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Prevention of surface encrustation of urological implants by coating with inhibitors

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Abstract

The encrustation of materials used for urological implants is as yet an unresolved problem. The crystallisation-inhibiting effect of the glycosaminoglycan heparin was used to reduce encrustation. Heparin was covalently bound to the surface of slotted-tube stents of tantalum and stainless steel using a spacer molecule. To verify the inhibition of crystallisation processes, reproducible in vitro tests and in vivo tests using the rat as animal model were carried out. The in vitro and in vivo experiments showed that the heparin coating has a significant influence on the encrustation of the surface. After 7 days in vitro and 120 days in vivo, heparin coated stents were free of encrustation, whereas the uncoated reference stents were extensively covered. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Encrustation; Crystal inhibition; Ureteral stent; Urethral stent; Coating; Glycosaminoglycans

1. Introduction

Urethral and ureteral stents, catheters and other urological implants are frequently affected by encrustation due to their permanent contact with urine. Crystals and deposits consisting of the ionic and organic components of urine cover the surface of the implant [1]. The formation of encrustation is promoted by a high urinary pH, the product of infections. Encrustation leads to a higher risk of infection, and a vicious circle begins [2]. Thus, the encrustation of urological implants does not only cause their failure by obstructing the lumen [3,4], but it also presents a focus for infection [5]. Encrustation of the diverse materials used in urological implants is as yet an unresolved problem and requires an urgent solution.

The fact that glycosaminoglycans, which are natural constituents of human urine, act as crystal growth inhibitors has been known for a long time. These macromolecular substances are able to bind to urine components by a mechanism that blocks their relevant crystal growth sites [6–8]. Thus, the blocked urine components are excluded from participation in crystallisation processes. So far the activity of macromolecular inhibitors has been studied only in solution; it is intended to employ the crystallisation-inhibiting effect of glycosaminoglycans at solid surfaces.

The inhibitor heparin has the strongest inhibiting effect of all glycosaminoglycans [6]. Furthermore, the bonding of heparin to different materials as an antithrombogenic coating is already well known [9]. Heparin was therefore selected to verify experimentally the inhibition of crystallisation processes by surface coating with glycosaminoglycans.

2. Method and materials

2.1. Substrates

Expandable stents of tantalum with an outside diameter of 1.60 mm were used as substrates for the in vitro experiments. For the in vivo evaluation, stents of stainless steel with an outside diameter of 0.79 mm, adapted for the rat as animal model, were tested.

Heparin is covalently bound to the surface by a spacer molecule (Fmoc-p-Bz-Phe-OH), whereby a high long-term stability is achieved. The relevant functional groups

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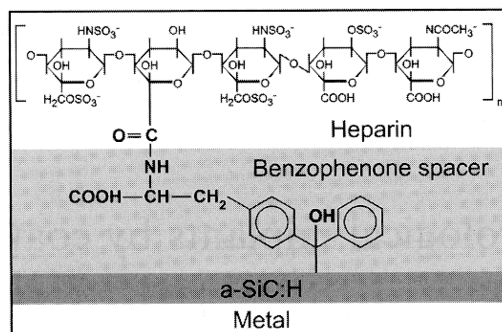

Fig. 1. Immobilisation of heparin over a benzophenone spacer and amorphous siliciumcarbide.

of the inhibitor are not blocked or deformed by this method.

To achieve covalent immobilisation of Heparin, the stents were coated with an intermediate layer of amorphous siliciumcarbide (a-SiC) by a plasma enhanced chemical vapour deposition (PECVD) procedure making the binding of the phenylalanine spacer by a C–C-linkage possible (Fig. 1). This was achieved by dipping the stent into a solution of the spacer in dimethylformamide and exposing to UV light at a wavelength of 365 nm. The Fmoc protection group of the spacer is then removed by piperidine in order to bind heparin at the amino group, which is then free. The coating with heparin is done by dipping the stent into an aqueous solution of carbodiimide and heparin. The surface loading achieved by the procedure (45 mIU/cm^2) was analysed by a standard chromogenic heparin test (Berichrom Heparin-Testkit, Behringwerke OWLD11).

2.2. *In vitro model*

Urine in vivo is often subject to fluctuations of the pH value and the concentration of ionic components. In order to analyse the performance of the coating under reproducible conditions, in vitro investigations were carried out. Further, the influence of mechanical deformation of the coating on the inhibition can be studied, deformations that might occur during dilatation of a stent. This is not possible in the case of the in vivo rat model, since stents of a suitable size are too small to have an expandable design.

In the experimental setup used for rinsing, the stents were introduced after the coating process into a silicone tube with an inside diameter of 4 mm and expanded in situ by a balloon catheter from 1.6 mm to approximately 4.2 mm outside diameter. Electrolyte flow through the tube and the stents was achieved by pumping artificial urine using a Heidolph tube pump with a flow rate of 12 ml/s from a reservoir (1 l). The reservoir was included in a Paratherm-U5 heating bath to maintain a constant temperature of 37°C.

Table 1
Concentrations of cations and anions in the artificial urine

Cations		Anions	
Na^+	181 mmol/l	Cl^-	226 mmol/l
Ca^{2+}	5.75	PO_4^{2-}	32.3
K^+	64.7	SO_4^{2-}	20.8
Mg^{2+}	3.85	$C_2O_2^{2-}$	0.381
NH_4^+	73.1	Citrate	3.21
		OH^-	27.6
(pH 6.0)			

Artificial urine (Table 1) was employed containing the inorganic constituents of human urine in comparable concentrations [10]. In order to ensure a worst-case condition the artificial urine contains no inhibitors. Thus, maximum crystallisation potential is achieved. Furthermore, the pH value of the artificial urine is continuously increased during the test period by contact with the air of the lab. This causes an infection of the artificial urine and the production of urease [2]. The pH value is measured continuously. In this way the effectiveness of the heparin coating is also proved at increased urinary pH.

2.3. *In vivo evaluation*

For in vivo evaluation the rat model was selected since rat urine has very similar composition and crystallisation behaviour to human urine [11,12]. The optimal technique for implantation is the direct insertion of the stents into the ureter over an executed incision. The rats were divided into 5 groups consisting of 4 animals in each. In the left ureter of each animal a heparin coated stent was implanted, while in the right was placed an uncoated stent. The explantation took place in groups after 7, 30, 50, 80 and 120 days. The animals were nourished normally during the implantation period.

2.4. *Analytical methods*

In vitro and in vivo tested stents were analysed by means of optical microscopy, raster electron microscopy (ISI-SX-40) and EDX-analysis (EDAX 9100). The quantity of phosphorus and calcium measured by EDX-analysis was chosen as the criterion for encrustation, since these elements are contained in all relevant crystals: struvite (NH_4MgPO_4), brushite ($CaHPO_4$), hydroxy apatite ($Ca_5(PO_4)_3OH$) and calcium phosphate ($Ca_3(PO_4)_2$).

3. Results and discussion

The in vitro and in vivo experiments show that the heparin coating prevents encrustation. Surfaces coated

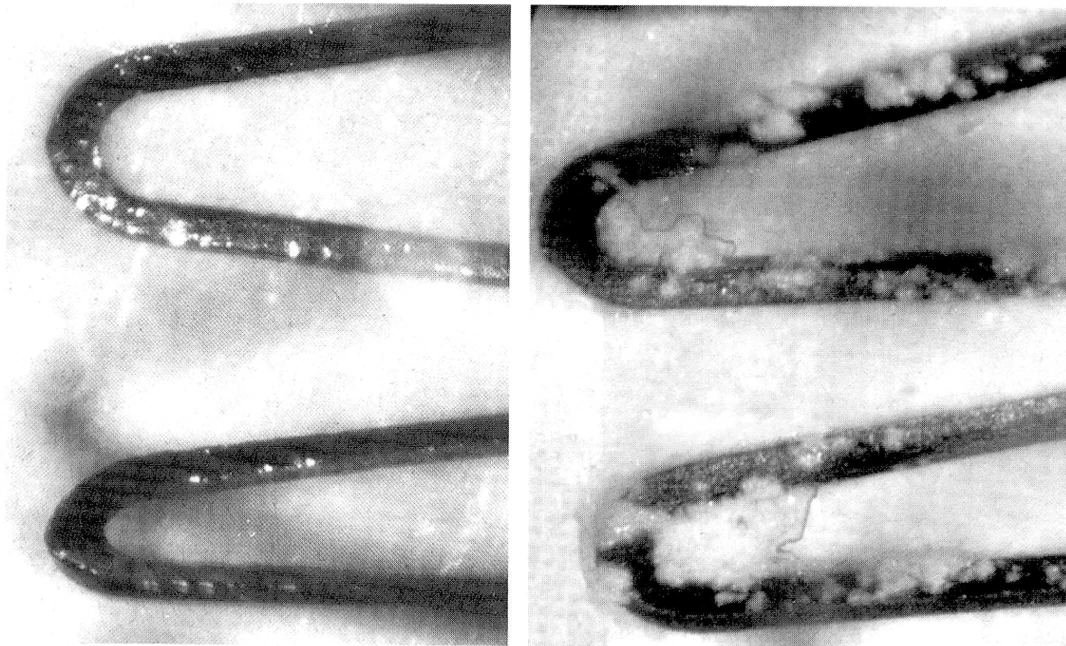

Fig. 2. Left: 164 h in vitro tested heparin coated stent (optical microscope, 50 ×). Right: 164 h in vitro tested uncoated stent (optical microscope, 50 ×).

with heparin are not covered by crystals even after several weeks of urine rinsing.

3.1. In vitro model

The 164 h (7 days) in vitro tested heparin coated stents show no encrustation in optical microscopical investigations, even at high magnifications (Fig. 2, on the left). The small light spots on the surface are reflections of the light source of the optical microscope. On the uncoated stents the process of encrustation is clearly observed under optical microscopy after the same period of time; in those areas of low flow rate, crystals formed in considerable amounts (Fig. 2, on the right).

Through the infection of the urine the pH value increased continuously from 6.0 to 7.0 during the rinsing procedure.

In order to detect and analyse low level optically invisible crystalline deposits, EDX-analysis was performed. In the case of the heparin coated stents the spectrum shows no pronounced peaks after a gate time of 60 s (Fig. 3, above). The measured $K_{\alpha 1}$-line of sulphur is caused by the sulphates contained in heparin.

In the case of the uncoated stents the $K_{\alpha 1}$-lines of phosphorus and calcium are clearly resolved as are also, although to smaller extent, the $K_{\beta 1}$-lines of magnesium and calcium (Fig. 3, below). The $K_{\alpha 1}$-line of sulphur originates in the sulphur contained in some crystals.

The EDX-measurements confirm the results of the investigations by optical microscopy. On the heparin coated stents no ionic deposits are detectable, whereas

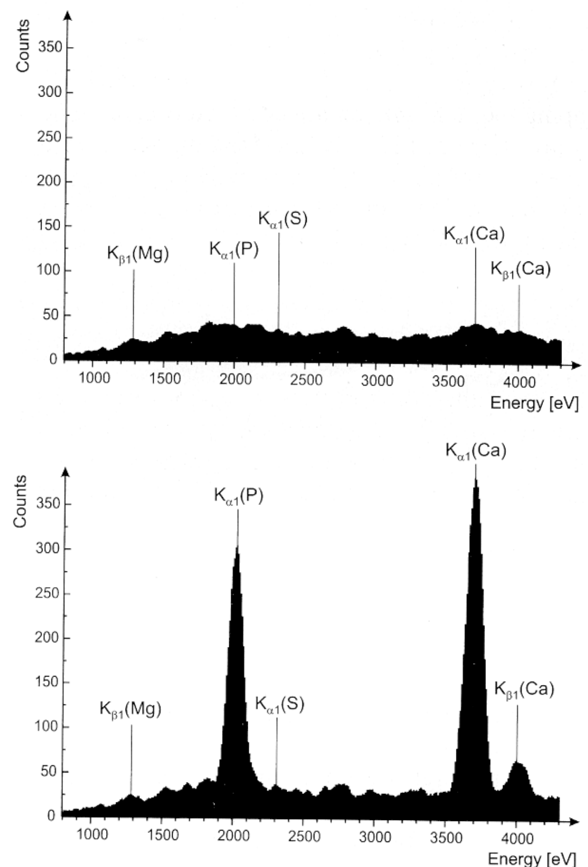

Fig. 3. Above: EDX counting rate of a heparin coated, 164 h in vitro tested stent. Below: EDX counting rate of an uncoated, 164 h in vitro tested stent.

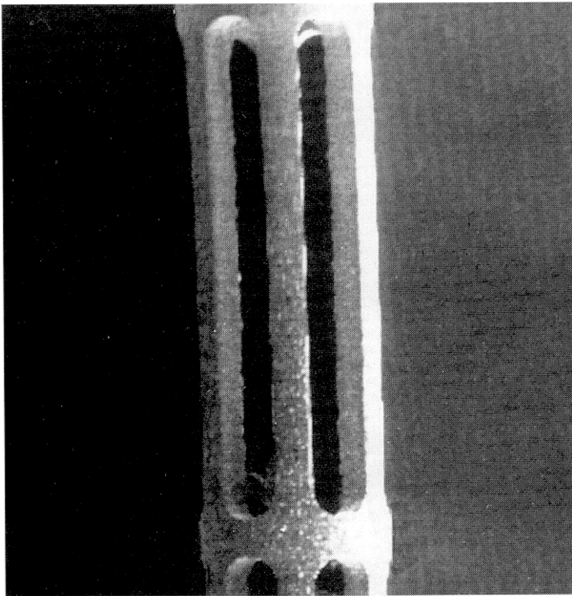

Fig. 4. Heparin coated stent after an implantation period of 120 days (REM, 40 ×).

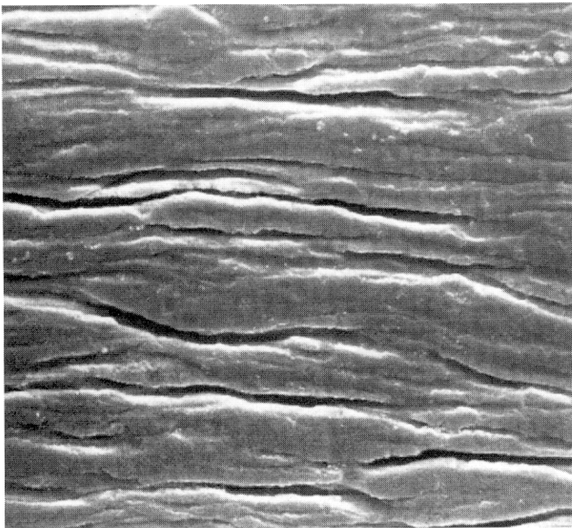

Fig. 5. Heparin coated stent after an implantation period of 120 days (REM, 1000 ×).

considerable amounts of phosphorus and calcium, the main components of encrustations, are present on the uncoated stents.

Due to the higher crystallisation potential the period of one week in vitro corresponds to a period of several months in vivo. Rinsing experiments with full urine of Tunney et al. [13] showed that after 14 days encrustation could be detected on none of the tested materials. Using artificial urine crystal deposits were already visible after 24 h.

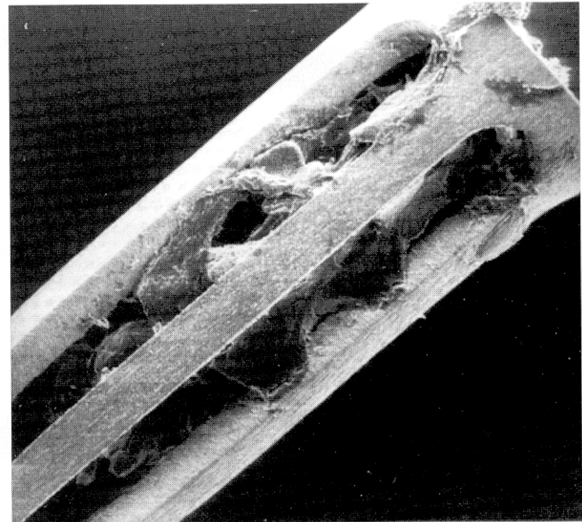

Fig. 6. Uncoated stent after an implantation period of 120 days (REM, 60 ×).

3.2. *In vivo evaluation*

Over the same implantation period, a substantial reduction in crystallisation is demonstrated on the stent surfaces coated with heparin when compared to controls (Figs. 4–7). With an increased implantation period from 7 to 120 days the encrustation of the uncoated stents enlarges. After the longest implantation period of 120 days, two of the 20 uncoated stents were completely obstructed by encrustation. The coated stents show deposits in much smaller amount. These deposits consist to some extent of urothelial cells, which deposited during the implantation period. None of the 20 coated stents was obstructed.

The histopathological analysis of the resected ureters following the implantation period showed no differences concerning the incorporation of coated and uncoated stents. Floride inflammatory modifications were not present. These results show the excellent biocompatibility of the heparin coating, which is important for the clinical application.

From the results of the in vivo evaluation, the encrustation inhibiting effect of the heparin coating in biological urine was proven. Assuming a similar effect to the macromolecular inhibitors when in solution, the crystallisation promoting characteristics of surfaces seem to vanish if the surface is coated with glycosaminoglycans [14]: Components of the urine which adsorb on an uncoated surface will still have free growth sites (1 in Fig. 8) which may lead to agglomeration of urine components and crystallisation occurs (2 in Fig. 8). If the implant is covered with glycosaminoglycans, however, then components of the urine can only adsorb in a way, that blocks their growth sites and prevents further crystal growth (3 in Fig. 8).

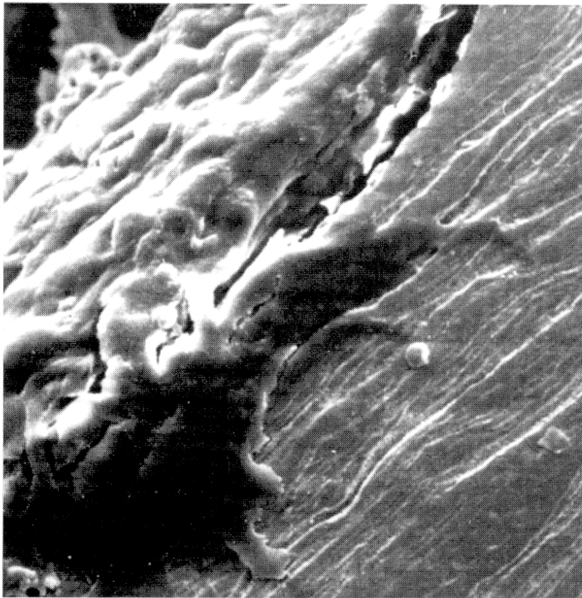

Fig. 7. Uncoated stent after an implantation period of 120 days (REM, 800 ×).

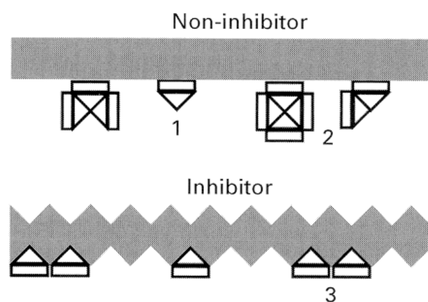

Fig. 8. Blocking of the growth sites of components of the urine by immobilised inhibitors.

4. Conclusion

The beneficial experience of heparin coating in the inhibition of encrustation is significant. For the first time, a coating has been found which is able to prevent encrustation for long periods and at different pH values. The clinical application in the form of encrustation inhibited ureteral and prostatic stents is a first example of the possibilities which the heparin coating offers.

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