Diamond-Like Carbon Coatings on Ureteral Stents—A New Strategy for Decreasing the Formation of Crystalline Bacterial Biofilms?

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Purpose: Any catheter material placed in the urinary tract provides a surface for bacterial colonization and, therefore, it is susceptible to encrustation with crystalline bacterial biofilm. Encrustation and blockage by biofilms remain a major complication in patient care. Most patients with indwelling ureteral stents experience irritative symptoms related to these implants and many experience discomfort.

Materials and Methods: Plasma deposited diamond-like amorphous carbon coatings are well-known for their excellent biocompatibility. A low temperature, low pressure plasma enhanced chemical vapor deposition technology was developed especially for coating polymeric medical implants with diamond-like carbon. We investigated the ability of diamond-like carbon to decrease the formation of crystalline bacterial biofilm as well as stent related side effects and discomfort. Diamond-like carbon coated ureteral Double-J® stents were tested in vivo.

Results: In 10 patients with heavy encrustation, different underlying diseases and a stent removal frequency of less than 6 weeks due to encrustation a total of 26 diamond-like carbon coated stents were successfully tested for their ability to decrease the extent of crystalline biofilm formation. There was a 2,467-day period of experience with diamond-like carbon coated stents. No primarily stent related complications occurred. No crystalline biofilm formation was observed in vivo. Excellent and facile handling, a less painful replacement procedure and high tolerance of application were reported by physicians and patients. Due to low friction the coated stents could be placed and removed much more easily than standard stents. The frequency and severity of symptomatic urinary tract infections were distinctly decreased.

Conclusions: Diamond-like carbon coating is a new strategy to improve the surface properties of ureteral stents. This novel surface effectively decreases friction, encrustation tendencies and biofilm formation.

Key Words: ureter, stents, biofilms, carbon

For more than 2,500 years urinary catheters have been applied in medicine.1 They are one of the oldest medical-technical aids and globally around 100 million units are currently sold annually. To ensure unobstructed urine transport catheters are routinely placed in the urinary tract from bladder to renal pelvis (ureteral stent2), or for direct drainage of the kidney or bladder (fistula catheter). Approximately two-thirds of the urinary flow occurs around the stent and a third occurs through the lumen of the stent.3 A wide spectrum of indications exists for applying ureteral stents and catheters.4 However, despite this extensive experience complications related to catheterization and stenting are frequent.5 About 35% to 40% of hospital acquired (nosocomial) infections are urinary tract infections, of which 90% are catheter associated since up to 100% of patients show at least asymptomatic bacterial colonization of the urinary tract after 30 days of an indwelling stent.6

Any catheter material placed in the urinary tract, such as indwelling transurethral catheters and ureteral stents, provides a surface for bacterial colonization and, therefore, it is susceptible to the formation of a persistent bacterial biofilm with increasing indwelling time (fig. 1). After planktonic bacteria become sessile this biofilm forms rapidly. It is mainly composed of a mixture of microbial cells (± 15% in volume) embedded in a self-produced matrix composed of extracellular polymeric substances, consisting primarily of polysaccharides (± 85% in volume).4,7 A crystalline bacterial biofilm evolves when crystal salt deposits became incorporated into the noncrystalline organic matrix. The crystals, which precipitate from urine, mostly consist of Ca and Mg phosphate, while Ca oxalate or uric acid can also be found.8

Catheter associated morbidity and complications, such as encrustation, luminal blockage, urinary tract infection, acute pyelonephritis, urolithiasis, vesicoureteral reflux or hydronephrosis, are frequent side effects of long-term urinary drainage. After an indwelling time of only a few weeks most patients are affected by at least 1 of these complications. Up to 80% of patients with internal ureteral stents experience irritative symptoms, hematuria, fever, significant and symptomatic bacteriuria, persistent discomfort or decreased libido.”

Dominant risk factors of progressive encrustation on ureteral stents are the surface properties of the stent material as

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Key Words: ureter, stents, biofilms, carbon
well as disturbed renal excretions of lithogenic substances. For example, hypercalciuria, hyperoxaluria and hypocitraturia can occur in patients with recurrent urolithiasis as a result of a metabolic disease, and in patients with end stage tumor as a result of the catabolic state of the metabolism.

Ureteral stents are rapidly colonized by adherent bacteria and depending on indwelling time up to 100% of patients show ureteral stent colonization. The pathogens are dominated by Escherichia coli and Enterococcus species, and to a minor extent by Staphylococcus species, Pseudomonas and Candida. In contrast to indwelling bladder catheters, whose biofilms are dominated by urease producing and, thus, urinary pH affecting pathogens, eg Proteus mirabilis, no direct causative link between biofilm formation and crystalline encrustation has been proved for these bacteria. In cases of particularly altered renal excretions due to underlying disorders of, for example intestinal metabolism, Ca-oxalate crystals can also form on the stent surface even under infection-free conditions at moderate urinary pH.

Bacterial biofilms decrease or inhibit the total effect of antibiotic agents, resulting in multiple antibiotic resistance that is difficult to treat. Inevitably a cycle of recurrent urinary tract infections develops. Life threatening consequences evolve when crystal and bacteria accumulation leads to full stent blockage, urine tailback, hydronephrosis, pyelonephritis and urosepsis.

Ureteral stents for long-term application should have an indwelling time of at least 6 months. However, due to encrustation tendencies as well as potential occlusion they must normally be removed as early as 6 to 8 weeks.

Various concepts for ureteral stent and urinary catheter materials are in use, such as silicone, polyethylene, polyurethane, biodegradable materials and drug delivery materials, as well as coatings such as silver, heparin, polytetrafluoroethylene and phosphorylcholine. These materials and coatings should 1) prevent or at least prolong biofilm formation, 2) decrease stent related traumatic or inflammatory complications and 3) increase wearing comfort for the patient. However, such high demands are hardly matched by existing products despite several efforts at improvement.

Of currently known biomaterials DLC a-C:H stands out due to variability in composition, and remarkable physical and chemical properties in the field of microbiology and medicine. Various investigations proved its excellent compatibility in respect to blood and bone cells. DLC is a thermodynamically meta-stable state of carbon, in which diamond-like (sp3-hybridized) and graphite-like (sp2-hybridized) bonding coexist with a large fraction of sp2 bonds. They can be prepared by miscellaneous deposition methods, eg ion deposition, sputtering, pulsed laser deposition and plasma enhanced chemical vapor deposition, which use accelerated hydrocarbon ions (plasma) as film forming particles. In general they are characterized by high mechanical hardness and chemical inertness. Depending on deposition conditions the properties of DLC films can be adjusted for several applications, eg to enhance the wear and corrosion resistance of precision cutting and machining tools. DLC is used as a protective coating on magnetic hard disks and optical glasses.

We investigated the ability of DLC thin films prepared by an especially adapted deposition method to prevent the initial attachment of bacteria and, therefore, effectively abort the formation of an infection activating crystalline biofilm. In vivo treatment attempts were performed to test the usefulness of DLC coated surfaces of ureteral stents for decreasing encrustation tendencies as well as friction.

**MATERIALS AND METHODS**

**Coating Process**

A plasma enhanced chemical vapor deposition process was developed especially for coating medical implants and surgical instruments. This process is particularly suitable for DLC deposition on several materials, even temperature sensitive polymer materials, eg PUR, polyethylene, silicone and latex. It allows a multitude of novel applications of a-C:H.

The typically 25 to 100 nm carbon coatings can be deposited on almost any substrate with a radio frequency activated (13.56 MHz) hydrocarbon gas plasma using acetylene (C2H2) for the current experiments. The chemical composition of the thin films can be varied by adding fluorine or nitrogen containing gases with different partial pressures, eg CF4, CH4, N2 and NH3, or by evaporation of alloying elements, eg vanadium, titanium and silicon, during the deposition process. By this process the volume and surface properties of the DLC layer, including elasticity, free surface energy, inner tension, hardness, friction and biocompatibility, can be specifically adapted.

Deposition conditions are characterized by low temperature and low gas pressure (temperature approximately 25°C and pressure = 1 – 5 × 10^-3 mbar). The film forming particle flux is adjusted by varying total gas pressure and high frequency power, and it is monitored by faraday cup particle flux is adjusted by varying total gas pressure and high frequency power, and it is monitored by faraday cup measurement. Based on several trial experiments an ion energy of 20 eV or less and an ion current density of 0.1 mA/cm² or less were chosen. This allowed not only a compact structure (hardness approximately 10 GPa, where steel is approximately 4 to 7 GPa) of the amorphous layer, but also excellent elastic properties, preventing the stripping off of a layer from the highly flexible stent material (E module approximately 60 GPa). These parameter settings ensured a moderate layer increase at the lowest possible thermal load of the substrate.

Layer thickness was determined by profilometry and ellipsometry on reference samples (Si wafer). Hydrophobicity (surface energy) was measured with a video based optical contact angle measuring device. Surface energy resulted in a slightly hydrophilic surface. The contact angle in the
surface/water/air system was determined with the sessile drop method and it was approximately 63 degrees.

The current investigations were performed on ureteral Double-J stents, which are a commonly applied and technologically highly challenging product to ensure long-term urinary drainage. Commercially available uncoated stents of equal length and shape were provided by several suppliers. All tested stents were composed of aliphatic PUR. This type of stent is routinely used at our clinic. After coating with DLC the stents were re-sterilized and packed.

In Vivo Treatment Attempts

In 10 carefully selected patients, including 1 male and 9 females, with different disease patterns and different indications for stenting DLC coated ureteral stents were tested for their ability to decrease the extent of crystalline biofilm formation (see Table). These patients were classified as having heavy encrustation since each experienced severe discomfort from recurrent stent encrustation for whatever individual reasons, resulting in a stent removal frequency of less than 2 months. Most patients had required permanent artificial urinary drainage for more than 2 years. Thus, for these patients an extensive database of experience existed with respect to encrustation frequency and the tolerance of common ureteral stents of all common surface types. These data could serve as the control because patient health status remained stable or poor, or even became worse during DLC coated stent application. Therefore, the experience gained with DLC coated stents could be compared with that previously gained with uncoated common ureteral stents in the same patient group.

All tests were performed in accordance with the relevant guidelines and regulations of the University of Bonn. Informed consent was obtained from all patients. The most frequent reasons for previous stent removal with a frequency of higher than 3 months were massive encrustation and blockage combined with symptomatic acute urinary tract infections caused by E. coli, Enterococcus faecium, E. faecalis, Enterobacter cloacae, Staphylococcus species, Klebsiella pneumoniae, Candida lusitanae, P. mirabilis and Torulopsis glabrata. During DLC stent indwelling patient urine was regularly investigated microbiologically for bacteriuria and causative organisms.

The table shows patient data and detailed indications for stenting. At our clinic tumor is by far the most frequent cause of long-term artificial urinary drainage and stone formers requiring permanent ureteral stents are relatively rare. The latter group is in general more prone to stent encrustation than other patients. However, in an advanced stage of tumor progression metabolic derangement can lead to urinary compositions that finally show comparably high lithogenicity. This is similar to that in urine from patients with severe urolithiasis related to metabolic disorders such as hypercalciuria, hyperoxaluria or hypocitraturia. When justifiable and possible, cystoscopic views were obtained of the bladder before stent removal to show the lower end curl(s) of the catheter and document the actual in vivo situation. This ensured that an encrustation-free catheter surface after removal was not an erroneous result of the removal procedure, but clearly related to the DLC surface.

Physicians applying the stents were assessed for their experience with stent placement and removal, and stent related morbidities that may occur during indwelling. Also, patients were surveyed for stent associated discomfort or other strikingly negative symptoms experienced in relation to DLC coated stents.

RESULTS

In 10 patients with recurrent stent encrustation, including some with such a history for many years, a total of 26 DLC coated stents were tested for the ability to prevent encrustation, and decrease stent related side effects and discom-

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<th>Patient data and indications for stenting</th>
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<td>VR—F—65</td>
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<sup>*</sup> Concentrations less than 10<sup>5</sup>/ml.
fort. There was a 2,467-day period of experience (see table). Mean stent indwelling time was 97 days (range 10 to 365).

In none of the patients did a primarily stent related complication occur. In all cases exceptionally facile handling; a less painful replacement procedure and high tolerance for application were reported by physicians and patients. Due to low friction the coated stents could be placed and removed much more easily than the stents that patients had previously experienced. The frequency and severity of acute symptomatic urinary tract infections were distinctly decreased.

Even after a multiple of the indwelling time of previously used commercial stents no crystalline biofilm formation was cystoscopically observed on the surface of DLC coated stents (figs. 2 and 3). In all cases the coated surface remained free of encrustation. Only patient H. U. had a soft mucoid covering on the stent bladder side curl, which was easy to wipe, after an indwelling time of 77 days. Unfortunately this period was characterized by massive and progressive worsening of health status, which developed into almost full metabolic derangement. Infra-red spectroscopic analysis of the mucus revealed that the substance was composed of 100% protein. No crystalline fraction was found.

In patients not primarily affected by urolithiasis the guidewires easily passed the stent lumen without any blockage related problems during replacement. In patient R. F. with urolithiasis this procedure was associated with the usual difficulties.

DISCUSSION

DLC (a-C:H) coatings are characterized by a number of clinically relevant advantages. Due to their chemical composition and the substrate saving plasma deposition process the a-C:H layers are fully biocompatible.

In the current study we used PUR stents. However, the coating can be applied to all currently known materials used for urinary stents and catheters, eg silicone and latex or proprietary modifications of polymers, eg C-Flex®, Silitec® or Tecoflex®. The surface properties of the coated stents are then identical because the DLC coating covers the entire surface.

In vivo treatment attempts clearly revealed an effective decrease in in vivo biofilm formation and encrustation tendencies in 9 patients with severe tumor and 1 with renal tubular acidosis related to pathologically altered urinary composition. The ultrasmooth carbon surfaces are characterized by highly decreased friction and consequently there is high patient tolerance, eg a decreased frequency of hematuria. In addition to decreased encrustation, greater comfort and, thus, improved quality of life for the patient are important aspects, considering the long list of stent associated morbidities and related discomfort normally caused by standard stents.

The possibility of performing particular adjustments to the physicochemical properties of the surface in respect to, for example, surface free energy and hardness offers the promising opportunity of producing indication specific optimum coatings, resulting in fewer replacements and optimized performance. Thus, DLC coated ureteral stents and indwelling transurethral catheters can provide great relief.
to patients with urinary disorders. However, the reason for the shown positive effects of DLC coatings must still be clarified. Generally preventing bacterial attachment, stopping bacterial growth, disrupting interbacterial communication or dissolving the biofilm matrix can be considered possible mechanisms for decreasing biofilm formation and encrustation.20 Because DLC is chemically inert, the latter 2 options seem less probable. In conclusion, the potential of the entire spectrum of the different DLC coatings on urological catheters remains to be identified in further systematic investigations.

ACKNOWLEDGMENTS

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<th>Abbreviations and Acronyms</th>
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<tr>
<td>a-C:H = amorphous carbon</td>
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<tr>
<td>DLC = diamond-like carbon</td>
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<td>IR = infrared</td>
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<td>PUR = polyurethane</td>
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