



**HAL**  
open science

## Blood Compatible Bioactive Polymers

Stéphane La Barre, Catherine Boisson-Vidal

► **To cite this version:**

Stéphane La Barre, Catherine Boisson-Vidal. Blood Compatible Bioactive Polymers. Donald Wise, Joseph Greisser, Debra Tranlolo, Mario Cattaneo, Kai-Uwe Lewandrowski, Michael Yaszemski. Fundamentals and Vascular and Carrier Applications, Humana Press, pp.25-48, 2000, Biomaterials Engineering and Devices: Human Applications, ISBN 0-89603-858-0. hal-00982849

**HAL Id: hal-00982849**

**<https://hal.science/hal-00982849>**

Submitted on 24 Apr 2014

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**BIOMATERIALS ENGINEERING  
AND DEVICES: *HUMAN APPLICATIONS***

---

VOLUME 1

***Fundamentals and  
Vascular and Carrier Applications***

*Edited by*

**Donald L. Wise, PhD**

*Northeastern University  
Boston, MA*

**Joseph D. Gresser, PhD**

*Cambridge Scientific, Inc.  
Cambridge, MA*

**Debra J. Trantolo, PhD**

*Cambridge Scientific, Inc.  
Cambridge, MA*

**Mario V. Cattaneo, PhD**

*Cambridge Scientific, Inc.  
Cambridge, MA*

**Kai-Uwe Lewandrowski, MD**

*Massachusetts General Hospital  
Boston, MA*

**Michael J. Yaszemski, MD, PhD**

*Mayo Clinic  
Rochester, MN*

HUMANA PRESS



TOTOWA, NEW JERSEY

# Blood-Compatible Bioactive Polymers

*Stéphane La Barre and Catherine Boisson-Vidal*

## 1. Introduction

The rise of biotechnology and the impressive development of substitutive therapies have contributed over the past 15 yr to providing new kinds of materials designed to specifically interact with biological systems: These were termed "biomaterials." The biomaterials family includes polymers, metals, ceramics, reconstituted biological material (such as collagen derivatives), carbon, composites and elastomers (1). Their chemical structures and physical characteristics apply to diverse medical applications. The wide applicability of these techniques is reflected in the recent surge of general interest for the so-called "intelligent materials," a popular metaphor for new families of environment-responsive materials.

For more than 20 yr, polymers (especially polyvinylchloride and polyurethanes) have been widely used in a number of structural implants (artificial heart, hip prostheses, intraocular lenses), external assistance (contact lenses, removable dental prostheses, renal dialyzers) or disposables (cardiopulmonary bypasses, diagnostic and therapeutic catheters, oxygenators, hemodialyzers, tubings, and blood bags). Their ability to replace defective organs and blood vessels, totally or in part, has improved the living conditions and life expectancies of many patients.

Many of these biomaterials are in contact with blood and, despite their extensive applications in medicine, and the numerous investigations they are subjected to, they are not inert to blood compo-

nents. Some undesirable secondary effects indeed occur when blood proteins and cells interact with polymers, and lead to the formation of interfacial thrombi. Until now, direct contact between a biomaterial and blood could be sustained for a short period of time, and typically required the administration of some heparin-based anticoagulant and antithrombotic treatment to prevent thrombosis. Unfortunately, the prolonged use of heparin is not recommended, especially when patients are subjected to hemorrhages. Many methods have been proposed to avoid blood coagulation and thrombus formation without drug administration, e.g., timed release of adsorbed anticoagulant agents onto the surface of implantable polymers. The limited time efficiency of these methods restricts their applicability to short-term use (1).

Another approach has been explored through an attempt to replicate nature by generating bioactive polymers that mimic precise components of selected molecules, e.g., the active site of heparin. A crucial step in the design of such materials is the understanding of the mechanisms involved during the contact of the foreign surface with the living body. For a few years now, chemists, biochemists, physicists, and biologists have been trying to decipher the recognition mechanisms involved in specific interactions between biological molecules, especially blood constituents, at the macromolecular scale (i.e., receptor–ligand, enzyme–substrate, enzyme–allosteric protein). These events, indeed, play a key role in enzymatic and regulation mechanisms.

From: *Biomaterials Engineering and Devices: Human Applications*, Volume 1  
Edited by D. L. Wise, et al. © Humana Press, Inc., Totowa, NJ

Following these various investigations (2–16), a new generation of polymers has been developed. The design of these materials obeys the principle of molecular specificity recognition of the polymer by the living body. This principle is based on the lock-and-key mechanism, which is ubiquitous in all living systems. Proteins are the agents of biological specificity, and, as such, they are capable of differentiating between molecules that have similar, yet not identical, structures. Thus, the antibody (Ab) generated following the intrusion of a foreign protein will recognize the same protein in any subsequent intrusion. This specific recognition process results from the tertiary structures of proteins. The architecture of protein molecules is such that one or more sites, involving both specific spatial arrangements and chemical structures, are present, and can interact specifically with complementary sites on target molecules.

The basic idea behind the authors' approach is to design a synthetic molecule having structural features similar in geometry and chemical character to those of the natural molecule of interest. In the case of enzyme–substrate reactions, for example, when a synthetic substrate is used, the natural recognition mechanism is bypassed to some extent, and the enzyme binds preferentially to the synthetic substrate. The synthetic polymer models developed in this laboratory, i.e., cross-linked dextran or insoluble polystyrene (PS), are functionalized with chemical functions randomly distributed on the macromolecular backbone. They contain a number of binding sites consisting of particular sequences of the functional groups that are specifically recognized by one or more biomolecules. Hence, the specificity of these biomaterials mostly results from the probability of emergence of biospecific sites, through the random substitutions of chemical functions on the surface of the polymer. The structure to function ratio is a key parameter in this concept, named the concept of statistical specificity and statistical heterogeneity (17).

Original experiments carried out at Laboratoire de Recherches sur les Macromolécules have repeatedly given substance to the above concept. Over the past 20 yr, generations of senior and student investigators have encountered biospecificity, using diverse biological models and tech-

niques on complete libraries of statistical copolymers, and attempted to identify the mechanisms underlying their interactions. Their most significant findings are detailed in this chapter. However, before introducing this principle, some background information on the events that occur when blood contacts a foreign surface, such as a polymer, is necessary.

## 2. Blood Compatibility and Physicochemical Properties of Polymers

When living tissue is in contact with a biomaterial, surface reactions are triggered that are identical to the immune rejection that occurs after grafting operations or transplants. The sequence of events initiates with the absorption of blood proteins on the surface of the polymer, followed by the activation of the coagulation system and thrombus formation, finally resulting in the activation of the immune system. The nature of these reactions depends on the physicochemical and biological properties of the material used. The essential and fundamental property of biomaterials is biocompatibility, measured as the degree of inertia toward the above reactions sequence.

### 2.1. How Should Biocompatibility Be Defined?

Biocompatibility has been aptly defined by Anderson as “the ability of a material to perform with an appropriate host response in a specific application” (18), or by Rattner and Hoffman as “the exploitation of proteins and cells in the body to design a specific goal,” (19), according to their respective concerns.

Biocompatibility will depend on the dynamic pathobiology of cells and humoral components that interact with a polymer, and also on the physical and chemical properties of the latter. Time is irrelevant, inasmuch as a biomaterial can be biocompatible within a short time period, but not for extended use. To be biocompatible, a biomaterial must be nonthrombogenic, must not induce an activation of the immune system, nor be toxic and carcinogenic, and finally should not disturb the blood flow, intrinsically or via waste products, after being in contact with blood (1,20–24). The

temperature, time of contact, blood flow rate, as well as biomaterial surface area (interface free energy, surface energy, charge), and mechanical properties must be taken into account.

## 2.2. Sequence of Events Resulting from Blood-Polymer Contact

Blood is an eminently heterogeneous tissue made up of cells, proteins, mineral salts, and water. All these components, especially proteins and cells, can interact with the foreign surface, be adsorbed, change shape, and be broken into fragments that can have new biological activity, e.g., with the coagulation factors (25–30). Contact between blood and foreign surface causes the activation of different biological systems, triggering processes that lead to the following observable sequence of events:

1. The initial nonspecific adsorption of the protein on the surface of the polymer is very rapid, and occurs within 1–3 min after contact is established (25,27–32). Some of these adsorption processes are partially or totally reversible, each adsorbed intermediate differing from the other by a change in the thickness and/or the structure of the adsorbed layer (29,32–34). Moreover, competitive adsorption of the proteins occurs, the more abundant (low surface-affinity proteins) being adsorbed initially, and later being displaced by the less-abundant species (high surface-affinity proteins) (30,31,35).
2. Second is the deposition of formed blood elements, adhesion, deformation, and release reaction of platelets induced by this adsorption of blood proteins (22,26,36–39).
3. At the same time, the activation of the blood coagulation systems occurs (Fig. 1), followed by the activation of the complement system, and both cellular and humoral immune responses (18,40–43).
4. At a later stage, the formation of thrombin is able, first, to induce platelet aggregation and release, then to hydrolyze soluble fibrinogen (FIB) into soluble fibrin polymer, which leads to the formation of thrombi released into the blood as emboli.

Such events never occur with blood in contact with vascular endothelium lining the vessel lumen

and heart chambers. The endothelial cells are naturally anticoagulant, and exert direct control over the blood coagulation mechanism.

This set of events control the healing process inside porous polymer networks, the formation of fibrous capsules around polymeric prostheses, and the calcification of the polymer matrix over a long-term exposure (27,37,44–46). In some cases, this may provide a suitable smooth blood-compatible surface (19,45,47–49). Several parameters, including shear rates and roughness, favor the coagulation process at the interface between blood and polymer, because smooth polished surfaces are less thrombogenic than rough and irregular ones (29,47,50).

Attention was directed first to parameters that control the coagulation process at the substrate-tissue interface, but thrombotic occlusions and stenoses are not the only disturbances to avoid. The protein coating which initiates at the onset on the biomaterial surface can itself lead to considerable disturbances.

## 2.3. Protein Adsorption

Protein adsorption is the major event that occurs within a few minutes following initial contact between material and blood, because it rules the subsequent behavior of the biomaterial. Good biocompatibility requires that reversible adsorption of proteins and interactions with the polymer surface be minimal. Understanding the process of protein adsorption is essential for developing a systematic method of controlling thrombogenesis at blood-material interfaces.

Proteins can adsorb onto polymer surfaces through a variety of interaction mechanisms. Depending on the chemical nature of both proteins and polymers, these interactions may be strong, reversible, and present a high affinity for the surface. The extent to which proteins are adsorbed and the composition of the resulting layer are conditioned by various physical parameters of the polymer, including (i) free energy, surface charge, wettability, and surface texture (22,27,51–54), (ii) the experimental conditions, such as ionic strength and pH (55–57), (iii) the shear rate and blood flow (33,50–52,58–60), (iv) the time of contact, (v) and, finally, the different binding sites of proteins (22,25,27–30,61–64).

For instance, hemodialysis is often associated

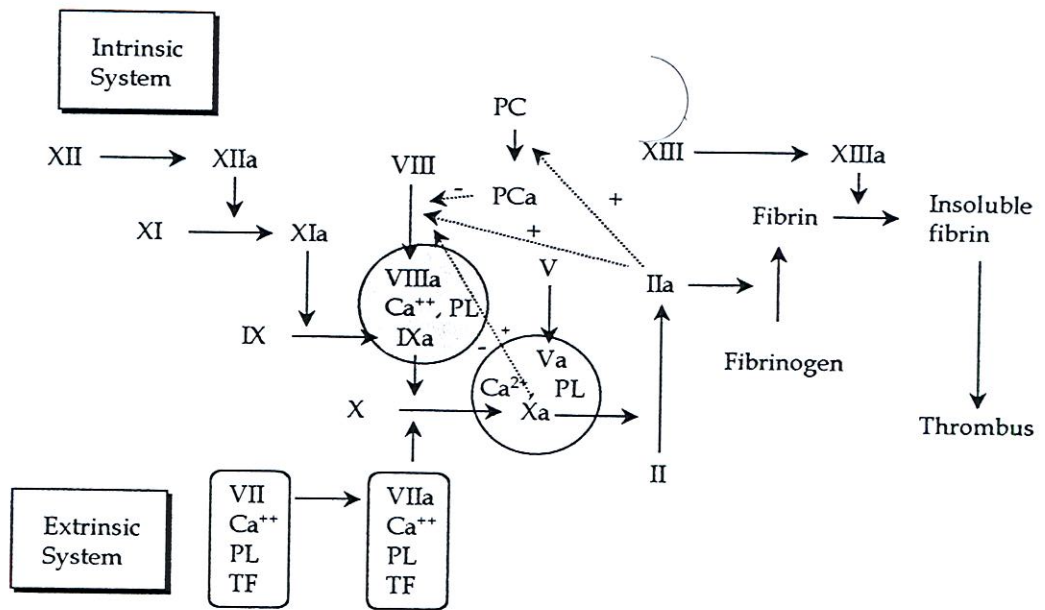


Fig. 1. Overview of blood coagulation. The diagram outlines the major factors and the sequence of reactions involved in the coagulation and fibrinolytic processes. Blood coagulation involves a sequence of reactions in which zymogens (designated with Roman numerals) are converted to active proteases (suffix a), by cleavage of one or more peptide bonds. At each stage, a protease from the preceding step, a zymogen, a nonenzymatic protein cofactor  $\text{Ca}^{2+}$ , and an organizing surface (such as platelets) are involved. The final protease generated in this sequence of reactions is thrombin, which converts fibrinogen (FIB) to fibrin monomers by cleaving fibrinopeptides from the amino terminal ends of the molecule. Removal of fibrinopeptides allows fibrin monomers to gel. Subsequently, the protein factor XIIIa catalyzes the crosslinking of adjacent fibrin monomers, to strengthen the clot. The solid arrows indicate pathways resulting in acceleration of blood; broken arrows represent inhibitory mechanisms (156). TF, tissue factor; PL, phospholipids; PC, protein C; PCa, activated protein C.

with the undesirable effects related to protein adsorption on clinically used membranes. Several studies carried out on the characterization of the adsorbed protein layer on artificial surfaces, after contact with patient plasmas before and after a hemodialysis session, allowed the identification of several proteins in vitro (26,29,65-67) or ex vivo (68-70). The deposited protein layers are very complex. Hydrophobic surfaces were initially considered a superior blood interface, until evidence showed that protein adsorption and platelet aggregation are generally lower on highly hydrophilic surfaces (22,32,49,51,52,54,56,63,71).

Early studies have focused on the roles of fibrinogen (FIB) and albumin in thrombogenesis, as the major constituents of the protein layer (29,65-67). Bovine serum albumin was shown to be easily adsorbed without denaturation onto

hydrophilic surface, and to induce an increase in the antithrombogenic character of the material (38,47,72). It induces an inhibition of the activation of the complement system when adsorbed on surfaces, such as poly(ethylene glycol) grafted cellulose membranes (56,73). In addition, these materials inhibit FIB adsorption and reduce platelet aggregation (61,72). Knowing that FIB adsorption mediates platelet adhesion, part of the research focused on developing materials that bind albumin preferentially to FIB (42). Polymer surfaces were modified toward an increase in albumin affinity, which itself improved blood compatibility. In this manner, polyurethane and silicone-polyurethane copolymers were shown to adsorb preferentially to FIB (23,51,52,58,74).

Modern antithrombogenic materials can be designed either by grafting anticoagulant agents, such as heparin, onto the surface of polymers,

or by covalent substitution of suitable chemical groups on the polymeric backbone. Whatever the technique, having a starting material endowed with improved biocompatibility (e.g., high albumin/FIB affinity ratio) is a valuable asset.

A step forward was made with the improvement of biocompatibility of new polymers as an alternative to the classical surface grafting of anticoagulant agents, such as heparin. Heparin is the oldest antithrombotic drug (known for more than 40 yr), and is still used in emergency anticoagulation therapy. Because of its antithrombotic properties, it was the first biologically active molecule to be immobilized on a polymer surface.

### 3. Biospecific Random Copolymers

#### 3.1. Classical Anticoagulant Materials:

##### *An Overview*

Equilibrium in blood is maintained chiefly through the action of various circulating natural inhibitors of the procoagulant enzymes, the most important being antithrombin (AT), which inhibit thrombin through the formation of a covalent complex. This reaction is catalyzed by heparin or heparin analogs present in the endothelial tissue (75–79). This major property of heparin is responsible for the widespread use of the mucopolysaccharide as an anticoagulant drug. Besides its anti-clotting effect, heparin has long been known to inhibit the activation of the complement system *in vitro* (76,80–82). In addition, it is endowed with numerous other biological effects, e.g., antibacterial and antiviral (76). The molecule can be linked to a polymer through covalent or ionic binding, thereby conferring its intrinsic anticoagulant properties to the grafted biomaterial.

The first attempt, carried out in 1963 (83), was to immobilize heparin by electrostatic binding, through its anionic groups. Subsequently, heparin was preferably surface-bound through quaternary ammonium groups (8,23). The drawback was that heparin rapidly eluted when the tubings were exposed to circulating blood. Treatment with glutaraldehyde stabilized the ionic binding of heparin, and it further reduced thrombogenicity of small-diameter polyurethane polytetrafluoroethylene (PTFE) grafts (84).

Another approach was to covalently graft the mucopolysaccharide to biomaterials, following

the example of polypropylene in oxygenators or polymethacrylate in intraocular lenses (42,46,53,84–91). As a rule, heparin-bound surfaces adsorb minimal amounts of protein (e.g., albumin, immunoglobulin G [IgG]), and are antithrombogenic (42,88,92–94). Reduced activation of the complement via the classical pathway is observed in relation to a high affinity for C1q (42,90,95). This surface heparinization technique indeed lowered the adsorption of FIB on the inner surface of treated grafts in *ex vivo* experiments performed on dogs (84,96). Long-term loss of this activity, however, resulted from prolonged exposure to the blood flow, as the mucopolysaccharide was progressively released and degraded (88,97–99), thereby establishing the risk of clot formation. Treatments attempting to address this problem, through improved heparin stabilization or tighter binding to the polymer, resulted in partial loss of useful properties (56,85,88,100).

One innovative idea explored the coupling of heparin to prostaglandins coated on polymers. These heparin–prostaglandin materials exhibit low fibrin formation and platelet aggregation, both *in vitro* and *in vivo* (59,101).

Aside from their specific limitations, in all the above examples, the enhancement of biocompatibility is dependent on the complement system's downregulating properties of heparin through its binding to the C3a fragment, and subsequent unavailability of the latter (40).

#### 3.2. The Enzyme–Substrate Model

The formation of an enzyme–substrate complex illustrates the ability of a protein to recognize a substrate molecule as a whole or in part, with greater or lesser specificity, thanks to well-defined sites on its available surface. Labile bonds are then formed between the enzyme's exposed receptor site and, according to size, the target molecule or the areas of matching conformation on a larger macromolecular substrate. As an example, chemotherapy has designed decoy molecules of natural substrates to act as specific inhibitors of key enzymes involved in microbial diseases. As long as the structural analog is retained in the catalytic pocket by weak-energy noncovalent bonds, the reactivity of the enzyme is considerably lowered. Competition situations may occur between the natural substrate and its inhibitor to occupy the

corresponding enzyme surface site. Such is the case of sulfamides, especially that of sulfanyl amide, a functional analog of paraaminobenzoic acid.

Ligand recognition is the result of individual energy contributions from binding structural epitopes. Consensus sequences emerging from combinatorial chemistry libraries (obtained from systematic mutational analysis) may be informative in cases in which the binding sites are located on the surface of the protein, and tend to act independently (102). The protein must be of suitable size, and the binding epitopes must not be part of the catalytic site, nor even partly buried (103), if one is to get realistic energy values. Alternative strategies are needed to detail the role of the specificity sites (i.e., how much energy they contribute to binding, and whether or not their contribution is additive) to benefit studies of structure–function correlation and rational drug design. Libraries generated from combinatorial chemistry (104) have been used to identify consensus sequences for binding specificity. Substrates generated in this manner can also be used to probe the molecular environment of the specificity sites of the enzyme to determine how they contribute to recognition in the transition state. A practical application of this approach is reported for several examples, conception of anticoagulant surfaces, supports for the removal of selected antibodies or antiproliferative agents.

### 3.3. Novel Copolymers

A novel approach may address the quest to improve the design of drugs and medical devices, and may possibly bring new information or confirm specific mechanisms of interaction with target components of living systems. Novel copolymers are achieved by random substitution of selected chemical functions, either on preformed macromolecular chains, or on corresponding monomers prior to copolymerization. Interaction studies with various blood components may reveal specific binding affinities for a given protein, coagulation factor, coagulation inhibitor, cell receptor, extracellular matrix, item of the complement system, Ab, or growth factor, with or without biological activities. Systematic screenings of novel copolymer libraries show that biospecificity is composition-dependant within a given family.

Biospecific sites thus emerge as combinations of motifs (substituted or unsubstituted) on the polymer chain, offering a topologically suitable match unique to each interacting species. Optimal specificity is defined by the synthetic key (on one member of a given novel copolymer family) that best fits the natural lock (on one biological component). This revisitation of Fisher's concept is the essence of the concept of statistical specificity and statistical heterogeneity (16).

The stability of such systems is directly influenced by the physicochemistry of the medium, which may destroy the tertiary structures of proteins or preclude the occurrence of given intermediate conformations. Protein chains, according to thermal agitation, may adopt extended configurations of great flexibility, e.g., spinning motions through space. In brief, the final conformation of a protein in solution in a given medium is the result of prevailing energies.

### 3.4. BIORACS with Heparin-like Character

When designing structural analogs, a starting point in the search for biospecificity is the recognition of the minimal structural requirements responsible for the activity of interest in the natural molecule. Only one-third of the heparin chains (essentially made up of alternating glucosamine and uronic acid residues (76,79,105,106)) possess a specific AT-binding sequence, identified as a pentasaccharide segment that contains a single trisulfated D-glucosamine residue, which has been fully synthesized (76,79,107–109) (Fig. 2).

Synthetic analogs were created in our laboratories, by covalent binding of sulfonate and carboxyl groups on various polymer matrices (dextran [Pharmacia, France], crosslinked PS, [Bio-Rad, France], crosslinked dextran [Sephadex, Pharmacia, France], polyethylene (PE)–PS graft copolymers; see Fig. 3), in order to mimic the natural catalytic sites known to confer its anticoagulant and antithrombotic properties to heparin (2,4,8,110–114). They were made soluble or insoluble according to their prospective *in vivo* use, e.g., as transfusion additives or as implants, or *in vitro* as solid-phase affinity sorbents. Structure vs activity tests over the past few years have yielded ever-improving anticoagulant activities. These synthetic heparin-like polymers were



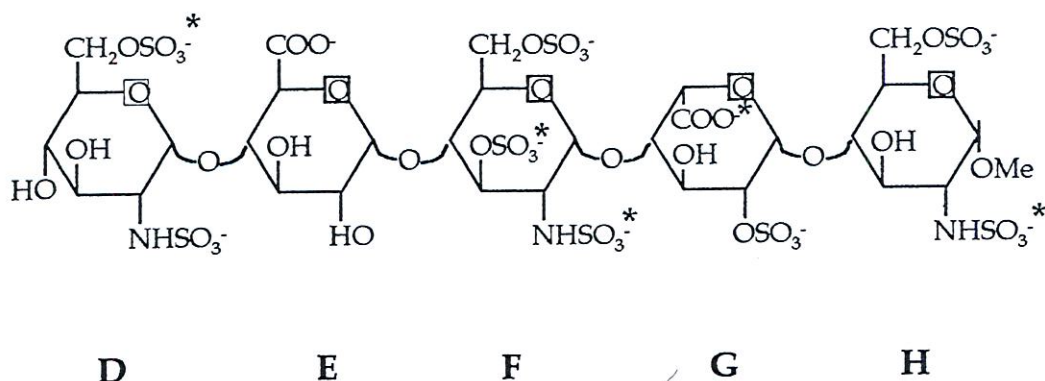


Fig. 2. Schematic representation of the pentasaccharide unit structure of heparin that binds to AT. The groups marked with asterisks in the heparin pentasaccharide, DEFGH, are critical for productive binding to AT (79).

shown to exhibit anticoagulant properties similar to heparin itself, i.e., they catalyze the inhibition of the serine proteases of the blood coagulation cascade by the natural plasma inhibitors, AT and heparin cofactor II. This anticoagulant activity depends mostly on the type of the substituents, on the degree of substitution, and on the net charge borne by the polymer.

Measurable heparin-like activities were obtained with aspartyl groups on reticulated insoluble PS (2). Advantage was made of this observation to produce tubings of small diameter made of surface-treated PE-PS graft copolymers. In the case of large-diameter synthetic grafts vessels, the high blood flow rate prevents the formation of thrombi, unlike what is observed on smaller diameters tubings. Despite the efforts lately devoted to design any antithrombogenic small-diameter, implantable vessel, none has proven satisfactory for long-term use. Grafting of aspartyl and sulfonate groups onto the lumen surface of PE tubings were performed with the aim of obtaining structurally improved small-diameter versions. Treated inner surfaces showed no fibrin networks, compared to untreated sulfonated tubings (7,115,116).

The arginyl sulfamide residue, protected by a terminal methyl ester group, presents a definite AT character (3,117,118). AT inhibits thrombin by forming a covalent complex, in which an arginyl function of the reactive center loop of the inhibitor is bound to the enzyme-active site, in the manner of a substrate. The initial idea was to mimic part of the active site of AT, through the

random substitution of arginyl derivatives. These polymers showed up as antithrombic, but failed to catalyze the inhibition of thrombin by AT. One of the polymer samples exhibited a high affinity for thrombin ( $K_{\text{aff}} = 10^7 \text{ M}^{-1}$ ), which turned out to be specific, thus implying reversible ionic binding. This particular resin was substituted with 10% arginyl methyl ester; little, if any, affinity for thrombin was recorded with more-substituted samples. This resin was used as stationary phase for thrombin purification in liquid affinity chromatography, both low and high performance (6,119). The authors successfully purified thrombin from a prothrombin complex in just one step, with an excellent 90% yield with a specific activity of about 3000 National Institute of Health Units (NIHU)/mg.

The soluble functionalized dextrans, carboxyl-methyl dextrans benzylamide sulfonate (CMDDBS), catalyze thrombin inhibition via AT (4,113,120) and heparin-cofactor II (121), depending on their composition. They have weaker anticoagulant effects than heparin. Their anticoagulant activity is related to their high content of carboxymethyl (CM) and sulfonated (S) groups (4,114,120). Carboxy methyl dextran (CMD) does not exhibit any anticoagulant activity in the absence of benzylamide sulfonate groups (Fig. 4). Moreover, an increase is shown with increasing molecular mass, up to a 40 kDa threshold (120). These polysaccharides, just like heparin, are able to inhibit thrombin generation after stimulation by both contact-activated and thromboplastin-activated systems

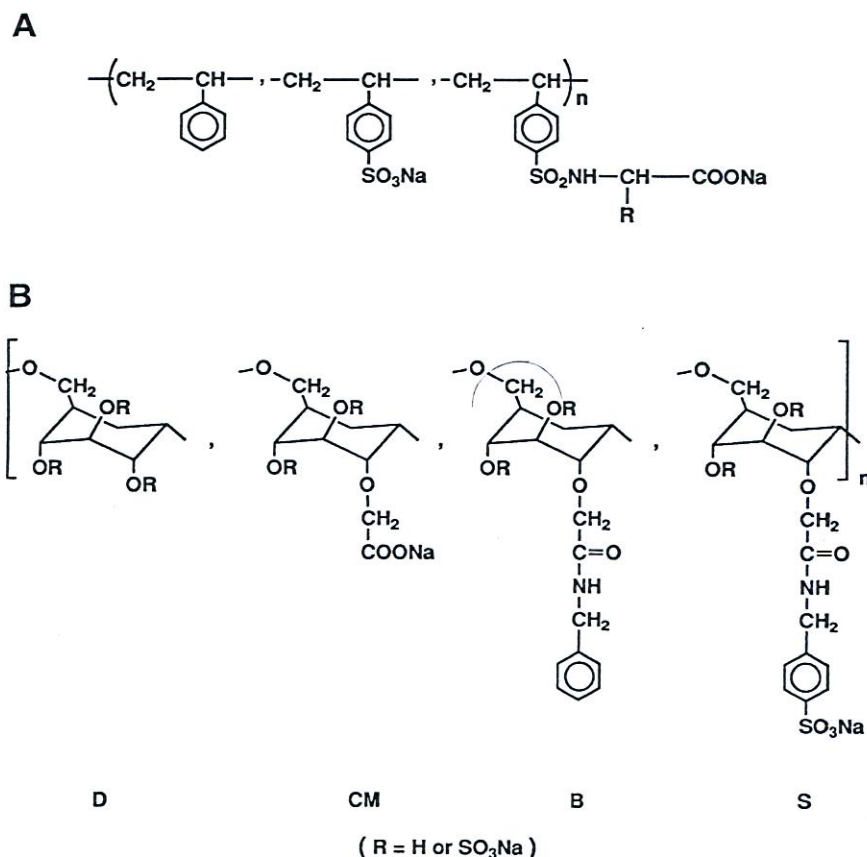


Fig. 3. Structures of the derivatized dextrans and crosslinked PS $\alpha$  (A). The PS beads are crosslinked with 3% w/w of divinyl benzene. The substituent chemical groups are randomly distributed along the PS chains. R is the side chain of standard  $\alpha$ -amino acids (2,3,5,8,9,14). It is well recognized that the polymer surface may differ in composition from the bulk. In order to address the issue of surface vs bulk composition, low-resolution X-ray photoelectron spectroscopy (XPS) was performed on the resins. The sampling depth, at a take-off angle of 90 degrees, is typically approx. 10 nm, and all elements, with the exception of hydrogen and helium, are detected with high sensitivity (131). (B) CMDBS, where R is a sulfonate group. The CMDBS family is schematically described as composed of some D-glucosyl units (D) bearing methylcarboxylate groups (CM): Some of the latter or derivatized as benzylamides (B), which are then sulfonated to give sulfonated B groups (S). The first and most determining step for their synthesis is the dextran carboxymethylation (4).

(122). The results suggest the formation of a complex between CMDBS and thrombin, inducing a direct thrombin inhibition. CMDBS, like the natural analog hirudin extracted from leeches, may interact with thrombin at the anion binding exosite I, or at the specific pocket of the enzyme, rather than by its active site (122). Consequently, CMDBS can be considered as models carrying active sites resembling structural elements identi-

fied in various biological substances implied in blood coagulation.

These polysaccharides were investigated as potential plasma expanders. However, they possess very slight but effective heparin-like properties, and they also inhibit the activation of both complement pathways (120,123). Their capacity to inhibit formation of the classical and the alternate C3 convertases in purified system is similar

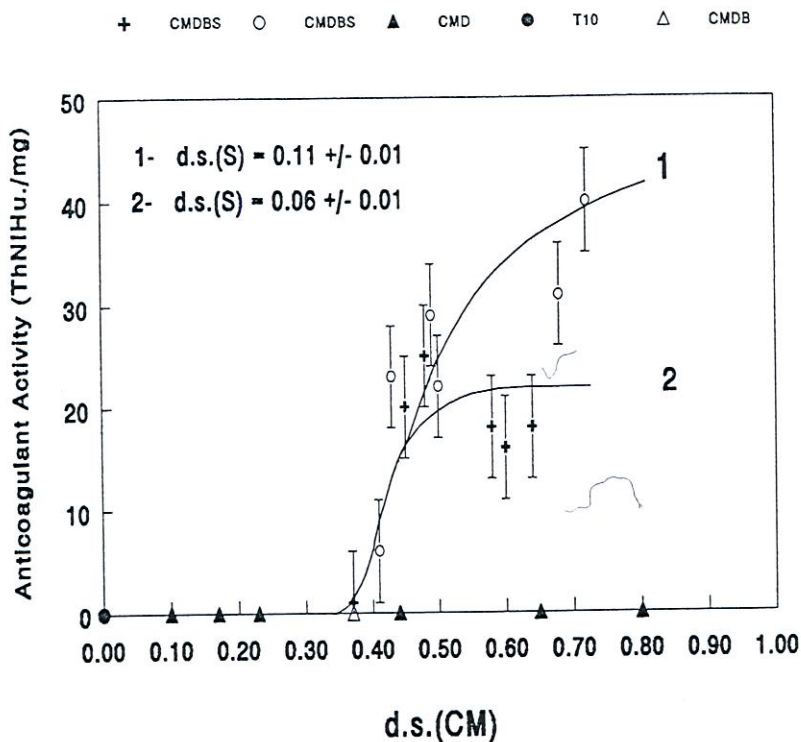


Fig. 4. Dependence of CMDBS anticoagulant activity on the content of CM and S groups. The anticoagulant activity was determined by measuring thrombin clotting time of poor-platelet human plasma, in the presence of various amounts of polymer and of 5 NIH units/mL human thrombin (113). It is expressed as the number of thrombin NIH units inactivated by 1 mg polymer. The two series of CMDBS are characterized by S content of 0.06 and 0.11 (s.d.); each series with different CM content, ranges from s.d. = 0.37 to 0.72. Neither CMD nor CMDB derivatives exhibit anticoagulant activity. CMDBSs do not reveal significant anticoagulant activity when their CM groups content is found below d.s. 0.35–0.40; beyond this value, the activity increases sharply to reach a maximum value for a CM content of d.s. = 0.72. The higher the S content, the higher the maximum of biological activity (4,114,120).

to that of heparin, while in whole serum this capacity is superior to that of the mucopolysaccharide. The composition dependence is different for the C3b binding sites than for the anticoagulant sites, which suggests that the structures of the sites are different (Fig. 5). The anticoagulant and complement inhibitory activities of the different dextran derivatives are also different from one another (120). The complement-activating ability of crosslinked dextran (Sephadex) is suppressed by coupling CM groups to the surfaces (8,124). Since the activation of the complement system is an essential step in the immune response, these CMDBS's can be considered as nonsteroidal anti-inflammatory drugs. They are interesting because

they can be degraded by dextranase in *in vitro* assays, and possess a very low immunogenicity (120,125).

### 3.5. BIORACS and Antibodies

Some bioactive polymers were designed to interact specifically with selected Abs. Autoimmune diseases are disorders in which the immune system produces auto-Abs to an endogenous antigen (Ag), with consequent injury to tissues. It usually results from the failure of the immune system to distinguish between foreign Ags and the body's own molecules, and sometimes from systematic bacterial infestation of intestinal blood. The pathologies caused by the production of Ig

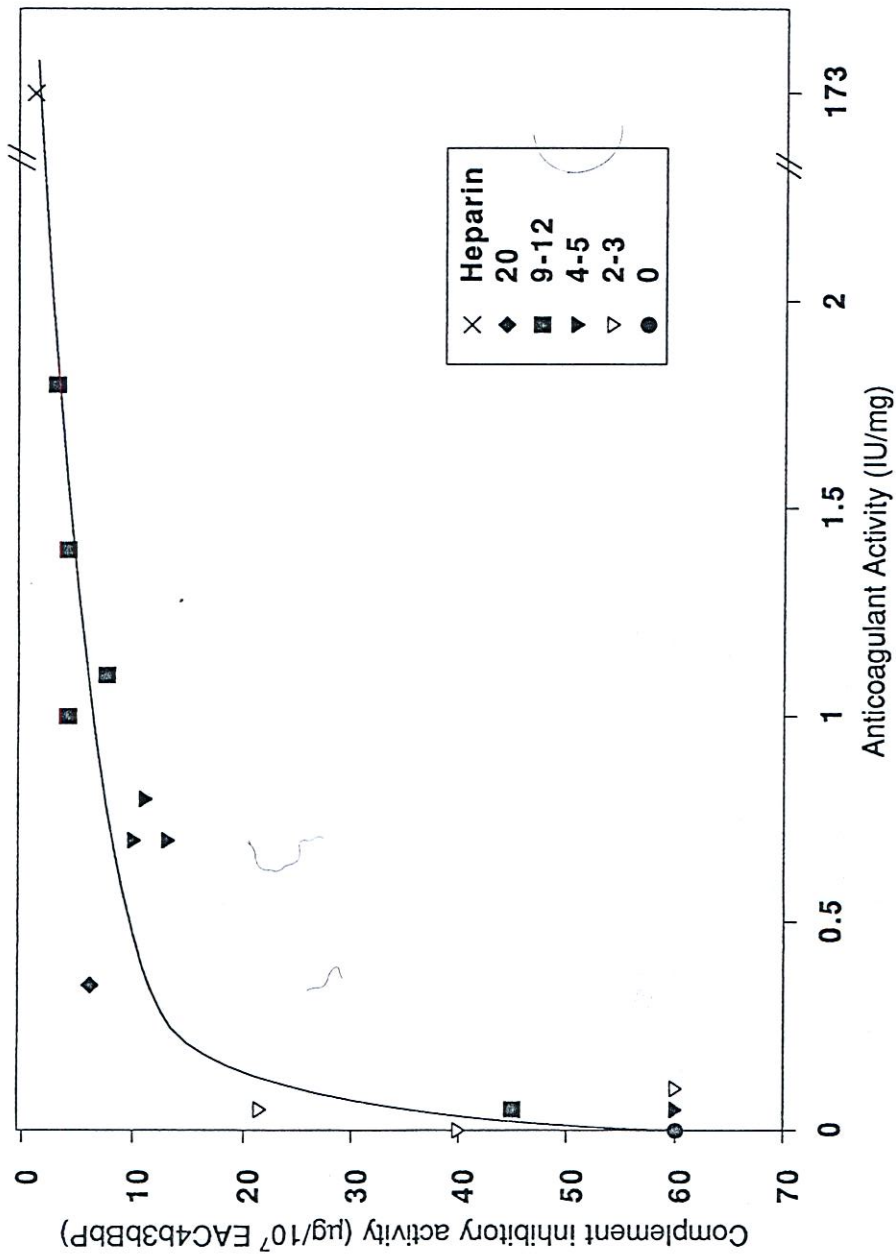


Fig. 5. Anticomplementary activity vs the specific anticoagulant activity of dextran derivatives, with different contents in glucosyl units substituted with benzylamide sulfonate (S%) (123). The anticoagulant activity is expressed as the number of thrombin NIH units inactivated by 1 mg polymer. It is compared to that of a standard heparin (hog intestine heparin, batch H108, Institut Sanofi-Choay, France, 173 IU/mg), which allows the determination of the specific anticoagulant activity (IU/mg polymer). The anticomplementary activity of the dextran derivatives is expressed as the input of polymer which inhibits 50% convertase formation on 10<sup>7</sup> EAC4b,3b. The effect of CMDBS on the formation of P-stabilized alternative pathway C3 convertase sites (C3b, Bb, P) is assessed by assembling the convertase on sheep erythrocytes bearing C3b in the presence or in the absence of increasing amounts of tested dextran derivatives.

auto-Abs are often evolvable and debilitating. The specificity of the Ab–Ag recognition mechanism can indeed be taken advantage of in producing synthetic ligands capable of inactivating the Ig proteins.

Abs or Igs are produced by plasma cells, when B-cells are exposed to a particular Ag (exogenous proteins, toxins). They are mostly composed of polypeptide chains divided into a crystallizable fragment with conserved regions, and a highly variable Ag-binding fragment (Fab), which contains the specific binding site toward a given Ag. In either case, BIORACS interact specifically with the Abs through their Fab fragment. The random substitutions of the different chemical groups lead to the formation of sequences that possess high affinity for sites in the Fab region.

### 3.5.1. Abs Antifactor VIII

Insoluble PSs were functionalized to specifically interact with Abs antifactor VIII (anti-fVIII). The anti-fVIII are predominantly of the IgG type (IgG4 and IgG1 subclasses). They appear in approx 15–20% of severely affected hemophilic A patients, and they neutralize the procoagulant activity of the factor VIII (fVIII). The level of anti-fVIII inhibitor present in the plasma of an affected patient represents only a small proportion of total plasma IgG. Various therapeutic approaches have been proposed to deplete plasma of these Abs, but they remain unsatisfactory to date (126). The treatment strategies depend on recorded inhibitor levels, and on patient's response to treatment. Among the most common treatments are plasmapheresis and eparation of plasma over a protein A Sepharose column, combined with injection of massive doses of fVIII (127). Protein A immunoadsorption exploits the affinity that IgG has for this protein, extracted from *Staphylococcus aureus*. This treatment typically reduces the Ab titers, but depletion of the patient's total IgG occurs, and requires replenishment by infusion.

A bioactive polymer able to develop a specific interaction with anti-fVIII, and to minimize the nonspecific interaction with normal IgG and other plasmatic proteins, was prepared (5,128). Following the experiments of Shima et al. (129), cross-linked PS were grafted with tyrosyl residues and the methyl ester derivatives, because they are

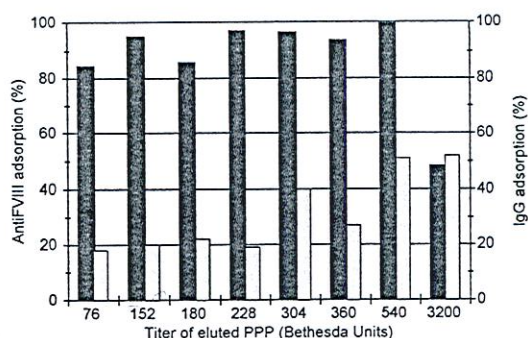


Fig. 6. In vitro removal of anti-FVIII from several hemophilic A plasmas onto a functionalized polystyrene resin substituted with 18% of tyrosyl methyl ester sulfamide groups. Dark-shaded bars show adsorption of anti-fVIII expressed as the percentage of the total inhibitors present in the eluted plasma and light-shaded bars show adsorption of IgG present in the eluted plasma (14,130,131). Two mL hemophilic A plasma, with inhibitors, were loaded onto 1 mL gel, corresponding to 750 mg dry polymer, and circulated for 90 min at 37°C at a flow rate of 0.1 mL/min. The amount of adsorbed anti-fVIII is high (approx 90% of initial anti-fVIII concentration), and increases with increasing plasma concentration, up to 540 Bethesda Units/mL. The binding capacity of the support is about 1540 Bethesda Units/mL gel (corresponding to 2050 adsorbed Bethesda Units/g) at saturation.

involved in the recognition of the epitope of the fVIII molecule by anti-fVIII (14,130). Other resins were prepared using the aspartic acid–tyrosyl dipeptide. The adsorption of the F(ab')<sub>2</sub> IgG fragments onto PS beads is generally strong and irreversible, presenting a peak value at the vicinity of the isoelectric point of the proteins in solution (57). Resins derivatized with both sulfonate and tyrosine methyl ester sulfamide groups were found to exhibit a high affinity for the anti-fVIII Ab ( $K_{\text{aff}} = 10^8\text{--}10^9\text{ M}^{-1}$ ), and the binding capacity of the resins appeared to vary with the percentage of benzene rings bearing the tyrosine methyl ester sulfamide groups, with maximum capacity appearing at approx 20% substitution. The affinity of the resins for total IgG appeared to be low (about  $10^5\text{ M}^{-1}$ ) (14,130,131). The modified sulfonated PS resins are able to selectively adsorb the anti-fVIII-inhibitory Ab from hemophilic plasma (Fig. 6). The adsorption of total IgG amounted to

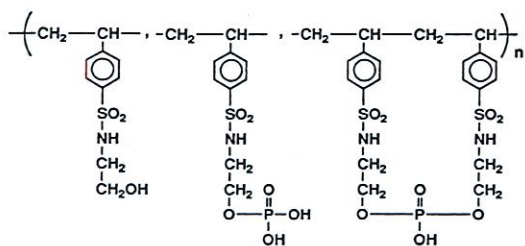


Fig. 7. Structure of phosphorylated crosslinked PS derivatives. They are prepared following a three-step procedure: chlorosulfonation of PS condensation with ethanolamine, and phosphorylation with phosphorus oxychloride in dry dimethyl phosphate.

no more than 40% of the initial concentration. No changes in plasmatic proteins concentration were detected. The binding capacity of the best support is about 1540 Bethesda Units per mL of gel (Bethesda Units/mL), corresponding to 2050 adsorbed Bethesda Units/g at saturation. The Bethesda Unit is the concentration of anti-fVIII Ab able to inhibit 50% of fVIII in 1 mL of normal plasma following a 2 h incubation at 37°C.

### 3.5.2. Antilupic Abs

Another example is related to phosphorylated PS derivatives (Fig. 7) (9,132). These insoluble polymers have been developed in order to interact specifically with systemic lupus erythematosus anti-DNA and/or antiphospholipid (anti-PL) Abs. Lupus results from the presence of autoimmune Abs in the sera of patients. These Abs form stable complexes with DNA and/or PLs like cephalin or cardiolipin. Such polymers act as DNA-like polymers. Adsorption experiments performed with both anti-DNA abs and normal Igs showed high-affinity constants of phosphorylated PS resins for anti-DNA Abs ( $4 \cdot 10^9 M^{-1}$ ), and low-affinity constants for normal IgG ( $2 \cdot 10^5 M^{-1}$ ). The adsorption of the DNA is of Langmuir type and varies according to phosphorylated units substitution, with a maximum for a substitution rate in the range of 30%, and a minimum above 60%. In contrast, the adsorption of anti-PL Ab is maximum at a substitution rate of approx 70%. This is true even when both anti-DNA and anti-PL Abs are present in the sera. The difference in the recognition of the resins by these two Abs is

attributed to a different surface distribution of the phosphate groups.

These observations demonstrated that the Abs at large (anti-fVIII, anti-PLs or anti-DNA) bind to the resins via their Ag-binding regions with high-affinity constants. Using selected resins, the ultimate goal is to develop a new method to remove unwanted Abs, by extracorporeal circulation from hemophiliac A patient in the case of anti-fVIII, and from SLE serum in the case of anti-DNA and anti-PLs.

### 3.6. BIORACS and Cells

The probability of rapid and aseptic local wound closure is expected to increase with the action of factors regulating re-epithelialization and angiogenesis (133). In cardiovascular surgery, since vascular endothelium represents a unique nonthrombogenic surface, one could expect that surface endothelialization would improve tolerance to grafts, and further increase their anti-thrombogenic activity. The polymers are traditionally pretreated with different biological substances (fibronectin [FN], albumin, laminin, collagen), in order to minimize nonspecific adsorption, and to increase cell attachment (66,134–136). Several workers showed that not only endothelial cells (ECs), but also smooth muscle cells (SMCs) migrate and proliferate following the implantation of polymer grafts (PTFE, Dacron) (48).

Interactions between cells and polymers may involve mechanisms of a nature different than that described for plasmatic proteins, as witnessed by an abundant literature (1,49,133,137). Binding to cell constituents can be achieved in a variety of ways, via one of the proteoglycans of the cellular matrix, or via a well-expressed membrane receptor or growth factor.

As a rule, cell adhesion and growth both are functions of the nature of the substrate, of the hydrophobic or hydrophilic character of its surface, and, of course, of the type of cell. Prokaryotic cells adhere poorly on hydrophilic surfaces, and eukaryotic in vitro cell growth is not favored on hydrophobic surfaces (49,138). In general, hydrophilization of the substrate (e.g., treated PS, glass) is beneficial to the growth of most cell lines (139), with exceptions including 3T3 cells and hydrophobic ethyl methacrylate surfaces (49). ECs

likewise adhere better to, and thrive on, the more hydrophobic surfaces, as witnessed by the preferred trend:  $-\text{CH}_2\text{OH} > -\text{CO}_2\text{Me} > -\text{CH}_3 > \text{CF}_3$  polymers (137,140).

From the above *in vitro* studies, it was postulated that polymers could be synthesized which developed specific interactions with cell membrane receptors (12,13,141), either directly (13,141) or indirectly, i.e., via an adherence protein adsorbed on the polymer (12).

### 3.6.1. BIORACS as Modulators of SMCs, ECs, and Other Cell Types

Culturing human umbilical vein endothelial cells (HUVECs) onto insoluble BIORACS necessitates the mediation of the adherence protein, FN. Because FN adsorbs differently from one substrate to another, HUVEC growth can be stimulated or discouraged. FN-precoated PS, comprising only S groups, favor HUVEC growth; defined substitution rates in aspartyl residues on the same resin, and with the same FN treatment, will totally obliterate any colonization (142). This dramatic effect is directly linked to the change in conformation of the adsorbed FN. With the merely sulfonated resins, the RGDS cell attachment domain is made available, and it has been suggested that, in the aspartyl sulfamide family of PS, loss of adherence may be caused by this sequence no longer being presented (142). The authors believe that one or more heparin domains may also participate in the binding to the S-treated PS, in addition to the RGDS sequence. Thence, protein conformational changes, which follow adsorption on the aspartyl sulfamide family, are likely to alter the critical stabilizing interactions necessary to allow proper orientation of the heparin-binding domains, possibly modifying their very structure. With respect to cells, it was found that the COOH terminal of FN heparin-binding domain promoted EC adhesion by a RGDS-independent mechanism (143,144).

Soluble BIORACSs may be internalized and/or degraded, and operate via unique intracellular mechanisms. For example, they may act directly on specific factors, like transcription factors. Heparin, as a natural sulfated mucopolysaccharide, can modulate (inhibit or stimulate) cell proliferation *in vitro* or *in vivo* (37). It has been shown

that, in some cases, this growth regulation was associated with the presence of growth factors in the medium. The soluble CMDBS were demonstrated to regulate the proliferation of different cell types, depending on the nature and distribution of the affixed chemical groups (16). Some dextran derivatives behaved as heparin mimics, regarding the protection, stabilization, and potentiating effects of the fibroblast growth factors 1 and 2, but with no significant anticoagulant activity (11). By themselves, the derivatives may accelerate repair in tissues, such as skin, bone muscle, and cornea, where regeneration is sought (145–147). The CMDBS family's heparin-like behavior and tissue-growth-regulating properties have been verified with a number of other cell types (148–150). A low anticoagulant derivatized dextran, for example, has the ability to inhibit SMC growth (10,149).

Independently, CMDBSs are able to modulate the expression of collagen biosynthesis in vascular cells (151–153). They accelerate the accumulation of a collagenous matrix, yet lowering its concentration in type III collagen. A potent anti-inflammatory capacity is noted in these low-anticoagulant forms (123,125). A dual effect (decrease in cell proliferation and synthesis of extracellular matrix) is thus noted, each depending on the substitution ratio of key chemical functions. Benzylamide groups, for instance, are required to enhance the antiproliferative effect. Also, those CMDBSs that exhibit the highest anticoagulant activity are not the most active. These data are consistent with the fact that the specific sequences required for anticoagulant activity are different from those conferring the antiproliferative activity (10,149). In contrast to this, the same CMDBS composition stimulates the proliferation of HUVEC cells *in vitro* (150). This latter effect, which cannot otherwise be attributed to the presence of growth factors in the culture medium, is again associated with the distribution of benzylamide sulfonate groups.

The antiproliferative growth-promoting activities appear to be independent, the CMDBS acting as an agonist of a natural effector in a situation, and as an antagonist in another (e.g., antiproliferative activity on SMCs and no effect on HUVEC growth). Preliminary structural analysis of these

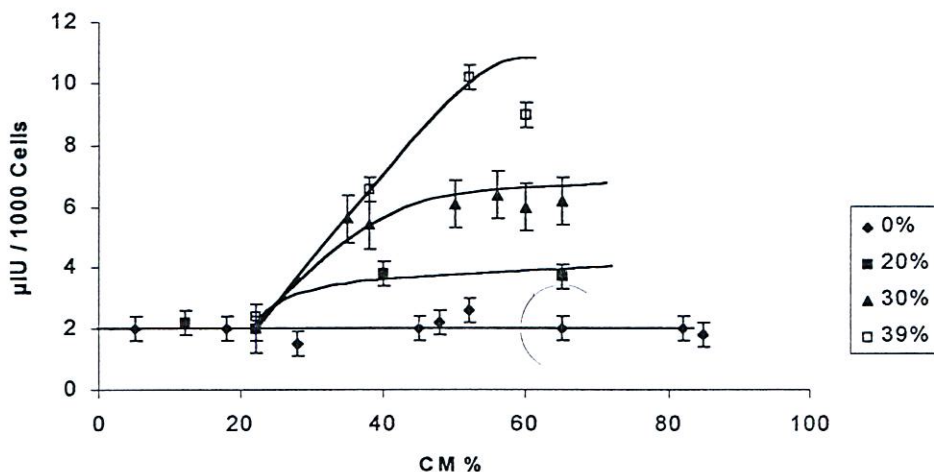


Fig. 8. Insulin secretion by RINm5F cells expressed in micro international units (U) of insulin per 1000 cells vs the percentage of carboxymethyl dextran units (CM%) for different crosslinked dextran beads bearing various benzylamide groups (B%) (13). RINm5F cells are insulin-secreting cell lines derived from a radiation-induced rat insulinoma. The insulin secreted by the cells controls insulin secretion, when RINm5F cells are cultured on crosslinked derivatized dextran beads (13). In the absence of benzylamide groups, beads derivatized with carboxylic groups do not stimulate RINm5F cells; the stimulating effect increases with increasing content of benzylamide glucosyl units. The effect is fivefold greater than the insulin-secretion effect induced by soluble secretory agents.

sulfated polysaccharides is under investigation, in order to determine the smallest sequence necessary and sufficient for cell-CMDBS interaction.

### 3.6.2. BIORACS as Modulators of Bacterial Proliferation

The emergence of strains of the common infectious bacteria *Staphylococcus aureus* and *Streptococcus pyogenes*, which have become completely resistant to the antibiotics used in hospital environments, necessitates the use of complementary approaches, in order to avoid untreatable septic shocks following surgical infections. Bacterial adhesion and colonization of biomaterial surfaces, following the implantation of cardiovascular devices, is initiated and encouraged by adherence proteins found in plasma and extracellular matrix, e.g., FN, vitronectin, and laminin. There are indications that observed antiadhesive activities of bacteriophobic CMDBS were exerted at the *S. aureus* binding site of FN (154). Finally, the aspartyl-substituted family of PSs, which hindered HUVEC proliferation through inducing appropriate conformational changes to the inter-

mediate FN layer (142), could make choice experimental candidates toward designing solid bacteriophobic substrata.

### 3.6.3. BIORACS as Modulators of Insulin Secretion

In this example, the PS derivatives and cross-linked dextran derivatives were used as substrates for growing RINm5F cells, an insulin-secreting cell line derived from an irradiation-generated rat insulinoma (13,141). A direct interaction is observed, the inhibition of the insulin secretion being correlated to the substitution rate of sulfonate groups on the PS sulfonate microcarriers, and with the cells growing normally. The same effect is observed on crosslinked dextran derivatives (Fig. 8). By contrast, the ester derivatives of selected sulfamide-linked amino acid families of PS exhibit a stimulating effect on insulin secretion. In the case of the sulfonated PS control microbeads, the secretion is independent of the net charge of the polymer, but when BIORACS are deposited on the cells, an acute and reversible inhibition was shown (13,141). In this case, a



small population of cells are in contact with the beads. This result fits a direct ligand-receptor-type interaction. The nature of the interaction is still unknown, yet it is believed to result from the secretion of a chemical messenger.

### 3.7. First Monte Carlo Simulations

The authors have validated numerical models of the polymer interactions. These models use Monte Carlo algorithms. The two examples below refer, respectively, to a water-soluble substance, and to an insoluble one.

#### 3.7.1. Example 1

The specific interactions of CMDBS with thrombin and AT and their heparin-like character, suppose that short sequences spanning different units are intervening. The authors therefore postulated that the probability of finding such sequences in CMDBS depends on both the chain structure and the unit distribution. The latter property is fostered by the kinetic peculiarities of the chemical reactions used. The kinetics of the initial carboxymethylation reaction, and the structure elucidation of the resulting compounds by joint 1D and 2D proton-<sup>13</sup>C nuclear-magnetic resonance spectroscopy, provided nine rate coefficients of carboxymethylation. They were modeled using Monte Carlo algorithms, with simple assumptions on the subsequent benzylamidification and sulfonation steps (Fig. 9) (155). Structure-function relationships indicated that the anticoagulant activity of these compounds can indeed be explained in terms of unit distribution along the polymer chain.

#### 3.7.2. Example 2

DNA-like copolymers were obtained by phosphorylation of hydroxylated PS derivatives. Monte Carlo simulations of the distributions of phosphodiester (PDE) groups, along the macromolecular chain, calculate the frequency of particular PDE arrangements found in the active sites. The results showed that these sites are made up of PDE groups separated by distances that closely match those between the same groups along a generatrix of the DNA double-cylinder helix (Fig. 10) (16).

These findings offer exciting prospects for "à

la carte" manufacturing of BIORACS, with the assistance of computer-generated algorithms.

## 4. Conclusion

It is possible to statistically induce, on standard polymer matrices, the formation of interaction sites specific to a wide range of molecular components of living systems. Using combinatorial chemistry, suitable chemical groups can be substituted randomly on the polymeric backbone: In favorable cases, the distribution of the motifs will mimic the active site of a natural effector. The most typical example is heparin, a multipurpose protein active in blood environment. During the interaction between heparin and AT, the active site is known to involve carboxyl, and oxygen- and nitrogen-sulfate groups.

Grafting amino acids, other than arginyl residue, onto PS resins confers heparin-like properties, regarding the ability to catalyze the inhibition of thrombin by its natural inhibitor, AT. Agonistic (binding + bioactivity) or antagonistic (competitive binding only) effects of BIORACS are now demonstrated in many experiments involving various key components of the blood and immune system, or cell lines or microorganisms of medical importance.

Computer simulations are now increasingly used to predict the biological properties of biomaterials, hence opening new and faster ways to design a specific substance in response to clinical needs.

## Acknowledgments

This paper draws from the 20 yr collective work performed in the Laboratoire de Recherches sur les Macromolécules (UMR CNRS 7540) under the leadership of M. Jozefowicz. Key researchers involved included T. Avramoglou, C. Boisson-Vidal, M. P. Carreno, F. Chaubet, J. Champion, C. Douzon, D. Gulino, J. Jozefonvicz, D. Labarre, D. Letourneur, M. Mauzac, V. Migonney, B. Montdargent, D. Muller, G. Pavon, F. Pflüger, and H. Serne. This research was supported by funds from the Centre National de la Recherche Scientifique and the Ministère de la Recherche et de l'Enseignement Supérieur, France.

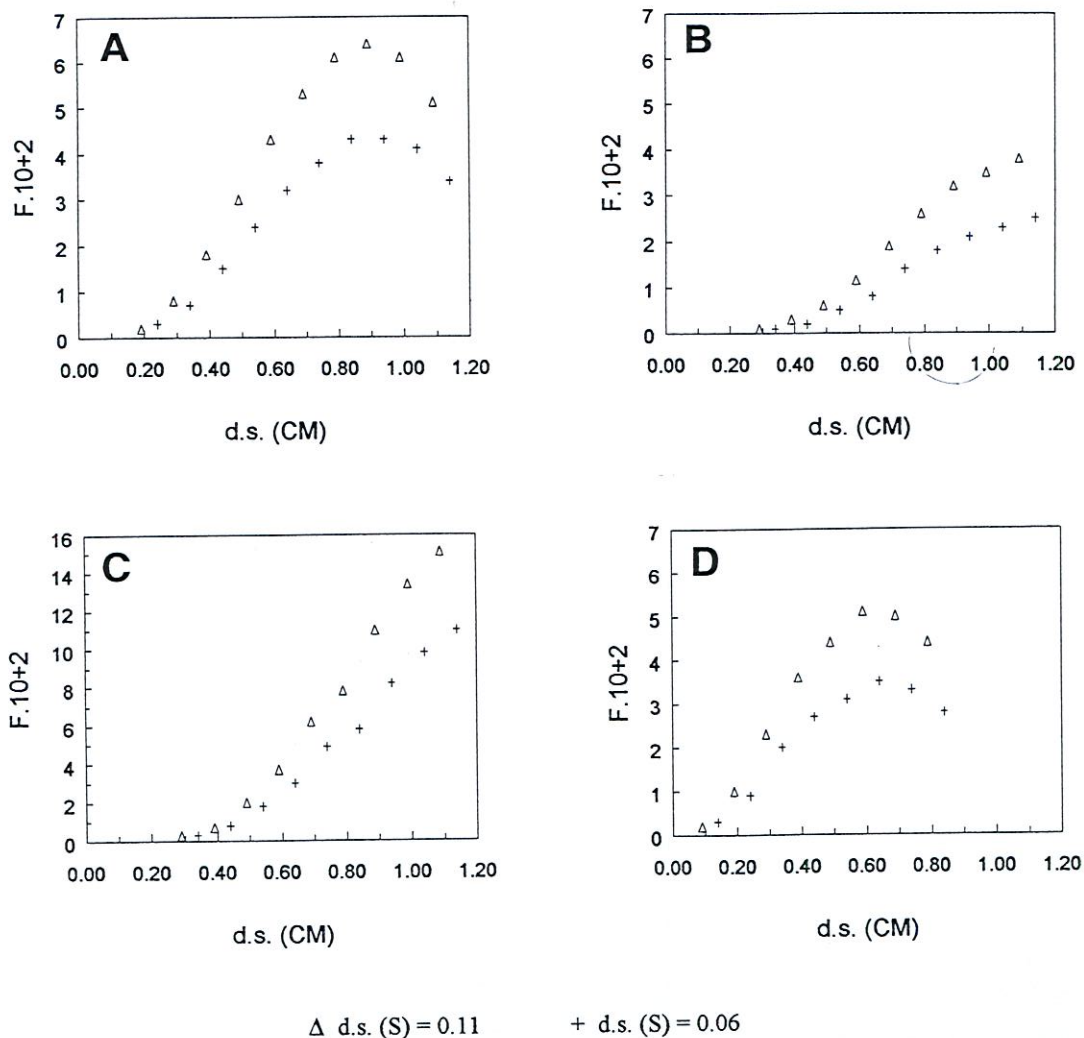


Fig. 9. Results of the Monte Carlo simulations. Dependence of fraction  $F$  of CMDBS chains, containing at least one pentad on the content of CM and S groups. (A) CM1DCM2CM1S1; (B) CM1DCM2CM1S2; (C) CM1CM1CM2CM1S1; (D); CM1DCM2DS1.  $F$  is a fraction of chains bearing at least one assumed active site. The existence of a maximum activity similarly requested the incorporation of D and/or monocarboxymethylated units in a supposed active site. Various triads, tetrads, and pentads were tested as possible active site, each consisting of D, monocarboxymethylated units (CM1), dicarboxymethylated ones (CM2), and sulfonated CM1 or CM2, respectively, S1 or S2 (the latter containing one CM and one S groups) in all cases, regardless of the position of the substituent. From the totality of sequences tested, the pentad, (A) CM1DCM2CM1S1, apparently deserves a preference. The calculated curves present similarities with the experimental ones, in their essential features: a threshold of CM value, a rise and a maximum belonging to almost the same CM region (155).

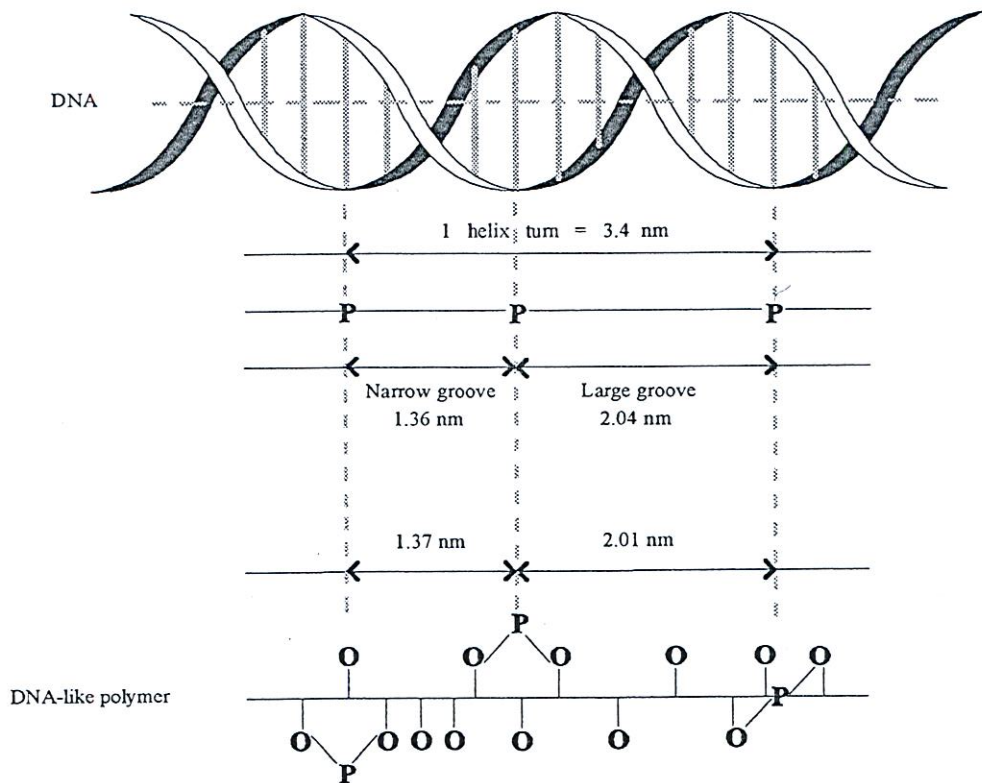


Fig. 10. Schematic representations of phosphorylate crosslinked PS derivatives DNA-like sites and DNA helix turn. Interdistances between PDE in DNA-like sites were computed by use of a Silicon Graphics Indigo 2 workstation and the Cerius 2 molecular modeling software (16). The PDE interdistances in the DNA-like sites are, respectively, of 1.37 and 2.01 nm, in good agreement with the actual distances of PDE, separated by the narrow groove and the large groove along a generatrix of the DNA double helix, i.e., 1.34 and 2.04 nm, as determined by crystallographic data.

## References

- 1 Sharma CP and Szycher M (eds) *Blood Compatible Materials and Devices* 1991; Technomic, Lancaster, UK.
- 2 Fougnot C, Jozefonvicz M, Samama M, and Bara L. New heparin-like insoluble materials: part I and part II. *Ann Biomed Eng* 1979; 7: 429–450.
- 3 Boisson C, Gulino D, Jozefonvicz J, Fischer AM, Tapon-Breaudière J, and Bros A. Antithrombic properties of insoluble modified polystyrene. Part I. *Thromb Res* 1984; 34: 269–276.
- 4 Mauzac M and Jozefonvicz J. Anticoagulant activity of dextran derivatives. Part I: synthesis and characterization. *Biomaterials* 1984; 5: 301–304.
- 5 Messaikeh H, Belattar N, Gulino D, Sultan Y, and Jozefonvicz J. Adsorption of human antibodies to factor VIII:C by various derivatives of insoluble polystyrene, in *Biological and Biomechanical Performances of Biomaterials* 1986; Christel P, Meunier A, Lee JC (eds), Elsevier, Amsterdam, pp. 321–327.
- 6 Yu XJ, Fischer AM, Muller D, Bros A, Tapon-Breaudière J, and Jozefonvicz J. Affinity chromatography of thrombin on modified polystyrene resins. *J Chromatogr* 1986; 376: 429–435.
- 7 Migonney V, Fougnot C, and Jozefonvicz M. Heparin-like tubings I. Preparation, characterization and biological *in vitro* activity assessment. *Biomaterials* 1988; 9: 145–149.
- 8 Labarre DJ. Heparin-like polymer surfaces: control of coagulation and complement activation by insoluble functionalized polymers. *Int J Artif Organs* 1990; 13: 651–657.

- 9 Letourneur D, Douzon C, and Jozefowicz M. Synthesis and characterization of phosphorylated polystyrene derivatives for use in chromatography: DNA-like and phospholipid-like behavior. *J Polym Sci Part A: Polym Chem* 1991; 29: 1367–1377.
- 10 Avramoglou T and Jozefowicz J. Derivatized dextran inhibition of smooth muscle cell proliferation. *J Biomater Sci Polym* [Edn.] 1991; 3: 149–154.
- 11 Tardieu M, Gamby C, Avramoglou T, Jozefowicz J, and Barritault D. Derivatized dextrans mimic heparin as stabilizers, potentiators and protectors of acidic or basic FGF. *J Cell Physiol* 1992; 150: 194–203.
- 12 Najab-Benhayoum M, Serne H, Jozefowicz M, Fischer AM, Brisson C, and Sultan Y. Human umbilical vein endothelial cell culture on heparin-like microcarriers. *J Biomed Mater Res* 1993; 27: 511–520.
- 13 Oturan N, Serne H, Reach G, and Jozefowicz M. RINm5F cell culture on Sephadex derivatives. *J Biomed Mater Res* 1993; 27: 705–715.
- 14 Dahri L, Boisson-Vidal C, Muller D, and Jozefowicz J. Tyrosyl sulfamide derivatives as ligands for affinity adsorption of antiFVIII antibodies. *J Biomater Sci Polym Edn.* 1994; 6: 695–705.
- 15 Siali R, Correia J, Pavon-Djavid G, and Jozefowicz M. Biofunctional polystyrene derivatives exhibit a high affinity for the Epidermal Growth Factor receptor (EGF-R) Part I. *J Mater Sci Mater Med* (in press).
- 16 Jozefowicz M and Jozefowicz J. Randomness and biospecificity: random copolymers are capable of biospecific molecular recognition in living systems. *Biomaterials* 1997; 18: 1633–1644.
- 17 Andrade JD. Needs, problems and opportunities in biomaterials and biocompatibility. *Clin Mater* 1992; 11: 19–23.
- 18 Anderson JM. Inflammatory response to implants. *ASAIO* 1988; 11: 101–107.
- 19 Ratner BD and Hoffman TA. Surface grafted polymers for biomedical applications, in *Synthetic Biomedical Polymers, Concepts and Practice*, 1980; (Szycher M and Robinson WJ, eds), Technomic, Westport, CT, pp 133–151.
- 20 Black J. Systemic effects of biomaterials. *Biomaterials* 1984; 5: 11–14.
- 21 Merritt K. Role of medical materials, both in implant and surface applications, in immune response and in resistance to infection. *Biomaterials* 1984; 5: 47–54.
- 22 Andrade JD, Nagaoka S, Cooper SL, Okano T, and Kim SW. Surface and blood compatibility, current hypothesis. *J Am Soc Artif Intern Organs* 1987; 10: 75–84.
- 23 Barbucci R, Benvenuti M, and Tempesti F. Modifications to polymer surfaces to improve blood compatibility, in *Polymeric Biomaterials* 1993; (Dimitriu S, ed), Dekker, New York, 199–232.
- 24 Banerjee R, Nageswari K, and Puniyani RR. Hematological aspects of biocompatibility. *J Biomater Appl* 1998; 12: 57–76.
- 25 Kim SW and Lyman DJ. Interface of polymers with blood. *Appl Polym Symp* 1973; 22: 289–300.
- 26 Mason RG, Shermer WH, Zucker WH, and Brinkhous KM. Platelets and plasma protein reactions with foreign surfaces, in *Erythrocytes Thrombocytes, Leucocytes: Recent Advances in Membrane and Metabolic Research*, 1973, (Gerlach E, Moser K, Deutsch E, Wilmanns W, eds), Georg Thieme, Stuttgart, Germany, 263–266.
- 27 Vroman L, Adams AL, Klings M, Fischer GC, Munoz PC, and Solensky RP. Reactions of formed elements of blood with plasma proteins at interfaces. *Ann NY Acad Sci* 1977; 283: 65–76.
- 28 Baier RE. Organization of blood components near interfaces. *Ann NY Acad Sci* 1977; 17: 283–295.
- 29 Vroman L. Importance of surfaces in contact phase reactions. *Semin Thromb Hemost* 1987; 13: 79–85.
- 30 Brash JL. Role of protein adsorption in the response of blood to foreign surfaces, in *Biocompatible Polymers, Metals and Composites* 1991; (Szycher M, ed), Technomic, Lancaster, PA, 3–25.
- 31 Chan BMC and Brash JL. Adsorption of fibrinogen on glass, reversible aspect. *J Colloid Interface Sci* 1981; 82: 217–223.
- 32 Brash JL. Mechanism of adsorption of proteins to solid surfaces and its relationship to blood compatibility, in *Biocompatible polymers, Metals and Composites* 1983; (Szycher M, ed), Technomic, Lancaster, PA, 35–52.
- 33 Pitt WG and Cooper SL. FTIR-ATR studies of the effect of shear rate upon albumin adsorption onto polyurethaneurea. *Biomaterials* 1986; 7: 340–347.
- 34 Ramsden JJ. Puzzles and paradoxes in protein adsorption. *Chem Soc Rev* 1995; 24: 73–78.
- 35 Vroman L and Adams AL. Identification of rapid changes at plasma–solid interfaces. *J Biomed Mater Res* 1969; 3: 43–67.
- 36 Uniyal S, Brash JL, and Degterev IA. Influence of red blood cells and their components on protein adsorption, in *Biomaterials: Interfacial Phenom-*

- ena and Applications, 1982; (Cooper SL and Pepas NA, eds), Advances in Chemistry Series, 277-285.
- 37 Cazenave JP. Interaction of platelets with surface, in *Blood-Surface Interactions: Biological Principles Underlying Haemocompatibility with Artificial Materials*, (Cazenave JP, Davies JA, Kazatchkine MD, van Aken WC, eds), Elsevier, New York, 89-105.
  - 38 Ito Y, Sisido M, and Imanishi Y. Adsorption of plasma proteins and adhesion of platelets onto novel polyetherurethaneureas: relationship between denaturation of adsorbed proteins and platelet adhesion. *J Biomed Mater Res* 1990; 24: 227-242.
  - 39 Horng BL, Hergenrother RW, Silver JH, Lin FJC, Brodhagen TW, and Cooper SL. *Ex vivo* blood compatibility of silicone containing biomaterials. *J Mater Sci Mater Med* 1994; 5: 207-213.
  - 40 Janatova J, Cheung AK, and Parker CJ. Biomedical polymers differ in their capacity to activate complement. *Complement Inflamm* 1991; 8: 61-69.
  - 41 Ikada Y, Kohijiya S, Yamashita S, and Fukumura H. Activation of complement in human serum by polyurethane having PEO-PTMO-PEO as soft segment. *J Mater Sci Mater Med* 1991; 2: 110-117.
  - 42 Kirschfink M, Kovacs B, and Mottaghy K. Extracorporeal circulation: *in vivo* and *in vitro* analysis of complement activation by heparin-bonded surfaces. *Circ Shock* 1993; 40: 221-226.
  - 43 Berger M, Broxup H, and Sefton MV. Using ELISA to evaluate complement activation by reference materials. *J Mater Sci Mater Med* 1994; 5: 622-627.
  - 44 Basse-Cathalinat B, Baquey C, Llabador Y, and Fleury A. Direct *in vivo* study of flowing blood-artificial surface interactions: an original application of dynamic isotopic techniques. *Int J Appl Radiat Isot* 1980; 31: 747-756.
  - 45 Anderson JM and Kottke-Marchant M. Platelet interactions with biomaterials and artificial devices, in *Anonymous CRC Critical Reviews in Biocompatibility*, 1985; CRC, Boca Raton, 111-204.
  - 46 Ip WF, Zingg W, and Sefton MV. Parallel flow arteriovenous shunt for the *ex vivo* evaluation of heparinized materials. *J Biomed Mater Res* 1985; 19: 161-178.
  - 47 Lyman DJ, Knutzen K, McNeill B, and Shibatani K. Effects of chemical structure and surface properties on the coagulation systems. *Trans Amer Soc Artif Intern Organs* 1975; 21: 49-54.
  - 48 Clowes AW, Kinkman TR, and Reidy MA. Mechanism of arterial graft healing. A rapid transmural capillary ingrowth provides a source of intimal endothelium and smooth muscle in porous PTFE prostheses. *Am J Pathol* 1988; 123: 220-230.
  - 49 Horbett TA, Waldburger JJ, Ratner BD, and Hoffman TA. Cell adhesion to series of hydrophilic-hydrophobic copolymers studied with a spinning disc apparatus. *J Biomed Mater Res* 1988; 22: 383-404.
  - 50 Offeman RD and Williams MC. Shear induced hemolysis: effects of blood chemistry and shear surface. *Biomater Med Dev Art Org* 1976; 4: 49-55.
  - 51 Lee R and Kim SW. Adsorption of protein onto hydrophobic polymer surfaces: adsorption isotherms and kinetics. *J Biomed Mater Res* 1974; 8: 251-259.
  - 52 Chuang HYK, King WF, and Mason RG. Interaction of plasma proteins with artificial surfaces: protein adsorption isotherms. *J Lab Clin Med* 1978; 92: 483-496.
  - 53 Arnander C, Sjoback DB, Frebelins S, Larsson R, and Swedenborg J. Long term stability *in vivo* of a thromboresistant heparinized surface. *Biomaterials* 1987; 8: 496-499.
  - 54 Fujimoto K, Tadokoro H, Ueda Y, and Ikada Y. Polyurethane surface modification by graft polymerization of acrylamide for reduced protein adsorption and platelet adhesion. *Biomaterials* 1993; 14: 442-448.
  - 55 Morrissey BW and Stromberg RR. Conformation of adsorbed blood proteins by infrared bound fraction measurements. *J Colloid Interface Sci* 1974; 46: 152-156.
  - 56 Park KD, Okano T, Nojiri C, and Kim SW. Heparin immobilization onto segmented polyurethane-urea surfaces: effect of hydrophilic spacers. *J Biomed Mater Res* 1988; 22: 977-992.
  - 57 Ortega-Vinuesa JL and Hidalgo-Alvarez R. Study of the adsorption of F(ab')<sub>2</sub> onto polystyrene latex beads. *Colloids Surfaces B: Biointerfaces* 1993; 1: 365-372.
  - 58 Brash JL and Samak QM. Dynamics of interactions between human albumin and polyethylene surface. *J Colloid Interface Sci* 1978; 65: 495-504.
  - 59 Ebert CD, McRea J, and Kim SW. Controlled release of antithrombotic agents from polymer matrices, in *Controlled Release of Biactive Materials* 1980; (Baker J, ed), Academic, New York, 107-121.
  - 60 Lok BK, Cheng YL, and Robertson CR. Protein adsorption on crosslinked polydimethylsiloxane

- using total internal reflection fluorescence. *J Colloid Interface Sci* 1993; 91: 104–116.
- 61 Lyman DJ, Klein KG, Brash JL, Fritzinger BK, Andrade JD, and Bonomo FS. Platelet interaction with protein coated surfaces: an approach to thromboresistant surfaces. *Thromb Diath Haemorrh* 1970; 42: 109–113.
  - 62 Vroman L, Adams AL, Fischer GS, and Munoz PC. Interaction of high molecular weight kininogen, factor XII and fibrinogen in plasma at interfaces. *Blood* 1980; 55: 156–159.
  - 63 Horbett TA. Adsorption of proteins from plasma to a series of hydrophilic-hydrophobic copolymers. II. Compositional analysis with the pre-labeled protein technique. *J Biomed Mater Res* 1981; 15: 673–695.
  - 64 Caix J, Migonney V, Baquay A, Fougnot C, Minnot AP, Beziade A, et al. Control and isotopic quantification of affinity of antithrombin III for heparin like surface. *Biomaterials* 1988; 9: 62–64.
  - 65 Brash JL and Thibodeau JA. Identification of proteins adsorbed from human plasma to glass bead columns: plasmin-induced degradation of adsorbed fibrinogen. *J Biomed Mater Res* 1986; 20: 1263–1275.
  - 66 Bale MD, Mosher DF, Wolfarth L, and Sutton RC. Competitive adsorption of fibronectin, fibrinogen, immunoglobulin, albumin and bulk plasma proteins on polystyrene latex. *J Colloid Interface Sci* 1988; 125: 516–525.
  - 67 Rapoza RJ and Horbett TA. Postadsorptive transitions in fibrinogen: Influence of polymer properties. *J Biomed Mater Res* 1990; 24: 1263–1287.
  - 68 Vaziri ND, Tookey J, Paule P, Alikkain S, and Hung E. Alterations in hemostatic parameters during hemodialysis with dialyzers of different membrane composition and flow design. *Am J Med* 1987; 83: 411–418.
  - 69 Mulzer SR and Brash JL. Identification of plasma proteins adsorbed to hemodialyzers during clinical use. *J Biomed Mater Res* 1989; 23: 1483–1504.
  - 70 Parzer S, Balcke P, and Mannhalter C. Plasma protein adsorption to hemodialysis membranes: studies in an *in vitro* model. *J Biomed Mater Res* 1993; 27: 455–463.
  - 71 Brundstedt MR, Ziats NP, Schubert M, Stack S, Rose-Caprara V, Hiltner A, and Anderson JM. Protein adsorption and endothelial cell attachment and proliferation of PAPI-based additive modified poly(ether urethane ureas). *J Biomed Mater Res* 1993; 27: 499–510.
  - 72 Munro MS, Eberhart RC, Maki NJ, Brink BE, Okada T, Tamada Y, and Fry WJ. Thromboresistant alkyl derivatized polyurethanes. *J Am Soc Artif Intern Organs* 1983; 6: 65–75.
  - 73 Corretge E, Kishida A, Konishi H, and Ikada I. Grafting of poly(ethylene glycol) on cellulose surfaces and the subsequent decrease of the complement activation, in *Polymers in Medicine III*, 1988; (Chiellini E, Guisti P, Migliaresi C, Nicolais L, eds), Plenum, New York, 61–72.
  - 74 Ikada Y. Comparison of surface modification of polymers by different methods. *Radiat Phys Chem* 1992; 39: 509–511.
  - 75 Rosenberg RD, Oosta GM, Jordan RE, and Gardner WT. Interaction of heparin with thrombin and antithrombin. *Biochem Biophys Res Commun* 1980; 96: 1200–1208.
  - 76 Heparin: Chemical and Biological Properties. Clinical Applications. (Lane DA and Lindahl U, eds), Edward Arnold, London.
  - 77 Pratt CW and Church FC. Antithrombin: structure and function. *Semin Hematol* 1991; 28: 3–9.
  - 78 Serpins: Structure, Function and Biology 1996; (Landes PGW, Gettins PA, Patston ST, Olson RG eds), Austin, TX.
  - 79 Desai UR, Petitou M, Björk L, and Olson ST. Mechanism of heparin activation of antithrombin. Role of individual residues of the pentasaccharide activating sequence in the recognition of native and activated states of antithrombin. *J Biol Chem* 1998; 273: 7478–7487.
  - 80 Kazatchine MD, Fearon DT, Metcalf DD, Rosenberg RD, and Austen KF. Structural determinants of the capacity of heparin to inhibit the formation of the human amplification C3 convertase. *J Clin Invest* 1981; 67: 223–228.
  - 81 Linhardt RJ, Rice KG, Kim YS, Engelken JD, and Weiler JM. Homogenous structurally defined heparin-oligosaccharides with low anticoagulant activity inhibit the generation of the amplification pathway C3 convertase *in vitro*. *J Biol Chem* 1988; 263: 130,090–130,096.
  - 82 Sahu A and Pangburn MK. Identification of multiple site of interaction between heparin and the complement system. *Mol Immunol* 1993; 30: 679–685.
  - 83 Gott VL, Whiffen JD, and Dutton RC. Heparin binding on colloidal graphite surfaces. *Science* 1963; 142: 1297–1298.
  - 84 Esquivel CO, Bjorck CG, Bergentz SE, Carson SN, and Rothman U. Reduced thrombogenic characteristics of expanded polytetrafluoroethylene and polyurethane arterial grafts after heparin bonding. *Surgery* 1984; 95: 102–107.

- 85 Ebert CD and Kim SW. Immobilized heparin: spacer arm effects on biological interactions. *Thromb Res* 1982; 26: 43–57.
- 86 Evangelista RA and Sefton MV. Coating of two polyether-polyurethanes and polyethylene with a heparin-poly(vinyl alcohol) hydrogel. *Biomaterials* 1986; 7: 206–211.
- 87 Ito Y, Sisido M, and Imanishi Y. Synthesis and antithrombogenicity of anionic polyurethanes and heparin-bound polyurethanes. *J Biomed Mater Res* 1986; 20: 1157–1177.
- 88 Larsson R, Larm O, and Olsson P. Search for thromboresistance using immobilized heparin. *Ann NY Acad Sci* 1987; 516: 102–115.
- 89 Han DK, Jeong SY, and Kim SW. Evaluation of blood compatibility of PEO-grafted and heparin-immobilized polyurethanes. *J Biomed Mater Res* 1989; 23: 211–228.
- 90 Mollnes TE, Videm V, Riesenfeld J, Garred P, Svennevig, JL, Fosse E, Hogasen K, and Harboe M. Complement activation and bioincompatibility: the terminal complement complex for evaluation and surface modification with heparin for improvement of biomaterials. *Clin Exp Immunol* 1991; 86: 21–26.
- 91 Marconi W, Benvenuti F, and Piozzi A. Covalent bonding of heparin to a vinyl copolymer for biomedical applications. *Biomaterials* 1997; 18: 885–890.
- 92 Blezer R, Fouache B, Willems GM, Lindhout T. Activation of blood coagulation at heparin-coated surfaces. *J Biomed Mater Res* 1997; 37: 108–113.
- 93 Sánchez J, Elgue G, Riesenfeld J, and Olsson P. Inhibition of the plasma contact activation system of immobilized heparin: Relation to surface density of functional antithrombin binding sites. *J Biomed Mater Res* 1997; 37: 37–42.
- 94 Ritter EF, Kim YB, Reischl HP, Serafin D, Rudner AM, and Klitzman B. Heparin coating of vascular prostheses reduces thromboemboli. *Surgery* 1997; 122: 888–892.
- 95 Nilsson UR, Larm O, Nilsson B, Storm KE, Elwing H, and Nilsson EK. Modification of the complement binding properties of polystyrene: effects of end-point heparin attachment. *Scand J Immunol* 1993; 37: 349–354.
- 96 Boerboom LE, Olinger GN, Karas BJ, Lindon JN, Rosen LZ, Thyagarajan, K, and Tu R. Heparinization of biological vascular graft reduces fibrin deposition. *Int J Artif Organs* 1993; 16: 263–267.
- 97 Mori Y, Nagaoka S, and Masubuchi Y. The effects of released heparin from heparinized hydrophilic polymer (HRSD) on the process of thrombus formation. *Trans Amer Soc Artif Intern Organs* 1978; 24: 736–745.
- 98 Goosen M and Sefton MV. Properties of heparin-poly(vinyl alcohol) hydrogel coating. *J Biomed Mater Res* 1983; 17: 359–373.
- 99 Plate G, Hollier L, and Glociczki P. Overcoming failure of venous vascular prostheses. *Surgery* 1984; 96: 503–510.
- 100 Ip WF and Sefton MV. Patency of heparin-PVA coated tubes at low flow rates. *Biomaterials* 1989; 10: 313–317.
- 101 Jacobs H, Okano T, Lin JY, and Kim YS. PGE1-Heparin conjugate releasing polymers, in *Controlled Release Series 1. Advances in Drug Delivery Systems*, 1988; Anderson JM and Kim SW (eds), Elsevier, New York, 313–321.
- 102 Tsiang M, Jain AK, Dunn KE, Rojas ME, Leung LLK, and Gibbs CS. Functional mapping of the surface residues of human thrombin. *J Biol Chem* 1995; 270: 16,854–16,863.
- 103 Carter P and Wells JA. Dissecting the catalytic triad of a serine protease. *Nature* 1988; 332: 564–568.
- 104 Devlin JJ, Panganiban LC, and Devlin PE. Random peptide libraries: a source of specific protein binding molecules. *Science* 1990; 249: 404–406.
- 105 Mikhailov D, Linhardt RJ, and Mayo KH. NMR solution conformation of heparin-derived hexasaccharide. *Biochem J* 1997; 328: 51–61.
- 106 Ersdal-Badju E, Lu AQ, Zuo YC, Picard V, and Bock SC. Identification of the antithrombin III heparin binding site. *J Biol Chem* 1997; 272: 19,393–19,400.
- 107 Petitou M, Duchaussoy P, Lederman I, Choay J, Sinay P, Jacquinet JC, and Torri G. Synthesis of heparin fragments. A chemical synthesis of the pentasaccharide O-(2-deoxy-2-sulfamido-6-O-sulfo-alpha-D-glucopyranosyl)-1->4-O-(beta-D-glycopyranosyluronic acid)-(1->4)-O-(2-deoxy-2-sulfamido-3,6-di-O-sulfo-alpha-D-glucopyranosyl)-(1->4)-O-(2-O-sulfo-alpha-L-idopyranosyluronic acid)-(1->4)-2-deoxy-2-sulfamide-6-O-sulfo-D-glucopyranose decasodium salt, a heparin fragment having high affinity for antithrombin III. *Carbohydr Res* 1986; 147: 221–236.
- 108 Yates EA, Santini F, Guerrini M, Naggi A, Torri G, and Casu B. <sup>1</sup>H and <sup>13</sup>C NMR spectral assignments of the major sequences of twelve systematically modified heparin derivatives. *Carbohydr Res* 1996; 294: 15–27.
- 109 Walenga JM, Jeske WP, Bara L, Samama MM, and Fareed J. Biochemical and pharmacologic rationale for the development of a synthetic heparin pentasaccharide. *Thromb Res* 1997; 86: 1–36.

- 110 Fougnot C, Dupillier MP, and Jozefowicz M. Anticoagulant activity of amino acid modified polystyrene resins: influence of the carboxylic acid function. *Biomaterials* 1983; 4: 101–104.
- 111 Fougnot C, Jozefowicz M, and Rosenberg RD. Affinity of purified thrombin or antithrombin III for two insoluble anticoagulant polystyrene derivatives. *In vitro* adsorption studies. *Biomaterials* 1983; 4: 294–298.
- 112 Fougnot C and Rosenberg RD. Adsorption of purified thrombin or antithrombin for two insoluble anticoagulant polystyrene derivatives: II Competition with the other plasma proteins. *Biomaterials* 1984; 5: 89–93.
- 113 Fischer AM, Mauzac M, Tapon-Bretonnière J, and Jozefowicz J. Anticoagulant activity of dextran derivatives. Part II: Mechanism of thrombin inactivation. *Biomaterials* 1985; 6: 198–202.
- 114 Chaubet F, Champion J, Maïga O, Mauray S, and Jozefowicz J. Synthesis and structure-anticoagulant property relationships of functionalized dextrans: CMDDBS. *Carbohydr Polym* 1995; 28: 145–152.
- 115 Migonney V, Fougnot C, and Jozefowicz M. Heparin-like tubings II. Mechanism of the thrombin-antithrombin III reaction at the surface. *Biomaterials* 1988; 9: 230–234.
- 116 Migonney V, Fougnot C, and Jozefowicz M. Heparin-like tubings III. Kinetics and mechanism of thrombin, antithrombin III and thrombin-antithrombin complex adsorption under controlled-flow conditions. *Biomaterials* 1988; 9: 413–418.
- 117 Boisson C, Gulino D, and Jozefowicz J. Affinity of antithrombin III for insoluble modified polystyrene. *Thromb Res* 1987; 46: 101–108.
- 118 Boisson C, Jozefowicz J, and Brash JL. Adsorption of thrombin from buffer and modified plasma to polystyrene resins containing sulfonate and sulfamide arginyl methyl ester groups. *Biomaterials* 1988; 9: 47–52.
- 119 Fischer AM, Yu XJ, Tapon-Bretonnière J, Muller D, Bros A, and Jozefowicz J. Thrombin purification by one-step preparative affinity chromatography on modified polystyrenes. *J Chromatogr* 1986; 363: 95–100.
- 120 Crepon B, Maïllet F, Kazatchkine MD, and Jozefowicz J. Molecular weight dependency of the acquired anticomplementary and anticoagulant activities of specifically substituted dextrans. *Biomaterials* 1987; 8: 248–253.
- 121 Maaroufi RM, Jozefowicz M, Tapon-Bretonnière J, Jozefowicz J, and Fischer AM. Mechanism of thrombin inhibition by heparin cofactor II in the presence of dermatan sulphates, native or oversulphated, and a heparin-like dextran derivative. *Biomaterials* 1997; 18: 359–366.
- 122 Mauray S, De Raucourt D, Chaubet F, Maïga-Revel O, Sternberg C, and Fischer AM. Comparative anticoagulant activity and influence of thrombin generation of dextran derivatives and of a fucoidan fraction. *J Biomater Sci Polym Edn* 1998; 9: 373–387.
- 123 Mauzac M, Maïllet F, Jozefowicz J, and Kazatchkine MD. Anticomplementary activity of dextran derivatives. *Biomaterials* 1985; 6: 61–64.
- 124 Carreno MP, Labarre D, Jozefowicz M, and Kazatchkine MD. Ability of sephadex to activate human complement is suppressed in specifically substituted functional sephadex derivatives. *Mol Immunol* 1988; 25: 165–171.
- 125 Thomas H, Maïllet F, Letourneur D, Jozefowicz J, and Kazatchkine MD. Effect of substituted dextran derivative on complement activation *in vivo*. *Biomaterials* 1995; 16: 1163–1167.
- 126 Aledort L. Inhibitors in hemophilia patients: current status and management. *Am J Pathol* 1994; 47: 208–217.
- 127 Gail M. Treatment of factor VIII inhibitors: products and strategies. *Semin Thromb Hemost* 1993; 19: 13–24.
- 128 Boisson-Vidal C, Messaïkeh H, and Jozefowicz J. 1991. Absorption of human antibodies to factor VIII:C to insoluble modified polystyrene from plasma. *J Mater Sci Mater Med* 1991; 2: 193–196.
- 129 Shima M, Fulcher CA, Mahoney SDG, Houghten RA, and Zimmerman TS. Localization of the binding site for a factor VIII activity neutralizing antibody to amino acid residues Asp1663-Ser1669. *J Biol Chem* 1989; 261: 225–229.
- 130 Dahri L, Boisson-Vidal C, Regnault V, Muller D, Sultan Y, and Stoltz JF. Synthetic sorbents for removal of factor VIII inhibitors from haemophilic A plasma. *J Chromatogr B Biomed Sci Appl* 1995; 664: 47–54.
- 131 Cornelius RM, Dahri L, Boisson-Vidal C, Muller D, Jozefowicz J, and Brash JL. Adsorption of IgG and anti-Factor VIII inhibitor antibody from haemophilic plasma to derivatized polystyrenes. *Biomaterials* 1997; 18: 429–436.
- 132 Imbert E, Souïrti A, Menard V, Pfüger F, Jozefowicz M, and Migonney V. DNA-Like and phospholipid-Like phosphorylated polystyrenes: Characterization, distribution of functional groups, and calcium complexation properties. *J Appl Polym Sci* 1994; 52: 91–97.



- 133 Pierschbacher MD, Polarek JW, Craig WS, Tschopp JF, Sipes NJ, and Harper JR. Manipulation of cellular interactions with biomaterials toward a therapeutic outcome: a perspective. *J Cell Biochem* 1995; 56: 150–154.
- 134 Lydon MJ, Minnett TW, and Tighe BJ. Cellular interactions with synthetic polymer surfaces in culture. *Biomaterials* 1985; 6: 393–402.
- 135 Clark RAF, Folkvord JM, and Nielsen LD. Either exogenous or endogenous fibronectin can promote adherence of human endothelial cells. *J Cell Sci* 1986; 82: 263–280.
- 136 Grinnell F. Fibronectin adsorption on material surfaces. *Ann NY Acad Sci* 1987; 516: 280–290.
- 137 Margel S, Vogler EA, Firment L, Watt T, Haynie S, and Sogah DY. Peptide, protein and cellular interactions with self assembled monolayer model surfaces. *J Biomed Mater Res* 1993; 27: 1463–1476.
- 138 Smetana K. Cell biology of hydrogels. *Biomaterials* 1993; 14: 1046–1050.
- 139 Schakenraad JM, Busscher HJ, Wildevuur CRH, and Arends J. Influence of substratum surface free energy on growth and spreading of human fibroblasts in the presence and absence of serum proteins. *J Biomed Mater Res* 1986; 20: 773–784.
- 140 Van Wachem PB, Beugeling T, Mallens BWL, Dekker A, Feijen J, Bantjes L, and Van Aken WG. Deposition of endothelial fibronectin on polymeric surfaces. *Biomaterials* 1988; 9: 121–123.
- 141 Aubert N, Reach G, Serne H, and Jozefonvicz M. Reversible morphological and functional abnormalities of R1Nm5F cells cultured on polystyrene beads. *J Biomed Mater Res* 1987; 21: 585–601.
- 142 Stanislawski L, Serne H, Stanislawski M, and Jozefonvicz M. Conformational changes of fibronectin induced by polystyrene derivatives with a heparin-like function. *J Biomed Mater Res* 1993; 27: 619–626.
- 143 Gill PJ, Silbert CK, and Silbert JE. Effects of heparin sulfate removal on attachment and reattachment of fibroblasts and endothelial cells. *Biochemistry* 1986; 25: 405–410.
- 144 Visser MR, Vercelotti GM, McCarthy JB, Goodman JL, Herbst TJ, Furcht LT, and Jacob HS. Herpes simplex virus inhibits endothelial cell attachment and migration of extracellular matrix proteins. *Am J Pathol* 1989; 134: 223–230.
- 145 Fredj-Reygobellet D, Hristova LD, Ettaiche M, Meddahi A, Jozefonvicz J, and Barritault D. CMDBS, functional analogue of heparan sulfate as a new class of corneal ulcer healing agents. *Ophthalmic Res* 1994; 26: 325–331.
- 146 Meddahi A, Benoit J, Ayab N, Sezeur A, and Barritault D. Heparin-like polymers derived from dextran enhance colonic anastomosis resistance to linkage. *J Biomater Sci Polym Edn* 1996; 31: 293–297.
- 147 Aamiri A, Butler-Browne GS, Martelly I, Barritault D, and Gautron J. Influence of a dextran derivative on myosin heavy chain expression during rat skeletal muscle regeneration. *Neurosci Lett* 1995; 201: 243–246.
- 148 Bagheri-Yarmand R, Morere JF, Letourneur D, Jozefonvicz J, Israël L, and Crepin M. Inhibitory effects of dextran derivatives *in vitro* on the growth characteristics of premalignant and malignant human mammary epithelia cell lines. *Anti-cancer Res* 1992; 12: 1641–1646.
- 149 Letourneur D, Logeart D, Avramoglou T, and Jozefonvicz J. Antiproliferative capacity of synthetic dextrans on smooth muscle cell growth: the model of derivatized dextrans as heparin-like polymers. *J Biomater Sci Polym Edn* 1993; 4: 431–444.
- 150 Letourneur D, Champion J, Slaoui F, and Jozefonvicz J. *In vitro* stimulation of human endothelial cells by derivatized dextrans. *In Vitro Cell Dev Biol* 1993; 29: 67–72.
- 151 Senni K, Borchiellini C, Duchesnay A, Pellat B, Letourneur D, and Kern P. Antiproliferative polysaccharides modulate distribution and phenotypic expression of collagens by gingival fibroblasts. *J Biomed Mater Res* 1998; 40: 164–169.
- 152 Benazzoug Y, Logeart D, Labat-Robert J, Robert L, Jozefonvicz J, and Kern P. Derivatized dextrans modulate collagen synthesis in aortic smooth muscle cells. *Biochem Pharmacol* 1995; 49: 847–853.
- 153 Logeart D, Letourneur D, Jozefonvicz J, and Kern P. Collagen synthesis by vascular smooth muscle cells in the presence of antiproliferative polysaccharides. *J Biomed Mater Res* 1996; 28: 501–508.
- 154 Vaudaux P, Avramoglou T, Letourneur D, Lew DP, and Jozefonvicz J. Inhibition by heparin and derivatized dextrans of *Staphylococcus aureus* adhesion to fibronectin-coated biomaterials. *J Biomater Sci Polym Edn* 1992; 4: 89–97.
- 155 Krentsel L, Chaubet F, Rebrov A, Champion J, Ermakov I, Bittoun P, et al. Anticoagulant activity of functionalized dextrans. Structures analyses of carboxymethylated dextran and first Monte Carlo simulations. *Carbohydr Polym* 1997; 33: 63–71.
- 156 Bourin MC and Lindahl U. Glycosaminoglycans and the regulation of blood coagulation. *Biochem J* 1993; 289: 313–330.