Coronary stent coatings

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Coronary Artery Disease 1994, **5**:590–596

Keywords: endovascular prosthesis, coatings, biocompatibility, thrombosis

Stents should be regarded as intravascular foreign bodies, and therefore two equally important issues, blood compatibility and tissue compatibility, need to be addressed when designing or modifying stents. An implant is considered 'blood-compatible' when it induces only mild activation of coagulation proteins and platelets, and is considered 'tissue-compatible' when it does not interfere with wound healing and does not induce either excessive cell proliferation or chronic inflammation. Given that thrombosis is an integral and essential part of wound healing, blood and tissue compatibility are closely inter-related.

Modification of stents with respect to blood and tissue compatibility can be achieved by changing the stent material. This can, however, influence the mechanical behavior of the stent, making it either too strong or too weak. It is only the outer layer of the stent that interacts directly with the blood and later the surrounding tissue and it is therefore sufficient to change only the outer few micrometers of the stent by applying a thin coating of another material. Given our current understanding of thrombosis, the possibility for extensive in-vitro and in-vivo testing, and the availability of a multitude of materials and techniques for changing the surface characteristics of devices, our best chance of success in the near future lies in reducing thrombogenicity. Our efforts at present are therefore mainly directed at modifying those stent characteristics that influence blood compatibility by using stent coatings.

Shortly after implantation, and until incorporation in the arterial wall, the stent interacts with the vessel-wall surface and the bloodstream. Clinical studies employing angioscopy and histology indicate that endothelialization of the stent is complete within 2–3 months [1,2]. It is assumed that, during this period, the patient is at risk of thrombotic stent occlusion. In clinical practice, thrombotic occlusion occurs only within 2–3 weeks of stent implantation [3], under the current stringent anticoagulant regimen, which requires review. It is not known how or to what extent the reduction of systemic anticoagulation will influence the duration and intensity of the period at risk with the advent of local treatment, because cyclic variation of anticoagulant treatment is suspected of inducing both thrombosis and restenosis [4].

Once the thrombotic reaction to the stent has been pacified, it is thought that the acceptance of the stent as an implant is largely determined by the stent–tissue interaction. This is only partially true, however, because early thrombotic deposits remain present for a considerable length of time [1]. It therefore seems that influencing these early events may be of long-term benefit. Furthermore, preliminary data from our laboratory suggest that factors originating from the blood may still interact with processes inside the vessel wall because of defects in the endothelial cell barrier [5].

This paper is divided into three parts. The first part deals with general principles governing blood in contact with a foreign (stent) surface. The second part deals with the several approaches to changing stent characteristics, with emphasis on stent coatings. In the third part, experimental data illustrating each of the approaches will be presented.

Stent thrombogenicity

The main determinants of stent thrombogenicity are the stent itself, the recipient vessel wall, and the circulating blood. Stent factors may include geometry or physicochemical and topographic surface features. Lesion complexity and content, vessel diameter, and, of course, implantation trauma are characteristics relevant in the recipient vessel wall. The circulating blood is the principal source for adsorption of proteins, activation of complement, coagulation, and cellular adhesion factors, platelets, and

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white blood cells. Both lesion complexity and the adsorption of elements from the blood may be secondary to those factors involved in acute coronary syndromes and inflammation, such as platelet hyperaggregability and increased fibrinogen content.

Three phases in the thrombotic response

Immediately after implantation, a complex interaction between stent, damaged vessel wall, and flowing blood commences [6], which can be divided into three phases: a proteinaceous, a cellular, and an organizational response (Fig. 1).

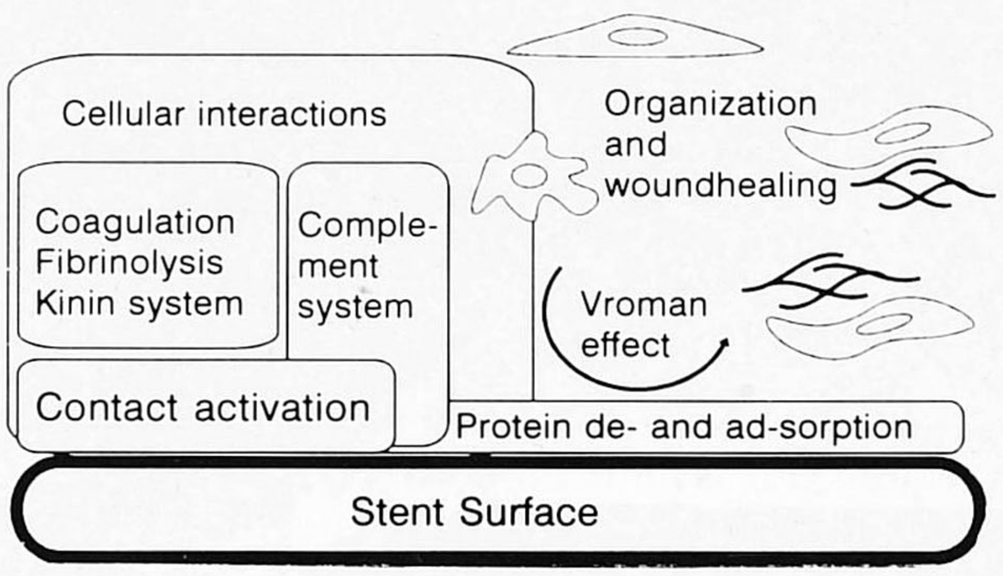


Fig. 1. Schematic representation of the events that take place during and after stent implantation, comprising the proteinaceous, cellular, and organizational response.

The proteinaceous response

The deposition of plasma proteins depends on variables such as plasma concentration of the protein, surface charge and wettability of the stent material, surface chemistry, and topography. These characteristics generate differences in temporal and spatial adsorption patterns; a protein that initially adheres to a surface because of its high plasma concentration can later be displaced by other proteins that have a higher affinity for the stent, the so-called Vroman effect [7]. It can therefore be expected that a stent surface will initially be covered by albumin, other abundant transport proteins, and gamma globulins. This layer will then increasingly be displaced by other proteins such as fibrinogen, high-molecular-weight kininogen, complement factors, and so forth. These processes are important for the phases that follow because so-called 'bland proteins', such as albumin, will have a different influence on events from, for example, fibrinogen [8]. Albumin will decrease platelet activation of some surfaces but at the same time will also decrease the speed of endothelialization, whereas fibrinogen will activate platelets but will accelerate endothelialization. It is important to note that it is often the distances between the adsorbed proteins that are of biologic importance rather than their overall density [9].

The cellular response

This initially consists of platelet-rich aggregates, but, within 1 h, leukocytes (mainly neutrophils) are found to have infiltrated the platelet-rich clot. Cytotoxic substances produced by leukocytes and platelets may leech from the clot and lyse endothelial cells. All three cell types thereby provide a continued source of chemotactic, procoagulant, and growth-promoting factors [e.g. cytokines, platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), tumor necrosis factor (TNF), adenosine diphosphate (ADP), and thrombin], although the relative importance of each of these factors is not yet clear. During the next few hours, polymorphs and monocytes infiltrate the growing thrombus mass. These processes are influenced by the proteinaceous response; fibrinogen, immunoglobulins, complement factors, and coagulation factors all influence platelet activation and aggregation as well as neutrophil and macrophage activation. The importance of the multitude of factors traditionally thought to influence thrombosis, complement activation, inflammation, and their inter-relation, is illustrated by, for instance, patients with afibrinogenaemia who still have normal platelet aggregation.

The organization response

Thrombus organization, i.e. clot retraction, fibrocellular proliferation, matrix formation and remodelling, is one of the last phases in the interaction between the foreign surface and the blood. The cells (including overgrowing endothelial cells and fibromuscular cells) displace the adsorbed plasma proteins using cell-derived adhesion ligands (e.g. glycoproteins). The endothelial cells thereby create their own matrix upon which to grow and remain attached. This phase in the response of the organism to the implant is probably initiated within 2 days, while the earlier cellular response is still underway. Depending on the type of implant, this organization may take from weeks to months [10]. Large synthetic grafts are known to have incomplete endothelialization, and even in porcine coronary segments that are completely endothelialized within 1 week of stent implantation, neointimal remodelling remains active until at least 12 weeks after the procedure [11].

How to control stent thrombosis

Several techniques for controlling the thrombogenicity of stents are available [12]. These can roughly be divided into active or passive prevention of thrombosis, bypassing thrombosis or shortening the period for thrombotic complications, or

both, by accelerating and enhancing the quality of wound healing.

Prevention of thrombosis

Thrombosis can be prevented passively by creating an inert stent surface that improves those surface characteristics that influence thrombosis (e.g. charge, wettability, and topography). It is possible to polish surfaces, chemically to modify the surface by attaching groups like polyethylene oxide, or to deposit thin films of inert material like teflon or polyurethanes. A second method is to couple an active component to the stent surface in order to prevent thrombosis, e.g. prostaglandins [13], heparins [14,15], other thrombin inhibitors, or enzymes such as ADPase [16].

Bypassing thrombosis *ex vivo* or surface disguising

One way of controlling thrombosis is to mimick an already completed thrombotic response. This can be achieved by creating a controlled thrombus *in vitro* (preclotting), because polymerized and stabilized fibrin is no longer thrombogenic. Disguising the surface with plasma proteins such as albumin, gamma globulins, or phospholipids may also limit thrombus formation by skipping certain phases in the proteinaceous response.

Accelerating wound healing

During thrombus organization, the endothelial cells create their own matrix upon which to grow and remain attached. Given that the normal endothelium is non-thrombogenic, depositing a layer of substrate for endothelialization can shorten the period considered to constitute a risk for subacute thrombosis. Another technique used is 'cell seeding' of normal or genetically engineered endothelial cells in order to accelerate re-endothelialization.

Experimental studies

Prevention of thrombosis

Biogold

Biogold is an example of a passive polymer coating applied to the Wallstent (Fig. 2). The Biogold coating [17] is prepared using the plasma gas discharge technique. An approximately 30 nm thick layer, composed of hydrocarbons with one to six carbon atoms, may be attached to the stent filaments. This coating both smoothes the surface and changes its chemistry. Experimental studies [11] showed that the Biogold coating protected against early thrombotic occlusion caused by stainless steel self-expanding stents.

Heparin

As heparan sulphate, a heparin-like molecule, is synthesized by endothelial cells [18], heparins may be regarded as 'natural surface conditioners'. Heparin has therefore been one of the most extensively explored substances for coating synthetic surfaces. The principal anticoagulant mechanism of heparin is mediated by its interaction with antithrombin III [19], which accelerates the inactivation of thrombin and other coagulation factors. In addition, heparin has been reported to be highly compatible with platelets as well as granulocytes and macrophages [20–22]. In a study designed to test short- and long-term patency of stents with a high dose of covalently attached heparin in pig coronary arteries [14], thrombotic events (stent thrombosis within 48 h leading to sudden death or myocardial infarction), as observed in approximately 30% of the non-coated stents, were absent in animals that received a coated stent. This observation is in accordance with those made using other heparin-coated stents [23,24].

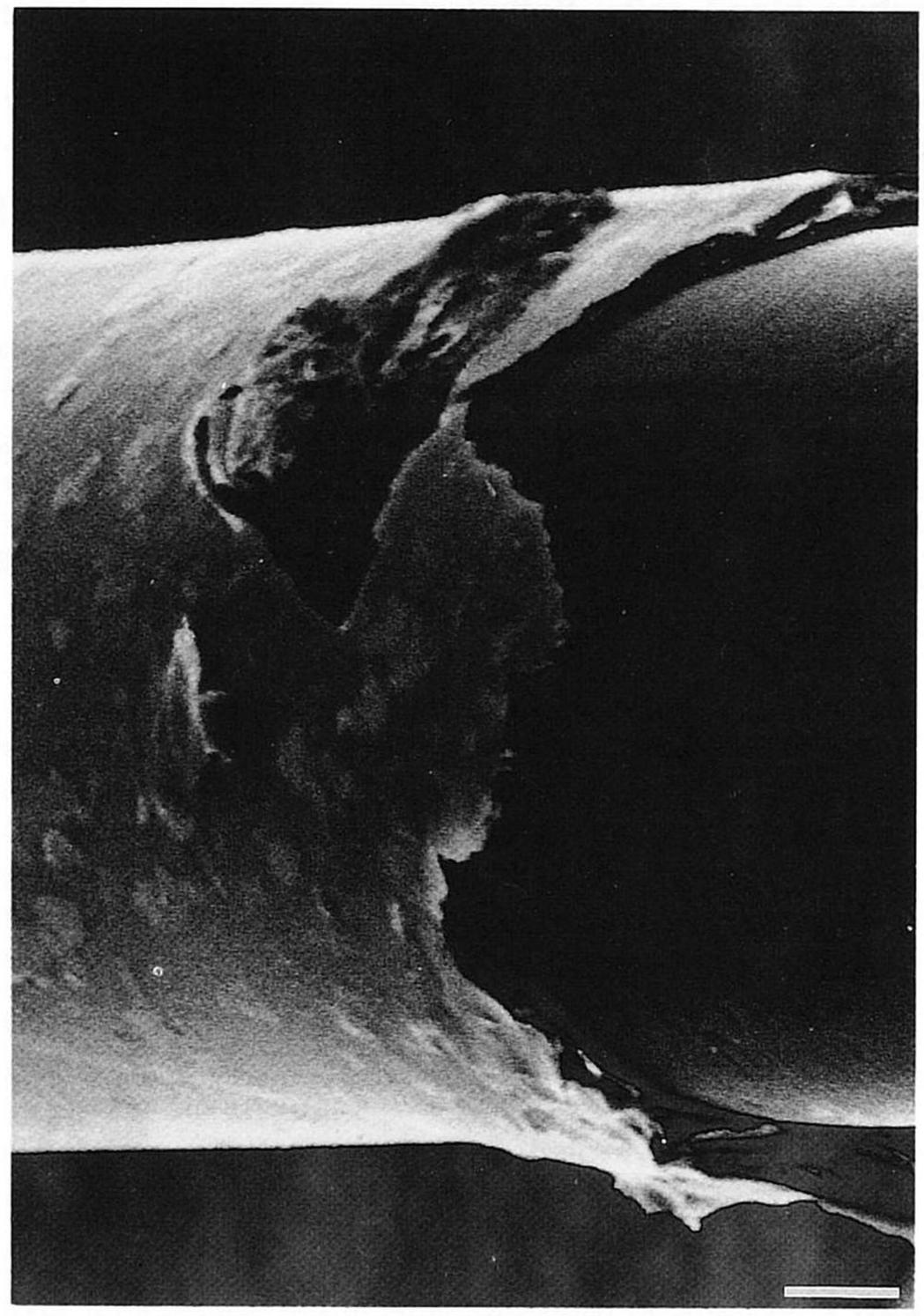


Fig. 2. Scanning electron micrograph of a Biogold-coated Wallstent, showing the thin polymer layer covering the metal wire. Bar = 9 μm. Published with permission [11].

Mimicking thrombosis

Controlled thrombosis

As an extension to the technique of preclotting, cryoglobulins and thrombin can be used to deposit thin layers of polymerized fibrin onto a surface (Fig. 3).

This technique was used to create full casts [25] or thin wire coatings [26] for the Wiktor stent. The stent surface can be coated homogeneously and is able to withstand handling and balloon expansion. Preliminary in-vivo experiments, however, have shown that fibrin formation is a critical step, which, if excess thrombin cannot be removed, can induce acute thrombotic complications. Combining this coating with heparan sulphate eliminates this unwanted thrombogenicity, probably by thrombin inactivation.

Accelerating wound healing

Extracellular matrix

We developed a method for depositing thin layers of extracellular matrix material onto the surface of stents (Fig. 4a). This material, which is present in the normal vascular wall, is a suitable substrate on which the endothelial cells can grow. In-vivo implantation was performed in a small number of pigs to show the potential of such a coating. Although it is difficult to say whether this technique would increase the rate of endothelialization in humans, we showed that the thickness of the neointima had decreased in these pigs. Thrombus remnants were not observed within the neointimal layer (Fig. 4b), suggesting an attenuation in the thrombotic process as well.

Endothelial cell seeding

The technique of endothelial cell seeding, first tested *in vitro* on stents by van der Giessen *et al.* [27], may be used to enhance the rate of endothelialization. It was shown that, even after exposure to blood flow, considerable retention (approximately 50%) of endothelial cells is achieved [28]. Although activated endothelial cells can be procoagulant, by seeding genetically engineered cells that express anticoagulant molecules, thrombosis could in theory be prevented. The cell-seeding technique has been tested *in vivo* in large synthetic grafts [29]. Results from clinical trials using this technique were inconclusive concerning the advantage of seeded versus non-seeded grafts. Co-culture with smooth muscle cells might help retain the endothelial cells and increase their potential for replication and migration [30].

Stent coatings and subacute occlusion

When wound healing is well underway, a thrombotic event sometimes causes acute occlusion as late as 2–3 weeks after implantation. Which elements are important in these subacute occlusions is not clear, but may be associated with subtherapeutic anticoagulation therapy or other factors involved in acute coronary syndromes [31,32]. Macrophages and neutrophils, for instance, may be stimulated to express procoagulant factors such as tromboxane A_2 and tissue factor to induce thrombosis [33].

Ongoing thrombus growth or separate event

It is unclear whether subacute thrombosis is a separate event or the continuous growth of a primary thrombus. This information is important because it determines whether coatings will be successful in preventing subacute thrombosis. If subacute thrombosis is a separate event, it is certain that, by the time the problem arises, the stent will already be covered by thrombus or granulation tissue, or both, hindering the coating from preventing thrombosis. Even a

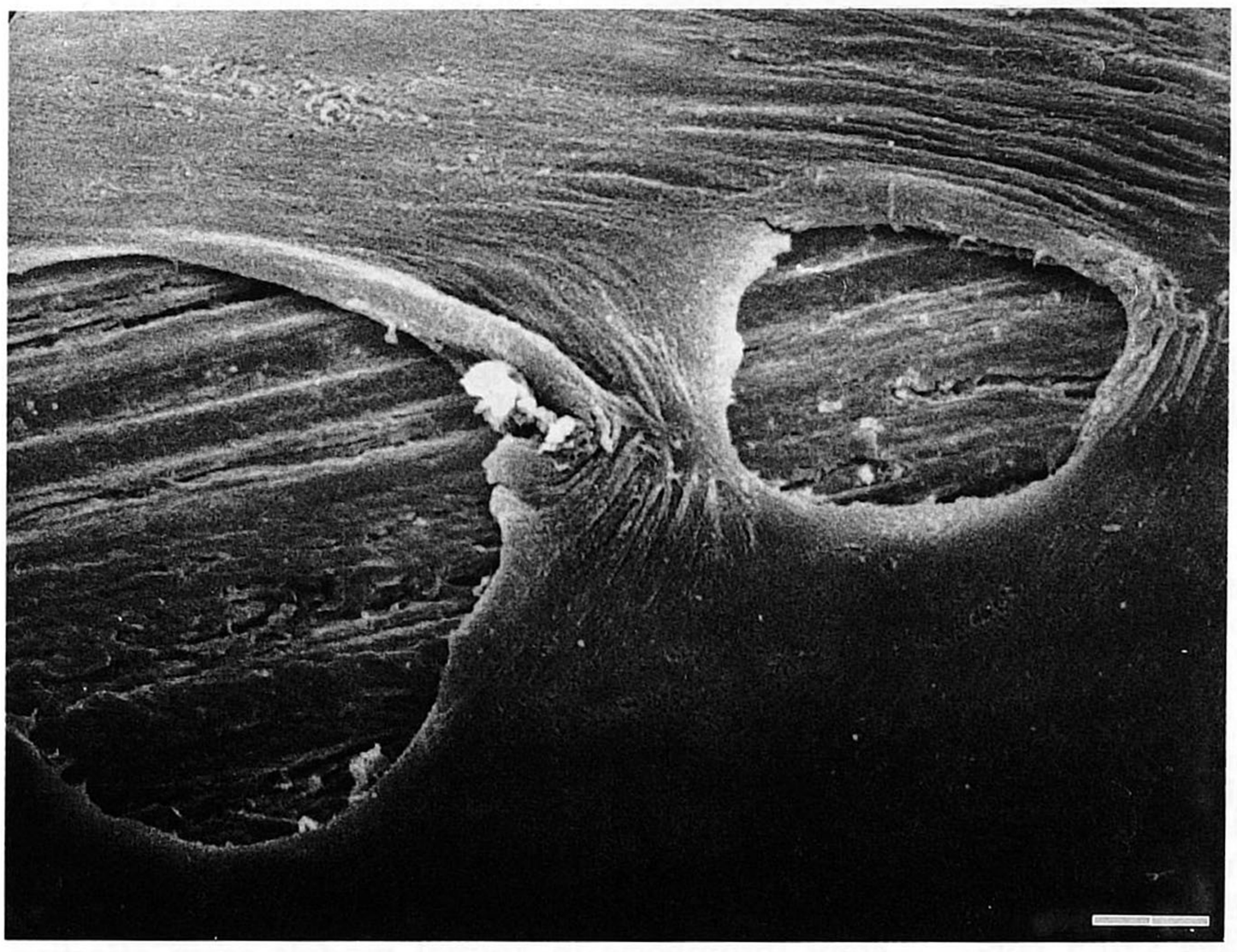
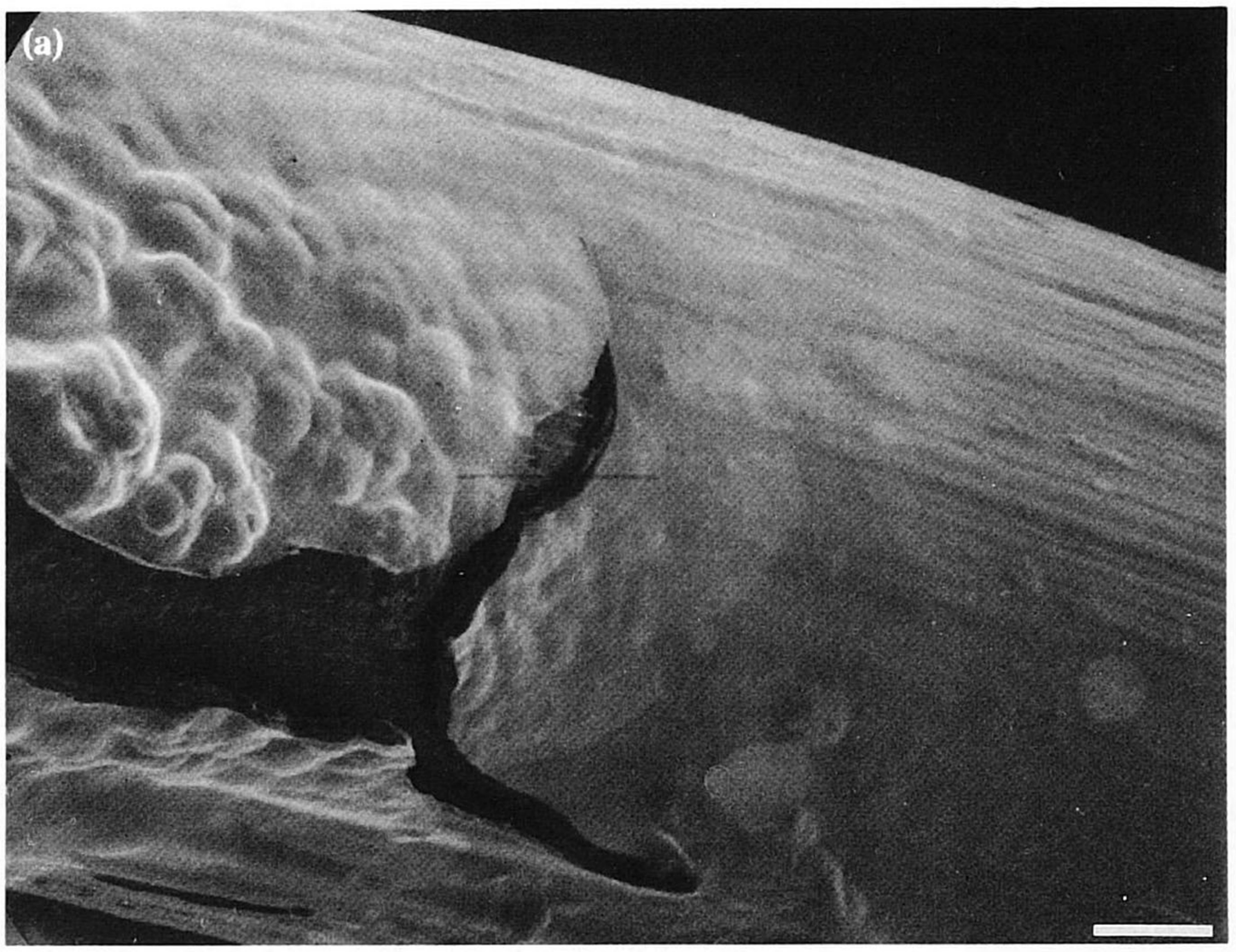


Fig. 3. Scanning electron micrograph of a fibrin-coated Wiktor stent, showing the thin fibrin layer covering the metal wire. Bar = 6 μm.



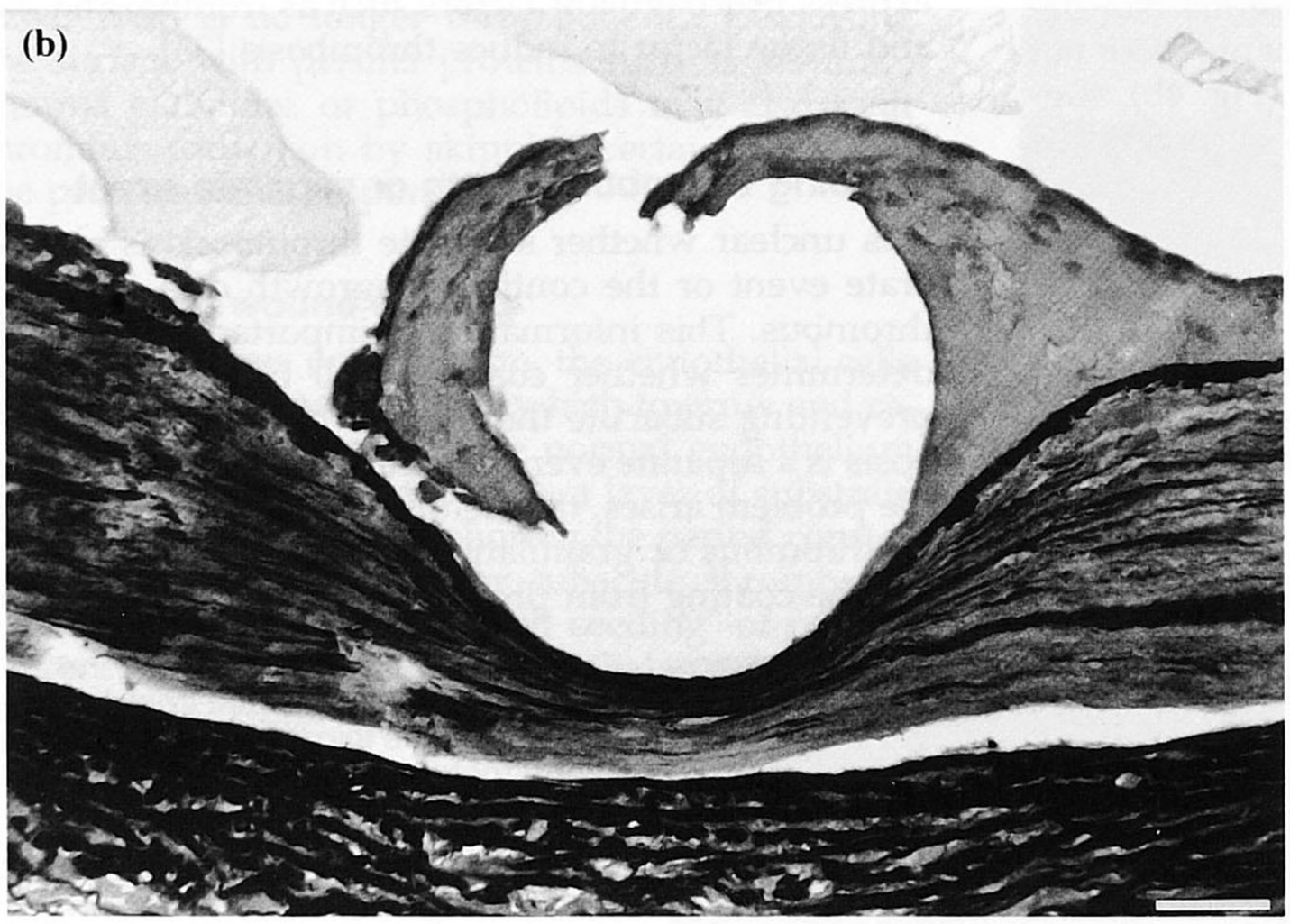


Fig. 4. (a) Scanning electron micrograph of an extracellular matrix-coated Wiktor stent, showing the thin layer covering the metal wire. Bar = 20 μm. **(b)** Light microscopy, 1 week after implantation of an extracellular matrix-coated stent in normal porcine coronary arteries. The extracellular matrix material is covered directly with endothelial and smooth muscle cells, whereas thrombus remnants are not observed. Resorcin–Fuchsin stain. Bar = 30 μm.

drug-releasing stent coating would have trouble preventing secondary thrombosis through this protein barrier.

Timing of subacute occlusion

Most thrombotic occlusions occur in the first 3 weeks after stenting. Although very little data are available on a day-to-day basis, the period during which thrombotic occlusion occurs in several reports [3,34,35] may be summarized in a graph (Fig. 5). This graph, which combines both elective stenting and stenting for bail-out, gives the impression that there is a rise in the percentage occlusion during the first few days with a maximum at days 3 to 5, corresponding to the change from intravenous heparin to oral anticoagulants. From then on, subacute occlusion gradually decreases. Obviously, damage is not an important determinant in the timing of subacute occlusion, because there is no difference between elective and bail-out stenting.

Conclusion

It is important to realize that it is not easy to separate blood and tissue compatibility. We have seen, however, that several techniques for reducing occlusive stent thrombosis in animal models seem effective,

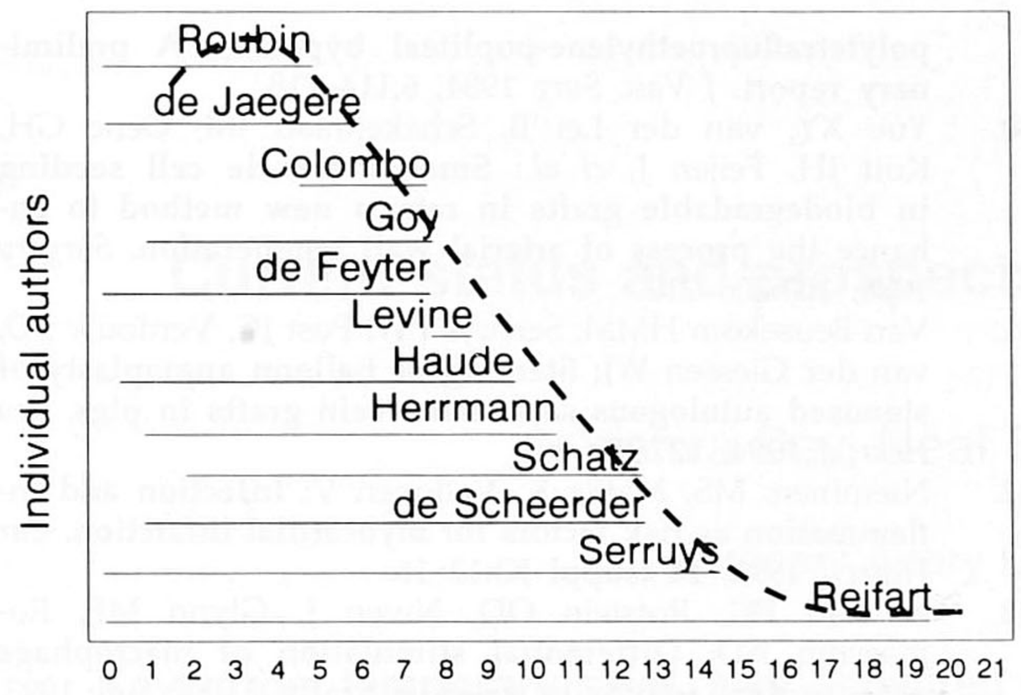


Fig. 5. Schematic summary of the timing of subacute occlusion (in days) after stent implantation [3], ordered according to the last day on which subacute thrombosis (SAT) was observed. Dotted line indicates a probable distribution of SAT, with a maximum between 3 and 5 days.

such as the Biogold coating, the heparin coating, and the Basement Membrane coating, as demonstrated in the examples shown in this paper. Whether these approaches will stand the test of clinical trials will be demonstrated by the recently started Benestent-II trial, which compares heparin-coated and non-coated Palmaz–Schatz stents.

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- • Of special interest
- •• Of outstanding interest

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