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International Journal of Antimicrobial Agents 23S1 (2004) S67–S74

INTERNATIONAL JOURNAL OF Antimicrobial Agents

www.ischemo.org

Bacterial biofilm formation on urologic devices and heparin coating as preventive strategy

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Abstract

In the process of endourological development a variety of foreign bodies have been invented besides urinary catheters, on which biofilm can be formed. Bacteria in the biofilm are less susceptible to antibiotics. An additional problem of medical biomaterials in the urinary tract environment is the development of encrustation and consecutive obstruction. The most promising prevention strategy for bacterial biofilms is the production of materials with anti-adhesive surfaces such as heparin. Although heparin-coated ureteral stents are expensive, they justify their cost. Our studies show that such devices are protected against incrustation and biofilm formation for a longer period of time: 6–12 months, both in vitro and in vivo.

Keywords: Biofilm formation; Antimicrobial susceptibility; Incrustation; Obstruction; Heparin coating

1. Introduction

It is known that bacterial biofilms can colonise the surfaces of both tissues and implanted medical devices. The process of biofilm formation and the impact on the development and clinical course of infectious diseases, however, are still poorly understood. Effective preventive and therapeutic strategies still need to be developed for device-associated infections. By definition, a biofilm is an accumulation of microorganisms and their extracellular products forming a structured community on a surface.

It is evident that with the steadily increasing number of biomaterial devices used in urology for urinary drainage (catheters, ureteral and prostatic stents) as well as implants for replacement of lost body functions (sphincters and other continence devices, penile prosthesis), biofilm formation and device infection is an issue of growing importance. In addition, new tissue surfaces are created by using bowel segments for partial or complete replacement of the lower urinary tract. According to a North American survey, permanent urinary catheters and stents are the most common biomaterial implants comparable to contact and intraocular lenses or hip and knee implants [1].

2. Mechanism of biofilm formation

The formation of biofilm generally consists of several main steps: the first step is the deposition of the microorganisms, next follows their attachment by microbial adhesion and anchorage to the surface by exopolymer production. After this process their growth, multiplication and dissemination can be observed (Fig. 1) [2–8].

The initial event in this process is bacterial adhesion and the deposition of a host urinary component on the surface of the biomaterial leading to the formation of a conditioning film. This film consists of proteins, electrolytes and some unidentified molecules [2,5]. The types of components that form the conditioning film depend on the surface characteristics (chemistry, charge and hydrophobicity). Many of the protein molecules in the conditioning film play an active role in the bacterial adhesion process. The conditioning film does not cover the entire implant surface

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0924-8579/$ – see front matter
doi:10.1016/j.ijantimicag.2003.12.007

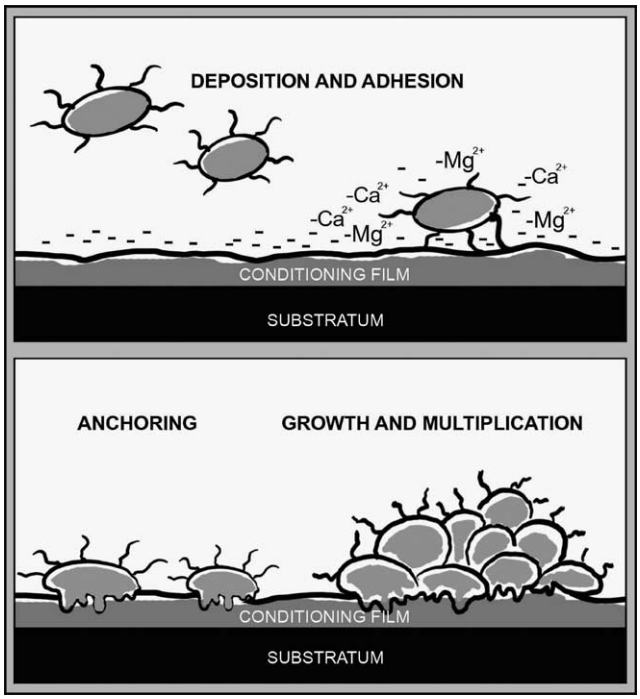

Fig. 1. Formation of biofilm.

completely, but rather forms a "mesh-like" covering [9]. Several factors are thought to influence bacterial adhesion to foreign body surfaces, such as biomaterial surface characteristics, bacterial surface features and the behaviour of microorganisms and the presenting clinical condition [4,5].

The biofilm is usually built up of three layers (Fig. 2). The linking or conditioning film is attached to the surface of a tissue or biomaterial, the biofilm base consisting of microorganisms and the surface film acts as an outer layer where planktonic organisms can be released free-floating and spread to the surrounding compartments [2,4,6,8]. The development of the biofilm is shown in Fig. 3a and b.

3. Antimicrobial susceptibility of biofilms

Bacteria within the biofilms differ both in behaviour and in phenotypic form from the planktonic, free-floating bacteria. Conventional clinical microbiology can detect only the planktonic, free-floating bacteria, which are absolutely different from bacteria enclosed in the biofilm [2,4,10].

The failure of antimicrobial agents to treat biofilms has been attributed to a variety of mechanisms [2–4,6–8,11,12]. In general, organisms encapsulated in the biofilm grow more slowly than the planktonic organisms, probably because the encapsulated bacteria have a decreased nutrient and oxygen supply leading to a decreased metabolic rate and, as a consequence, to a decreased antimicrobial susceptibility. This may lead to a less susceptible genotype selecting a resistant population. Furthermore, antimicrobial binding proteins are poorly expressed in these slow-growing biofilm bacteria.

The biofilm matrix itself often delays or impedes the diffusion of antibiotic molecules into the deeper layer of the film (*extrinsic resistance*).

Bacteria within the biofilm are phenotypically different from their planktonic counterparts. Antimicrobial agents would therefore frequently fail to eradicate them. Bacteria within a biofilm activate many genes, which change their surfaces and other molecular targets, reducing the susceptibility to antimicrobial agents (*intrinsic resistance*). It is suggested that these phenotypic changes are more important for antimicrobial resistance than the external resistance mechanisms such as biofilm matrix or glycocalyx.

Bacteria within a biofilm can analyse the external environment, develop interbacterial communication and may transfer genetic information and plasmids within biofilms. As a consequence, bacteria in biofilms may survive the use of antibacterial agents at concentrations 1000–1500 times higher than needed to eradicate planktonic bacteria of the same species.

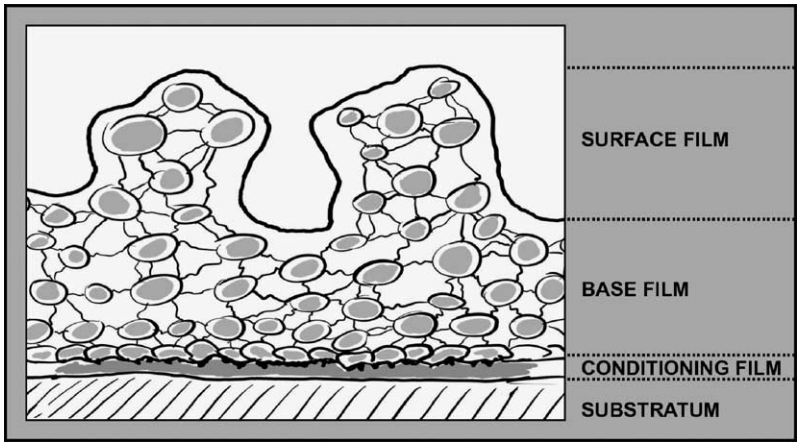

Fig. 2. Composition of the biofilm.

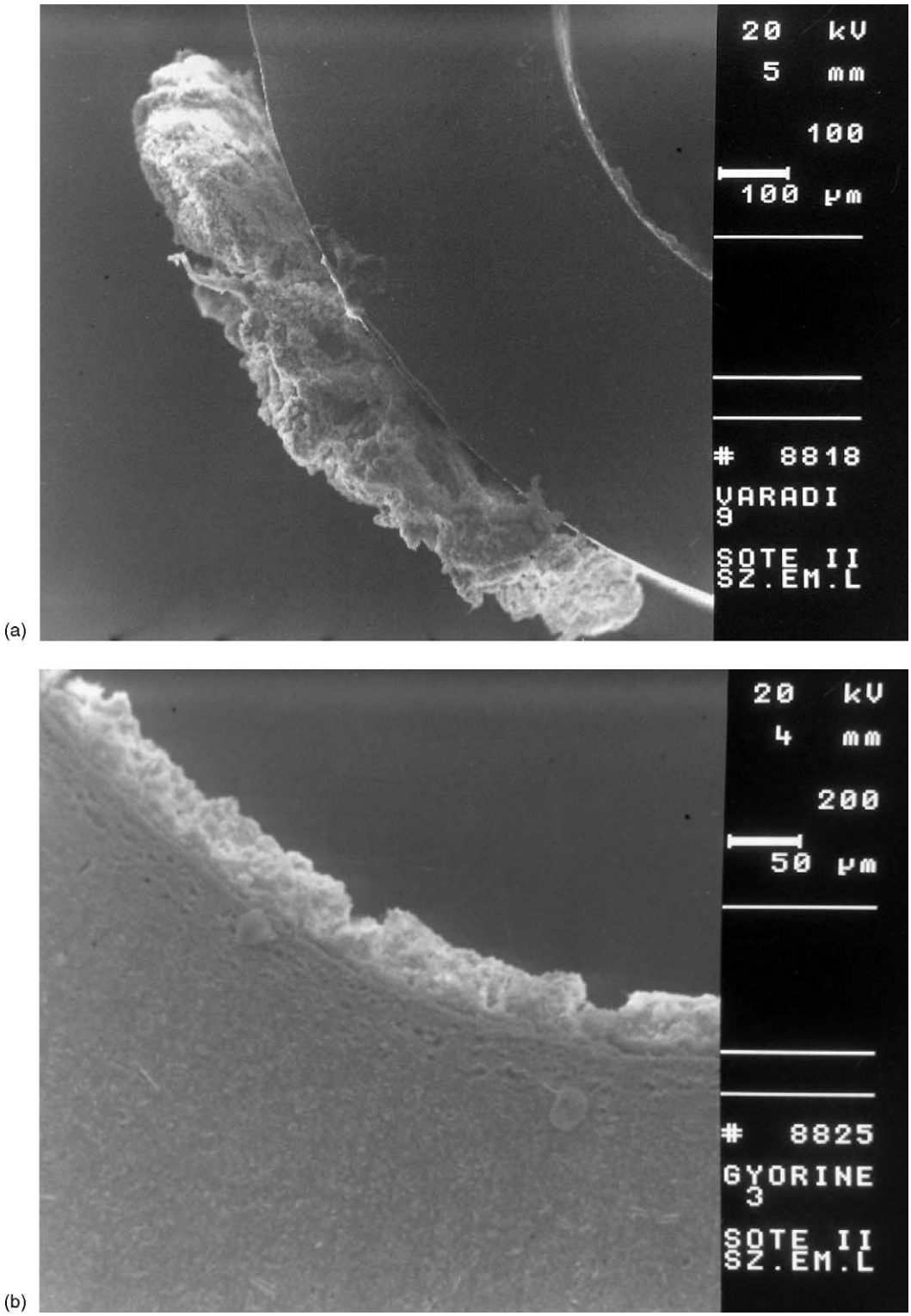

Fig. 3. Scanning electron microscope of a developing biofilm. Formation of the biofilm on the (a) outer surface of the polyurethane stent and (b) inner surface of the polyurethane stent.

4. Management of biofilm infection

Urine cultures of planktonic bacteria and the definition of their antimicrobial susceptibility may contribute to the failure of eradicating chronic bacterial infections with biofilms. Antimicrobial treatment may be effective in "young" biofilms that developed within 24 h or less [2,4,5,13,14]. Wollin et al. demonstrated that ciprofloxacin and ofloxacin

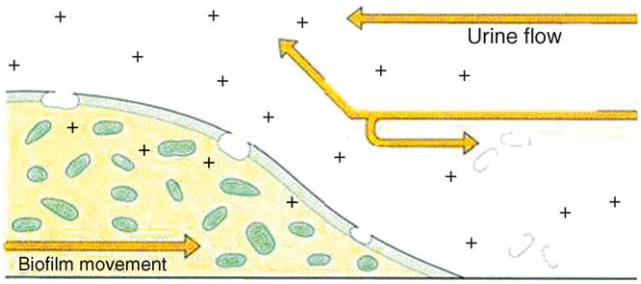

Fig. 4. Effect of antibiotics (+) on biofilm progression (adapted from [14]).

rapidly reached a high urinary concentration and were able to penetrate into conditioning bacterial biofilms and onto the stent surface. Significantly greater amounts of both antibiotics were absorbed onto the biofilm than to the stent surface [15]. It was also demonstrated that ciprofloxacin concentrations on biofilms surrounding urinary stents were significantly higher than ofloxacin concentrations [5,10,16,17]. Other studies showed that ciprofloxacin and ofloxacin might prevent microbial adhesion and biofilm formation for a short period of time [1,13,18–21]. Goto demonstrated that other drugs such as trimethoprim-sulphamethoxazole and tobramycin were less potent against biofilms compared to the fluoroquinolones [5,10]. According to Kumon, a combination therapy with fluoroquinolones and macrolides or fosfomycin seems to be most effective against biofilm infections [2,4,13,22]. Most researchers believe that antibiotics can only slow down the progress of biofilm formation by eliminating unprotected planktonic bacteria and stopping or reducing the metabolic activity of bacteria on the biofilm surface (Fig. 4) [2,8,10,14]. However, during an acute febrile phase of a biofilm infection, antimicrobial therapy is reasonable and essential because the planktonic and not the biofilm bacteria are responsible for febrile reactions [2].

5. Biofilms in catheter-associated urinary tract infections

An additional problem of medical biomaterials in the urinary tract environment is the development of encrustation and consecutive obstruction. When the drained urinary tract becomes infected by urease-producing bacteria such as *Proteus mirabilis*, the bacterial urease generates ammonia from urea and elevates the pH of the urine. In this alkaline environment, crystals of magnesium ammonium phosphate (struvite) and calcium phosphate (hydroxyapatite) are formed and trapped in the organic matrix surrounding the cells. Progression of these encrustations eventually blocks the catheter lumen [16,19,23–26]. Clinical experience and laboratory studies have shown that all types of catheters currently available are vulnerable to blockage by crystalline *P. mirabilis* biofilms [23,27,28]. The complications resulting from catheter encrustation seriously compromise patient care. The crystalline deposits can be hard and abrasive and can traumatise the bladder mucosa and urethra. Obstruction of urinary flow through the catheter may cause either incontinence due to leakage of urine around the catheter or painful distention of the bladder due to urinary retention. Bacteriuria is always found in these patients, therefore retention and vesico-ureteral reflux may facilitate ascending infection of the urinary tract, culminating in episodes of pyelonephritis, septicaemia and shock [29,30]. Thus, undetected catheter blockage may lead to life-threatening complications [12,30,35]. Several studies reported that up to 50% of patients undergoing long-term catheterisation will require unscheduled catheter replacement because the flow of urine has been blocked by crystalline deposits [24,31,32].

Current approaches in preventing catheter blockage by encrustation (i.e. replacement of the catheter, changing the type or size of catheter, increasing fluid intake, administration of cranberry juice or acidifying drugs and washing the bladder/catheter with acidic, antiseptic or saline solutions) are frequently ineffective [33]. Recurrent catheter blockage gives patients the reputation as “blockers” [6,23,30]. Effective procedures to prevent encrustation are definitely needed.

Since the significance of biofilm formation has been appreciated as the main problem of all implants and biomaterial devices, modification of the biomaterial surface was regarded the most promising prevention strategy for bacterial biofilms. A variety of techniques have been designed for this purpose, including the controlled release of antimicrobial agents or antiseptics (such as minocycline, rifampicin, gentamicin, nitrofurantoin) incorporated in the device material, surface coatings with silver and other metals, surface modifications to change or increase hydrophobicity or to create functional groups with intrinsic antimicrobial activity, and anti-adhesive surfaces such as heparin and phosphorylcolin [2,6,7,13,16,28,34,35].

Heparin with its antithrombogenicity and its strong electronegativity that repells cellular organisms is an excellent candidate for an anti-adhesive stent coating. In 1987, Ruggieri et al. showed a 90% reduction of bacterial adhesion on urinary catheter surfaces by heparin coating [36]. Hildebrandt et al. demonstrated the reduction of stent encrustation by heparin coating in an experimental setting [37].

6. An in vitro examination of the ability of heparin-coated catheters to resist encrustation by crystalline *P. mirabilis* biofilm

The ability of three catheter types to resist encrustation and blockage by crystal-generating urine cultures of *P. mirabilis* isolated from patients encrusted catheters was examined in a laboratory model of the catheterised bladder [28]. Catheters (14 French) were inserted aseptically through a section of silicone tubing. They were attached to a glass outlet at the base of the vessel into a 200 ml glass chamber maintained at 37 °C. The catheter balloon was

inflated with water securing the catheter in position and sealing the outlet from the vessel "bladder". The catheter was then attached to a drainage tube and reservoir bag. Sterile pooled human urine was supplied to the bladder via a peristaltic pump. Thus, a residual volume of about 30 ml was collected in the vessel below the level of the catheter eyehole. As urine was supplied to the model the overflow drained through the catheter into the collecting-bag.

The three catheters tested were a latex catheter with hydrophilic coating, a silicone catheter and a heparin-coated silicone catheter. After inoculation of the sterilised urine with the *P. mirabilis* strain, the organisms were allowed to establish themselves in the model for 1 h. The peristaltic pump was then switched on and fresh urine supplied to the bladder at 0.5 ml per minute. The models were operated until the catheters were blocked with encrustation. Low vacuum scanning electron microscopy (REM) was performed to visually assess the extent of encrustation at catheter cross-sections 1, 4, 10 and 30 cm from the tip.

Whereas the hydrogel-coated latex catheter was blocked after an average of 28.1 h in four experiments, time until blockage was significantly longer for the heparin-coated catheter (58.2 h) and silicone catheter (54.8 h) in the setting described. However, all three types of catheters were vulnerable to *Proteus* blockage.

Encrustation (REM) was observed only on hydrogel-coated and silicone catheters especially around the eyeholes and balloon but no encrustation was found on heparin-coated

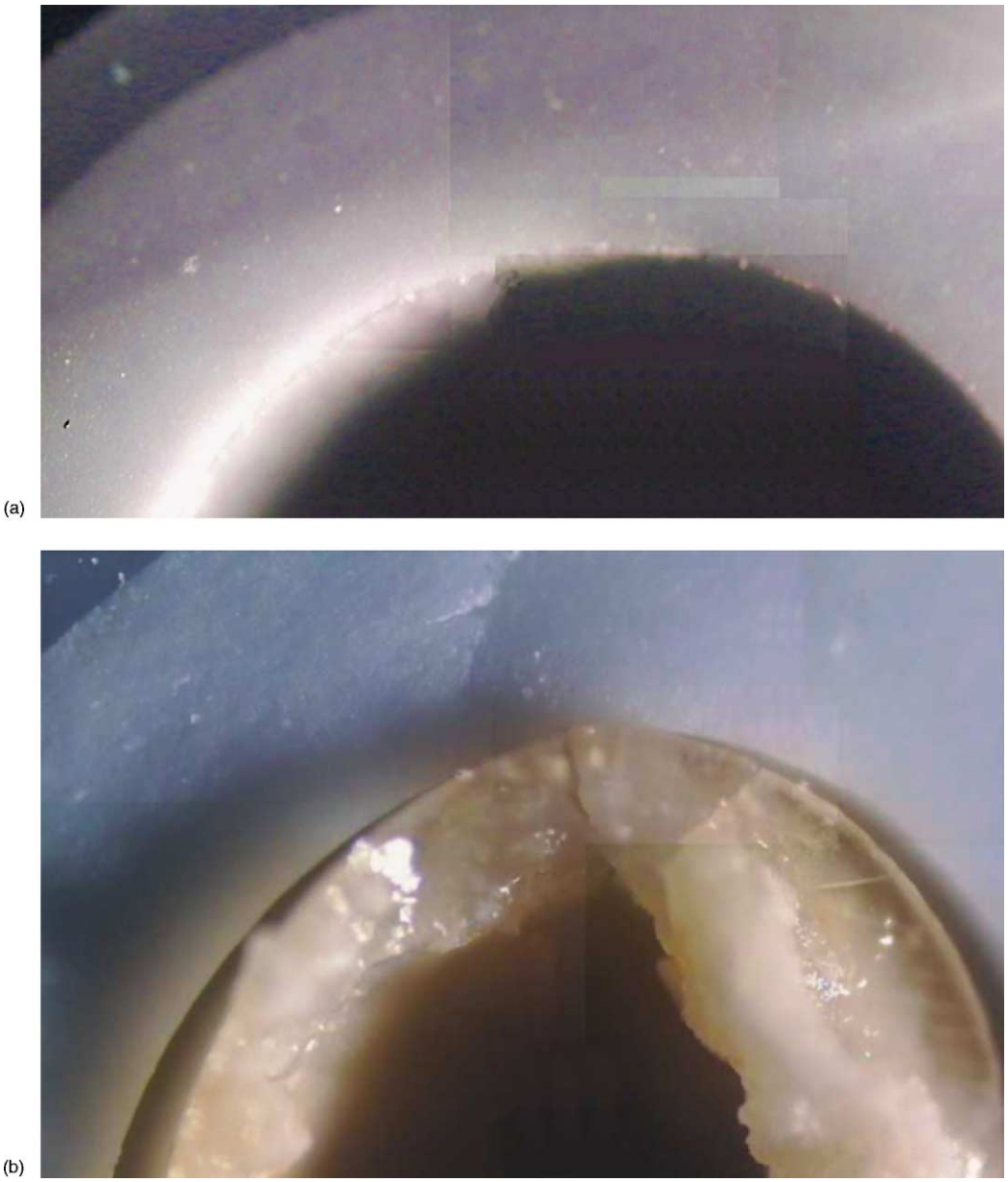

Fig. 5. (a) A heparin-coated ureteral stent which remains unaffected by biofilm formation and incrustation. (b) An uncoated ureteral stent with a biofilm formed on its inner surface.

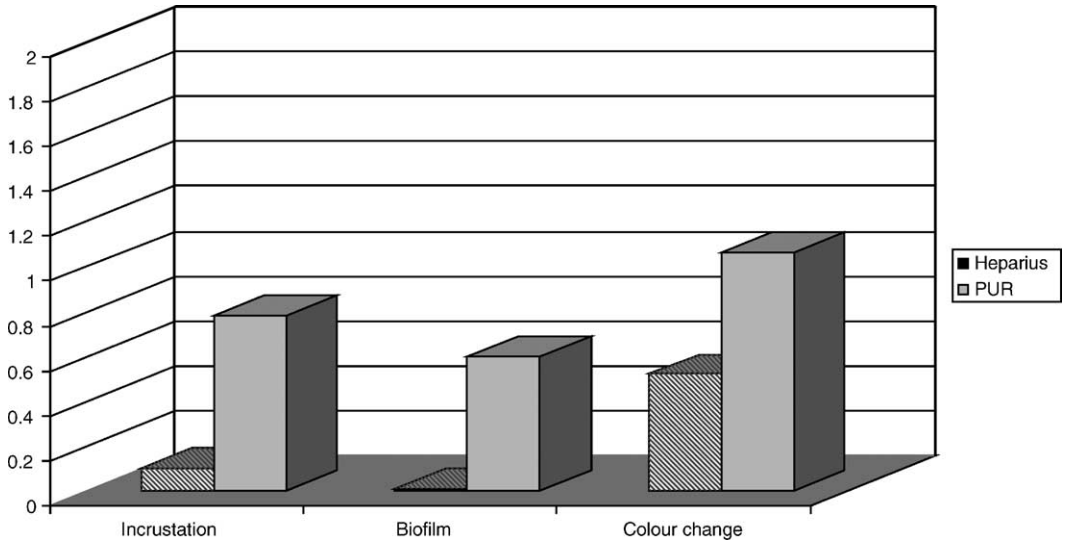

Fig. 6. Incrustation, biofilm formation and colour change of heparin-coated ureteral stents (Heparius) and uncoated polyurethane stents (PUR) within a 6-week observation period. *Y*-axis is—0: no change, 1: moderate change and 2: significant change.

catheters; the blockage was caused by plugs of clear gel-like material.

7. Clinical evaluation of encrustation and biofilm formation on heparin-coated ureteral stents and nephrostomy tubes

In a pilot study the encrustation of heparin-coated ureteral stents was compared to uncoated polyurethane stents. Twenty heparin-coated and 20 uncoated stents were inserted into obstructed ureters in a prospective randomised study under sterile conditions and left indwelling for periods between 2 and 6 weeks. The stents were then removed under sterile conditions, sealed in sterile covers and sent for electron-microscopic evaluation. Nephrostomy tubes were used in two patients with permanent bilateral external urinary drainage suffering from frequent encrustation obstruction of their silicone catheters that resulted in repeated emergency visits. In these patients a heparin-coated and an uncoated nephrostomy tube were used simultaneously for either side so that direct comparison of encrustation status was possible.

Electron microscopy showed a significant difference between heparin-coated and uncoated ureteral stents. Fig. 5a and b demonstrate the two types of stents that react to biofilm formation in different ways. Two weeks after the insertion, two types of deposits could be detected on the surfaces of the uncoated stents—amorph anorganic deposits consisting of mineralised crystals and another of bacterial biofilms. Heparin-coated stents were unaffected by encrustations. After 6 weeks of indwelling time, all uncoated stents showed varying degrees and forms of deposits. Within the limited observation period of this pilot study none of the uncoated stents became totally obstructed. The heparinized nephrostomy tubes remained unaffected for the whole 6–8 weeks indwelling periods, whereas uncoated tubes got obstructed within 2–3 weeks.

This pilot study showed that no biofilms were detectable on heparin-coated stents whereas significant biofilms were demonstrated in 33% of uncoated stents. Mild incrustation was observed in 10% of heparin stents compared to significant incrustation in 50% of uncoated stents, and incrustations/biofilms were demonstrable on uncoated stents as early as 2 weeks after implantation (Fig. 6).

8. Extended indwelling times for heparin-coated ureteral stents

In 10 patients with permanent ureteral stent drainage, heparin-coated stents were left indwelling for 6–8 months (Group I). In all patients bacteriuria was demonstrable at the time of heparin-coated stent insertion; from previous stents. In three patients with uretero-enteral anastomosis stricture in an ileal conduit, heparin-coated stents were left for 1 year (Group II).

No obstruction/blockage of the stents was observed during this time in group I. On REM none or only minimal encrustations were found after this prolonged indwelling time.

In the difficult bacteria-exposed Group II situation, the silicone stents were found to be obstructed after 7 weeks while the hydrogel-coated stents in 5 months, whereas all of the heparin-coated stents were unaffected after 12 months of indwelling time.

Heparin-coated ureteral stents are more expensive than standard stents. However, with longer indwelling times and reduction of the number of stent exchange procedures, the total costs for heparin stents should be reduced compared to

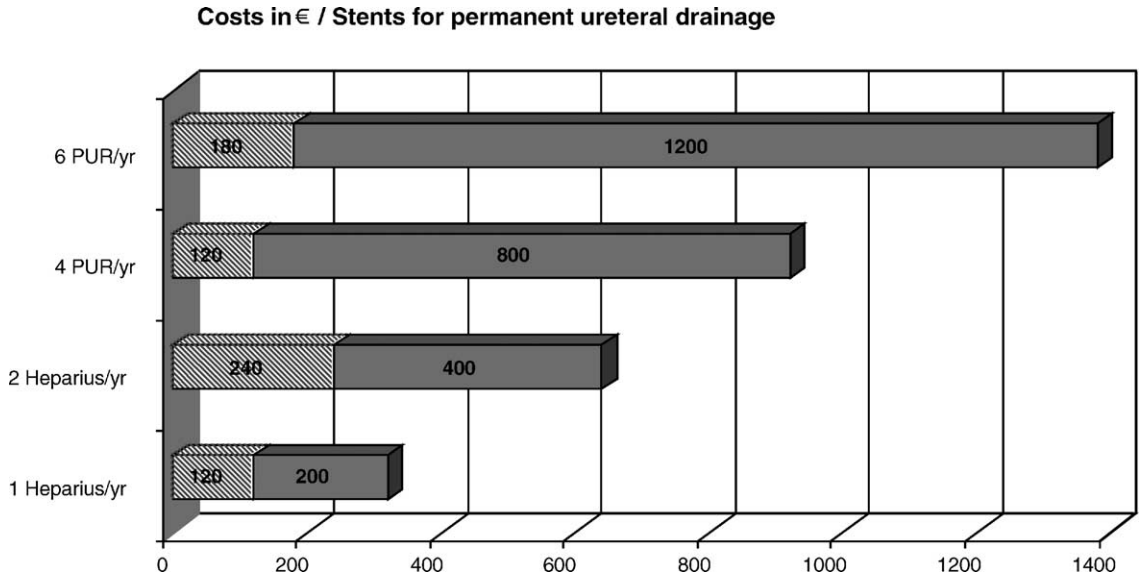

Fig. 7. Costs per year for heparin-coated ureteral stents (Heparius) and uncoated polyurethane stents (PUR).

other stents. Heparin-coated stents cost four times the price of uncoated stents; the ratio is 2.3 for nephrostomy tubes and 6.5 for urethral catheters. If a stent exchange procedure is calculated at € 200 (a very conservative estimate), the costs per year for various stents and variable indwelling times are demonstrated in Fig. 7.

Similar calculations have been made for nephrostomy tubes and urethral catheters to show the advantage of heparin-coated devices. Each institution has to check its own regime with regard to cost effectivity, but the excellent qualities of the heparin-coated urologic drainage devices add a new possibility to the urologic armamentarium. Besides the possible cost reduction for permanent urinary drainage, significant reduction of patients morbidity due to less obstruction, less emergency visits and less invasive procedures are compelling arguments.

9. Conclusion

Further research in the field of biofilm physiology, development and function is mandatory. Mechanisms for prevention and control of biofilm formation and catheter encrustation have to be found. Heparin coating seems one possible solution but further development of catheter materials resisting bacterial colonisation is to be continued.

The future goal is to define easier methods for diagnosing and quantifying biofilm infection and to develop antimicrobial agents, which are effective against bacteria enclosed in the biofilm. It is also important to identify molecular targets of biofilm bacteria as well as the urinary components that are involved in biofilm formation. An ideal surface device to resist protein has to be developed. Bacterial adhesion and the interaction between the biomaterial surface and urine also need to be defined.

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