Silicon Carbide as an Anti-Thrombogenic Stent Coating: An Example of a Science-Based Development Strategy

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Summary

Coronary stenting has been proven to be a feasible and successful strategy in the therapy of coronary heart disease. However, even if the rate of clinical complications could be significantly reduced due to improvements in the mechanical properties of modern stent designs, there is further need and potential for enhancing clinical success by controlling the interactions between the artificial implant and the biological environment through the use of a suitable stent coating. Based on a physical model of the phase boundary between the surface of the implant and the blood protein fibrinogen as a precursor of thrombus formation, the electronic requirements of an anti-thrombogenic implant surface have been derived. This model of contact activation has been proven through several experiments with semiconducting, amorphous, hydrogen-rich, phosphorous-doped silicon carbide (a-SiC:H) matching the electronic requirements.

Key Words

Stent, thrombogenicity, contact activation, fibrinogen, fibrin, surface coating

Introduction

The most significant progress in interventional cardiology has been intracoronary stenting. Compared to balloon angioplasty, the rate of restenosis could be significantly reduced through additional stent implantation. However, despite improvements in the mechanical properties of modern stent designs, considerable complication rates, especially due to long-term restenosis, are still limiting therapeutic success.

The dominant mechanism of late restenosis is assumed to be the proliferation of smooth muscle cells resulting in neointimal hyperplasia. It is suspected that this proliferation is triggered by interaction processes at the interface between the implant and the biological environment. Thus, different mechanisms related to the stent surface, such as corrosion of the implant, inflammatory response of the vessel wall, and thrombus formation, may affect the clinical outcome of the procedure. The requirement of an "ideal stent" surface, as stated in Figure 1, can be summarized with the requirement that the surface behave like a "magic hat" in order to avoid short- and long-term complications related to the interaction processes between the implant and the patient's cells and proteins.

However, materials like 316L stainless steel, which is used for the bulk of the stent due to its superior mechanical properties, suffer from relatively poor hemo- and biocompatibility. Therefore, within the scope of a "hybrid design", the bulk of the stent should be coated with a different material tailored to reduce unwanted reactions of cells and blood proteins. Using the example of fibrinogen, which plays a crucial role in thrombus formation, the development of an anti-thrombogenic stent coating is described in this article:

- First, research results will be presented to show that the beginning of thrombus formation is caused by an electron transfer process that converts the fibrinogen molecule into fibrin. This conversion process is related to the release of the fibrinopeptides, which is analogous to the action of thrombin in the clotting cascade.

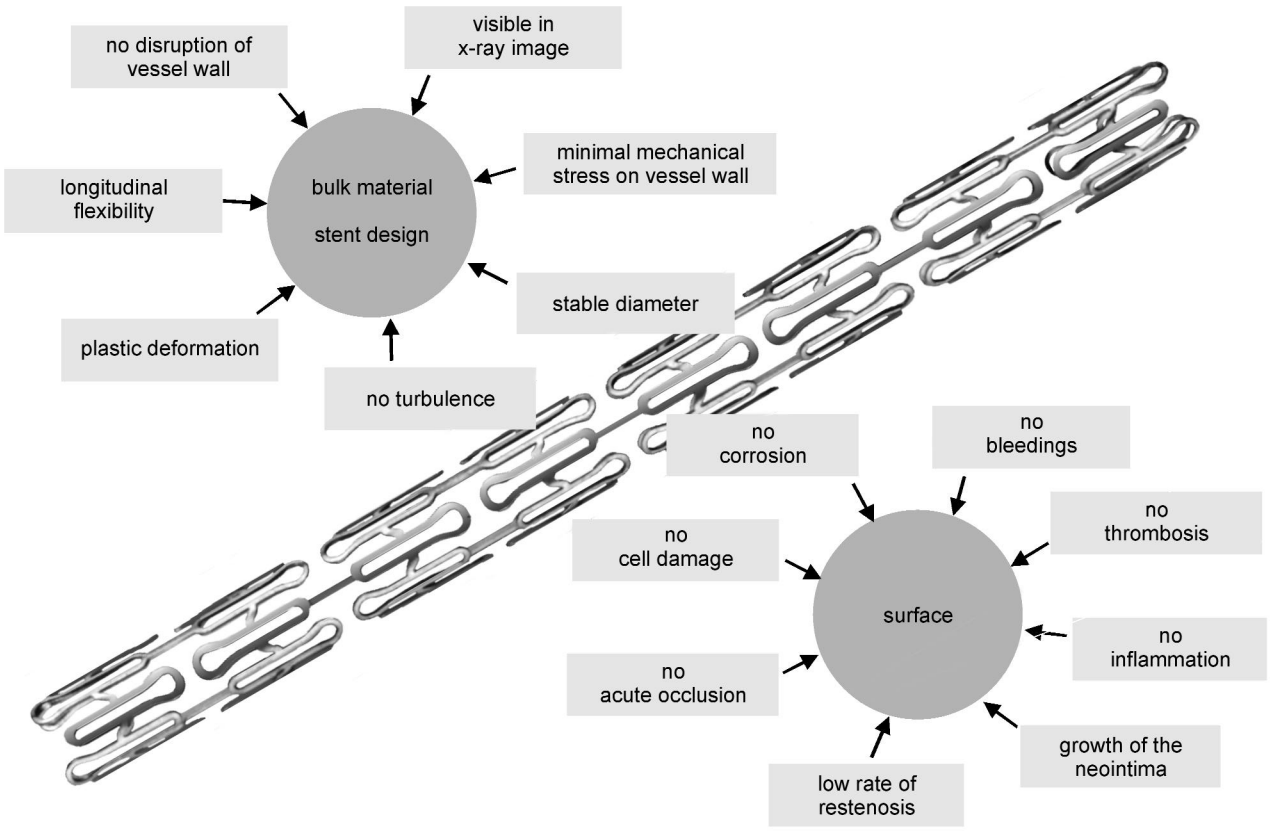


Figure 1. The requirements for an ideal coronary stent.

- Next, a physical model of the initial electron transfer process will be presented. This model helps to specify the electronic requirements for an antithrombogenic surface in a general manner.
- Finally, experimental results are presented in brief, showing that fibrin formation is significantly reduced at the a-SiC:H surface as compared with 316L stainless steel; thus, the electronic model of contact activation is proven.

Fibrin Formation is Triggered By an Electron Transfer Process

It has been known for a long time that thrombus formation is related to electronic phenomena. Brattain, who was awarded the Nobel Prize in 1956 for the invention of the bipolar transistor, together with Sawyer and Boddy, studied the physical nature of contact activation of blood in a classical electrochemical setup using platinum electrodes [1,2]. Through the electrolysis of blood, they showed that clotting takes place only at the anode, which was the first indication for the participation of electrons in the interaction between blood and metal electrodes. In experiments with germanium electrodes, Baurschmidt subsequently proved that an electron transfer from the fibrinogen molecule to the germanium electrode causes the irreversible formation of a fibrin layer at the surface of the electrode [3].

In order to confirm these qualitative observations regarding the nature of the fibrinogen-biomaterial interaction, the exchange current caused by the conversion of fibrinogen into fibrin was measured and quantified by cyclic voltammetry [4]. In this electrochemical method, a triangular-shaped potential is applied to a working electrode in contact with an electrolytic solution containing fibrinogen, and the resulting cell current is measured with a potentiostat. Electron transfer processes are identified as peaks or shoulders in the plot of the current vs. the potential. Figure 2 shows a series of three successive voltammograms obtained with a semiconducting SnO_2 electrode. As an electrolyte, human fibrinogen (Sigma, type IV from human plasma, 60 % protein, 95 % clottable) was

dissolved at a concentration of 2.5 mg/ml in TRIS-HCl-buffered saline solution (pH 7.4, 50 mM $CaCl_2$). To rule out disturbing interactions, the electrodes were located in separate cell compartments connected by Luggin-capillaries. All potentials were measured versus an Ag/AgCl reference in contact with the main cell via a salt bridge filled with 3M KCl. A platinum wire was used as a counter electrode.

In the cyclic voltammograms, the additional exchange current caused by the presence of fibrinogen in the electrolyte is clearly recognizable as a shoulder (Figure 2, circles). Other electrochemical processes, which occur because the SnO_2 electrode is also in contact with the fibrinogen-free electrolyte, do not disturb the current due to the presence of fibrinogen (Figure 2, squares): Both the hysteresis — caused by capacitive currents that charge the phase boundary — as well as the current above 0.9 V — due to the development of oxygen — show smaller currents in the potential range of interest. The increase in the current-potential curve at a potential of about 0.6 V vs. Ag/AgCl reference is significant in comparison with the fibrinogen-free electrolyte. The positive sign of this current shows that electrons are transferred from the fibrinogen to the electrode. Thus, the fibrinogen molecule is oxidized at the positively polarized SnO_2 electrode. Furthermore, the asymmetrical shape of the voltammogram is a clear indication of the correlation of the observed electron current with an irreversible electrochemical process.

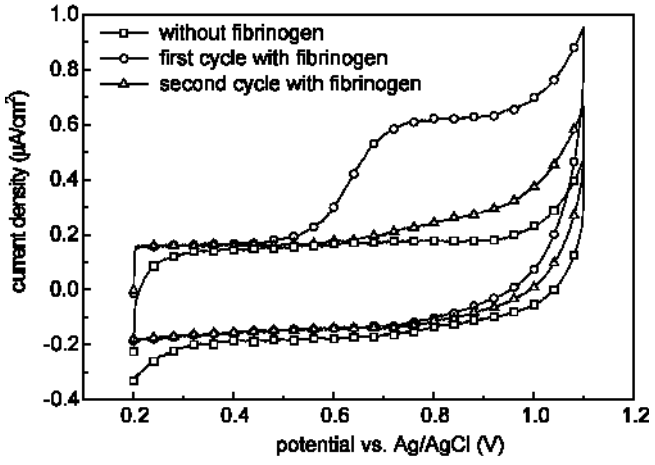


Figure 2. Cyclic voltammograms obtained before and after addition of fibrinogen to the electrolyte; potential rise 10 mV/s, TRIS-HCl-electrolyte (pH 7.4), 2.5 mg/ml human fibrinogen.

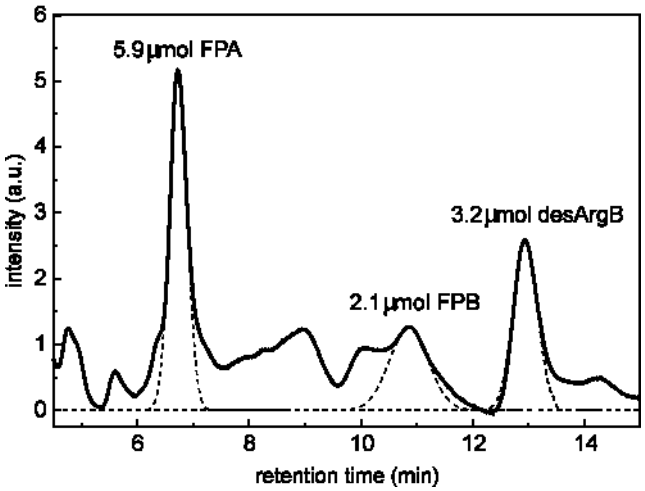


Figure 3. HPLC spectrum of fibrinogen containing electrolyte (TRIS-HCl-buffer, pH 7.4, 2.5 mg/ml human fibrinogen); 316L stainless steel has been in contact with electrolyte for 15 hours (900 cycles) under an external potential load (0.6V - 0.9 V vs. Ag/AgCl reference, gradient 10 mV/s).

The cyclic voltammogram obtained in the second cycle shows the same characteristics as the voltammogram obtained in the first cycle after the addition of fibrinogen to the electrolyte (Figure 2, triangles). The only difference is a significantly smaller current density (0.1 µA/cm² compared to 0.4 µA/cm²) due to the fibrin formation at the SnO_2 electrode in the first cycle, resulting in a partial passivation of the electrode.

The results of these electrochemical investigations prove the electronic nature of the interaction between fibrinogen molecules and solids. To correlate the observed exchange current with the release of peptides from fibrinogen, the electrolytes used in the electrochemical experiments were analyzed by means of reversed phase high-performance liquid chromatography (HPLC). The examined electrolytes were pretreated by boiling them for 2 min, then centrifuging and filtering them with a 0.22 µm micro filter before injecting them into the RP12 column of the HPLC unit. The composition of the mobile phase is held constant over time (70 % water, 20 % acetonitrile, 10 % methanol, 0.05 % TFA) and a steady flow of 1 ml/s is used. Figure 3 shows a typical HPLC spectrum of an electrolyte that has been in contact with a 316L stainless steel electrode under electrical load (0.6 V - 0.9 V vs. Ag/AgCl reference, gradient 10 mV/s) for 900 cycles (15 hours). In the HPLC spectrum, peaks

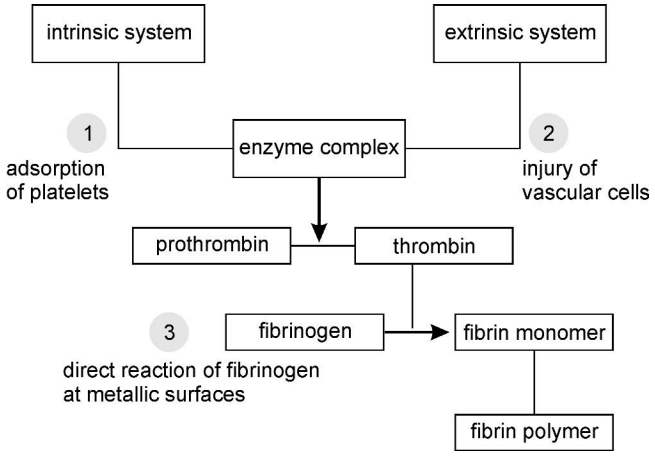


Figure 4. Schematic diagram of the plasmatic coagulation cascade. After stent implantation, thrombus growth via fibrin formation is triggered by 1) the "intrinsic system" due to platelet adsorption, 2) the "extrinsic system" caused by even a small injury of the vessel wall, or 3) directly when fibrinogen comes in contact with metallic surfaces.

correlated with the fibrinopeptide A (FPA), the fibrinopeptide B (FPB) and the desarginine form of fibrinopeptide B (desArgB) are clearly resolved. The appearance of the desarginine form of fibrinopeptide B is a well-known effect in in-vitro studies with fibrinogen [5]. The amount of FPA equals the sum of FPB and desArgB showing symmetrical cleavage of the fibrinopeptides. The quantitative analysis reveals that under electrical load, 40 % of all fibrinogen molecules present in the electrolyte were activated during the 900 cycles by electron transfer processes at the electrode surface.

From these experimental findings, it can be concluded that an electron transfer from the fibrinogen molecule to the implant is responsible for the formation of fibrin as a first step to thrombus growth. The fibrin formation is accompanied by the cleavage of the fibrinopeptides. Thus, with regard to thrombus formation, a metallic surface behaves quite similarly to thrombin. As a consequence, looking at the plasmatic coagulation cascade (Figure 4), fibrin formation is not only triggered by the extrinsic and intrinsic system, but also directly via the surface of an implant.

Physical Model of the Fibrinogen-Implant Interface

Based on the experimental results outlined above, a physical model of the whole system encompassing the implant's surface as well as the fibrinogen molecule is needed to derive the requirements of an anti-thrombogenic surface. Because the electronic properties of both the protein and the implant obviously play a dominant role in the reaction behavior of fibrinogen, the following section presents a few basics of the electronic properties of solids and macromolecules containing a large number of atoms.

Electronic Properties of Solids and Macromolecules Containing a Large Number of Atoms

The consideration begins with the well-known electronic structure of single atoms being spaced apart from each other. According to Niels Bohr (1913), each atom is characterized by discrete electronic energy levels [6]. Starting from the deepest energy level, the electronic states are "filled" with a number of electrons corresponding to the atomic number of the specific element (Figure 5a). The electrons with a higher level of energy are farther away from the atomic nucleus than the electrons with a lower level of energy.

The distribution of electronic states changes significantly if the atoms approach each other and the outer electrons begin to interact. According to an experimentally proven theory put forward by Enrico Fermi (1926), it is not possible for two electrons to occupy the same electronic state at the same position [6]. As a consequence of the interaction between the outer electrons, the energy levels that are farther away from the atomic nucleus are redistributed across a range of energies, thus forming so-called energy bands (Figure 5b). The resulting distribution of electronic states depends on the nature of the interacting atoms and the location of all the single atoms in space, thus being characteristic for the specific solid or molecule. In general, the electronic nature of such a system, which consists of a large number of atoms, can be classified into one of three distinct groups according to the resulting electronic behavior (Figure 5c):

- In a *metal*, the energy band containing the electrons with the highest energy is not completely filled. By absorbing thermal energy, electrons can easily change into neighboring electronic states within this band, resulting in a good electric conductivity even at room temperature.
- In a *semiconductor*, the energy band containing electrons with the highest energy, also known as the valence band, is completely filled. Between this valence band and the adjacent empty electronic band

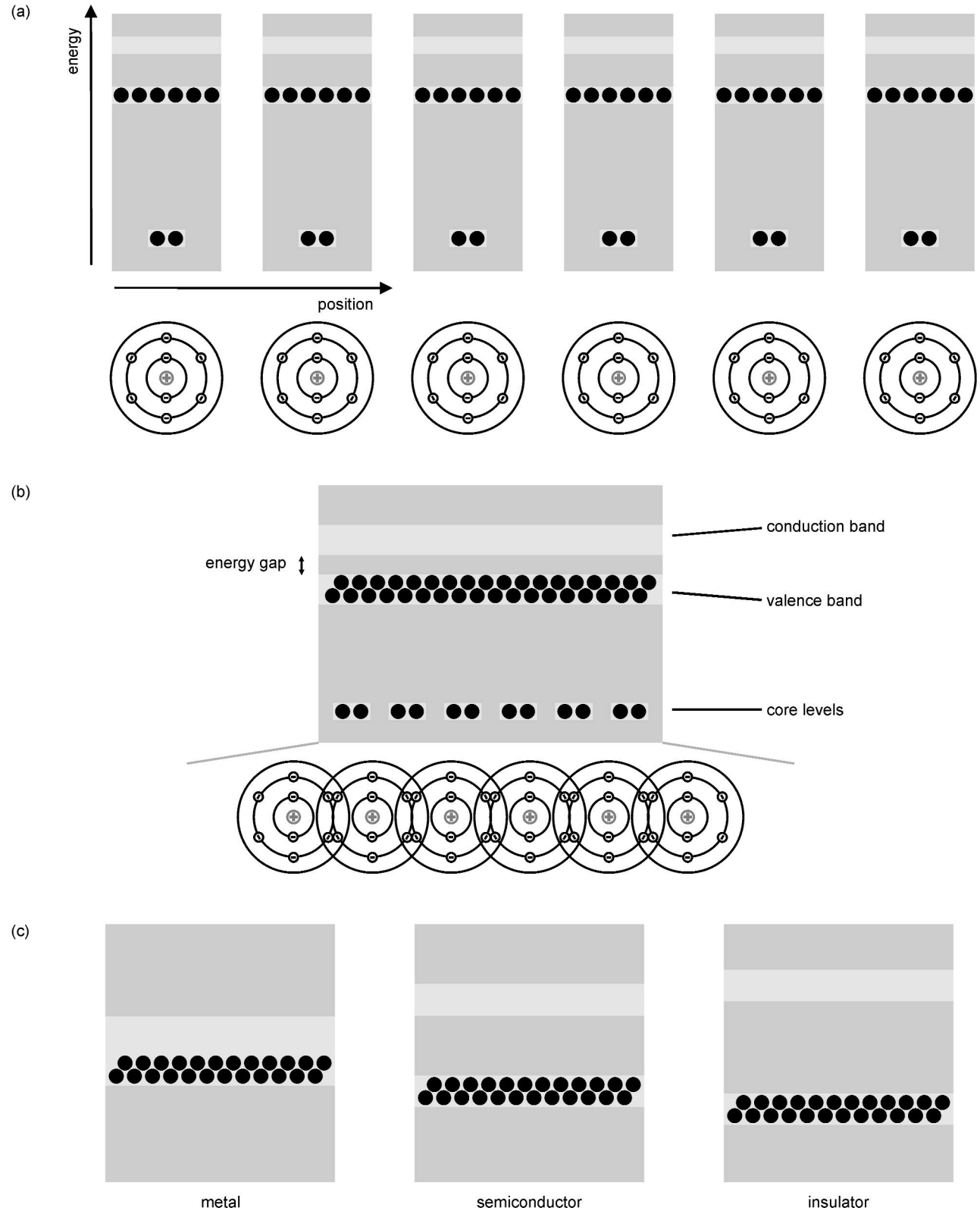


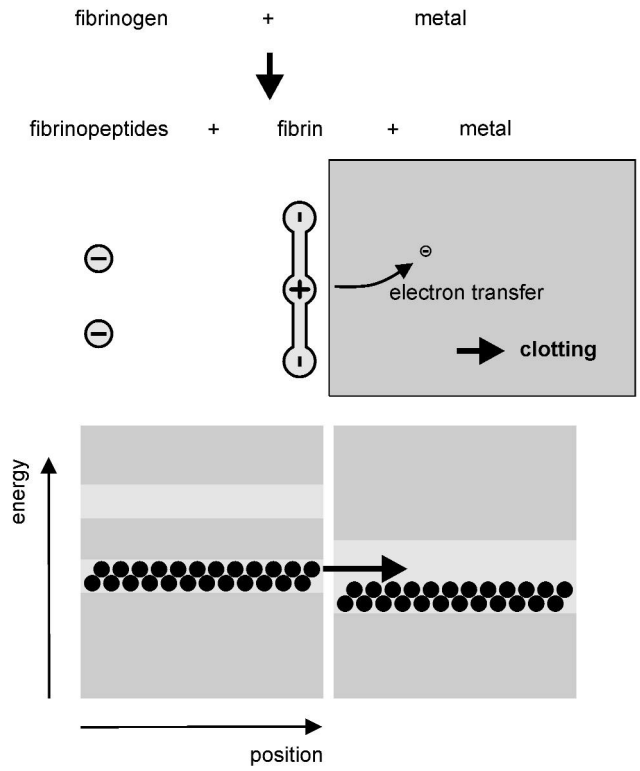
Figure 5. Schematic diagram of electronic energies: a) Discrete energy levels in single atoms spaced apart from each other. b) Formation of a band structure in systems containing a large number of interacting atoms. c) Electronic classification of solids and large molecules in metals, semiconductors and insulators.

— the conduction band - there is an energy gap for which there are no available electron states. As a result, electrons in the valence band of a semiconductor are hindered in occupying states in the conduction band through absorption of thermal energy. Thus, the conductivity of semiconductors is significantly smaller than the conductivity of metals.

- If the band gap exceeds the range of a few eV, the material is designated as an *insulator*. The energy gap in an insulator is so big that electrons only rarely gather enough energy to occupy states in the conduction band through thermal activation. Because of the absence of mobile electrons, insulators have an extremely low conductivity.

The Electronic Model of Contact Activation

Based on these characteristic distributions of electronic states in solids and macromolecules, it is possible to describe the experimental observations regarding the fibrin formation due to electron transfer processes between the fibrinogen molecule and a solid in a physical model. But what are the electronic properties of the fibrinogen molecule? First considerations regarding the electronic structure of proteins date back to



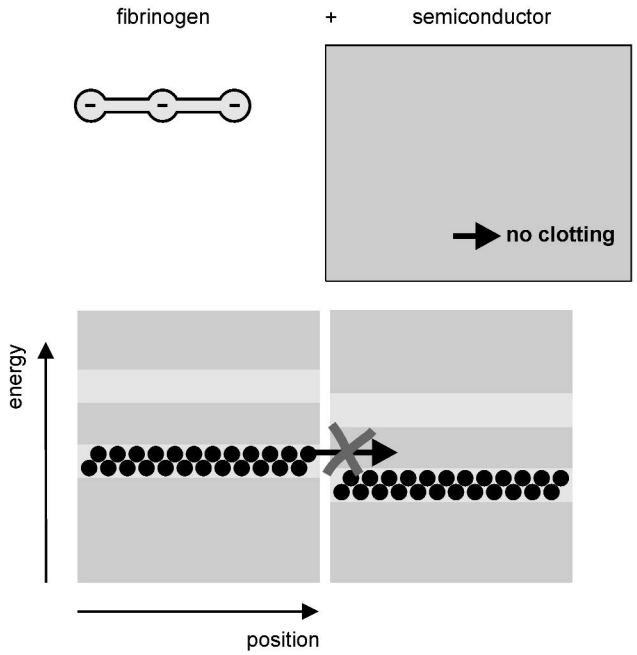


Figure 7. Electronic model of the contact fibrinogen-semiconductor.

Szent-Györgyi [7] who formulated the hypothesis that proteins have semiconducting properties. Baurschmidt confirmed this hypothesis experimentally and showed the size of the energy gap to be 1.8 eV for the hydrated fibrinogen molecule in solution [3]. Thus, the fibrinogen molecule has similar electronic properties to a classic semiconductor like silicon.

In Figure 6, the contact between fibrinogen and a metallic surface is described in terms of the electronic model. In this case, an electron transfer from the completely filled valence band of the fibrinogen macromolecule to the metal is possible because the metal provides a huge number of empty electronic states in the energy range of the valence band of fibrinogen. That is, electrons in the fibrinogen molecule are able to occupy empty electronic states with the same energy in the metal. Thus, an electron transfer is possible and a conversion of fibrinogen into fibrin triggered by the metallic surface takes place.

However, looking at the energy diagram of the fibrinogen molecule, the strategy to avoid this unwanted electron transfer becomes evident: the energy diagram of the implant must not have any empty electronic states within the range of the valence band of the fibrinogen (Figure 7). In this case, the electron transfer process from fibrinogen to the implant is inhibited and, there-

fore, fibrin formation is inhibited as well. A detailed investigation shows that the semiconducting surface of the implant has to match other specific electronic requirements with regard to electric conductivity (> 10^{-3} S/cm; band bending < 200 mV), density of trace electronic states within the band gap (< 10^{17} $eV^{-1}cm^{-3}$), as well as the distance between the Fermi Energy of the system and the edge of the valence band of the implant surface (> 1.4 eV).

a-SiC:H as an Anti-Thrombogenic Surface Coating

Starting with a detailed investigation of the interactions between fibrinogen molecules and the surfaces of implants, it has been possible to identify and quantify the relevant electronic properties of an anti-thrombogenic material. With this knowledge, a material and a deposition process have been developed that allow the coating of the stent's bulk material, which consists of 316L stainless steel.

The material is silicon carbide in an amorphous, heavily phosphorous-doped, hydrogen-rich modification (a-SiC:H). The deposition of a thin film (80 nm) of a-SiC:H is performed by means of a plasma-enhanced chemical vapor deposition technique (PECVD) [8]: the gaseous agents silane (SiH_4), methane (CH_4) and phosphine (PH_3) are cracked in a plasma forming chemically activated species. These activated molecules are able to react at the stent's surface, forming an a-SiC:H-coating that completely covers the bulk structure. In order to achieve a homogenous thickness in the deposited film, the stents are rotated around their longitudinal axis during the PECVD deposition process.

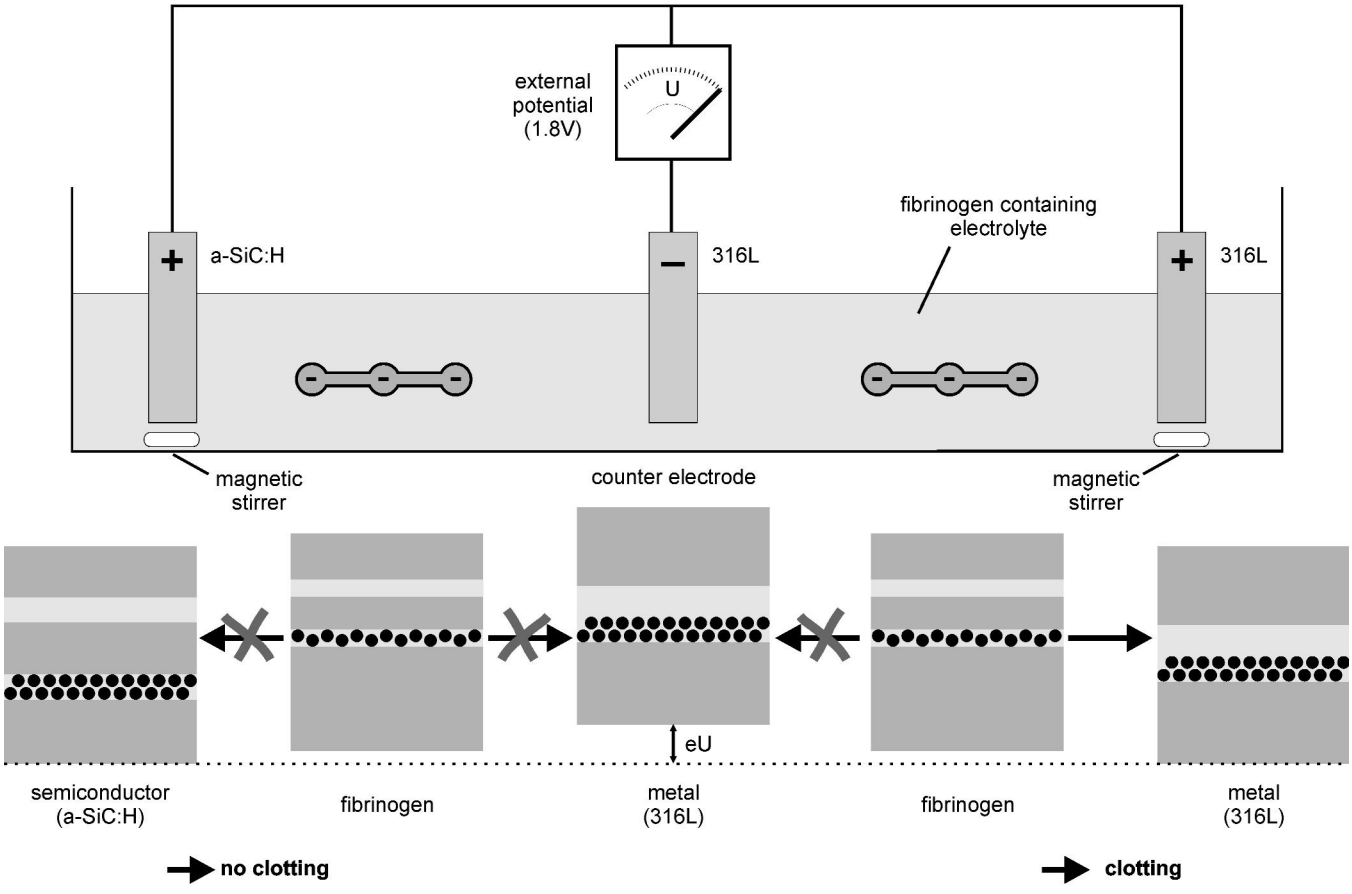


Figure 8. The upper part shows the experimental setup used for the simultaneous electrolysis of a fibrinogen solution (human fibrinogen dissolved at a concentration of 2.5 mg/ml in TRIS-HCl-buffered saline solution) on an uncoated and an a-SiC:H-coated 316L stainless steel tube. The potential of both test electrodes is held constant over time at + 1.8 V (anodic) versus a common 316L stainless steel counter electrode (cathodic). In the lower part the electronic structure of the single system is depicted (see text).

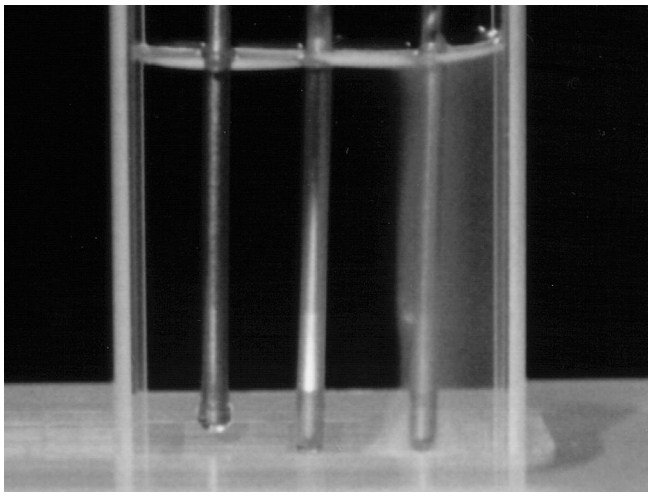


Figure 9. Image of the experimental outcome after 20 minutes of electrolysis. In contrast to the a-SiC:H-coated tube shown on the left, a fibrin thrombus has formed on the uncoated 316L stainless steel tube as shown on the right.

With silicon carbide coated specimens, various tests have been performed in vitro to prove the physical

molecules by providing a semiconducting surface with the specific requirements stated above.

a-SiC:H-Coated 316L Stainless Steel Tubes
In order to compare a-SiC:H-coated to uncoated 316L

depicted in Figure 8 was used, which is in fact very similar to that of Brattain, Sawyer and Boddy [1,2].

In the same type of electrolyte used for the previously

dissolved at a concentration of 2.5 mg/ml in TRIS-HCl-

an uncoated 316L stainless steel tube, an a-SiC:H-coated 316L stainless steel tube, and a common 316L

fibrin formation, a constant common potential of + 1.8 V was applied to both test electrodes; that is, the

the platinum counter is cathode-poled.
Figure 9 shows the results after 20 minutes. On the

fibrin formation occurred and no changes are detectable. On the uncoated 316L stainless steel tube

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ent experimental outcome is observed. In the course of

covers the surface of the electrode, has formed. The distribution of electronic states in the fibrinogen

potential load of + 1.8 V is depicted in the lower part of Figure 8. As metals, the stainless steel counter and

valence band. The fibrinogen molecule and the a-SiC:H coated tube as semiconductors have a band

conduction band. Due to the external potential load, the

and silicon carbide coated electrodes is shifted to lower electron energies by an amount of 1.8 eV versus the

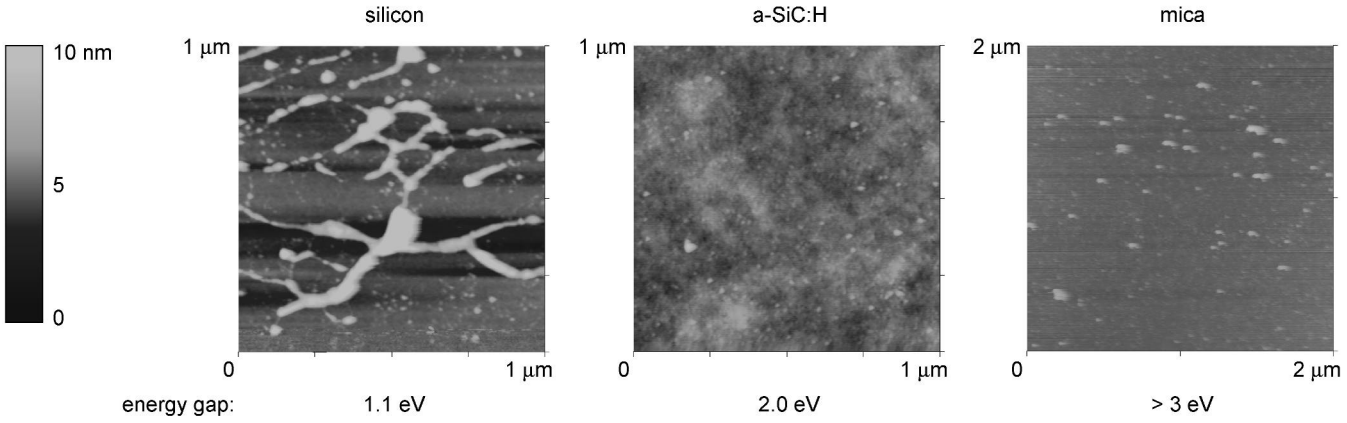

Figure 10. Scanning force microscopy images done in the "tapping mode" of Si-, a-SiC:H- and mica-surfaces incubated with fibrinogen solution (6 µg/ml). The energy gaps of the materials are 1.1eV (Si), 2.0eV (a-SiC:H), and > 3eV (mica), respectively.

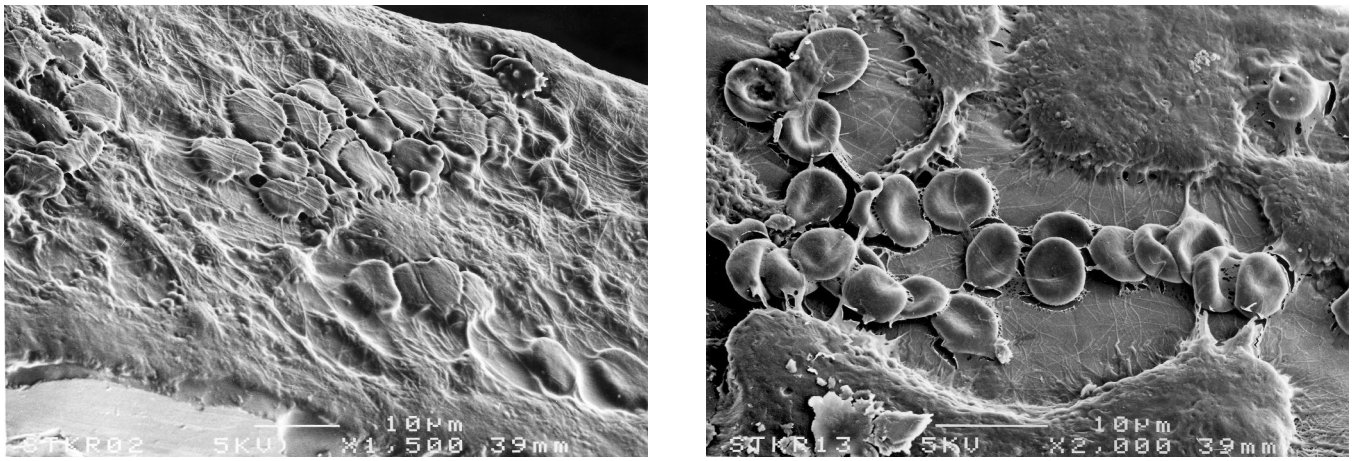

Figure 11. Scanning electron microscopy of implant surfaces exposed to circulating blood: a) 316L stainless steel surface showing areas with thrombi and erythrocytes, but not densely packed and not extensively covered with fibrin.

of the electronic model for the experimental situation, the interpretation of the experimental outcome is quite straightforward. At the 316L electrode, a large number of empty electronic states is opposite to occupied states in fibrinogen resulting in electron transfer processes, i.e., fibrin formation. In contrast, at the a-SiC:H coated electrode, occupied states in fibrinogen are opposite to the band gap with no empty electronic states even under anodic potential load, i.e., fibrin formation is inhibited. At the stainless steel counter, no fibrin formation is observed because the electronic states are shifted to higher energies due to the cathodic load, resulting in a situation where occupied states are opposite to occupied states. Thus, this experimental observation is a direct confirmation of the electronic model of protein activation in solids. While on the silicon carbide coated specimen, with its optimized semiconducting surface, contact activation of fibrinogen is inhibited even under external anodic potential load, the electron transfer resulting in fibrin formation is not prevented on the uncoated specimen.

Scanning Force Microscopy Study of Fibrin Formation on Surfaces with Different Electronic Properties

Further experimental support for the electronic model of contact activation is provided by scanning force microscopy (AFM) measurements. Figure 10 shows the result of an AFM study of semiconducting materials with different band gaps after exposure to a 6 µg/ml fibrinogen solution [9]. On silicon, wide fibrin networks were imaged (Figure 10, left panel). In contrast, only single molecules and small clusters of fibrin were observable on a-SiC:H (Figure 10, middle panel) and mica (Figure 10, right panel) under the same experimental conditions. The different electronic properties of the three semiconducting materials provide the explanations for these observations: On silicon, which has the smallest energy gap (E_{gap} = 1.1 eV), the electron transfer is possible and conversion to fibrin occurs [4]. Conversely, on the substrates mica (E_{gap} > 3 eV) and a-SiC:H (E_{gap} = 2.0 eV), the gaps are large enough to extend across the range of the fibrinogen valence band, so that no tunneling into free electronic states is possible, and no conversion of fibrinogen into fibrin occurs. Thus, the scanning force microscopy images give an indirect indication for the validity of the electronic model of contact activation at the molecular level.

Scanning Electron Microscopy Study of Stent Surfaces With and Without a-SiC:H-Coating after Exposure to Circulating Human Blood

In further experiments, two groups of coronary stents (group A: 316L stainless steel uncoated; group B: 316L stainless steel coated with a-SiC:H) have been exposed to circulating human blood for 15 min, and scanning electron micrographs were taken of the surfaces of both groups [10,11]. While a dense fibrin network with incorporated blood cells is found on the metallic surface (Figure 11, left panel), only single thrombi and erythrocytes are observed on the a-SiC:H-coated surface (Figure 11, right panel).

Discussion and Conclusions

In the last decade, coronary stenting has become a well-established therapy for coronary artery disease. However, in up to 30 % of all stent procedures, the process of restenosis leads to a re-narrowing of the vessel within several months. Optimization of the geometrical stent design with regard to mechanical properties only resulted in limited success in reducing the restenosis rate.

It is our conviction that further improvement in the clinical outcome of interventional stent procedures is only possible by stringent application of sound scientific methods. Further significant reduction of the restenosis rate to values smaller than 10 % even under difficult conditions requires a detailed understanding of the various interaction processes between the implant and the surrounding biological environment. Based on experimental data, scientific models have to be established and continuously improved in order to establish the dominant reaction partners in the processes that lead to the remaining major challenge: restenosis. On the basis of these models, which document the behavior of the complete system including the stent as well as the (pathologic) vessel structure and blood components, technological solutions have to be developed that will lead to medical products that aid the physician in effective therapy of the disease.

The development of a-SiC:H as an anti-thrombogenic stent coating has been presented as an example of the scientific method. The aim was to reduce thrombus formation on the stent surface for two reasons: On the one hand, thrombus formation may lead to the short-term complication of acute or subacute occlusion of the stented vessel. On the other hand, blood cells like leukocytes and platelets that are incorporated to a large degree in a growing fibrin network on the stent surface are suspected of contributing to the long-term complication of restenosis.

In the process of thrombus formation, the irreversible conversion of fibrinogen into fibrin plays a dominant role. Triggered by early results like those of Sawyer and Brattain, which provided evidence that foreign-body induced clotting of blood is related to electronic phenomena, theoretical considerations led to the electronic model of contact activation. According to this model, fibrin formation is initiated by an electron transfer process when fibrinogen is in close contact with a metallic surface. This electron transfer can be inhibited by providing a semiconducting surface that is characterized by the specific electronic requirements as stated above.

Amorphous, hydrogen-rich, phosphorous-doped, silicon carbide (a-SiC:H) was found to match all the electronic requirements of the model. Thus, the model could be verified using metallic substrates covered by a thin a-SiC:H film deposited by a plasma-enhanced chemical vapor deposition (PECVD) process. The reaction behavior of a-SiC:H films has been extensively studied in electrochemical experiments as well as in investigations on the molecular level using scanning force microscopy (AFM). Today, more than 40,000 commercially available stents with a-SiC:H coating are implanted worldwide and the clinical results obtained so far appear to be promising.

However, a-SiC:H is not the end of stent development. Further improvements in clinical results must be the goal. But the development procedure of the anti-thrombogenic a-SiC:H coating is a good example to elucidate the effectiveness of a development strategy based on systematic research methods. It is our conviction that further progress in the reduction of the complication rate after stent implantation will depend on an ongoing commitment to a science-based development strategy instead of "trial and error", even if — or better yet, because — the interaction processes to be modeled will be of increasing complexity.

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