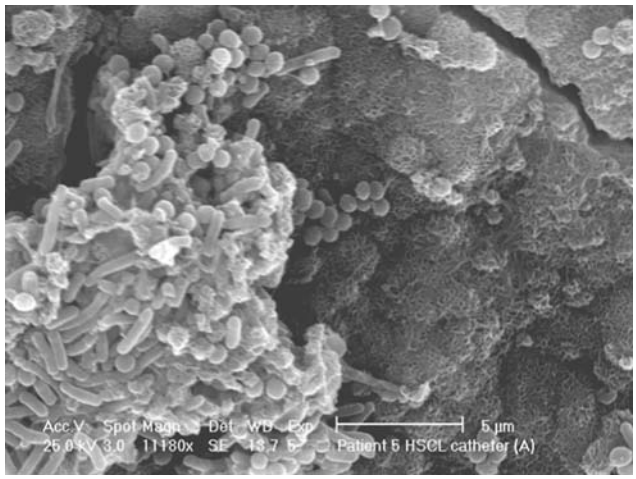

Fig. 5 The crystalline biofilm around the eye-hole of the catheter removed from patient 5. The micrograph shows bacteria colonizing the microcrystalline aggregates typical of calcium phosphate that have formed a foundation layer on the catheter surface

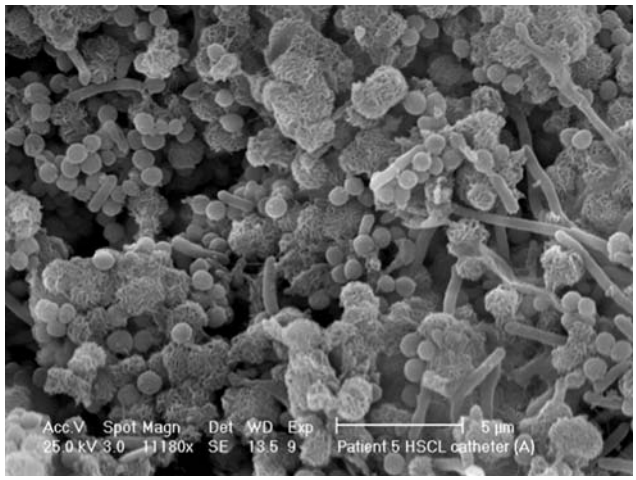

Fig. 6 Bacilli, cocci and microcrystalline aggregates of calcium phosphate in the crystalline biofilm around the eye-hole of the catheter from patient 5

The urine that had drained through the catheters was collected from the drainage bags at 30 min and 24 h. No silver was detected in any of the urine samples obtained from three replicated experiments.

Discussion

The electron micrographs presented in Figs. 1, 2, 3, 4, 5, 6, 7, 8 illustrate the structure of the crystalline bacterial biofilms that can develop on silver/hydrogel-coated latex catheters in patients undergoing long-term catheterization. These observations also provide an insight into how bacteria manage to colonize the silver surfaces. In patients, who are prone to catheter encrustation and blockage, it is clear

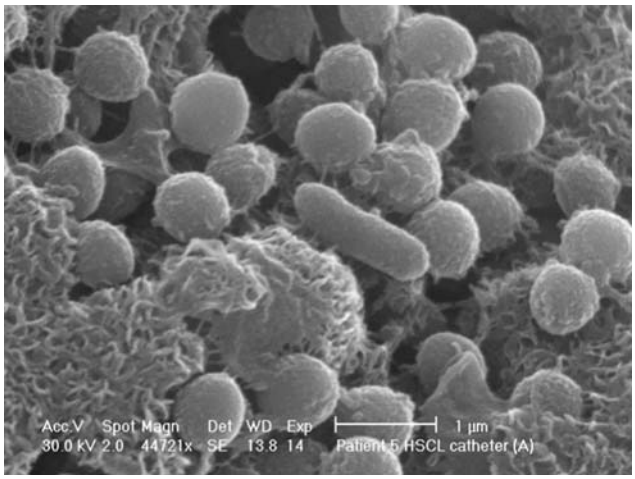

Fig. 7 Micrograph illustrating the microcrystalline structure of the aggregates typical of apatite in the biofilm around the eye-hole of the catheter removed from patient 5

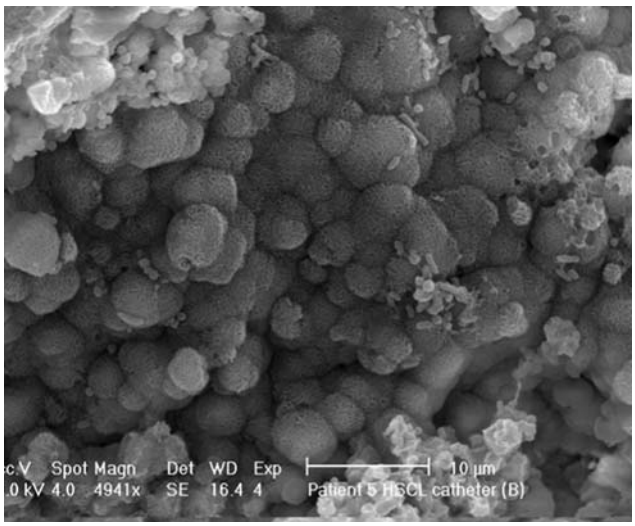

Fig. 8 The microcrystalline foundation layer of the biofilm on the luminal section of the catheter removed from patient 5

that layers composed of aggregates of microcrystalline material are deposited on the catheter surfaces. Bacteria then attach to these foundation layers and grow into mature biofilms, shielded from the underlying silver.

These observations confirm the conclusions from previous laboratory studies in the model of the catheterized bladder on the vulnerability of the silver/hydrogel catheters to encrustation by *P. mirabilis* crystalline biofilms [5, 6]. Recently we also reported the results of experimental work in the laboratory model investigating the early stages of the formation of these biofilms on antimicrobial catheters [11]. In patients, who suffer from recurrent catheter encrustation, the usual management policy is to simply replace the blocked catheter. Under these circumstances, fresh catheters are placed directly into urine cultures of *P. mirabilis* at alkaline pHs containing crystals of calcium and magnesium phosphates. We used scanning electron microscopy to study the early stages of crystalline biofilm formation under these circumstances on a range of catheter types, including the silver/hydrogel devices. After only 1 h, in the models, the catheter surfaces were found to be covered by a layer of microcrystalline material. The material forming this "foundation layer" is similar in structure to that found deposited on patient's catheters (Figs. 5, 6, 7, 8) and to the crystalline forms of calcium phosphate involved in the formation of the dentin of teeth [12]. X-ray microanalysis confirmed that this material was indeed composed largely of calcium and phosphate. Bacterial colonization of this foundation layer became visible at 4 h with micro-colonies of cells attaching to the crystals. By 18 h, the eyelets and lumenal surfaces of all these catheters were comprehensively covered by densely populated crystalline *P. mirabilis* biofilm [11]. The results presented in Figs. 1, 2, 3, 4, 5, 6, 7, 8 suggest that crystalline biofilm can form in the same way on catheters in vivo.

The work quoted [1, 2] to support the assertion that the silver catheters resist colonization by bacterial biofilms is an in vitro study in which the attachment of radio-labelled cells from suspensions in a dilute laboratory minimal liquid medium was measured on to sections of the silver/hydrogel-coated latex catheters [13]. It would have been more appropriate to have performed the tests with cells suspended in urine for two reasons. Firstly, when catheters come into contact with a body fluid such as urine, they can rapidly acquire a conditioning film of protein which could significantly change the surface properties of the devices [14]. Secondly, in dilute minimal media, urease producing bacteria would not generate alkaline conditions and phosphate crystals would not form. Whatever, after just 2 and 18-h exposure, the sections were removed and "rinsed vigorously" presumably with the intention of washing off loosely bound cells, before assessing the amount of radioactivity that had been retained on their surfaces. These rinsing techniques are very difficult to control and standardize. The extent to which they remove attached cells is variable and difficult to assess. The data presented in this paper claims to show that bacterial adhesion to the silver catheters was lower than that to control catheters. An alternative explanation is that the apparent reduction in radioactivity was brought about because the vigorous rinsing also removed some of the surface hydrogel coating (and thus any cells attached to it) from the silver catheters. To eliminate this possibility, hydrogel-coated latex catheters should have been used as controls. Unfortunately, all-silicone catheters were used. The silicone surface of the control sections (not being coated in hydrogel) would not of course be affected in this way. Critical analysis thus leads to the inevitable conclusion that it is not possible to be certain about the extent to which bacterial adhesion is inhibited by the

silver alloy on these hydrogel latex-based catheters from the data presented in this paper.

The clear lesson from the results reported from the experimental study [11] and those presented in Figs. 1, 2, 3, 4, 5, 6, 7, 8 is that in order to prevent catheter encrustation, it is essential to prevent the pH of the urine rising above the pH at which crystals form. If antimicrobials are to be incorporated into catheters to achieve this, they must diffuse out from the catheter surface and reduce the viable cell populations of the urease producing bacteria that elevate the pH and trigger crystal formation. In the case of the IC catheter, if there was any silver at all eluting into the urine, it was at concentrations of less than 0.05 μg/ml. Urease producing bacteria such as *P. mirabilis* are quite capable of growing and generating the alkaline conditions necessary to precipitate calcium phosphate crystals from this urine [6, 11]. An alternative silver catheter produced from silicone impregnated with nano-particulate silver has recently been described [15]. It will be interesting to know whether this catheter is capable of producing urinary concentrations of silver that are bactericidal. Chakravarti et al. [16] showed that it was possible to generate urinary concentrations of silver that inhibited *P. mirabilis* induced encrustation by passing an electric current through silver electrodes attached to catheters. The effect was temporary however as the particular silver electrodes used disintegrated after about 150 h.

Conclusions

Electron microscopy has revealed the structure of the crystalline bacterial biofilms that form on the silver/hydrogel-coated Foley catheters in patients undergoing long-term catheterization. The bacteria adhere to crystalline formations that cover the catheter surface and produce biofilms shielded from the underlying silver. These observations, on catheters taken from patients together with the results from experimental work in laboratory models, suggest that if antimicrobials are to be incorporated into catheters to prevent encrustation, they must diffuse out from the catheter surface and reduce the viable cell populations of the urease producing bacteria that elevate the urinary pH and trigger crystal formation. In the case of the IC catheter, the concentrations of silver eluting into urine are not sufficient to prevent crystalline biofilm formation.

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