Hydrogenated amorphous carbon coatings on implants drastically reduce biofilm formation and water permeation

Falk Bernsmann¹, Norbert Laube¹*, Gerhard Baldsiefen² and Mattia Castellucci³

¹ NTTF Coatings GmbH, Maarweg 32, D-53619 Rheinbreitbach, Germany
² Institute of Thin Film Technology, Kaiserslautern University of Technology, Maarweg 32, D-53619 Rheinbreitbach, Germany
³ Università degli Studi di Trieste, piazzale Europa 1, I-34127 Trieste, Italy

E-mail: norbert.laube@nttf-coatings.de

Abstract. Inflammations and crystalline bacterial biofilms (encrustations) remain a major complication in long-term artificial urinary tract drainage. To solve this problem we present urological implants with coatings made of amorphous hydrogenated carbon (a-C:H) that show excellent protection from encrustation in-vitro as well as in-vivo. Part of the success of a-C:H coatings is attributed to their ability to act as a diffusion barrier between an implant and the body, which prevents leaching of solvents from polymeric implants. To further enhance their barrier properties a-C:H coatings are combined with parylene coatings to develop diffusion-barrier multilayer coatings with a total thickness between 0.2 µm and 0.8 µm. The combination of the two types of coatings leads to a reduction of water diffusion by a factor of up to ten with respect to uncoated 25 µm thick polyimide sub-strates. The diffusion of water vapour from a controlled atmospheric pressure chamber through coated foils to a vacuum chamber is measured in a custom-built device.

1. Introduction

1.1. Modifications on implants and other biomedical devices

Thanks to the progress of medical technology, more and more sophisticated implants like in-situ blood-pressure sensors, cardiac pacemakers or retinal implants are developed. Also “routine” implants such as indwelling urological stents and catheters, the latter in principle in use since ancient times [1], have been undergoing continual improvement since that time. The limiting factors on implantation times are governed by the (undesired) interactions of the implant surface with the body and vice versa. In particular the build-up of bacterial and/or crystalline biofilms or mucus deposits, as well as the attachment of debris, often leads to the need of early implant removal. Furthermore, many implant bulk materials, for example silicon or polyurethane, do not withstand the corrosive body environment, or they can leach toxic substances, such as heavy-metal ions or plasticisers. In both examples, improvements of the implant’s biocompatibility can only be achieved with an optimization of the implant/body interface: in the specific cases by prevention of bacteria adhesion and by encapsulation (Table 1). Tailor-made thin film coatings, which neither alter the implant’s surface topography to an undesired extent nor hinder its functionality, are needed. Concerning encapsulation traditional polymers and even common high barrier coatings like poly-p-xylylenes (parylenes) are often not sufficient to provide the necessary diffusion-tightness.

* To whom any correspondence should be addressed.
Table 1: Properties of implants and other medical devices that can be adapted by coatings to complement the bulk materials’ properties.

| Chemical Properties | Task | Problem Area (Examples) | Example of Use |
|---------------------|------|-------------------------|----------------|
|                     | Diffusion barrier | Irritations and inflammations due to migration of substances out of plastic materials / polymers | Urological implants |
|                     |                   | Entry of body-fluids into electrically active implants | Neuroimplants |
|                     |                   | Avoidance of uncontrolled migration of reactive materials | Prostate seeds |
|                     | Corrosion prevention | Sterilization of metallic medical devices | Increase of life-time of, e.g., surgical instruments and endoscopes |
|                     | Control of adhesion of bacteria, cells, proteins and biomolecules | Biofilm formation and growth | Cardiological and urological implants |
|                     |                   | Contact lenses with low protein adhesion | |
|                     | Functionalization with biomolecules | Active molecules no longer have to be incorporated into the basic (bulk) material (e.g. polymers) | Coatings of implants with growth- and adhesion factors to stimulate tissue formation (orthopédics, trauma surgery, dentistry) |
|                     |                   | Generation of anchor molecules for the binding of locally acting drugs (e.g. „drug-eluting“ stents, chitosan) | |
|                     |                   | Fabrication of anti-bacterial but non-cytotoxic surfaces on urological catheters and stents | |
| Physical Properties | Increase of resistance of wear and tear, hardness | Early implant- or instrument failure | Orthopedic and dental implants |
|                     | Wear debris gets into bloodstream / body | Increase of scratch resistance of optical lens systems | |
|                     | Optimizing of tribological properties (coefficient of friction) | Friction too high or too low | Gilding properties of implants and implant-guide wires |
|                     | Variation of topography | (Micro-)Roughness | Nano-structuring of dental implants for improvement of osseointegration |
|                     | Adhesion of unwanted and non-adhesion of desired substances or microorganisms, respectively | Hydrophobicity | „Super-hydrophilic” surfaces allow for instantaneous wetting of the implants with blood, thereby significantly improving e.g. osseointegration |
|                     | Adjacent wettability | Adhesion of unwanted and non-adhesion of desired substances, resp. | Lotus effect, „easy-to-clean”-surfaces |
|                     | Optical properties | Reduced (micro-)roughness | Antiinfection- and antifogging-coatings on optical lenses |
|                     | Electrical conductivity | Non-biocompatible electrodes | Cardiac pacemakers, neuroimplants |
|                     | Radioactivity | Local radiotherapy | Radiotherapeutic procedures at locally limited cancer |
| Biological Properties | Increase of body-compatibility | Implant failure / rejection | Vessel supports (stents) |
|                     | Hemocompatibility | Adhesion of blood components, blood coagulation | Artificial heart valves, dialysis-tubes, bone implants |
|                     | Cell adhesion | Adhesion | Burn wound covering |
|                     | Antibacterial activity | (Implant-associated) Infections | Implant coatings with photo-catalytically active titanium dioxide |

1.2. Crystalline biofilm formation: a common problem in urology

Urologic indwelling catheters and especially ureteral stents are among the most frequently used medical implants. A wide spectrum of indications for artificial urinary drainage exists in order to ensure unobstructed transport of urine, which can be compromised for example due to a tumour applying a confining pressure on the ureter (Figure 1).

However, unpleasant side-effects of artificial urinary drainage are frequent. In most cases they are triggered by bacteria entrained into the urinary tract already during catheter placement or migrating on the inner or outer part of the indwelling implant’s surface. In fact, most patients with an indwelling urinary catheter for 30 days or longer develop bacteriuria [2]. Complications of intermediate (2-6 weeks) and long-term (> 6 weeks) catheterization include pain, urgency, discomfort and bladder irritability (bladder spasms and contraction) as well as chronic renal inflammation, chronic pyelonephritis, nephrolithiasis, cystolithiasis, recurrent (mostly) polymicrobial urinary tract infections with symptomatic pyelonephritis, bacteraemia, sepsis and death [3, 4, 5, 6, 7, 8, 9].

These serious complications can be caused by the formation of organic biofilms and deposition of inorganic salts on the implants’ surfaces thereby often provoking fatal functional failure. Within only a few hours in-situ, a conditioning film composed of urinary proteins forms, and subsequently bacteria start to attach to the surface of the implants. After only a couple of days the surfaces of the urinary stents and catheters are colonized by bacteria forming an infectious bacterial biofilm. Consecutive deposition of insoluble inorganic salts - as a consequence of bacteria-metabolism-related rise in urinary pH - leads to the formation of sharp-edged crystals (Figure 2).

A bacterial biofilm can be considered as a chronic urinary tract infection, which, under varying antibiotic therapy, may easily result in therapeutically hard to reach multi-resistant bacterial strains. For this reason the catheters and stents have to be replaced at the first indication of complications. In the
extreme case, the intracorporeal indwelling time of a standard implant may be only a few days. Early replacement is unpleasant to the patients and produces high economic costs. In the US, the overall annual cost for medical intervention of catheter-associated urinary tract infections alone amount to $424 million to $451 million [10] and catheter-associated bacteremia is estimated to cost approximately $2,900 per episode [11].

Figure 1: Left: Typical urological implants for different use (a: suprapubic nephrostomy catheter; b, d: suprapubic bladder catheters; c: nephrostomy catheter; e, f, g: transurethral bladder catheter; h, i: ureteral stents). Right: Ureteral stents are implanted in the ureter between kidney and bladder to ensure unrestricted urinary outflow (computer tomography (CT) showing a large ureter-confining tumour requiring permanent stent placement).

Figure 2: From left to right: Typical confining encrustation of an ureteral stent after only a few weeks of indwelling, “forgotten” ureteral stent with huge mineral deposition (approx. 150 g in total) as an extreme example of crystalline bacterial biofilm (CT-view of the in-situ situation and explanted stent). The embrittled stent broke during explantation. Crystalline material on both stents is composed of Mg- and Ca-phosphates (90 % carbonate (hydroxyl-)apatite and 10 % struvite, and 50 % carbonate (hydroxyl-)apatite and 50 % struvite, respectively).

1.3. Hydrogenated amorphous carbon coatings

Amorphous carbon (a-C), also called diamond-like carbon (DLC) is a class of materials with a wide range of properties from graphitic to diamond-like that can be tuned by the carbon hybridisation and hydrogen content. DLC are generally characterised by good tribological properties, high chemical resistance and biocompatibility [12]. The typically 10-200 nm thick DLC-surface coatings described in this work were especially developed for medical and biological applications and belong to the sub-
group of hydrogenated amorphous carbon, a-C:H (approximately 20 at% hydrogen). Due to the processing temperatures below 50 °C, the a-C:H coatings are particularly suitable for flexible polymer-based medical implants; the maximum operating temperature is 600 °C. Despite a Vickers hardness of 1,000-3,000 HV and a friction coefficient against steel as low as 0.05, the surface conform coating is still very flexible and does not detach even after extreme flexing.

2. Experimental Part

2.1. Surface preparation

Different hydrogenated amorphous carbon coatings were deposited on polyurethane (PU) tubes taken from ongoing production of a stent manufacturer by plasma-enhanced chemical vapour deposition (PECVD) using a radiofrequency-(RF)-driven (13.56 MHz), inductively coupled plasma beam source (CCR COPRA®; stainless steel, 266 mm diameter) with a transverse magnetic field providing a high density plasma [13, 14, 15]. Deposition conditions are characterized by low temperature and low gas pressure (temperature approximately 25 °C and pressure of 0.1 Pa to 0.5 Pa). The film forming particle flux is adjusted by varying total gas pressure and high frequency power, and it is monitored by faraday cup measurement. With ion energies between 15 eV and 25 eV and ion current densities between 0.1 µA/cm² and 0.2 µA/cm², the energy flux density is reduced by two orders of magnitude with respect to the conditions typical for the production of diamond-like carbon films [16]. This allowed not only for a compact structure (Vickers hardness $H \approx 10$ GPa) of the amorphous layer, but also excellent elastic properties, preventing the stripping off of a layer from the highly flexible stent material (Youngs modulus $E \approx 60$ GPa) [17]. The ratio $E/H$ of 6 is in agreement with the constraint model of Angus et al. [18]. That means the carbon films are thermodynamically stable. The film forming parameter settings ensured a moderate layer growth at the lowest possible thermal load of the substrate.

In total, six different a-C:H coatings were tested against an uncoated polyurethane reference (B-PU). The thin films a-C:H-2, a-C:H-125 and a-C:H-Uro were deposited each from acetylene ($C_2H_2$-flow $\approx 30$ sccm) precursor gas at different gas pressures ($\approx 0.07$ Pa, $\approx 0.15$ Pa, $\approx 0.15$ Pa) and RF-powers (150 W, 125 W, 250 W). In order to achieve different surface wettabilities the gases O$_2$, NH$_3$ and CF$_4$ (gas flow $\approx 30$ sccm, each; RF-power $= 125$ W), respectively, were added during the coating process for the deposition of the other three thin films a-CO$_x$-H, a-C:H-NH$_3$ and a-C:H-F-2.

Multilayer coatings were deposited on polyimide foils (DuPont Kapton HN, nominal water vapour transmission rate $WVTR = 54$ g/day/m² at 37 °C) of 5 cm diameter and 25 µm thickness. Hydrogenated amorphous carbon coatings of 20 nm thickness were deposited using the same system mentioned before but with a different procedure: First, the samples were exposed to an oxygen plasma for 10 min at a pressure of 2 Pa and a RF power of 200 W for surface cleaning and activation. The a-C:H layers were deposited from acetylene ($C_2H_2$) within 10 min at a pressure of 0.7 Pa and a power of 107 W. Parylene C was deposited from 2-chloro-[2,2]-paracyclophane using a Gorham process [19] with a chamber pressure of 3 Pa, a vapouriser temperature of 150 °C and a reactor temperature of 720 °C.

The coating thickness was measured on silicon slides coated in the same processes as the polyimide foils either with an ellipsometer (Dr. Riss Ellipsometerbau EL X-02C, 633 nm wavelength, variable angle of incidence) for a-C:H coatings or with a profilometer (Veeco Dektak$^3$) for parylene coatings.

2.2. In-vitro experiments for determination of the encrustation tendency

The encrustation tendencies of the seven different surface types were tested in several runs. Each run consisted of eight coated (two coating types, four samples each) and four uncoated reference samples.

The samples were simultaneously incubated under defined reproducible conditions in the extended in-vitro crystallization model. This so-called “encrustator” device is a water bath thermostated flow-crystallizer model according to the mixed suspension mixed product removal method (MSMPR) [20] and it allows the simultaneous investigation of twelve test samples in artificial or native urines. A detailed description of the experimental procedure can be found elsewhere [21]. For better reproduci-
bility of the experiments, the systematic investigations presented here were performed using synthetic urines composed of dissolved salts and few low-molecular substances [22].

The common urinary tract infection is induced by urease-producing microorganisms. As urease splits urea into ammonia and CO₂, a typical pH rise in the urine is observed. The increase in the urine's pH provides the supersaturation required for the formation of phosphate salts. In vitro, this pH increase was triggered by direct addition of urease to the artificial urine. Two partial solutions, A (containing, inter alia, urea and albumin) and B (containing, inter alia, urease), were each fed at a constant rate into the encrustator's reaction chamber and mixed; at this moment, a potentially crystal forming solution exists. The constant addition of fresh urine avoids depletion of one of the lithogenic substances during incubation and maintains a quasi-constant urinary supersaturation within the reactor, allowing for salt formation over the entire incubation time. An overflow keeps the urine volume in the reaction chamber constant.

After 45 hours incubation time, morphology, amount and composition of the precipitations were determined. The salts obtained on the different stent surfaces were dried at room temperature (24 h), photographically documented, and then dissolved in 10 mL 1 N hydrochloric acid.

From the solution's Ca, Mg, and PO₄²⁻ concentrations, a qualitative and quantitative analysis of the crystalline material was performed, using a procedure previously evaluated by infrared spectroscopy and routinely used for geochemical rock characterization. By applying mass balance calculations, a standardized mineral paragenesis of the dissolved salts was evaluated. This computed mineralogical composition consists of the Ca-phosphates carbonate (hydroxyl-apatite and brushite, and of the Mg-ammonium-phosphate, struvite. These salts were determined in a number of investigations to represent the most abundant minerals found in biofilms formed on ureteral stents. They occur either as single phases or in paragenesis. Their (ideal) composition is assumed to be Ca₁₀(PO₄)₅CO₃(OH)₂, CaHPO₄ × 2H₂O, and MgNH₄PO₄ × 6H₂O, respectively [23, 24, 25].

For each run, a normalized amount of minerals for each surface type was calculated by dividing the molar concentrations of all minerals found on each surface type by those values found for the uncoated PU surface. This allows for both qualitative and quantitative analysis of the crystalline material.

### 2.3. Surface energy measurement

The surface properties determined in this study were motivated by the general ideas and thermodynamic models on the biofilm and mineralization determining surface properties [26, 27, 28] claiming hydrophilic/hydrophobic interactions as one of the key factors of bacterial adhesion and crystallization [29]. The surface free energies of the samples were determined according to the method of Owens, Wendt, Kaelble and Rabel [30] by optical contact angle measurements with three different test liquids (water, diiodomethane, ethylene glycol) using the sessile drop method and an OCA 15 plus (DataPhysics Instruments GmbH, Filderstadt, Germany) as described in [21].

### 2.4. Diffusion measurement

We have developed a device to measure the diffusion of various gases from a controlled environmental chamber through thin foils to a high vacuum chamber, where the diffused species were detected by a quadrupole mass spectrometer (Hiden Analytical Halo 201). The principle of the device is sketched in Figure 3. The sample foil was mounted on a perforated titanium sheet (0.1 μm thickness, 500 holes of 70 μm diameter) to avoid deformation and damage due to the pressure difference. The temperature of the sample and the environmental chamber were controlled with a computer program (National Instruments LabVIEW) by ceramic heaters. The humidity in the environmental chamber was automatically adjusted by the inflow of wet or dry gas.

Before starting a measurement the environmental chamber was flooded with argon and the high vacuum chamber was evacuated to a water partial pressure (pH₂O) of about 3 μPa. Once pH₂O has stabilised, the sample temperature was set to the desired value and the environmental chamber was filled with air at ambient pressure and 100 % relative humidity. An exemplary record of pH₂O during an experiment is shown in Figure 4.
Depending on the temperature, $p_{H_2O}$ stabilises at different equilibrium values. The difference in $p_{H_2O}$ ($\Delta p_{H_2O}$) between the equilibrium pressure and the background pressure measured at the same temperature without water in the environmental chamber was supposed to be proportional to the water vapour transmission rate ($WVTR$). The value of $\Delta p_{H_2O}$ measured with an uncoated polyimide foil of known $WVTR$ was used to obtain a calibration factor for calculating the $WVTR$ of coated foils from their $\Delta p_{H_2O}$. Once the $WVTR$ of uncoated ($WVTR_f$) and coated ($WVTR_{cf}$) foils was known, the $WVTR$ of the coating itself ($WVTR_c$) could be calculated as

$$\frac{1}{WVTR_c} = \frac{1}{WVTR_{cf}} - \frac{1}{WVTR_f}$$

**Figure 3:** Sketch of the diffusion-measurement device built for this work.

**Figure 4:** Partial pressure of water diffusing through a polyimide foil coated with 20 nm of a-C:H and 800 nm of parylene at increasing temperatures. The blue line represents the recorded values; the red lines represent the equilibrium pressures used to calculate the water vapour transmission rate.
3. Results

3.1. Surface free energy and crystal formation

Figure 5 and Figure 6 show the results obtained from the in-vitro crystallization experiments and those of the determinations of the free surface energies, respectively. The positive effect of any of the different a-C:H film compositions on the encrustation tendency is clearly shown. All types of a-C:H films tested show less encrustations than the commercially available uncoated “premium stent” (“B-PU”) claimed being optimized for “long-term indwelling”, which is taken as reference. According to the approach of [29], the slightly hydrophilic a-C:H-films (water contact angles between 53.3° (± 0.5°) and 69.2° (± 0.4°) compared to 30.9° (± 0.6°) for uncoated B-PU) are in particular suitable to hinder the adherence of microorganisms with “hydrophobic” properties.

Figure 5: Different surface types vs. normalized amount of phosphate salts found on these surfaces after 45 hours of incubation. The calculated fractions of the standardized mineral content are also given.

Figure 6: Different surface types in order of decreasing mineral amounts vs. surface free energy. The total surface free energies as well as their polar and disperse fractions are shown for each examined coating.
3.2. Water vapour permeation

Figure 7 shows the WVTR of polyimide foils coated with layers of hydrogenated amorphous carbon and parylene of two different thicknesses measured at foil temperatures from 35 °C to 70 °C. The two samples labelled “DLC” are a-C:H coatings deposited by the same process in two separate batches. The a-C:H coatings on top of the two thicknesses of parylene are also deposited in two separate batches. Since there are only slight differences in the properties of the a-C:H coatings from different batches, all of them are labelled “DLC” in figure 6 and figure 7 and supposed to be equivalent for the results’ interpretation.

As expected each additional layer reduces the WVTR. At 35 °C the best performing trilayer system made of 20 nm a-C:H, 800 nm parylene and another 20 nm a-C:H (DLC-P800-DLC) reduces the WVTR by a factor of ten compared to an uncoated foil (PI) while increasing its thickness by only 1/30. At higher temperatures the effect of the coating is less pronounced with a reduction in WVTR by a factor of six at 70 °C. This is probably due to the development of cracks because of the different thermal expansion coefficients of parylene (35⋅10^{-6}/K [31]), polyimide (20⋅10^{-6}/K according to supplier) and a-C:H (1⋅10^{-6}/K to 7⋅10^{-6}/K [32]).

The calculated WVTR of the coating systems without polyimide are represented in Figure 8 in comparison to nominal values of commercially available parylene C monolayers. Thin (20 nm) hydrogenated amorphous carbon monolayers have a much lower WVTR than the thicker parylene monolayers. Adding two a-C:H layers to a parylene layer leads to a reduction in WVTR by a factor of thirteen while increasing its thickness by only 5 % (P800) or 18 % (P225).

3.3. In-vivo experience

The presented a-c:H coated stents are on the market since four years. Over 15,000 ureteral stents were sold and to date no problems neither from the patients and urologists nor from the quality management system occurred. Patients as well as doctors report an increased quality-of-life and a facilitated placement procedure.

The complication-free indwelling time is considerably longer due to less frequent painful body implant interactions and significantly reduced bacterial biofilm formation.
4. Conclusions

The in-vitro investigations on differently composed a-C:H-films clearly revealed that a stent’s en-crustation tendency can be stepwise reduced. The especially developed a-C:H single-layer coatings create a durable and highly flexible bidirectional seal on ureteral stents, which has clinically proven to increase the patients’ quality-of-life as well as to decrease the formation of crystalline bacterial biofilms, thereby significantly prolonging complication-less indwelling times.

We have presented a custom-built device to analyse the gas diffusion through coated foils in a temperature and humidity controlled environment. Thanks to the detection with a mass spectrometer the same setup can be used to measure the diffusion of various gases in relatively short measurement times. Using standards the obtained values can be compared to other measurement devices. In case of water diffusion the detection limit in transmission rate is below 1 g/day/m². Due to the omnipresent water contamination in the vacuum system this value is relatively high. For other gases considerably lower detection limits can be reached.

The investigated a-C:H–parylene–a-C:H trilayer systems reduce water transmission through polyimide foils much better than parylene monolayers of comparable thickness. A trilayer system of 840 nm total thickness, for example, can reduce water transmission by a factor of ten compared to an uncoated polyimide foil. Both types of coatings are deposited by standardised procedures in industrial scale coating devices at substrate temperatures below 40 °C. Therefore the coating systems can easily be applied to a wide range of substrate materials.

The high variability in composition and related physical properties make low-temperature plasma-deposited a-C:H-films a promising concept for the improvement of implants of various bulk materials.

Showing at the same time biofilm and diffusion reducing qualities the potential of a-C:H-coatings may be superior to most other concepts of implant surface modification.
5. Outlook

No “perfect” surface coating will exist that solves all of the problems at the same time that may occur during the interaction between an implant and its surrounding body fluids. Developments towards more individualized solutions may be a strategy to reduce implant-related discomfort, inflammations, infections and more severe medical conditions.

Depending on deposition conditions, density and composition of a-C:H films can be varied in order to provide tailor-made surface solutions required for the particular environmental situation (i.e. the patient’s individual medical condition) or desired (active or passive) interaction. Using anchor-sites (e.g. amino-groups, -NH₂) the a-C:H coatings can be given specialized functions by controlled covalent binding of ‘therapeutically active’ molecules (e.g. topically effective drugs, proteins, DNA-fragments, bactericidal agents). More than three amino-groups per nm² can be created on a-C:H.

With regard to intra-luminal formation of bacterial and crystalline deposits, in particular at urethral catheters, liquor shunts or indwelling venous cannulae, coating of the implant’s inner surface with a-C:H may further rise the implant’s complication-free indwelling time. Development of inner a-C:H coatings for tubes with high aspect ratios is in progress.

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