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Bacterial biofilms in patients with indwelling urinary catheters

David J Stickler

SUMMARY

Bacteria have a basic survival strategy: to colonize surfaces and grow as biofilm communities embedded in a gel-like polysaccharide matrix. The catheterized urinary tract provides ideal conditions for the development of enormous biofilm populations. Many bacterial species colonize indwelling catheters as biofilms, inducing complications in patients' care. The most troublesome complications are the crystalline biofilms that can occlude the catheter lumen and trigger episodes of pyelonephritis and septicemia. The crystalline biofilms result from infection by urease-producing bacteria, particularly *Proteus mirabilis*. Urease raises the urinary pH and drives the formation of calcium phosphate and magnesium phosphate crystals in the biofilm. All types of catheter are vulnerable to encrustation by these biofilms, and clinical prevention strategies are clearly needed, as bacteria growing in the biofilm mode are resistant to antibiotics. Evidence indicates that treatment of symptomatic, catheter-associated urinary tract infection is more effective if biofilm-laden catheters are changed before antibiotic treatment is initiated. Infection with *P. mirabilis* exposes the many faults of currently available catheters, and plenty of scope exists for improvement in both their design and production; manufacturers should take up the challenge to improve patient outcomes.

KEYWORDS bacterial biofilms, *Proteus mirabilis*, urinary catheterization, urinary tract infection, urolithiasis

REVIEW CRITERIA

A comprehensive PubMed search of the English-language literature published between January 1980 and March 2008 was made for relevant articles using the Medical Subject Heading terms "biofilms" and "urinary catheterization". The reference lists of retrieved articles were evaluated for additional articles. Papers on catheter-associated urinary tract infections and bacterial biofilms collected during over 30 years working in this field were also reviewed.

CME

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Learning objectives

Upon completion of this activity, participants should be able to:

1 Differentiate bacteria that promote an initial infection vs chronic colonization among patients with long-term indwelling urinary catheters.
2 Describe the formation of crystalline biofilms among patients with urinary catheters.
3 Identify factors that promote the formation of biofilms with *Proteus mirabilis*.
4 Describe the prevention and treatment of urinary catheter-associated biofilms.

Competing interests

The author and the Locum Journal Editor N Siva declared no competing interests. The CME questions author CP Vega declared that he has served as an advisor or consultant to Novartis, Inc.

INTRODUCTION

The biofilm mode of growth is a basic survival strategy deployed by bacteria in a wide range of environmental, industrial and clinical aquatic settings.[1] Bacterial cells have a strong preference for life on surfaces rather than in planktonic suspension.[2] These cells have an array of adhesins in their cell walls that allow them to colonize many types of substrate, and, on contact with a surface, the cells secrete exopolysaccharides that secure their attachment. The bacteria then multiply to form microcolonies of cells that subsequently spread over the surface, forming populations embedded in a gel-like polysaccharide matrix (Figure 1). The cells in these biofilm communities are protected from environmental stresses, and this protection has particular advantages

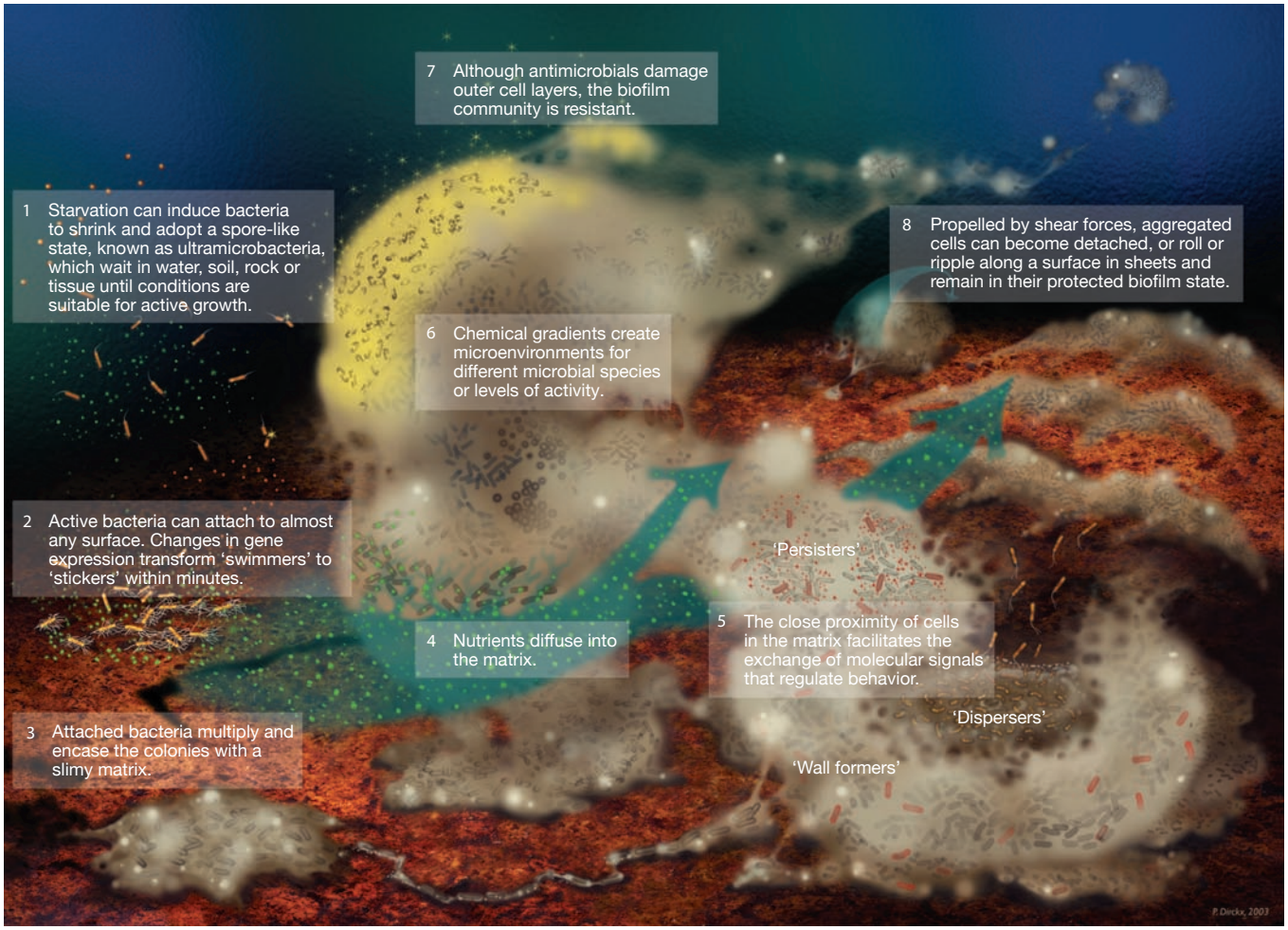

Figure 1 Conceptualization of biofilm development and dynamic behaviors. The figure was compiled from laboratory and natural observations of pure-culture (both Gram-positive and Gram-negative organisms) and mixed-culture biofilms. Image courtesy of P Dirckx, Center for Biofilm Engineering, USA. Permission obtained from Nature Publishing Group © Hall-Stoodley L *et al.* (2004) *Nat Rev Microbiol* **2:** 95–108.

for the bacteria in biofilms that develop *in vivo*. Microorganisms that are apparently fully sensitive to antibiotics and antiseptics in conventional laboratory testing methods become fully resistant in the biofilm mode *in vivo*.

CATHETER BIOFILMS

Prolonged urinary tract infections can facilitate the development of catheter biofilms. While indwelling (Foley) catheters are effective in relieving urinary retention and managing urinary incontinence, external bacteria have easy access to the bladder, and catheterization can often result in bacteriuria. The risk of urinary tract infection is related to the length of time the catheter is in place. Most patients catheterized for a week or less should escape infection, but for the many elderly and disabled patients who are catheterized for several months or years, bacteriuria is inevitable.[3,4]

Urinary tract infections in catheterized patients can occur in several ways. Organisms that colonize the periurethral skin can migrate into the bladder through the mucoid film that forms between the epithelial surface of the urethra and the catheter. In addition, contamination of the urine in the drainage bag can allow organisms to access the bladder through the drainage tube and the catheter lumen.[5,6] The initial bacteria that cause the urinary tract infections are usually *Staphylococcus epidermidis*, *Escherichia coli* or *Enterococcus faecalis*.[6,7] As time goes by, other species appear in the residual bladder urine, including *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Providencia stuartii*, *Morganella morganii* and *Klebsiella pneumoniae*.[3,8] The bacteria that

Table 1 The incidence of bacterial species isolated from 106 catheter biofilms.[17]

Species	Number (%) of catheters colonized by each species		
	All catheter biofilms	Mixed-species biofilms (76 catheters)	Single-species biofilms (30 catheters)
Pseudomonas aeruginosa[a]	38 (35.9)	31 (40.8)	7 (23.3)
Enterococcus faecalis	36 (34.0)	34 (44.7)	2 (6.7)
Escherichia coli	33 (31.1)	31 (40.8)	2 (6.7)
Proteus mirabilis[a]	32 (30.2)	26 (34.2)	6 (20.0)
Klebsiella pneumoniae[a]	19 (17.9)	18 (23.7)	1 (3.3)
Morganella morganii[a]	14 (13.2)	11 (14.5)	3 (10.0)
Providencia stuartii	11 (10.4)	9 (11.8)	2 (6.7)
Staphylococcus aureus[a]	11 (10.4)	10 (13.2)	1 (3.3)
Enterobacter cloacae	9 (8.5)	7 (9.2)	2 (6.7)
Klebsiella oxytoca[a]	9 (8.5)	8 (10.5)	1 (3.3)
Providencia rettgeri[a]	5 (4.7)	4 (5.3)	1 (3.3)
Coagulase-negative staphylococci[a]	5 (4.7)	4 (5.3)	1 (3.3)
Citrobacter species	4 (3.8)	4 (5.3)	0 (0.0)
Proteus vulgaris[a]	3 (2.8)	2 (2.6)	1 (3.3)

[a]Indicates species capable of producing urease. Table modified, with permission, from The Society for General Microbiology © Macleod SM and Stickler DJ (2007) *J Med Microbiol* **56:** 1549–1557.

present in the latter stages of urinary tract infection are difficult to eradicate with antibiotics while the catheter is in place.[8,9] As the infections are usually asymptomatic, and because of the danger of promoting antibiotic resistance, catheter-associated bacteriuria is generally not treated.[10–12] In patients with long-term indwelling catheters, catheter changes are commonly scheduled at 10–12-week intervals; contaminated urine can, therefore, be flowing through individual catheters for periods of 3 months at a time. Thus, catheters provide attractive sites for bacterial colonization: the biofilm bacteria thrive in their matrix gel and the gentle flow of warm nutritious urine. Enormous populations develop, and become visible to the naked eye as thick coatings. Biofilms containing 5×10^9 viable cells per centimeter can be found on long-term indwelling catheters removed from patients.[13] The biofilm populations, therefore, often outnumber those in the urine.

A variety of bacterial species colonize catheters, and many of these biofilms can induce serious complications.[10,13–17] Table 1 summarizes the bacterial species identified from a set of 106 catheter biofilms;[17] 14 species were commonly found. Isolated cases of single-species biofilms were observed, but most biofilms contained mixed bacterial communities containing up to five species. The most common species present in the mixed-population biofilms were *E. faecalis*, *P. aeruginosa*, *E. coli*, and *P. mirabilis*. In patients who develop bacteriuria during short-term catheterization, bacterial colonization of the catheter does occur.[16] The biofilms formed are generally sparse, and because the catheter is removed within a few days, they cause few problems. By contrast, long-term catheters become colonized by extensive biofilms, which can have profound effects on the health of the patient. By far the most troublesome biofilms are those that become crystalline in nature.[18,19] These biofilms can form on the outer surface of the catheter around the balloon and catheter tip, and can cause trauma to the bladder and urethral epithelia. On deflation of the retention balloon, crystalline debris from the biofilm can be shed into the bladder and initiate stone formation. The main complication, however, is blockage in the flow of urine through the catheter that results from the build up of the crystalline material on the lumenal surfaces (Figure 2). As a consequence, urine often leaks along the outside of the catheter and patients become incontinent, resulting in the increased need for nursing

assistance. In addition, blockage of the catheter can lead to retention of urine in the bladder and vesicoureteric reflux of infected urine; if the blockage is not detected and if the catheter is not changed, patients can suffer episodes of pyelonephritis and septicemia.[15,20]

About half the patients who undergo long-term catheterization will suffer the complication of catheter encrustation and blockage by bacterial biofilms at some time.[21–23] The welfare of many elderly and disabled patients is thus put at risk by the development of these biofilms, and considerable demands are made on the resources of the health-care service to manage the complications. An insight into the scale of the problem was given by a prospective study of 467 patients in community care in the UK. Over a 6-month period, 506 emergency referrals were recorded for these patients, mostly to deal with catheter blockage.[23]

CRYSTALLINE BIOFILMS

The crystalline deposits on catheters have a similar composition to infection-induced kidney and bladder stones. Struvite (magnesium ammonium phosphate) and a poorly crystalline form of apatite (a hydroxylated calcium phosphate, in which a variable proportion of the phosphate groups are replaced by carbonate) are the principle crystalline components.[24,25] Scanning electron microscopy has shown that large numbers of bacilli are associated with the crystals (Figure 3).[26] Culture techniques have confirmed the persistence of a range of bacteria. Notably, species capable of producing the enzyme urease are predominantly associated with crystallization.[27] Urease is, in fact, the driving force of crystallization: it hydrolyzes urea, leading to the formation of ammonium and carbonate ions and an increase in urinary pH. As the urine becomes alkaline, magnesium and calcium phosphate crystals are precipitated. Aggregates of this crystalline material accumulate in the urine and in the biofilm that develops on the catheter surfaces. The continued accumulation of crystalline bacterial biofilm blocks the flow of urine through the catheter.[14,28]

Several species commonly found in catheter biofilms produce urease (Table 1). In laboratory tests, urease can be detected in *P. aeruginosa*, *K. pneumoniae* and *M. morganii*, *Proteus* species, including *P. mirabilis*, some *Providencia* species and some strains of *Staphylococcus aureus* and coagulase-negative staphylococci. Of these species, *P. mirabilis* is most commonly isolated from the urine of patients suffering from recurrent catheter encrustation and blockage.[29,30] Furthermore, *P. mirabilis* is also the species most commonly recovered from patients' encrusted catheters.[27] The urease produced by *P. mirabilis* is a potent enzyme, and is able to hydrolyze urea several times faster than urease produced by other species.[31] Experimental work in laboratory

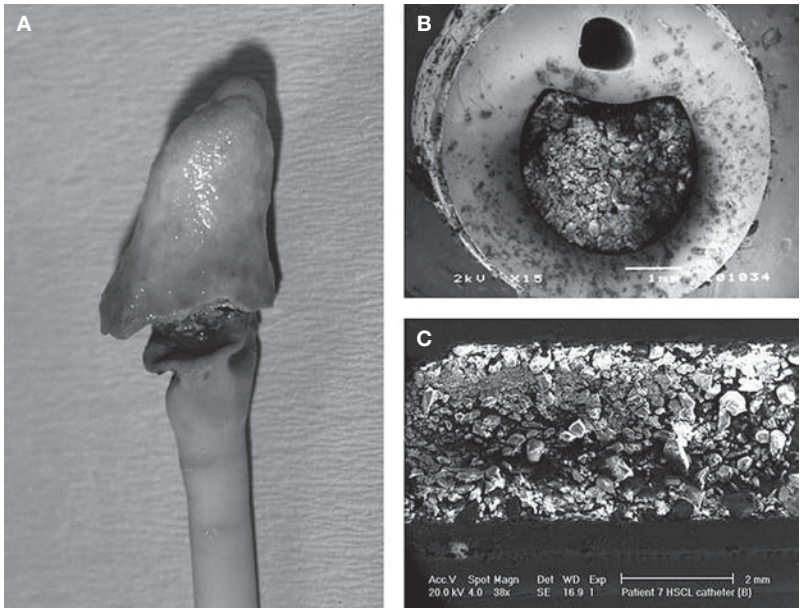

Figure 2 Examples of crystalline biofilms on blocked catheters taken from patients. (**A**) This image shows a catheter that had been indwelling suprapubically for 6 months. It was removed surgically. Crystalline material completely covered the eyehole and balloon of the hydrogel-coated latex catheter. Image kindly supplied by Professor Roger Feneley. (**B**) A cross-section of a silicone catheter that had been indwelling for 8 weeks. The image shows that the central lumen is occluded by crystalline biofilm. Permission obtained from Elsevier Ltd © Stickler DJ (1999) *Eur Urol Update Series* **5:** 1–8. (**C**) A longitudinal section of a silver–hydrogel-coated latex catheter that blocked after 11 days *in situ*.

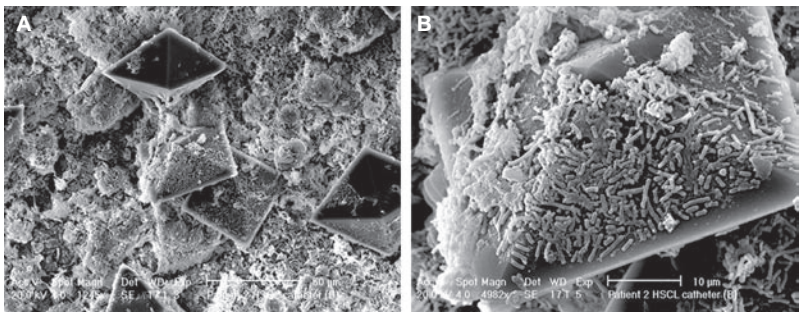

Figure 3 Scanning electron micrographs of encrustation on the surface of a silver–hydrogel-coated latex catheter that had been indwelling for 14 days. (**A**) This image shows the crystalline forms present in the biofilm. (**B**) In this higher-magnification image, the bacterial colonization of these crystals is illustrated.

models of the catheterized bladder has demonstrated that species such as *M. morganii*, *K. pneumoniae*, and *P. aeruginosa* fail to produce alkaline urine and do not produce appreciable encrustation on catheters.[32] In this laboratory work, the only species capable of producing alkaline urine and causing extensive encrustation were *P. mirabilis*, *Proteus vulgaris* and *Providencia rettgeri*. As these latter two species are only found in about 5–10% of catheter biofilms,[17] strong epidemiological and experimental evidence indicates that *P. mirabilis* is mainly responsible for the formation of crystalline biofilms on catheters.

PROTEUS MIRABILIS

P. mirabilis is not usually a pioneer colonizer of the catheterized urinary tract, and is not commonly found in patients undergoing short-term catheterization.[7] The longer the catheter is in place, however, the more likely it is to be found in the urine. In patients undergoing long-term catheterization, *P. mirabilis* has been isolated from around 40% of urine samples.[33] Sabbuba *et al.*[34] have developed a technique for genotyping *P. mirabilis* to help understand the epidemiology and pathogenesis of *P. mirabilis* catheter-associated urinary tract infections. Pulsed-field gel electrophoresis of restriction enzyme digests of DNA from *P. mirabilis* produced highly discriminatory genotypic profiles. The application of this technique established the remarkable stability of *P. mirabilis* strains in the catheterized urinary tract. The same genotype persisted in a patient's urinary tract despite many catheter changes, courses of antibiotic treatment, and even periods when the patient was not catheterized. Further investigation revealed that *P. mirabilis* was also present in the bladder stones that frequently form in these patients. Genotyping of pairs of *P. mirabilis* isolates from the encrusted catheters and bladder stones from the same patient demonstrated that, in each case, the strain of *P. mirabilis* was identical.[35] Genotyping also showed that the majority of patients were infected with genetically distinct strains. *P. mirabilis* is an enteric organism, and subsequent analysis showed that bacteria from fecal and catheter biofilm isolates from the same patients were identical.[36] These findings indicate that most long-term catheterized patients who suffer from catheter encrustation probably acquire *P. mirabilis* from their own fecal flora. These strains will eventually cause chronic colonization of the urine, catheters and bladder stones.

THE FORMATION OF *PROTEUS MIRABILIS* BIOFILMS

Biological factors

All types of Foley catheters, including silver-coated and nitrofurazone impregnated catheters, are vulnerable to colonization by crystalline biofilms.[37,38] At present, no effective technique is available to prevent the problem;[15,39,40] therefore, understanding the precise mechanisms that *P. mirabilis* uses to colonize, encrust and block catheters is important. *P. mirabilis* is considered to be an ingenious organism capable of initiating crystalline biofilms in a variety of ways. The first stage in the development of biofilms on implanted prosthetic devices usually involves the rapid coating of the device by a conditioning film of host proteins from the surrounding body fluids. These proteins provide receptors that bacterial cells attach to via fine, hair-like fimbriae (adhesins) that protrude from their surface.[41,42] This process probably happens on urinary catheters. Several different adhesins have been identified on *P. mirabilis* cells.[43,44] Examination of catheters removed from patients after short periods have revealed coatings of proteins such as fibrin.[16] Evidence also indicates that *P. mirabilis* cells can bind directly onto silicone surfaces:[45] bacilli seem to be able to bind to catheters whether they are coated in host proteins or not.

The involvement of genetic factors of *P. mirabilis* in biofilm formation has been reviewed by Jacobsen *et al.*[44] Although the ability of *P. mirabilis* to bind to the catheter is an important factor in the development of crystalline biofilms, the most important factor seems to be the ability of *P. mirabilis* to synthesize potent urease: experimental work has shown that urease-negative *P. mirabilis* mutants failed to form crystalline biofilms,[46] whereas mutants lacking flagella or the ability to swarm encrusted and blocked catheters at the same rates as the wild-type parent strain.[47]

Physical factors

In addition to the biological factors, powerful physical forces can initiate the development of crystalline biofilms. Scanning electron microscopy has revealed the rough, irregular nature of catheter surfaces.[48] Latex-based catheters have particularly uneven surfaces.[49] The manufacturing techniques used to produce the eyeholes tear through the latex and produce surfaces that must seem like rocky landscapes of craters and crevices

to bacteria (Figure 4). The roughness of the lumenal surfaces is exacerbated by the common occurrence of embedded diatom skeletons.[50] These skeletons come from the diatomaceous earth—a naturally occurring, soft, chalk-like sedimentary rock, which is easily crumbled into a fine powder—that is used to prevent the latex sticking to the metallic formers on which the catheters are produced. All-silicone catheters have smoother surfaces than latex catheters, but irregularities are still common around the eyeholes and where extrusion manufacturing techniques have produced striations on the lumenal surfaces.[49]

Eyeholes are particularly vulnerable to bacterial colonization. In experiments where catheters were removed from bladder models at various intervals after urine inoculation with *P. mirabilis*, scanning electron microscopy revealed that, within 2 h, bacterial cells were trapped in the crevices in the uneven surfaces of the eyelets.[49] Microcolonies of cells developed in the surface depressions, and then, with the rise in urinary pH, crystals started to form in the biofilm (Figure 5). Extensive crystalline biofilm developed and spread down the catheter lumen. The silica skeletons of the diatoms embedded in the latex were also attractive sites for bacterial colonization.[50] Blockage with extensive crystalline biofilm generally occurred at the eyehole or in the balloon region of the lumen.

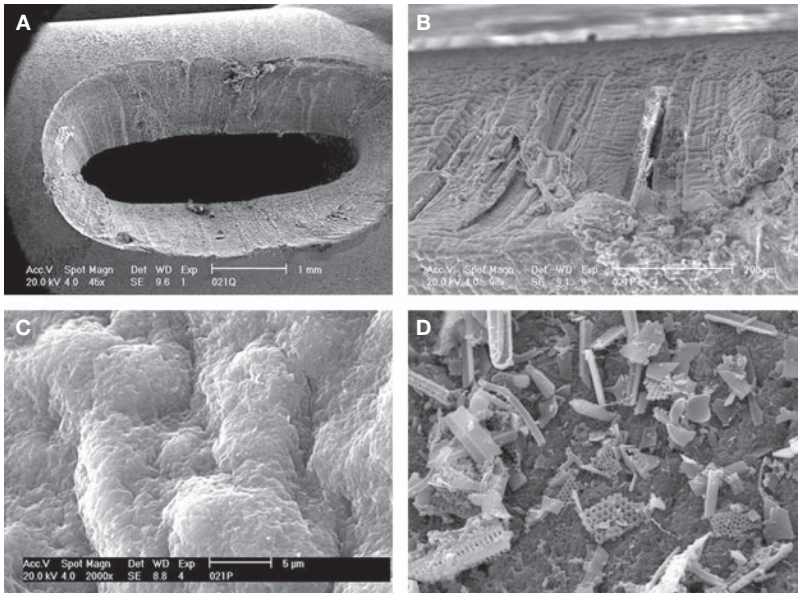

Figure 4 Scanning electron micrographs of the surfaces of unused hydrogel-coated latex catheters. (**A**) This image illustrates the rough surface produced by cutting the eyeholes. Permission obtained from Springer © Stickler DJ (2003) *Urol Res* **31:** 306–311. (**B**) This higher-magnification image also shows the rough surface of the eye holes. (**C**) This high-magnification micrograph shows the craters and crevices produced in the latex around the eyelet. The silica skeletons of diatoms can be seen on the irregular lumenal surfaces. (**D**) This image shows the presence of the diatom skeletons on the luminal surface of the catheter.

Chemical factors

The chemical environment also has a vital role in the development of crystalline biofilms. Brisset *et al.*[51] reported in 1996 that hydrophobic cells were more likely to colonize hydrophobic than hydrophilic surfaces, and that colonization was increased in alkaline urine. Experiments in parallel-plate flow cells have also shown that when urine cultures flow over smooth, flat, polymer films, the pH of the urine can be a major factor in determining the extent to which bacteria adhere. For example, some polymers with strongly electron donating, hydrophilic surfaces will resist colonization by cells until the pH of the urine rises. In the alkaline urine, however, macroscopic aggregates of cells and crystals will form, settle on the polymer surface, and initiate crystalline biofilm formation.[46]

The chemical environment can also affect the rate at which biofilms develop. A prospective study by Mathur *et al.*[52] in patients infected with *P. mirabilis* revealed that the time taken

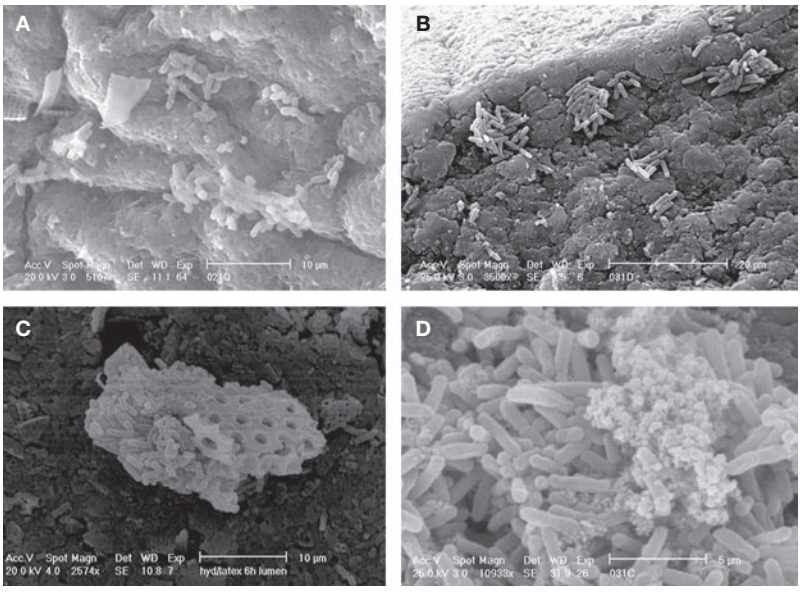

Figure 5 Electron micrographs illustrating the colonization of a hydrogel-coated latex catheter by *Proteus mirabilis* in a laboratory model of the bladder. (**A**) This image shows bacteria trapped in crevices in the surface of the eyeholes 2 h after incubation in the model. (**B**) Microcolonies of *P. mirabilis* develop at the eyehole 4 h after incubation. (**C**) Bacteria attach to a diatom skeleton embedded in the lumenal surface of the catheter 6 h after incubation in the model. (**D**) Biofilm develops at the eyehole 6 h after incubation in the model. Aggregates typical of apatite can be seen forming in the biofilm as the urine becomes alkaline. Permission obtained from Springer © Stickler DJ (2003) *Urol Res* **31:** 306–311.

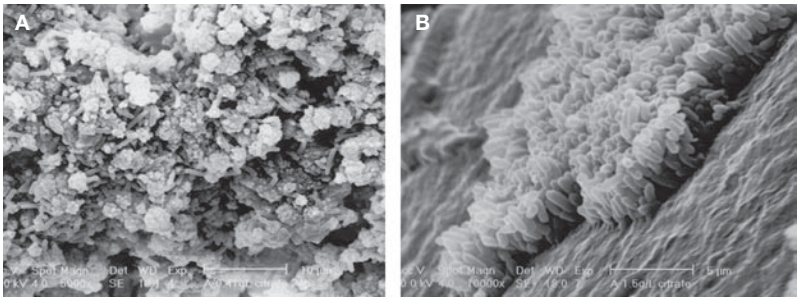

Figure 6 Electron micrographs of catheters removed from bladder models with varying citrate concentrations and nucleation pHs. (**A**) This micrograph shows a section of *Proteus mirabilis* crystalline biofilm on an all-silicone catheter that was removed 24 h after incubation in a bladder model supplied with urine containing 0.41 mg/ml citrate, which had a nucleation pH of 7.4. Permission obtained from The Society for General Microbiology © Stickler DJ and Morgan SD (2006) *J Med Microbiol* **55:** 489–494. (**B**) Micrograph of the surface of a silicone catheter removed 24 h after incubation from a model supplied with urine containing citrate at 1.5 mg/ml, which had a nucleation pH of 8.3. The biofilm is sparse and is composed of microcolonies of cells with no signs of crystalline material. Permission obtained from The Society for General Microbiology © Stickler DJ and Morgan SD (2006) *J Med Microbiol* **55:** 489–494.

for catheters to block varied from 2 to 98 days. This variation can be explained by the concept of the nucleation pH of urine (pHn). The pHn of a urine sample is the pH at which the urine becomes turbid, due to microcrystals of apatite and struvite coming out of the solution. The urine becomes turbid as the pH increases. Choong *et al.*[53] found that, for patients whose catheters were blocked by crystalline biofilms, the mean pHn of their urine was 7.58, while the mean pH of the voided urine was 7.85; these results clearly indicate that catheters become encrusted if the pH of the urine is greater than the pHn.

Further analysis[54] of the data from the study by Mathur *et al.*[52] showed that, in patients infected with *P. mirabilis*, the pHn of the urine was the most important factor in predicting the rate of catheter encrustation. The higher the mean pHn value, the slower the rate of encrustation, and the longer catheters took to block. As Mathur *et al.*[52] had shown that the pHn of any patient's urine varied from week to week, the authors of the later analysis suggested that manipulation of pHn might be possible; raising the pHn above urinary pH values would thus prevent catheter encrustation.[54] A study in healthy, noncatheterized volunteers demonstrated that dilution of the urine by increasing fluid intake, and increasing the urinary concentration of citrate (a chelating agent that can keep divalent metal ions, such as Ca^{2+} and Mg^{2+}, in solution) elevated the pHn to values that were rarely exceeded by the urinary pH of patients infected with *P. mirabilis*.[55] Subsequent experiments in a laboratory model of *P. mirabilis* infection confirmed that when the models were supplied with dilute, citrate-containing urine with a pHn >8.3, crystalline biofilms did not form (Figure 6).[56]

The advice for patients to increase their fluid intake by drinking steadily throughout the day[57] clearly has a sound basis in physiology and physical chemistry. The dilution of urine resulting from an increased fluid intake will elevate the pHn and slow the rate of catheter encrustation. If the citrate content of urine can also be elevated by encouraging patients to take, for example, lemon-based drinks, the rate of crystal formation should reduce further. These observations should encourage a clinical trial to examine the effect of increasing patient's fluid intake with citrate-containing drinks on the encrustation and blockage of catheters.

RECURRENT CRYSTALLINE BIOFILMS

In many patients who suffer recurrent catheter encrustation, the usual management involves simply replacing the blocked catheter with a new one. Fresh catheters are thus placed directly into urine cultures of *P. mirabilis* at alkaline pHs, which contain microcrystals of calcium and magnesium phosphates. Examination of the early stages of crystalline biofilm formation under these circumstances in a laboratory model has revealed a common sequence in the development of crystalline biofilm on all-silicone, silicone-coated latex, hydrogel-coated latex, and silver–hydrogel-coated latex catheters.[50] After only 1 h in the model, the catheter surfaces were covered by a microcrystalline layer. X-ray microanalysis confirmed that this material was composed largely of calcium and phosphate. Bacterial colonization of this foundation layer followed, with microcolonies of cells developing on the microcrystals (Figure 7). By 18 h, the eyelets and lumenal surfaces of all these catheters were comprehensively covered by densely populated, crystalline *P. mirabilis* biofilm. Examination of catheters removed from patients has confirmed that a microcrystalline foundation layer forms on catheters *in vivo* (Figure 8).[50]

FUTURE CATHETER DESIGN

The formation of *P. mirabilis* crystalline biofilms has important implications for the development of encrustation-resistant catheters. Attempts to