Optimization of hemocompatibility and functionality by a hybrid design of cardiovascular stents

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Abstract

Thrombogenesis at artificial surfaces can be described by means of an electron transfer reaction.[1] The inhibition of this oxidation process is the prerequisite for high hemocompatibility requiring semiconducting properties of the solid´s surface. However, due to the high brittleness of all known solid semiconductors, the requirements for high hemocompatibility and mechanical stability cannot be met by one material. Therefore, a hybrid design of implants is introduced as a new approach to improve the hemocompatibility of cardiovascular stents.

Key Words

Palmaz Schatz stent, amorphous silicon carbide, hemocompatibility, biocompatibility, hybrid design, coating, in vitro tests

Introduction

Despite sufficient biocompatibility, the hemocompatibility of the currently available coronary stents is still limited. Beside clinical, anatomical, and procedural factors,[2,3] and as a consequence of the inherent thrombogenicity, coronary stenting is associated with a significant number of acute (1% to 3%) and subacute thrombosis (9% to 18%).[4,5,6] The heavy anticoagulation necessary to avoid stent thrombosis, leads to major bleeding events and vascular complications in 8% to 16% [2,3] of patients, often requiring surgical repair.[7]

Different strategies, *e.g.*, intravascular ultrasound guided stent placement,[8] radial approach,[9] restriction of stent placement only in arteries ≥ 3 mm,[7,10] high pressure implantation techniques, and "overdilatation" [11] were suggested to overcome these major limitations. However, due to an increase in costs and decrease of the indication-spectra, they are generally not accepted as a satisfactory solution of the problem.

A new concept to directly address stent thrombosis is the improvement of the hemocompatibility of the materials themselves.

Background

The hemocompatibility of alloplastic materials is determined by their surface properties. Above all, smooth surfaces are necessary to avoid the activation of the clotting process via trapped corpuscular blood components. However, different materials with the same surface roughness affect the clotting system differently. Thus, thrombosis is also caused by electrochemical interactions of blood coagulation proteins with the surface of the implants.

The first analysis on this issue was performed by Sawyer, Brattain and Boddy,[12] who found a relationship between thrombosis and the electrochemical potential of materials. Additionally, the work of Baurschmidt proved that thrombogenesis at artificial surfaces is induced by an electron transfer from the protein solution to the solid surface.[13] During recent years, a physical model for this behavior was developed.[1] This model describes a third pathway for thrombogenesis by means of an electron transfer reaction. If an electron tunnels from a blood clotting protein — especially fibrinogen — to an alloplastic material, the protein changes its tertiary structure, which results in clotting.

This model allows derivation of the electronic or electrochemical requirements for high hemocompatibility as follows:

- To avoid degeneration of blood proteins, the electronic transfer current must be minimized, which correlates with a minimized density of electronic states at the transfer level.
- This requires semiconductivity properties of the surface of the implant with an energetic difference between the Fermi level and the upper balance band edge ($E_f - E_v$) of at least 1.6 eV.
- To stabilize the electrochemical equilibrium at the interface, the electrical resistance must be lower than 10^4 Ω cm.

On the one hand, this model explains the enhanced hemocompatibility of passivating metals (like titanium) in comparison to noble metals due to the semiconducting properties of most passivating oxides. On the other hand, defined semiconductors with taylored electronic properties should exhibit superior hemocompatibility.

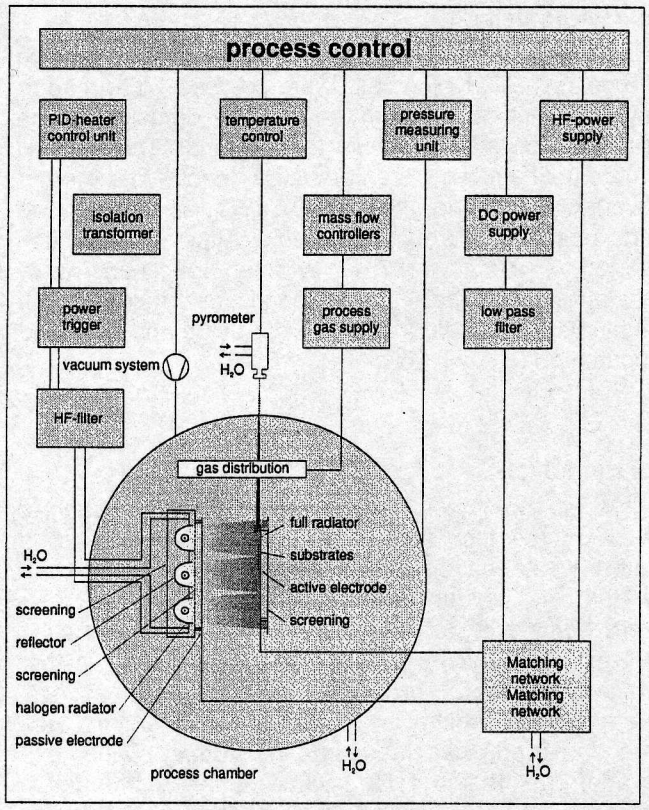

Figure 1. *Equipment for the deposition of amorphous silicon carbide.*

Materials and Methods

Concerning these theoretical aspects, hemocompatible materials must be semiconductors. However, due to the high brittleness of all known semiconductors, the requirements for high hemocompatibility and mechanical stability cannot be met by a single material. As a solution, materials with well known mechanical properties are coated with a thin semiconducting film, which represents a hybrid design. Thus, the substrate material is selected to meet the functional requirements, whereas the surface coating assures the hemocompatibility of the device.

A material, which meets all the electronic requirements mentioned above, is silicon carbide in an amorphous, doped and hydrogen-rich modification (a-SiC:H). This material is deposited using the plasma enhanced chemical vapour deposition (PECVD) technique. Figure 1 shows the technical performance of the PECVD equipment, which allows a-SiC:H deposition as well as different plasma physical pre-treatments necessary to enhance the adhesion of the coating.

The films were deposited by a plasma enhanced activation of a mixture of silane (10% diluted in hydrogen) and pure methane in a 13.56 MHz coupled plasma at 0.1 mbar. To achieve a low density of states in the band gap, the substrate temperature was held at 250 C. The n-doping was realized with phosphine, resulting in suitable electrical conductivities. The plasma activated gaseous components are deposited on the surface of the stents forming a homogeneous and defect-free coating. The process parameters with respect to biocompatibility as well as the hemocompatibility of amorphous silicon carbide were optimized.

By applying plasma physical pre-treatments prior to the deposition of a-SiC:H, the adhesion of the coating was improved, thus preventing spallations and defect formation during the dilatation of the stent.

To evaluate the suitability of amorphous silicon carbide as a coating for stents, the biocompatibility as well as the hemocompatibility of the coating were investigated using the following methods.

a) Cytotoxicity

Cytotoxicity evaluation was performed using mice fibroblasts L929. The cells were covered with an agar-overlay culture medium to prevent damage of the sensitive cells by the solid test material. The a-SiC:H coated substrates were contacted directly to the agar-overlay with saline and 0.04M $CuSO_4$ as the negative and positive control, respectively. After an incubation period of 24 h at 37 C, all test specimens were removed. The cell response to the material was evaluated after coloring with trypian blue and a second incubation (2 h, 37 C).

b) Hemolysis

Sheep erythrocytes were mixed with an extract of a-SiC:H coated samples (37 C, 72 h). As a reference, aqua dest. and saline were added to the erythrocyte solution, representing a 100% and a zero hemolysis degree, respectively. After incubating all test solutions for 2 h at 37 C, the hemolysis test was performed on the centrifugation excess. Hemoglobin, which is released when erythrocytes are damaged, was measured by adding caliumhexa cyanoferrat III and caliumcyanid to the centrifugation excess. The resulting chemical complex (hemoglobincyanid) enhances the optical absorption of the test fluid at 546 nm, which was quantified.

c) Mutagenicity (Ames test)

The degree of mutation caused by a-SiC:H was investigated on mutated salmonella typhimurium (TA98, TA100, TA1535, TA1537). The a-SiC:H coated substrates together with positive and negative control samples were contacted to the cell culture. After an incubation period of 3 days at 37 C, the degree of inverse mutations of salmonella typhimurium around the test substances was measured by the amount of histidin, indicating the mutagenic potential of the test material.

d) Growth of Endothelial Cells

Human endothelial cells were isolated from the umbilicalis vein and distributed with a density of 10000 per cm^2 on a culture medium. The growth behavior of these cells on a-SiC:H and on reference materials was evaluated after incubation periods of one, four, and seven days. After removing the cells from the culture medium with trypsin, their quantity was measured with a counter.

e) Hemocompatibility

The in vivo behavior was investigated by implanting three conventional Palmaz Schatz stents, which were coated with amorphous silicon carbide, in the arteria femoralis region of a pig for three days. To simulate worst-case conditions, no treatment against anticoagulation, as well as thrombocytic aggregation, was applied. After explantation, the specimens were histologically examined.

Clinical data

In 14 patients suffering from abrupt closure after conventional balloon angioplasty due to dissections with superimposed thrombotic occlusion, silicon carbide coated commercially available Palmaz-Schatz stents were implanted. After the procedure, the patients were treated with 500 U Heparin/hr until sheath removal the next day. Additionally, they received aspirin (320 mg daily) and ticlopidine (2x250 mg daily) without oral anticoagulation within the first month.

Results

The results of the in vitro tests are summarized in the following figures.

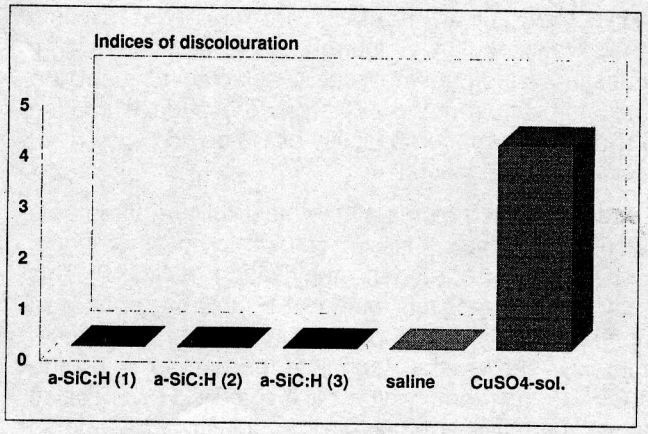

Figure 2. *Coloring zones around the test substrates indicating cytotoxicity.*

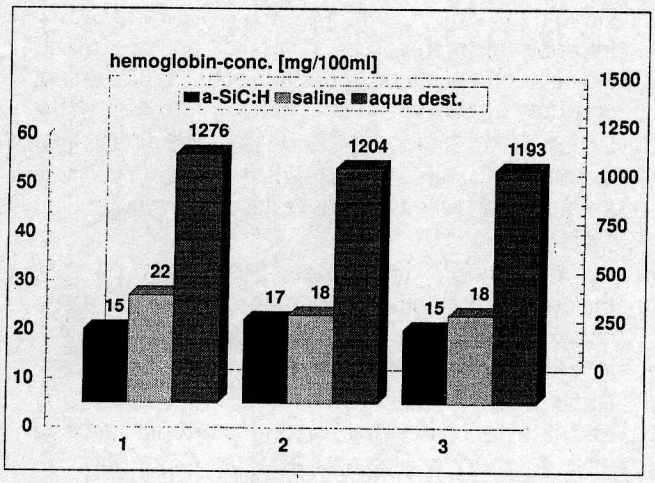

Figure 3. Concentration of hemoglobin in the centrifugation excess indicating damage to erythrocytes.

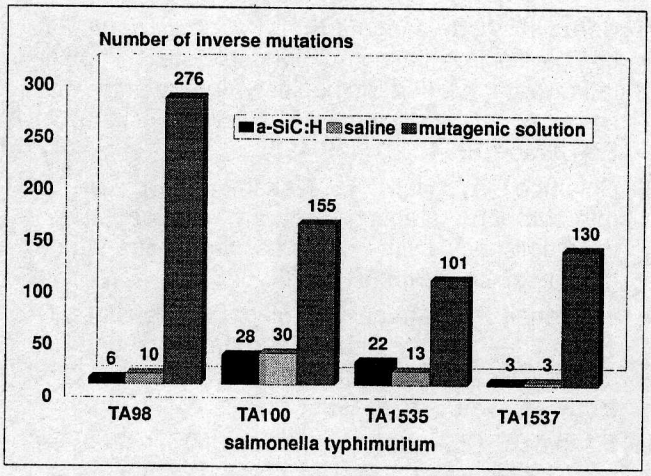

Figure 4. Number of inverse mutations of different cell cultures to assess mutagenicity.

Figure 5. Morphology of endothelial cells on a-SIC:H after growth period of 7 days.

In the cytotoxicitiy test (see Figure 2), the coloring indices from 0 to 5 represents graduate reactions from non-toxic (0 and 1) to moderately toxic (2 and 3) and toxic (4 and 5) behaviour. Therefore, amorphous silicon carbide does not cause any cytotoxic reaction to mice fibroblasts L929, whereas the positive and the negative reference show the expected behavior.

Figure 3 shows that the hemoglobin concen-tration, which was measured after interaction of sheep erythrocytes with the a-SiC:H extract, is similar to the concentration found after interaction with saline solution. Therefore, a-SiC:H does not provoke damage to red blood cells.

Concerning the Ames test, the number of mutagenic cells caused by a-SiC:H is similar to that obtained after contact with saline (see Figure 4). Therefore, no mutagenic potential can be attributed to a-SiC:H.

Human endothelial cells proliferate on amorphous silicon carbide, forming a complete covering of the surface as shown by scanning electron microscopy in Figure 5.

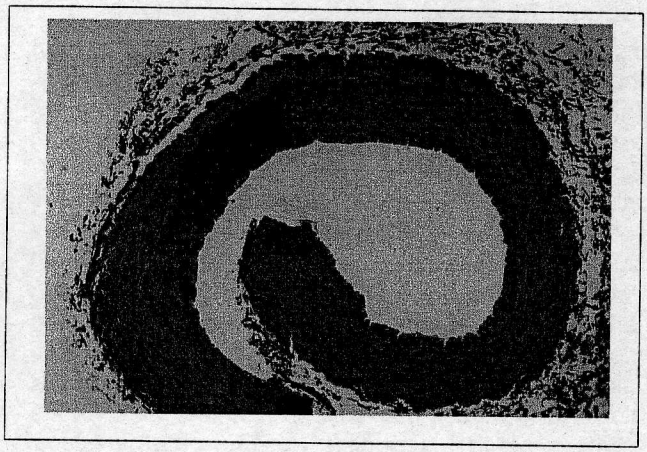

Figure 6a. Histology of an arterial blood vessel implantation of a-SIC:H coated Palmaz-Schatz Stents macroscopically

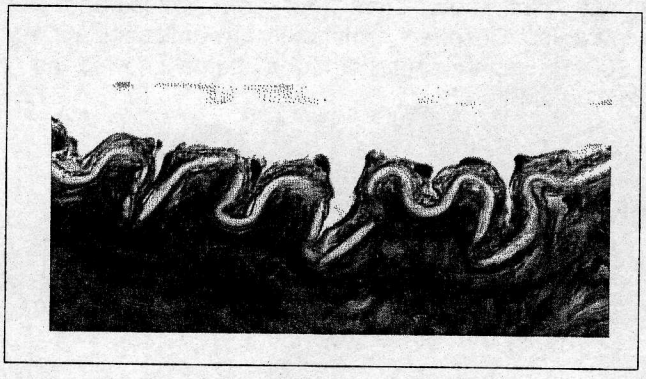

Figure 6b. Histology of an arterial blood vessel implantation of a-SIC:H coated Palmaz-Schatz Stents with higher resolution.

Quantitative evaluation shows the number of cells increasing by a factor of seven one week after cell seeding, indicating a fast cell growth compared to positive reference materials.

After a perfusion interval of three days, the lumina of all vessels, which were stented with amorphous silicon carbide coated Palmaz-Schatz stents, remained open. The histological evaluation of the vessel anatomy shows no signs of thrombus formation, macroscopically as well as microscopically (see figure 6a and 6b).

In the preliminary clinical trial the day after implantation, coronary angiography revealed open arteries in all patients without angiographic evidence for thrombus formation. The in-hospital and four-week outcome were both uneventful without clinical or electrocardographic signs for a subacute stent thrombosis in both patients. Due to the reduced anticoagulation, no bleeding complications occurred.

Discussion

Amorphous silicon carbide shows an ideal behavior concerning cytotoxicity, hemolysis and mutagenicity. Besides its excellent biocompatibility, the surface of the stented vessels stay free from any thrombus also indicating a high hemocompatibility. This was mainly attributed to the electronic properties of the amorphous silicon carbide surface layer, which considerably reduces the electronic transfer current from the protein solution to the alloplastic coating, thus preventing chemical reactions and protein degeneration. The biological evaluation of a-SiC:H demonstrated sufficient growth behavior of human endothelial cells, which indicates a rapid covering of the stent struts. Therefore, a fast reduction of the pharmaceutical regimen following stent implantation is expected.

The clinical data so far available in 14 patients with bail-out stent implantation after abrupt closure, are encouraging. Despite a remarkably low anticoagulation of 500 U Heparin per hour, the stents showed no evidence of thrombosis.

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