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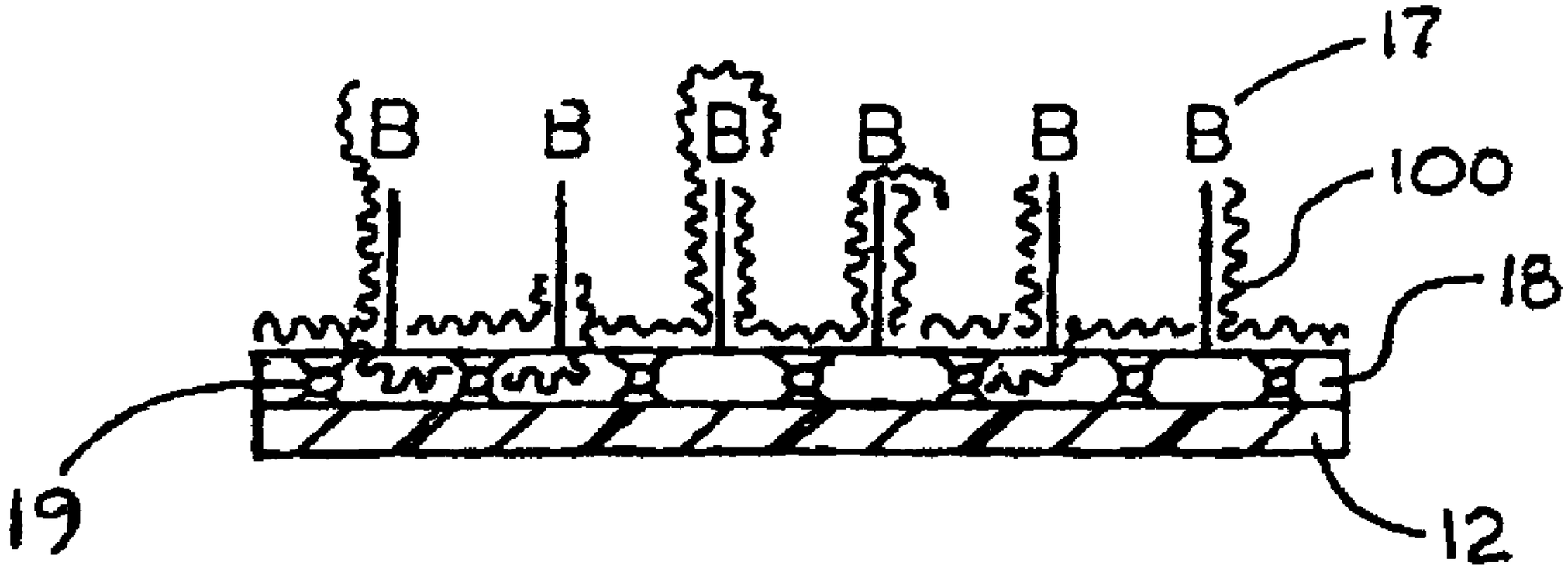

(57) Abrégé/Abstract:
The present invention relates to immobilized biologically active entities that retain significant biological activity following mechanical manipulation of a substrate material to which the entities are immobilized. The significant biological activity is retained through the contact of the coating material and the biologically active entities with a biologically compatible composition

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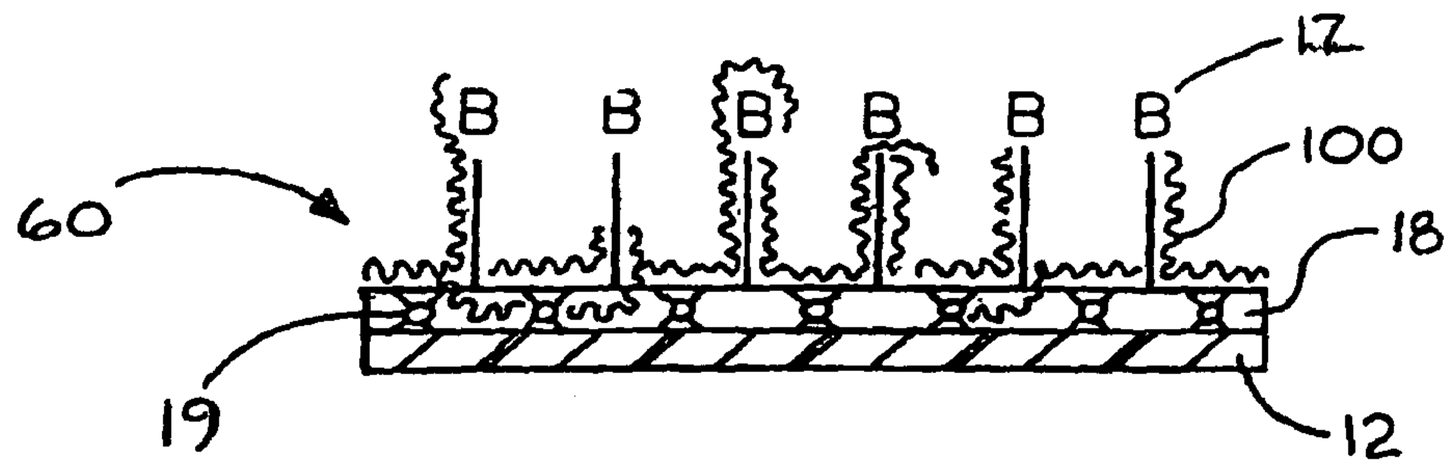

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Immobilized Biologically Active Entities Having A High Degree of Biological Activity Following Mechanical Manipulation or Sterilization

FIELD OF THE INVENTION

The present invention relates to substrate materials having immobilized biologically active entities that maintain their biological activity following exposure to conditions of elevated heat, high humidity, antibiotic agents, and/or mechanical stress. The present invention is particularly useful in the field of medical devices.

BACKGROUND OF THE INVENTION

In the field of medical devices, glass, polymeric, and/or metallic materials are common substrate materials. These materials can be used for diagnostic devices or extracorporeal devices. With the exception of glass, many of the materials can be used for implantable devices.

Immobilization of biologically active entities on substrate materials in a biologically active form involves an appreciation of the respective chemistries of the entity and the substrate material. Modification of the chemical composition of a substrate material is often required to immobilize a biologically active entity thereon. This is usually accomplished by treating surfaces of the substrate material to generate a population of chemically reactive elements or groups, followed by immobilization of the biologically active entity with an appropriate protocol. With other substrate materials, surfaces of a substrate material are covered, or coated, with a material having reactive chemical groups incorporated therein. Biologically active entities are then immobilized on the substrate material through the reactive chemical groups of the covering material. A variety of schemes for covering, or coating, substrate materials have been described. Representative examples of biologically active entities immobilized to a substrate material with a covering, or coating, material are described in U.S. Patent Nos.: 4,810,784; 5,213,898; 5,897,955; 5,914,182; 5,916,585; and 6,461,665.

When biologically active compounds, compositions, or entities are immobilized, the biological activity of these "biologics" can be negatively impacted by the process of immobilization. The biological activity of many of biologics is

dependent on the conformation (*i.e.*, primary, secondary, tertiary, etc.) of the biologic in its immobilized state. In addition to a carefully selected immobilization process, chemical alterations to the biologic may be required for the biologic to be incorporated into the covering material with a conformation that renders the biologic sufficiently active to perform its intended function.

Despite an optimized covering and immobilization scheme, additional processing, such as sterilization, can degrade the biological activity of the immobilized biologic. For implantable medical devices, sterilization is required prior to use. Sterilization may also be required for *in vitro* diagnostic devices having sensitivity to contaminants. Sterilization of such devices usually requires exposure of the devices to elevated temperature, pressure, and humidity, often for several cycles. In some instances, antibiotic agents, such as ethylene oxide gas (EtO) or vapor hydrogen peroxide are included in the sterilization process. In addition to sterilization, mechanical compaction and expansion, or long-term storage of an immobilized biologic can degrade the activity of the biologic.

There exists a need for medical devices having biologically active entities immobilized thereon that can be subjected to sterilization, mechanical compaction and expansion, and/or storage without significant loss of biological activity. Such a medical device would have biologically compatible compositions or compounds included with the immobilized biological entities that serve to minimize degradation of the biological activity of the entities during sterilization, mechanical compaction and expansion, and/or storage. In some instances, the additional biologically compatible compositions or compounds would increase the biological activity of some biologically active entities following a sterilization procedure.

SUMMARY OF THE INVENTION

The present invention relates to medical devices having substrate materials with biologically active entities immobilized thereon in combination with additional biologically compatible organic chemical compositions that enable the biologically active entities to retain significant biological activity following exposure of the immobilized entities to processing and storage conditions that would otherwise degrade the biological activity of the entities.

A suitable substrate material can be any material with a surface having reactive chemical groups that are capable of attaching, confining, or otherwise immobilizing a biologically active entity in a biologically active form to one or more surfaces of the substrate material. Substrate materials can also have a multiplicity of reactive chemical groups added to surfaces of the materials through the application of one or more covering compositions, or materials, to the surfaces. At least a portion of a covering material has chemical elements, groups, compounds, or components that are reactive to biologically active entities and serve to attach, confine, or otherwise immobilize a biologically active entity in a biologically active form to the covering material. In some embodiments, the biologically active entity can be reversibly immobilized.

At least one type of biologically active entity is chemically attached, confined, or otherwise immobilized to suitable reactive chemical groups on the substrate material and/or covering material. Following immobilization of a plurality of biologically active entities to at least a portion of a multiplicity of reactive chemical groups present on a substrate material and/or covering material, an additional biologically compatible organic composition is covalently or non-covalently combined with the biologically active entities, substrate, and/or polymeric covering material. The biologically compatible organic composition interacts with the biologically active entities and reactive chemical groups of the substrate material and/or covering material to prevent the biologically active entities from loosing biological activity under conditions that would otherwise significantly degrade the biological activity of the entities. These conditions include sterilization and storage. With expandable endoluminal medical devices, for example, mechanical compaction and expansion of such devices can also significantly degrade the biological activity of the entities.

In some cases, the additional biologically compatible organic composition seems to maintain the biological activity of the entities during sterilization, storage, and/or mechanical manipulation by limiting undesirable alterations to the entities often induced by sterilization, storage, and/or a mechanical manipulation process. The activity-diminishing alterations could include conformational changes to a biologically active entity obscuring an active site on the entity. The activity-diminishing alterations could also include interactions between neighboring biologically active entities. Rearrangements of biologically active entities with respect to a polymeric covering material are other possible activity-diminishing

alterations to the entities. Simple denaturation, or other degradation, of the biologically active entities could be another means by which the entities loose biological activity. As described in greater detail herein, immobilized biologically active entities sterilized, stored, and/or mechanically manipulated in the presence of the additional biologically compatible organic composition may retain a degree of biological activity significantly greater than a similar immobilized biologically active entity processed under the same conditions in the absence of the additional biologically compatible organic composition.

The additional biologically compatible organic composition can be removed from a sterilized medical device during post-sterilization processing or the composition can be removed by physiological processes of an implant recipient following deployment of the sterilized medical device at an implantation site.

Preferred biologically active entities reduce or inhibit thrombus formation on surfaces of a substrate and/or covering material. Glycosaminoglycans are preferred anti-thrombotic agents for use in the present invention, with heparin, heparin analogs, and derivatives being particularly preferred. Other preferred biologically active substances reduce undesirable cellular growth from tissue in which the present invention is implanted. Preferred anti-proliferative agents for use in the present invention include, but are not limited to, dexamethasone, rapamycin, and paclitaxel.

Accordingly, one embodiment of the present invention relates to a medical device comprising a substrate material, a polymeric covering material attached to at least a portion of a surface of said substrate material, a plurality of biologically active entities having anti-thrombin III binding activity covalently attached to at least a portion of said polymeric covering material, and a biologically compatible composition combined with said polymeric covering material, wherein said biologically active entities have an anti-thrombin III binding activity of at least 5 picomoles anti-thrombin III per square centimeter (pmol/cm^2) substrate material following sterilization or compaction and expansion of said substrate material. In other embodiments, the anti-thrombin binding activity is at least 6 picomoles anti-thrombin III per square centimeter (pmol/cm^2) substrate material, at least 7 picomoles anti-thrombin III per square centimeter (pmol/cm^2) substrate material, at least 8 picomoles anti-thrombin III per square centimeter (pmol/cm^2) substrate material, at least 9 picomoles anti-thrombin III per square centimeter (pmol/cm^2)

substrate material, or at least 10 picomoles anti-thrombin III per square centimeter (pmol/cm^2) substrate material. In some embodiments, the anti-thrombin III binding activity is at least 100 pmol/cm^2 picomoles anti-thrombin III per square centimeter (pmol/cm^2) substrate material.

In another embodiment, the invention relates to a medical device comprising a substrate material, a polymeric covering material attached to at least a portion of a surface of said substrate material, a first plurality of heparin molecules having anti-thrombin III binding activity end point attached to at least a portion of said polymeric covering material, and a biologically compatible composition combined with said polymeric covering material, wherein said first plurality of heparin molecules have an anti-thrombin III binding activity of at least 10 picomoles anti-thrombin III per square centimeter (pmol/cm^2) substrate material following compaction and expansion of said substrate material.

In another embodiment, the present invention relates to a sterilized medical device comprising a polymeric substrate material, a polymeric covering material attached to at least a portion of a surface of said substrate material, a plurality of heparin molecules having anti-thrombin III binding activity end point attached to at least a portion of said polymeric covering material, and a composition comprising a plurality of polyethylene glycol molecules combined with said polymeric covering material, wherein said first plurality of heparin molecules have an anti-thrombin III binding activity of at least 50 picomoles anti-thrombin III per square centimeter (pmol/cm^2) substrate material following compaction and expansion of said substrate material.

In yet another embodiment; the present invention relates to a medical device comprising a substrate material, a plurality of chemical entities having anti-thrombin III binding activity present on at least a portion of said substrate material, a first biologically compatible composition combined with said substrate material, and a second biologically compatible composition admixed therewith.

A further embodiment of the present invention relates to a medical device comprising a substrate material, a polymeric covering material attached to at least a portion of a surface of said substrate material, a plurality of chemical entities having anti-thrombin III binding activity present on at least a portion of said polymeric covering material, a first biologically compatible composition combined with said

substrate material, and a second biologically compatible composition admixed therewith.

In embodiments relating to non-covalently combined biologically compatible organic compositions, at least a portion of the organic composition or the second plurality of heparin molecules is often released from the sterilized or mechanically manipulated medical device within several hours when placed in a 0.15 M phosphate buffer solution having a temperature of about thirty-seven degrees centigrade and a substantially neutral pH. Presence of the released compounds can be detected in the buffer solution with routine assay techniques.

In embodiments relating to covalently combined biologically compatible organic compositions, the organic composition or the second plurality of heparin molecules is substantially retained on the sterilized or mechanically manipulated medical device following sterilization or mechanical manipulation.

In yet other embodiments, the covalently combined biologically compatible organic composition may be released from the polymeric covering material through reversal of a covalent bond. Presence of the compounds released by reversal of a covalent bond can be detected in a buffer solution with routine assay techniques.

In some embodiments, the biologically compatible organic composition may be admixed prior to mechanical manipulation and/or sterilization. In other embodiments, the biologically compatible organic composition may be admixed following mechanical manipulation or sterilization (*i.e.*, in an operating room). This is particularly useful when the organic composition may be degraded through mechanical manipulation or sterilization of a substrate or device utilizing the composition. This also permits the organic composition to be placed at particular locations on a substrate or device and at varying dosages.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic representation of a polymeric substrate material having a multiplicity of reactive chemical groups thereon.

Figure 1A is a schematic representation of a metallic substrate material.

Figure 2 is a schematic representation of a polymeric substrate material having a plurality of biologically active entities immobilized thereto.

Figure 3 is a schematic representation of a polymeric substrate material having a polymeric covering material with a multiplicity of reactive chemical groups thereon.

Figure 3A is a schematic representation of a metallic substrate material having a polymeric covering material with a multiplicity of reactive chemical groups thereon.

Figure 4 is a schematic representation of a polymeric substrate material having a polymeric covering material with a plurality of biologically active entities immobilized thereto.

Figure 4A is a schematic representation of a metallic substrate material having a polymeric covering material with a plurality of biologically active entities immobilized thereto.

Figure 5 is a schematic representation of a polymeric substrate material having a plurality of biologically active entities immobilized thereto and an additional biologically compatible composition combined therewith.

Figure 6 is a schematic representation of a polymeric substrate material having a polymeric covering material with a plurality of biologically active entities immobilized thereto and an additional biologically compatible composition combined therewith.

Figure 6A is a schematic representation of a metallic substrate material having a polymeric covering material with a plurality of biologically active entities immobilized thereto and an additional biologically compatible composition combined therewith.

Figure 6B is a schematic representation of a polymeric substrate material having a polymeric covering material with a plurality of biologically active entities immobilized thereto showing some of the biologically compatible composition

illustrated in Figure 6 having been released from the substrate material and polymeric covering material.

Figure 6C is a schematic representation of a metallic substrate material having a polymeric covering material with a plurality of biologically active entities immobilized thereto showing some of the biologically compatible composition illustrated in Figure 6A having been released from the substrate material and polymeric covering material.

Figure 7 is a schematic representation of a polymeric substrate material having three layers of polymeric covering material applied thereto with a plurality of biologically active entities immobilized thereto and an additional biologically compatible composition combined therewith.

Figure 7A is a schematic representation of a metallic substrate material having three layers of polymeric covering material applied thereto with a plurality of biologically active entities immobilized thereto and an additional biologically compatible composition combined therewith.

Figure 7B is a schematic representation of a polymeric substrate material having three layers of polymeric covering material applied thereto with a plurality of biologically active entities immobilized thereto showing some of the biologically compatible composition illustrated in Figure 7 having been released from the substrate material and polymeric covering material.

Figure 7C is a schematic representation of a metallic substrate material having three layers of polymeric covering material applied thereto with a plurality of biologically active entities immobilized thereto showing some of the biologically compatible composition illustrated in Figure 7A having been released from the substrate material and polymeric covering material.

Figure 8 is a bar graph illustrating how sterilization of unbound heparin does not significantly reduce the biological activity of the heparin.

Figure 9 is a bar graph illustrating the effect of a variety of biologically compatible organic compositions on the biological activity of end-point attached heparin immobilized to reactive chemical groups on a polymeric covering material during and after exposure of the immobilized heparin to an ethylene oxide sterilization regimen.

Figure 10 is a bar graph illustrating the ability of added heparin or dextran sulfate biologically compatible organic compositions to result in high levels of ATIII

binding activity of heparin immobilized to a polymeric covering material on a substrate during and after exposure of the immobilized heparin to an ethylene oxide sterilization regimen.

Figure 11 is a bar graph illustrating the ability of added dextran sulfate to maintain the biological activity of end-point attached heparin immobilized on a polyvinyl alcohol coated substrate during and after exposure of the immobilized heparin to an ethylene oxide sterilization regimen.

Figure 12 is a bar graph illustrating the ability of added glycerol to maintain the biological activity of end-point attached heparin immobilized on a polymeric covering material of a substrate following compaction and expansion of the substrate material.

Figure 13 is a bar graph illustrating the ability of added glycerol and heparin to maintain the biological activity of end-point attached heparin immobilized on a polymeric covering material of a substrate following mechanical compaction, exposure to an ethylene oxide sterilization regimen, and mechanical expansion of the substrate material.

Figure 14 is a schematic representation of a polymeric substrate material having a polymeric covering material with a plurality of biologically active entities immobilized thereto and reactive chemical groups thereon.

Figure 15 is a schematic representation of a metallic substrate material having a polymeric covering material with a plurality of biologically active entities immobilized thereto and reactive chemical groups thereon.

Figure 16 is a schematic representation of a polymeric substrate material having a polymeric covering material with a plurality of biologically active entities and an additional biologically compatible composition covalently combined thereto.

Figure 17 is a schematic representation of a metallic substrate material having a polymeric covering material with a plurality of biologically active entities and an additional biologically compatible composition covalently combined thereto.

Figure 18 is a schematic representation of embodiments of the present invention having a second biologically compatible composition combined therewith.

Figure 19 is a schematic representation of embodiments of the present invention having a second biologically compatible composition combined therewith.

Figure 20 is a schematic representation of a polymeric substrate material having a polymeric covering material with a plurality of biologically active entities

immobilized thereto, a first biologically compatible composition, and a second biologically compatible composition combined therewith.

Figure 21 is a schematic representation of a metallic substrate material having a polymeric covering material with a plurality of biologically active entities immobilized thereto, a first biologically compatible composition, and a second biologically compatible composition combined therewith.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to materials and devices having biologically active entities immobilized thereto that retain significant biological activity following sterilization, mechanical compaction and expansion, and/or storage conditions that would otherwise significantly decrease the biological activity of the entities. The biological activity of an immobilized biological entity subjected to such conditions may be positively influenced by the presence of at least one additional biologically compatible composition covalently or non-covalently combined with the biologically active entities. In most embodiments, the additional composition is an organic compound. In some embodiments, however, the biologically compatible composition is an inorganic compound. In preferred embodiments, the additional composition is a carbohydrate in the form of a polysaccharide. Preferred polysaccharides are glycosaminoglycans. Preferred glycosaminoglycans are heparin compositions, heparin analogs, and heparin derivatives.

Referring to Figures 1 and 2, some polymeric substrate materials (12) have multiplicities of reactive chemical groups (16) populating at least a portion of the surfaces of the substrate materials to which a plurality of biologically active entities (17) are attached, confined, or otherwise immobilized. Most biologically active entities (17) are covalently attached, or bound, to the substrate materials (12) through the reactive chemical groups (16). Surfaces of the polymeric substrate material (12) can be smooth, rough, porous, curved, planar, angular, irregular, or combinations thereof. In some embodiments, substrate materials with surface pores have internal void spaces extending from the porous surface of the material into the body of the material. These porous substrate materials have internal substrate material bounding the pores that often provides surfaces amenable to immobilizing

biologically active entities. Whether porous or non-porous, substrate materials can be in the form of filaments, films, sheets, tubes, meshworks, wovens, non-wovens, and combinations thereof.

Suitable substrate materials (12) for immobilizing biologically active entities (17) include biocompatible polymeric materials such as polyethylene, polyurethane, silicone, polyamide-containing polymers, and polypropylene. Full density or porous polytetrafluoroethylene is a suitable polymeric substrate material (12) if reactive chemical groups (16) are introduced in constituents of the polymeric material. Substrate materials with a multiplicity of reactive chemical groups that are part of the substrate material are referred to herein as "functionalizable materials." Following reaction of a biologically active entity with a functionalizable substrate material, the substrate material is considered functionalized and the biologically active entity immobilized. In order to maintain the biological activity of the immobilized entity during subsequent processing conditions, such as sterilization, mechanical compaction and expansion, or storage, an additional biologically compatible organic chemical composition is non-covalently combined with the functionalized material and immobilized entity.

Substrate materials can also have a multiplicity of reactive chemical groups added to surfaces of the materials through the application of one or more covering compositions, or materials, to the surfaces. At least a portion of a covering material has chemical elements, groups, compounds, or components that are reactive to biologically active entities and serve to attach, confine, or otherwise immobilize a biologically active entity in a biologically active form to the covering material. The covering material can be applied in the form of a solute, particle, dispersion, coating, or overlay and attached to the substrate material in a variety of ways including, but not limited to, covalent bonding, adsorption, such as, physisorption or chemisorption, and non-covalent bonding, such as hydrogen bonding or ionic bonding. In preferred embodiments, the covering material is applied in a solution and forms a continuous or discontinuous film layer on one or more surfaces of the substrate material upon removal of the solvent. The covering material can be applied in one or more layers. The chemical constituents of the covering material in each layer can be the same or different. In some embodiments, the covering material is cross-linked to itself or other covering materials in other layers. The cross-linking bonds can be covalent or ionic.

Substrate materials (12, 14) lacking reactive chemical groups on their surfaces (Figure 1A) (or lacking appropriately reactive chemical groups) are covered, at least in part, with a polymeric covering material (18) having a multiplicity of reactive chemical groups (16) thereon (Figures 3 and 3A) to which biologically active entities (17) can be attached, confined, or otherwise immobilized (Figures 4 and 4A). Most biologically active entities (17) are covalently attached, or bound, to the polymeric covering material (18) through the reactive chemical groups (16) of the covering material (18). The polymeric covering material (18) forms at least one layer on at least a portion of a substrate material (12, 14). In some embodiments, the polymeric covering material (18) is cross-linked (19) to itself or other layers (18a, 18b) of polymeric covering material (Figure 7 and 7a). The cross-linking can be covalent, ionic, or both. Substrate materials amenable to covering are glass, metals (14), ceramics, polymeric materials (12), particularly chemically inert polymeric materials such as polytetrafluoroethylene.

At least one type of biologically active entity having anti-thrombin III binding capability (17) is chemically attached, confined, or otherwise immobilized to suitable reactive chemical groups (16) on the substrate material (12, 14) and/or covering material (18).

Biologically compatible compositions (11, 15, 100) include, but are not limited to, antithrombotics, anticoagulants, fibrinolytic or thrombolytic agents, antibiotics, antimicrobial/antiseptic compounds, anti-viral compounds, antiproliferatives, cell adhesive compounds, cell anti-adhesive compounds, and anti-inflammatories. Antithrombotics of particular interest are glycosaminoglycans, particularly heparin, including derivatives and analogs thereof. Other anticoagulant agents include, but are not limited to, hirudin, activated protein C, and prostaglandins. Fibrinolytic or thrombolytic agents include, but are not limited to, streptokinase, urokinase, and tissue plasminogen activator (tPA). Examples of antibiotics include, but are not limited to, penicillin, tetracycline, chloramphenicol, minocycline, doxycycline, vancomycin, bacitracin, kanamycin, neomycin, gentamycin, erythromycin and cephalosporins. Examples of cephalosporins include cephalothin, cephapirin, cefazolin, cephalexin, cephradine, cefadroxil, cefamandole, cefoxitin, cefaclor, cefuroxime, cefonicid, ceforanide, cefotaxime, moxalactam, ceftrizoxime, ceftriaxone, and cefoperazone. Examples of antimicrobial/antiseptics include, but are not limited to, silver sulfadiazine, chlorhexidine, peracetic acid, sodium hypochlorite, triclosan,

phenols, phenolic compounds, iodophor compounds, quaternary ammonium compounds, chlorine compounds, heparin and combinations thereof. Examples of anti-viral agents include, but are not limited to, alpha.-methyl-1-adamantanemethylamine, hydroxy-ethoxymethylguanine, adamantanamine, 5-iodo-2'-deoxyuridine, trifluorothymidine, interferon, and adenine arabinoside. Cell adhesive compounds include, but are not limited to, fibronectin, laminin, collagen, vitronectin, osteopontin, RGD peptides, RGDS peptides, YIGSR peptides, and antibodies targeting cell surface antigens. Compounds that may resist cellular attachment include Poly HEMA, poly ethylene glycol, polysaccharides, polyvinylpyrrolidone, and phospholipids. Other biologically active entities include, but are not limited to, enzymes, organic catalysts, ribozymes, organometallics, proteins, glycoproteins, peptides, polyamino acids, antibodies, nucleosides, nucleotides, nucleic acids, steroidal molecules, antibiotics, antimicrobial compounds, antimycotics, cytokines, carbohydrates, oleophobics, lipids, pharmaceuticals, and therapeutics.

While a variety of biologically active entities (17) can be used in the present invention, as described above, entities capable of interacting with components of mammalian blood to prevent coagulation or thrombus formation on surfaces of a substrate material (12, 14) or covering material (18) by the blood components are most preferred. Many of these biologically active entities are oligosaccharides or polysaccharides. Some of the polysaccharides are glycosaminoglycans including glucosamine or galactosamine compositions. Preferred glycosaminoglycans are heparin compositions, heparin analogs, and heparin derivatives. Heparin is a complex glycosaminoglycan with many biological functions mediated by its binding to growth factors, enzymes, morphogens, cell adhesions molecules, and cytokines. The biological activity of heparin to function as an anticoagulant is based on the ability of heparin to act as a catalyst for thrombin and anti-thrombin III (AT III) binding. Most of the anti-coagulant activity of heparin is associated with a pentasaccharide sequence that facilitates this binding.

The most preferred heparin composition for immobilization in the present invention is a heparin composition having a free terminal aldehyde group made according the teachings of U.S. Patent No. 4,613,665, issued to Larm.

In the process of making heparin with a free terminal aldehyde group, the heparin is subjected to degradation by diazotation to

form a heparin fragment having a free terminal aldehyde group. The free terminal aldehyde group allows the heparin composition to be "end point attached" to primary amino groups of a substrate or polymeric covering material to form an imine which, by reduction, is converted to a secondary amine. End point attachment of the heparin composition permits the heparin to be immobilized in a conformation that most advantageously exposes the biologically active portion of the heparin composition to components of the blood responsible for coagulation and thrombus formation. When exposed to the blood components responsible for thrombus formation and coagulation, the optimally immobilized heparin interacts with the blood components to reduce or prevent thrombus formation or other coagulation events on surfaces of the substrate and/or covering material.

Other desirable biologically active entities (17) for use in the present invention include synthetic heparin compositions referred to as "fondaparinux™," compositions involving antithrombin III-mediated inhibition of factor Xa, antiproliferatives, and anti-inflammatories.

Despite an optimized immobilization scheme, the biological activity of a heparin-based biological entity is significantly decreased during sterilization, mechanical compaction and expansion, and/or storage of the entities (Figures 9, 11, 12, and 13). As discussed above, the decrease in biological activity of an immobilized biologically active entity may be caused by a variety of factors. Regardless of the mechanism by which the biological activity of an immobilized entity is decreased, addition of a biologically compatible organic composition covalently and/or non-covalently combined with the immobilized biologically active entity maintains the biological activity of the entity during and after sterilization, mechanical manipulation - such as mechanical compaction and expansion, and/or storage of the entities.

The additional biologically compatible organic composition can have biological activity or no biological activity. The additional biologically compatible organic composition can be a carbohydrate in the form of polyhydroxy aldehydes or ketones and their derivatives. These carbohydrates include monosaccharides, disaccharides, oligosaccharides, and polysaccharides, including glycosaminoglycans, glycosaminomannans, and storage polysaccharides such as dextran and its derivatives. Other biologically compatible organic compositions suitable for use in the present invention include acid mucopolysaccharides, amino

acids, polypeptides, proteins, glycoproteins, nucleosides, nucleotides, polynucleotides, or other biologically compatible aliphatic or aromatic compound, charged or uncharged, having a molecular weight less than about 100,000 MW.

Referring to Figures 5 - 6A, covered or uncovered substrate materials (14, 12, respectively) having biologically active entities (17) immobilized thereon have an additional biologically compatible composition (100) combined with the biologically active entities (17), the substrate material (12, 14) and/or the covering material (18). The biologically compatible composition is preferably organic. The biologically compatible organic composition can be applied to the immobilized biologically active entities, substrate, and/or covering material in a variety of ways. In a preferred embodiment, a suitable carbohydrate-based biologically compatible composition is dissolved in an aqueous solvent and the solution applied to the immobilized biologically active entities, substrate, and/or polymeric covering material by spraying, dip coating, immersing, rolling, spreading, or other deposition means. In appropriate systems, biologically compatible compositions can be dissolved in organic solvents and similarly applied.

A preferred embodiment of the present invention relates to a sterilized medical device for implantation, or other placement, at an anatomical site. Also preferred are sterilized medical devices for placement inside an anatomical structure delimiting a void space, or lumen, to reinforce the anatomical structure or maintain the void space delimited thereby. When these sterilized devices are used within a vascular structure, immobilized biologically active entities in the form of end point attached heparin interact with blood flowing through, or around, the devices to minimize or prevent formation of thrombus or other coagulation products on blood-contacting surfaces of the devices. In a preferred embodiment, the additional biologically compatible organic composition is a polyethylene glycol compound covalently combined with the substrate material and/or covering material. The covalently bound heparin is allowed to remain with the sterilized devices. The preferred sterilization method includes ethylene oxide gas.

The manufacturing of medical devices may require mechanical manipulation that often reduces the biological activity of an immobilized biologically active entity. The additional biologically compatible composition combined with the immobilized biologically active entities, substrate material, and/or covering material as described above, may also maintain the biological activity of the immobilized biologically active

entities following mechanical compaction and expansion of a medical device (Figures 12 and 13). Expandable stents and stent-grafts are medical devices for which improved biological activity of immobilized biologically active entities is particularly significant.

The present invention, therefore, provides sterilized devices having biologically active entities immobilized thereto where the biological activity of the immobilized entities is significantly retained during and after a sterilization process (Figures 9 – 11, and 13). Prior to sterilization, the devices can be mechanically manipulated, through compaction and expansion, for example, and retain significant biological activity (Figures 12 and 13).

Figure 14 schematically illustrates embodiments of the present invention (50) having a polymeric substrate (12) having a polymeric covering, or coating, material (18) cross-linked (19) thereon. Covering (18) has a plurality of immobilized biologically active entities "B" (17) attached thereto. Covering (18) also has a plurality of chemically reactive groups "R" (13) thereon to which a biologically compatible composition "S" (15) can be covalently attached (Figures 16 and 17). In some embodiments, the covalent bonds are reversible thereby rendering the biologically compatible composition "S" (15) releasable from the invention under appropriate conditions. Figures 15 and 17 schematically illustrate similar constructions (50) using a metallic substrate (14).

Figures 18 and 19 schematically illustrate embodiments of the present invention (70) having a polymeric substrate (12) or metallic substrate (14) having a polymeric covering, or coating, material (18) cross-linked (19) thereon. Covering (18) has a plurality of immobilized biologically active entities "B" (17) and a first biologically compatible composition "S" (15) covalently attached thereto. In some embodiments, the covalent bonds are reversible thereby rendering the biologically compatible composition "S" (15) releasable from the invention under appropriate conditions. In addition, this embodiment has a second biologically compatible composition "A" (11) admixed therewith.

Figures 20 and 21 schematically illustrate embodiments of the present invention (80) having a polymeric substrate (12) or metallic substrate (14) having a polymeric covering, or coating, material (18) cross-linked (19) thereon. Covering (18) has a plurality of biologically active entities "B" (17) immobilized thereto. A first biologically compatible composition (100) is combined with the biologically active

entities (17). In addition, this embodiment has second biologically compatible composition "A" (11) admixed therewith.

EXAMPLES

Except for Example 1, calculations of heparin activity on surfaces in the present invention were conducted using the surface area of only one side of the sample material, although the entire sample, including interstices, may have heparin immobilized thereon. The heparin activity was assayed by measuring the ability, or capacity, of the end-point attached heparin to bind a known quantity of anti-thrombin III (ATIII). The results were expressed as picomoles anti-thrombin III (ATIII) bound per square centimeter of substrate material (pmol ATIII/cm^2 substrate material). This assay is described by Larsen M.L., *et al.* in "Assay of plasma heparin using thrombin and the chromogenic substrate H-D-Phe-Pip-Arg-pNA" (S-2238) (Thromb. Res. 1978; 13:285-288) and Pasche, *et al.* in "A binding of antithrombin to immobilized heparin under varying flow conditions" (Artif. Organs 1991; 15:281-491).

ATIII binding activity per surface area of substrate material is defined as the number of picomoles of ATIII bound per apparent surface area of covered or uncovered substrate material. The apparent substrate surface area does not take into account multiple covered surfaces nor porosity considerations of a porous substrate material. If the substrate material is porous, the effect of porosity on surface area is not considered for these calculations. For example, the apparent surface area of a cylindrical tubular ePTFE vascular graft (which is made of a porous material) with end-point attached heparin immobilized on substrate material comprising the inner surface of the tubular graft is calculated as it is for any cylindrical geometry as $2\pi rL$: where r is the graft inner radius; L is the axial length; and π is the number pi. It is important to note that the porous nature of ePTFE and its effect on surface area is not accounted for herein. Accordingly, non-porous substrate materials that are cut into squares for analysis are taken to have a surface area of the length multiplied by the width.

Example 1

This example demonstrates retention of biological activity of unbound "neat" heparin following exposure of the heparin to an ethylene oxide (EtO) sterilization process.

In this example, unsterilized USP grade heparin-sodium in lyophilized powder form was obtained from Celsus Laboratories (Cincinnati, OH). Measured quantities of heparin were placed into CHEX-ALL® sterilization pouches (Long Island City, N.Y.) for testing. One group of heparin-containing pouches was exposed to EtO sterilization. Ethylene oxide sterilization was carried out under conditions of conditioning for one hour (1 hr), an EtO gas dwell time of one hour (1 hr), a set point temperature of fifty-five degrees centigrade (55 °C), and an aeration time of twelve hours (12 hr). Another group was subjected to the sterilization procedure in the absence of EtO. A third group was not exposed to the sterilization procedure.

Following the sterilization procedure, known quantities of heparin were removed from each pouch and tested for bio-activity with an ACTICHROME™ Heparin (anti-FXa) assay kit available from American Diagnostica Inc. (Stamford, CT). Bioactivity values for each heparin sample were expressed as international units of heparin per mass of heparin (IU/mg). International units of heparin are calculated based on Factor X_a inactivation by ATIII that is catalyzed by heparin. International units are therefore a measure of the ATIII binding activity of heparin. Any reduction in heparin activity is expressed simply as a reduction in the IU/mg for comparable heparin controls from the ACTICHROME test. Heparin exhibiting a reduction in activity is considered to have been deactivated to a degree by the sterilization process.

Figure 8 is a bar graph illustrating the effect of EtO sterilization on the anti-thrombin III (ATIII) binding activity of dry powdered heparin in an unbound state. Figure 8 shows the mean activity levels, expressed as IU/mg, for the heparin samples (n=3) in each group. Control heparin samples that did not undergo sterilization had a mean value of 138 IU/mg. Control heparin samples that underwent the sterilization process in the absence of EtO (*i.e.*, high humidity, high temperatures, etc.) had a mean value of 119 IU/mg. The heparin samples that underwent the sterilization process in the presence of EtO had a mean value of 123 IU/mg. The heparin samples exposed to the sterilization process in the absence of EtO had an fourteen percent (14%) decrease in activity compared to the unsterilized

control samples, while the samples exposed to the sterilization process in the presence of EtO had only an eleven percent (11%) decrease in activity. As seen from Figure 8, sterilization of unbound, neat, heparin powder in the presence or absence of EtO does not significantly reduce ATIII binding to the heparin when compared to unsterilized control samples. The anti-thrombin III binding activity of unbound, unsterilized, heparin is not significantly diminished by sterilization without EtO or sterilization with EtO. Therefore, degradation of the anti-thrombin III binding activity of immobilized heparin subjected to similar EtO sterilization conditions must be caused by a mechanism other than simple exposure to sterilization with or without EtO.

Example 2

This example describes the construction of an embodiment of the present invention in which heparin anti-thrombin III (ATIII) binding is not significantly diminished by exposure to EtO sterilization.

In accordance with U.S. Patent No. 6,653,457 an aldehyde modified heparin composition made according to U.S. Patent No. 4,613,665, which is incorporated herein by reference, was end-point attached to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material. An additional biologically compatible organic composition was incorporated within the covering material and bound heparin to enable the immobilized heparin to undergo EtO sterilization without significant loss in biological activity.

An ePTFE material in sheet form was obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration Media (GMM-406). A covering material in the form of a base coating was applied to the ePTFE material by mounting the material on a ten centimeter (10 cm) diameter plastic embroidery hoop and immersing the supported ePTFE material first in 100% isopropyl alcohol (IPA) for about five minutes (5 min) and then in a solution of LUPASOL® polyethylene imine (PEI) and IPA in a one to one ratio (1:1). LUPASOL® water-free PEI was obtained from BASF and diluted to a concentration of about four percent (4%) and adjusted to pH 9.6. Following immersion of the ePTFE material in the solution for about fifteen minutes (15 min), the material was removed from the solution and rinsed in deionized (DI) water at pH 9.6 for fifteen

minutes (15 min). PEI remaining on the ePTFE material was cross-linked with a 0.05% aqueous solution of glutaraldehyde (obtained from Amresco) at pH 9.6 for fifteen minutes (15 min). Additional PEI was added to the construction by placing the construction in a 0.5% aqueous solution of PEI at pH 9.6 for fifteen minutes (15 min) and rinsing again in DI water at pH 9.6 for fifteen minutes (15 min). The imine formed as a result of the reaction between glutaraldehyde and the PEI layer is reduced with a sodium cyanborohydride ($NaCNBH_3$) solution (5 g dissolved in 1 L DI water, pH 9.6) for fifteen minutes (15 min) and rinsed in DI water for thirty minutes (30 min).

An additional layer of PEI was added to the construction by immersing the construction in 0.05% aqueous glutaraldehyde solution at pH 9.6 for fifteen minutes (15 min), followed by immersion in a 0.5% aqueous solution of PEI at pH 9.6 for fifteen minutes (15 min). The construction was then rinsed in DI water at pH 9.6 for fifteen minutes (15 min). The resultant imines were reduced by immersing the construction in a solution of $NaCNBH_3$ (5 g dissolved in 1 L DI water, pH 9.6) for fifteen minutes (15 min) followed by a rinse in DI water for thirty minutes (30 min). A third layer was applied to the construction by repeating these steps. The result was a porous hydrophobic fluoropolymeric base material having a hydrophilic cross-linked polymer base coat on substantially all of the exposed and interstitial surfaces of the base material.

An intermediate chemical layer was attached to the polymer base coat in preparation for placement of another layer of PEI on the construction. The intermediate ionic charge layer was made by incubating the construction in a solution of dextran sulfate (Amersham Pharmacia Biotech) and sodium chloride (0.15 g dextran sulfate and 100 g NaCl dissolved in 1 L DI water, pH 3) at 60 °C for ninety minutes (90 min) followed by rinsing in DI water for fifteen minutes (15 min).

A layer of PEI, referred to herein as a "capping layer" was attached to the intermediate layer by placing the construction in a 0.3% aqueous solution of PEI (pH 9) for about forty-five minutes (45 min) followed by a rinse in a sodium chloride solution (50 g NaCl dissolved in 1 L DI water) for twenty minutes (20 min). A final DI water rinse was conducted for twenty minutes (20 min).

Aldehyde modified heparin was end point attached, or conjugated, to the PEI layer(s) by placing the construction in a heparin-containing sodium chloride salt solution (1.5 g heparin, 29.3 g NaCl dissolved in 1 L DI water, pH 3.9) for one

hundred twenty minutes (120 min) at sixty degrees centigrade (60 °C). A 2.86 mL volume of a 2.5% (w/v) aqueous $NaCNBH_3$ solution was added to the one liter (1 L) heparin solution prior to adding the samples. The samples were then rinsed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1 L DI water, pH 9.0) for twenty minutes (20 min), and finally in DI water for fifteen minutes (15 min) followed by lyophilization of the entire construction to produce dry heparin bound to the ePTFE material. The presence and uniformity of the heparin was determined by staining samples of the construction on both sides with toluidine blue. The staining produced an evenly purpled surface indicating heparin was present and uniformly bound to the ePTFE material.

By adding particular compounds or compositions to the heparin-bound construction, the biological activity of the heparin can be maintained following exposure to conditions that would otherwise decrease the biological activity of the heparin. The conditions include, but are not limited to, EtO sterilization, mechanical compaction and expansion, and storage.

The above-described constructions coated with a covering material were exposed to solutions of the following compounds to evaluate their stabilizing effect on the biological activity of the heparin bound to parts of the coating: USP grade calcium chloride (Fisher Scientific), USP grade heparin sodium (Celsus), polyethylene glycol (20,000 molecular weight, Sigma), DEAE dextran (500,000 molecular weight, PK chemical), dextran sulfate sodium salt (8,000 molecular weight, Sigma), and dextran (9,500 molecular weight, Sigma) at concentrations of 0.5 g per 100 ml DI water adjusted to pH 9.6. Dexamethasone was also utilized at 0.5 g per 100 ml ethanol with no pH adjustment. Each of these solutions is referred to herein as a "treatment solution." The effect of these various compounds on binding activity of heparin to anti-thrombin III
(ATIII) following EtO sterilization was expressed as picomoles anti-thrombin III bound per square centimeter (cm^2) substrate material. These data are summarized in Figure 9.

To expose a particular heparin-containing construction to a particular treatment solution, the construction was placed into a two liter (2 L) beaker and one hundred milliliters (100 ml) of treatment solution was added, sufficient to completely immerse the construction in the treatment solution. Each construction was exposed

to the treatment solution for one hour (1 hr) at sixty degrees centigrade (60 °C). The construction was removed from the solution and lyophilized prior to exposure to a sterilization procedure.

In preparation for EtO sterilization, each lyophilized construction was placed and sealed in a Tower DUALPEEL® Self-Seal Pouch (Allegiance Healthcare Corp., McGaw Park, IL). Ethylene oxide sterilization was carried out under conditions of conditioning for one hour (1 hr), an EtO gas dwell time of one hour (1 hr), a set point temperature of fifty-five degree centigrade (55 °C), and an aeration time of twelve hours (12 hr).

After EtO sterilization, each construction (including controls) was removed from its pouch and washed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1 L DI water, pH 9.0) for twenty minutes (20 min), and finally a rinse in DI water for fifteen minutes (15 min).

Samples approximately one square centimeter (1 cm^2) in size were cut from the construction and assayed for heparin activity by measuring the capacity of the end point attached heparin to bind ATIII. The assay is described by Larsen M.L., et al., in "Assay of plasma heparin using thrombin and the chromogenic substrate H-D-Phe-Pip-Arg-pNA (S-2238)." Thromb Res 13:285-288 (1978) and Pasche B., et al., in "A binding of antithrombin to immobilized heparin under varying flow conditions." Artif. Organs 15:281-491 (1991). The results were expressed as amount of ATIII bound per unit surface area substrate material in picomoles per square centimeter (pmol/cm^2). All samples were maintained in a wet condition throughout the assay. It is important to note that while the approximately one square centimeter (1 $cm^{2)}$ samples each have a total surface area of two square centimeters (2 cm^2) if both sides of the material are considered, only one surface on the sample (*i.e.*, 1 cm^2) was used for calculating ATIII heparin-binding activity in pmol/cm^2.

Figure 9 is a bar graph illustrating the effects various biologically compatible organic compositions non-covalently combined with heparin immobilized on a covered substrate material on the anti-thrombin III binding activity of the immobilized heparin following exposure of the immobilized heparin to EtO sterilization.

The anti-thrombin III binding activity to the immobilized heparin was expressed in picomoles ATIII bound per square centimeter of substrate material (pmol/cm^2). One set of control samples was not sterilized. Another set of control

samples was subjected to EtO sterilization in the absence of a biologically compatible organic composition non-covalently combined with the immobilized heparin and covering material. Each remaining bar represents the anti-thrombin III binding activity of immobilized heparin in the presence of the indicated biologically compatible organic composition non-covalently combined with the immobilized heparin and covering material. All bars represent mean values of n=3 samples, except for dextran sulfate with n=6 samples.

As can be seen from the bar graph, sterilized control samples showed a dramatic reduction in anti-thrombin III binding activity compared to unsterilized control samples. The anti-thrombin III binding activity of the unsterilized control samples was 103 pmol/cm^2 substrate material. The anti-thrombin III binding activity of the sterilized control samples was 66 pmol/cm^2 substrate material. EtO sterilization caused a thirty-six percent (36%) reduction in anti-thrombin III binding activity compared to the unsterilized samples.

The influence of the above described biologically compatible organic compositions non-covalently combined with the immobilized heparin and covering material on the anti-thrombin III binding activity following sterilization is summarized in the following paragraph.. Each biologically compatible organic composition was rinsed, as described earlier, from each construction before the anti-thrombin III binding activity was determined.

When heparin was added to the construction, the mean anti-thrombin III binding activity was 108 pmol/cm^2. Addition of dextran to the construction resulted in a mean anti-thrombin III binding activity of 98 pmol/cm^2 substrate material. When dextran sulfate was added to the construction, the mean anti-thrombin III binding activity was 134 pmol/cm^2 substrate material. Additionally, polyethylene glycol resulted in a mean anti-thrombin III binding activity of 129 pmol/cm^2 substrate material. Interestingly, these values are greater than the mean values for the unsterilized control samples at 103 pmol/cm^2 substrate material.

When inorganic calcium chloride ($CaCl_2$) was added to the construction, the mean anti-thrombin III binding activity of the immobilized heparin was 75 pmol/cm^2 substrate material. Addition of dexamethasone to the construction resulted in a mean anti-thrombin III binding activity of 42 pmol/cm^2 substrate material. DEAE dextran seemed to diminish the anti-thrombin III binding activity of the immobilized heparin with a mean activity of 5 pmol/cm^2 substrate material.

These results demonstrate the ability to maintain, or increase, the anti-thrombin III binding activity of end point attached heparin following EtO sterilization with an appropriate biologically compatible composition non-covalently combined with the immobilized heparin and covering material.

Example 3

This example describes the ability of an additional biologically compatible organic composition to produce a high anti-thrombin III (ATIII) binding activity of heparin end point attached to a polymeric covering material on a substrate material that is a component of an implantable medical device.

The implantable medical device used in this example was in the form of a nitinol wire reinforced tube made of a porous, expanded, polytetrafluoroethylene (ePTFE) material obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename VIABAHN® Endoprosthesis. The tubular device was fifteen centimeters (15 cm) in length and six millimeters (6 mm) in diameter.

The VIABAHN® Endoprosthesis was constrained within a delivery catheter and required removal from the catheter before immobilizing heparin thereon. Each catheter-constrained device was removed for processing by pulling a release cord attached to a constraining sheath and releasing the sheath from around the device. Once unconstrained, each device was expanded and used as a separate substrate material. Each substrate material (endoprosthetic device) was immersed in a PEI solution (5% in DI water) and IPA (USP grade) in a volume percent ratio of 30:70, respectively, for about twelve hours (12 hr) to place a polymeric covering material (18) on the substrate material (12). The polymeric covering material (18) had a multiplicity of reactive chemical groups (16) to which a plurality of aldehyde-modified heparin molecules (17) were eventually end point attached.

At least one additional layer of covering material (e.g. 18A, 18B of Figs. 7-7C) was placed on the first PEI layer (18). This was performed by placing each endoprosthetic device within a separate silicone tube and the tube connected to a peristaltic pump and solution-reservoir. This allowed an additional solution containing a covering material to be repeatedly passed through the center of the tubular medical device to coat primarily the inside surfaces of the device.

With each endoprosthesis contained within one of these dynamic flow systems, a covering material (18) in the form of an aqueous solution of 0.10% (pH

9.0) PEI and IPA in a volume percent ratio of 45:55, respectively, was passed through the device for about twenty minutes (20 min). Each device was then rinsed in DI water (pH 9.0) for five minutes (5 min) and the PEI layers cross-linked (19) by exposure to a 0.05% aqueous glutaraldehyde solution (pH 9.0) for twenty minutes (20 min). The devices were then rinsed again with an aqueous solution of PEI (0.10%, pH 9.0) for five minutes (5 min). The resultant imines were reduced with a sodium cyanborohydride solution (5 g in 1 L DI water, pH 9.0) for fifteen minutes (15 min) and rinsed in DI water for thirty minutes (30 min).

An intermediate ionic charge layer was placed on the cross-linked PEI layer(s) of each device by flowing a solution of dextran sulfate (0.15 g dextran sulfate and one hundred grams sodium chloride (100 g NaCl) dissolved in one liter (1 L) of DI water, pH 3) through the dynamic flow system and over the PEI layer at sixty degrees centigrade (60 °C) for about ninety minutes (90 min). This was followed by rinsing the system with DI water for fifteen minutes (15 min).

A "capping" layer (18b) of PEI was added to the ionically charged dextran sulfate layer (18a) by flowing an aqueous solution of PEI (0.075%, pH 9.0) through the dynamic flow system for about forty-five minutes (45 min) followed by a rinse in a sodium chloride solution (50 g NaCl dissolved in 1 L DI water) for fifteen minutes (15 min). The rinse was followed by a brief DI water flush for about two and a half minutes (2.5 min).

Aldehyde modified heparin was end point attached, or conjugated, to the PEI layer(s) by placing the construction in a heparin-containing sodium chloride salt solution (1.5 g heparin, 29.3 g NaCl dissolved in 1 L DI water, pH 3.9) for one hundred twenty minutes (120 min) at sixty degrees centigrade (60 °C). A 2.86 mL volume of a 2.5% (w/v) aqueous $NaCNBH_3$ solution was added to the one liter (1 L) heparin solution ten minutes (10 min) after beginning the step. A first rinse in DI water for fifteen minutes (15 min), was followed by a rinse in a boric acid solution (0.7 g NaCl, 10.6 g boric acid and 2.7 g NaOH dissolved in 1 L DI water, pH 9.0) for about twenty minutes (20 min), and a final rinse in DI water for fifteen minutes (15 min). The construction was then subjected to a lyophilization process. Staining of selected samples with toluidine blue produced a consistent purple surface indicating uniformly bound heparin.

Based on the results obtained in the studies described in Example 2, *supra*, USP grade heparin (sodium salt) and 8,000 MW dextran sulfate (sodium salt) at a

concentration of 0.5 g/100 ml DI water (pH9.6), were chosen as the preferred biologically compatible organic compositions to maintain, or stabilize, the anti-thrombin III binding activity of the immobilized heparin during and after EtO-sterilization.

For each preferred biologically compatible organic composition, sections of the endoprostheses having heparin end-point attached to a polymeric covering material were placed in plastic tubes containing a solution of said biologically compatible organic compositions (each at a concentration of 0.5 g/100 mL DI water, pH 9.6) and incubated at sixty degrees centigrade (60 °C) for one hour (1 hr). Each treated sample was removed from the plastic tube and exposed to a lyophilization process.

Each lyophilized sample was placed in an individual Tower DUALPEEL® Self Sealing Pouch (Allegiance Healthcare Corp., McGraw Park, IL) and sealed for EtO sterilization. Ethylene oxide sterilization was carried out under conditions of conditioning for one hour (1 hr), an EtO gas dwell time of one hour (1 hr), a set point temperature of fifty-five degrees centigrade (55 °C), and an aeration time of twelve hours (12 hr).

After EtO sterilization, each construction was removed from its pouch and washed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid 2.7 g NaOH and 0.7 g NaCl dissolved in 1 L DI water, pH 9.0) for twenty minutes (20 min), and finally a rinse in DI water for fifteen minutes (15 min).

Samples of substrate material from each EtO-sterilized device (approx. 0.5 cm long) were cut from each device and the immobilized heparin measured for biological activity using the above-described ATIII binding assay (Example 2). Samples were kept wet throughout the assay process. The results were expressed as picomoles of anti-thrombin III bound per area unit of substrate material (pmol/cm^2) as measured on the luminal surface of each device and not the entire surface area of the device (*i.e.*, both abluminal and luminal surfaces).

Figure 10 is a bar graph illustrating the effect of two separate biologically compatible organic compositions in the form of heparin and dextran sulfate on anti-thrombin III binding activity of heparin immobilized on a covered substrate material during and after exposure to an EtO sterilization regimen. Anti-thrombin III binding activity is expressed as picomoles of bound anti-thrombin III per square centimeter of substrate material. As seen from the results, the use of heparin and dextran sulfate

biologically compatible organic compositions resulted in high anti-thrombin III binding activity to immobilized heparin following EtO sterilization, with activities of 97 pmol/cm^2 substrate material and 91 pmol/cm^2 substrate material, respectively. All bars represent mean values of n=6 samples.

Example 4

This example describes construction of an embodiment of the present invention having an aldehyde modified heparin compound end point attached to a polymeric covering material that includes an ionically neutral first covering layer. The construction had heparin ATIII binding that was not significantly diminished by exposure to EtO sterilization.

The covering material used as a base coat in this construction was chosen to render a heparin-containing covering material, or coating, that had essentially no ionic charge. Polyvinyl alcohol and PEI were used as the covering materials.

In accordance with U.S. Patent No. 6,653,457,

an aldehyde modified heparin composition was bound to a covered substrate material. The substrate material (12) was expanded polytetrafluoroethylene (ePTFE) material. An additional biocompatible organic chemical composition (100) was incorporated into the heparin-containing covering material (18) of the construction to enable the heparin to undergo EtO sterilization without significant loss in biological activity.

An ePTFE substrate material in sheet form was obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration Media (GMM-406). A layer of covering material, or base coat, was applied to the ePTFE substrate material by mounting the material on a 10 cm diameter plastic embroidery hoop and immersing the supported ePTFE material in a solution of 100% IPA for about five minutes (5 min). This was followed by immersion of the ePTFE material in an aqueous two percent (2%) solution of USP grade polyvinyl alcohol (PVA) (Spectrum) for fifteen minutes (15 min). After a fifteen minute (15 min) rinse in DI water, the PVA layer was exposed to a solution of two percent (2%) aqueous glutaraldehyde and one percent (1%) hydrochloric acid (HCL) for fifteen minutes (15 min) to cross-link (19) the PVA (18), *in situ*. The construction was rinsed in DI water for fifteen minutes (15 min) followed by a second fifteen minute (15 min) DI water rinse. The resulting cross-linked PVA base coating had no net ionic charge.

Another layer of polymeric covering material (18a) was added to the construction by immersing the construction in an aqueous 0.15% solution of PEI (pH 10.5) solution for thirty minutes (30 min). The resultant imines were reduced by immersing the construction in an aqueous solution of sodium cyanborohydride solution (5 g/L in DI water, pH 10.5) for fifteen minutes (15 min). The construction was rinsed in DI water for fifteen minutes (15 min) followed by a second fifteen minute (15 min) DI water rinse.

A covered ePTFE substrate material having a multiplicity of reactive chemical groups thereon was immersed in the heparin solution (1.0 g heparin, 29.3 g NaCl dissolved in 1 L DI water, pH 3.9) for ninety minutes (90 min) at 60 °C. A 2.86 mL volume of a 2.5% (w/v) aqueous $NaCNBH_3$ solution was added to the 1 L heparin solution prior to beginning this step. A first fifteen minute (15 min) rinse in DI water, was followed by a rinse in an aqueous boric acid solution (0.7 g NaCl, 10.6 g boric acid, 2.7 g NaOH dissolved in 1 L DI water, pH 9.0) for about twenty minutes (20 min), and a final rinse in DI water rinse for fifteen minutes (15 min). The construction was then subjected to a lyophilization process. Samples of the construction were then stained with toluidine blue. The staining produced a consistent purple surface indicating uniformly bound heparin on the covered ePTFE material.

The construction was exposed to an aqueous treatment solution containing a biologically compatible organic composition (100) in the form of 8,000 MW USP grade dextran sulfate (sodium salt) (Sigma) by immersing the construction in 100 ml treatment solution (0.5 g of dextran sulfate/100 mL DI water, pH 9.6) at sixty degrees centigrade (60 °C) for one hour (1 hr). Following removal of the construction from the treatment solution, the construction was lyophilized.

Each lyophilized construction was placed in a Tower DUALPEEL® Self Seal Pouch (Alligiance Healthcare Corp., McGaw Park, IL) for EtO sterilization. Ethylene oxide sterilization was carried out under conditions of conditioning for one hour (1 hr), an EtO gas dwell time of one hour (1 hr), a set point temperature of fifty-five degrees centigrade (55 °C), and an aeration time of twelve hours (12 hr).

After EtO sterilization, each construction (including controls) was removed from its pouch and washed in DI water for fifteen minutes (15 min), a borate buffer solution (10.6 g boric acid, 2.7 g NaOH, 0.7 g NaCl dissolved in 1L DI water, pH 9.0) for twenty minutes (20 min), and finally a rinse in DI water for fifteen minutes (15 min).

Samples of the membrane (approx. 1 cm^2) with end-point attached heparin were cut and the immobilized heparin measured for anti-thrombin III binding activity using the above-described ATIII binding assay (Example 2). Samples were kept wet throughout the assay process. The results were expressed as picomoles of anti-thrombin III bound per unit of substrate surface area (pmol/cm^2 substrate material).

Figure 11 is a bar graph illustrating the effect of a biologically compatible organic composition in the form dextran sulfate on anti-thrombin III binding activity of end-point attached heparin immobilized on a porous expanded polytetrafluoroethylene substrate material and a covering material of polyvinyl alcohol and PEI, following EtO sterilization. The biological activity of the immobilized heparin was expressed as picomoles of anti-thrombin III bound per square centimeter of substrate material.

Unsterilized control samples had an anti-thrombin III binding activity of 150 pmol/cm^2 substrate material. The sterilized control samples had an anti-thrombin III binding activity of 93 pmol/cm^2 substrate material. Ethylene oxide sterilized samples treated with dextran sulfate had an anti-thrombin III binding activity of 115 pmol/cm^2 substrate material. This value was greater than the control values for EtO-sterilized devices which were not exposed to a dextran sulfate treatment solution (*i.e.*, 93 pmol/cm^2 substrate material), indicating the added dextran sulfate increased the biological activity of the immobilized heparin following EtO sterilization. Both of these constructions had anti-thrombin III binding activity values that were significantly lower than the non-treated, non-EtO-sterilized, controls (150 pmol/cm^2 substrate material).

As seen from the results, dextran sulfate significantly impacted the anti-thrombin III binding activity of the immobilized heparin attached to a construction with a polymeric covering material that includes an ionically neutral first covering layer, following EtO sterilization. All bars represent mean values of n=3 samples.

Example 5

This example describes the ability of an additional biologically compatible organic composition to maintain or increase the biological activity of biologically active heparin immobilized to a covered substrate material during and after imposition of a mechanical stress of sufficient magnitude to otherwise significantly reduce the biological activity of the entity.

In this example, implantable medical devices in the form of endoluminal prostheses were provided with a heparin-containing coating as described in Example 3, *supra*. Each prosthesis was in the form of a nitinol wire reinforced tube made of a porous, expanded, polytetrafluoroethylene (ePTFE) material obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename VIABAHN® Endoprosthesis. The tubular device was fifteen centimeters (15 cm) in length and six millimeters (6 mm) in diameter. The same process was utilized as detailed in Example 3 for forming a heparin-containing coating on the device.

For treatment with the biologically compatible organic composition (100), substrate material (12) of the endoluminal device was prepared with a polymeric covering material (18) having aldehyde modified heparin (17) end point attached to at least a portion thereof. Sections of the prepared device were placed in plastic tubes and incubated with a glycerol solution (5 mL Sigma-Aldrich SigmaUltra glycerol in 100 mL of DI water, pH 9.6) at sixty degrees centigrade (60 °C) for one hour (1 hr). Each treated device was removed from the plastic tube and exposed to a lyophilization process.

Each cylindrical endoprosthesis was placed over an intravascular delivery system and mechanically compressed until it was sufficiently compacted on the delivery system to be restrained with a constraining sheath. Devices made according to Example 3 can withstand the mechanical stresses associated with compaction of the endoprosthesis on the delivery system without significant loss in the activity of the heparin incorporated in the coating.

Glycerol was chosen as the non-covalently bound biologically compatible organic composition (100) to maintain the biological activity of the end point attached heparin (17) during diametrical compaction and expansion of each test endoprosthesis. Each control endoprosthesis device section did not have the non-covalently bound biologically compatible glycerol composition (100) included with the end point attached (i.e., covalently bound) heparin (17) and polymeric covering material (18). Each device was subjected to a lyophilization process.

To compress and compact the endoluminal devices on a delivery system, each endoprosthesis was pulled through a tapered funnel with a fixed diameter. Each endoprosthesis had six (6) sutures (Gore-Tex® CV-0, 0N05) sewn through one end to pull the devices through the funnel. Each device was pulled through the opening of a twenty-five milliliter (25 ml) pipet tip (Falcon®, product #357525) with a

diameter of about three millimeters (3 mm) and into a glass tube with a diameter of about 3.1 mm to hold it in the compacted state.

After compaction, each endoprosthesis was deployed in a 0.9% aqueous saline solution at thirty-seven degree centigrade (37 °C), rinsed and tested for anti-thrombin III binding activity as described herein. The results are shown in Figure 12. Each endoprosthesis was prepared for testing by washing in DI water for fifteen minutes (15 min), followed by a rinse in borate buffer solution (10.6 g boric acid 2.7 g NaOH, 0.7 g NaCl, dissolved in 1 L of DI water, pH 9.0) for twenty minutes (20 min) and a final fifteen minute (15 min) DI water rinse.

Samples of heparin-containing material from each endoprosthesis (approx. 0.5 cm long) were cut and the bound heparin measured for biological activity using the above-described anti-thrombin III (ATIII) binding assay (Example 2). Samples were kept wet throughout the assay process. The results were expressed as anti-thrombin III binding per unit of substrate surface area (pmol/cm^2 substrate material).

Figure 12 is a bar graph illustrating the effect of a glycerol composition with immobilized heparin on a covered substrate material following compaction and expansion. Results show that the addition of glycerol to immobilized heparin significantly improves the anti-thrombin III binding activity of the bound heparin following compaction and expansion of the immobilized heparin compared to similarly treated control samples not having the added glycerol. All vertical bars represent mean values of n=3 samples.

Heparin-immobilized to a polymeric covering material that did not receive the additional glycerol biologically compatible organic composition, and was diametrically compacted and expanded, showed a significant reduction in anti-thrombin III binding activity (85 pmol/cm^2) compared to similarly constructed and treated control materials not diametrically compacted and expanded (137 pmol/cm^2). When heparin-immobilized covered substrate materials were treated with a biologically compatible organic glycerol composition and exposed to the same mechanical manipulations as the untreated construction, the anti-thrombin III binding activity of the immobilized heparin remained similar to the control materials (129 pmol/cm^2).

Example 6

This example describes the effect of the addition of a biologically compatible organic composition on the ATIII binding activity of the coated medical device

described in Examples 3 and 5, subjected to compaction, expansion and EtO sterilization.

The implantable medical device used in this example was constructed in the same way as described in Example 3. The device was in the form of a nitinol wire reinforced tube made of a porous, expanded, polytetrafluoroethylene (ePTFE) material obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename VIABAHN® Endoprosthesis. The tubular device was fifteen centimeters (15 cm) in length and six millimeters (6 mm) in diameter. The same process was utilized as detailed in Examples 3 for forming a heparin-containing coating on the device.

For treatment with the biologically compatible organic composition (100), substrate material (12) of the endoluminal device was prepared with a polymeric covering material (18) having aldehyde modified heparin (17) end point attached to at least a portion thereof. The prepared device was placed in a plastic tube and incubated with a heparin and glycerol solution (0.5 g USP heparin and 5 mL glycerol dissolved in 100 mL of DI water, pH 9.6) at sixty degrees centigrade (60 °C) for one hour (1 hr). The choice of these compounds is a result of the outcome of Examples 2, 3 and 5. Each treated device was removed from the heparin and glycerol solution and exposed to a lyophilization process. Further processing and analysis of devices was identical to Example 5, *supra*.

Figure 13 is a bar graph illustrating the ability of a biologically compatible organic composition in the form of glycerol and heparin to maintain the biological activity of heparin immobilized to a polymeric covering material on a substrate material both during and after exposure to an EtO sterilization regimen and mechanic manipulation in the form of compaction and expansion of the substrate and polymeric covering material to which the heparin was immobilized. All vertical bars represent mean values of n=3 samples.

Heparin-immobilized covered substrate materials that did not receive the additional glycerol and heparin biologically compatible organic compositions and were exposed to EtO sterilization and diametrically compacted and expanded showed a significant reduction in anti-thrombin III binding activity (63 pmol/cm^2) compared to similarly constructed and treated control materials not subjected to EtO sterilization and diametrical compaction and expansion (158 pmol/cm^2). When heparin-immobilized covered substrate materials were treated with a biologically

compatible organic glycerol and heparin composition and exposed to the same EtO sterilization conditions and mechanical manipulations as the untreated construction, the anti-thrombin III binding activity of the immobilized heparin remained similar to the control materials (147 pmol/cm^2).

Example 7

This example demonstrates a relatively low anti-thrombin III binding activity of a commercially available heparin-coated medical device. The device was a fifty centimeter (50cm) long, six millimeter (6mm) diameter, sterilized, and packaged heparin-coated vascular graft available under the tradename FLOWLINE BIPORE® Heparin Coated Vascular Graft (Catalog Number 15TW5006N) from JOTEC GmbH (Hechingen, Germany). According to the manufacturer, the tubular vascular graft is made of an expanded polytetrafluoroethylene (ePTFE) material with heparin covalently and ionically attached to the luminal surface of the graft. The manufacturer states that the heparin is stably and permanently attached to the ePTFE. Surfaces of the heparin-containing graft are said to be anti-thrombotic.

Samples (0.5 cm long) of the heparin-containing vascular graft were obtained and tested as described Example 2, *supra*. As with the inventive materials, the anti-thrombin III binding activity of the vascular graft were expressed as picomoles anti-thrombin III binding activity per square centimeter of substrate material (pmol/cm^2). As in previous examples, only the luminal surface area of each device was measured, not the entire surface area of the device. The results of the ATIII binding assay showed that there was no anti-thrombin III binding activity despite the claims by the manufacturer that biologically active heparin was present on luminal surface of the vascular graft. It should be noted that the anti-thrombin III binding activity assay is capable of detecting anti-thrombin III binding activity at a level of approximately five picomoles per square centimeter substrate material (5 pmol/cm^2 substrate material) and above.

Example 8

This example describes the use of a peptide antibiotic agent as a biologically compatible organic composition in conjunction with biologically active heparin

immobilized to a covered, or coated, substrate material. The construction exhibited significant ATIII binding after exposure to EtO sterilization.

In this example, an ePTFE material in sheet form was obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration Media (GMM-406) and provided with a heparin-containing coating using a process substantially equivalent to Example 2.

The above-described construction was exposed to a solution of bacitracin (72,000 units/gram) at a concentration of 0.5 g per 100 ml deionized water (DI water) by immersing the construction in one hundred milliliters (100 ml) of the bacitracin solution for three hours (3 hr) at room temperature. The construction was removed from the solution and lyophilized prior to exposure to a sterilization procedure.

In preparation for EtO sterilization, each lyophilized construction was placed and sealed in a Tower DUALPEEL® Self-Seal Pouch (Allegiance Healthcare Corp., McGaw Park, IL). Ethylene oxide sterilization was carried out under conditions of conditioning for one hour (1 hr), an EtO gas dwell time of one hour (1 hr), a set point temperature of fifty-five degree centigrade (55 °C), and an aeration time of twelve hours (12 hr).

After EtO sterilization, the construction was removed from its pouch and washed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1000 ml of DI water, pH 9.0) for twenty minutes (20 min), and finally rinsed in DI water for fifteen minutes (15 min).

Samples of the membrane (approx. 1 cm^2) with end-point attached heparin were cut from the sterilized construction and the immobilized heparin measured for anti-thrombin III binding activity using the above-described ATIII binding assay (Example 2). Samples were kept wet throughout the assay process. The results were expressed as picomoles of anti-thrombin III bound per unit of substrate surface area ($pmol/cm^2$).

The sample treated with bacitracin and subsequently sterilized with ethylene oxide had an anti-thrombin III binding activity of 9 $pmol/cm^2$ (n=3).

Example 9

This example describes the addition of a biologically compatible organic composition to biologically active heparin immobilized to a covered, or coated, substrate material and previously exposed to EtO sterilization. A peptide antibiotic

agent was selected as the biologically compatible organic composition in this example. A construction treated in this way had significant heparin ATIII binding after exposure to EtO sterilization.

In this example, an ePTFE material in sheet form was obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration Media (GMM-406) and provided with a heparin-containing coating using a process substantially equivalent to Example 2.

In preparation for EtO sterilization, each lyophilized construction was placed and sealed in a Tower DUALPEEL® Self-Seal Pouch (Allegiance Healthcare Corp., McGaw Park, IL). Ethylene oxide sterilization was carried out under conditions of conditioning for one hour (1 hr), an EtO gas dwell time of one hour (1 hr), a set point temperature of fifty-five degree centigrade (55 °C), and an aeration time of twelve hours (12 hr).

After EtO sterilization, the construction was aseptically handled in a NUAIRE™ Biological Safety Cabinets, class II, type A/B3, model NU-425-600 (Plymouth, MN).

Sterilized samples approximately one square centimeter (1 cm^2) in size were cut from the construction and submerged in a filter-sterilized bacitracin solution (649.4 mg at 77000 units/g dissolved in 10 ml of 0.9% sodium chloride irrigation solution purchased from Hospira, Inc.) with a resultant concentration of approximately five thousand (5000) units per ml of USP grade 0.9% sodium chloride irrigation solution. Samples were exposed to the bacitracin solution for two minutes (2 min) at room temperature.

Samples were removed from the solution and washed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1000 ml of DI water, pH 9.0) for twenty minutes (20 min), and finally a rinse in DI water for fifteen minutes (15 min).

Samples of the sheet material (approx. 1 cm^2) with end-point attached heparin were cut and the immobilized heparin measured for anti-thrombin III binding activity using the above-described ATIII binding assay (Example 2). Samples were kept wet throughout the assay process. The results were expressed as picomoles of anti-thrombin III bound per unit of substrate surface area (pmol/cm^2).

The sample that was initially sterilized with ethylene oxide and subsequently treated with bacitracin had an anti-thrombin III binding activity of 185 pmol/cm^2 pmol/cm^2 (n=3). As these results indicate, a therapeutic agent can be admixed with

biologically active heparin immobilized to a covered substrate material after the entity has been sterilized without significantly reducing its biological activity.

Example 10

This example demonstrates biologically active heparin immobilized to a covered substrate material that was admixed with a biologically compatible organic composition, EtO sterilized, and finally treated with a peptide antibiotic agent. A construction treated in this way has significant heparin ATIII binding.

In this example, an ePTFE material in sheet form was obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration Media (GMM-406) and provided with a heparin-containing coating using a process substantially equivalent to Example 2.

The above-described construction was exposed to a solution of polyethylene glycol (20,000 molecular weight, Sigma) at a concentration of 0.5 g per 100 ml DI water adjusted to pH 9.6. The construction was placed into a beaker and one hundred milliliters (100 ml) was added to completely immerse the construction in the polyethylene glycol solution. The construction was exposed to the polyethylene glycol solution for one hour (1 hr) at sixty degrees centigrade (60 °C). The construction was removed from the solution and lyophilized prior to exposure to a sterilization procedure.

In preparation for EtO sterilization, the lyophilized construction was placed and sealed in a Convertors® Self-Seal Pouch (Cardinal Health, McGaw Park, IL). Ethylene oxide sterilization was carried out under conditions of conditioning for one hour (1 hr), an EtO gas dwell time of one hour (1 hr), a set point temperature of fifty-five degree centigrade (55 °C), and an aeration time of twelve hours (12 hr).

After EtO sterilization, the construction was aseptically handled in a NUAIRE Biological Safety Cabinet, class II, type A/B3, model NU-425-600 (Plymouth, MN).

Sterilized samples approximately one square centimeter (1 cm^2) in size were cut from the construction and submerged in a filter-sterilized bacitracin solution (649.4 mg at 77,000 units/g dissolved in 10 ml of 0.9% sodium chloride irrigation solution) with a resultant concentration of approximately five thousand (5,000) units per ml of USP grade 0.9% sodium chloride irrigation solution. Samples were exposed to the bacitracin solution for two minutes (2 min) at room temperature.

Samples were removed from the solution and washed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1,000 ml of DI water, pH 9.0) for twenty minutes (20 min), and finally a rinse in DI water for fifteen minutes (15 min).

Samples of the membrane (approx. 1 cm^2) with end-point attached heparin were cut and the immobilized heparin measured for anti-thrombin III binding activity using the above-described ATIII binding assay (Example 2). Samples were kept wet throughout the assay process. The results were expressed as picomoles of anti-thrombin III bound per unit of substrate surface area (pmol/cm^2).

The sample that was initially treated with polyethylene glycol, sterilized with ethylene oxide, and then treated with bacitracin had an anti-thrombin III binding activity of 195 pmol/cm^2 (n=3). Hence, a sterilized covered substrate material with a biologically active heparin immobilized thereto and a first biologically compatible organic composition (PEG) admixed therewith can be further treated with a second biologically compatible organic composition (bacitracin) following EtO sterilization and retain significant ATIII binding activity.

Example 11

This example demonstrates the covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the subsequent covalent attachment of a biologically compatible organic composition (aldehyde activated dextran) to the covering material. This composition was exposed to EtO sterilization and thereafter demonstrated significant biological heparin activity.

In this example, an ePTFE material in sheet form was obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration Media (GMM-406). This ePTFE material was provided with a heparin-containing coating using a process substantially equivalent to Example 2, however, the construction was stored in DI water after being coated rather than lyophilized.

The above-described construction coated with a covering material was exposed to an aldehyde-activated dextran (40,000 molecular weight, Pierce) solution (0.050 g aldehyde-activated dextran, 2.93 g NaCl dissolved in 100 ml DI water, pH 5.5) for one hundred twenty minutes (120 min) at sixty degrees centigrade (60 °C). A 0.286 mL volume of a 2.5% (w/v) aqueous $NaCNBH_3$ solution was added to the

one hundred milliliters (100 ml) aldehyde-activated dextran solution prior to adding the sample.

The construction was removed from the aldehyde-activated dextran solution and washed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1,000 ml of DI water, pH 9.0) for twenty minutes (20 min), and finally a rinse in DI water for fifteen minutes (15 min) followed by lyophilization of the entire construction to produce dry heparin bound to the ePTFE material.

In preparation for EtO sterilization, the lyophilized construction was placed and sealed in a Convertors® Self-Seal Pouch (Cardinal Health, McGaw Park, IL). Ethylene oxide sterilization was carried out under conditions of conditioning for one hour (1 hr), an EtO gas dwell time of one hour (1 hr), a set point temperature of fifty-five degree centigrade (55 °C), and an aeration time of twelve hours (12 hr).

Samples of the sterilized membrane (approx. 1 cm^2) with end-point attached heparin were cut and the immobilized heparin measured for anti-thrombin III binding activity using the above-described ATIII binding assay (Example 2). The results were expressed as picomoles of anti-thrombin III bound per unit of substrate surface area ($pmol/cm^2$).

The sample prepared as described in this example had a mean anti-thrombin III binding activity of 65 $pmol/cm^2$ (n=3). This example demonstrates that a biocompatible organic composition can, in addition to the covalently bound end-point attached heparin, be covalently attached to the coating layer while maintaining significant heparin activity following EtO sterilization.

Example 12

This example demonstrates the covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the subsequent covalent attachment of a biologically compatible organic composition (aldehyde activated polyethylene glycol, 1,000 molecular weight) to the covering material. This composition was exposed to EtO sterilization and thereafter demonstrated significant biological heparin activity.

In this example, an ePTFE material in sheet form was obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration

Media (GMM-406). This ePTFE material was provided with a heparin-containing coating using a process substantially equivalent to Example 2, however, the construction was stored in DI water after being coated rather than lyophilized.

The above-described construction coated with a covering material was exposed to an aldehyde activated PEG (1,000 molecular weight, Nanocs) solution (0.20 g PEG, 3.90 g NaCl dissolved in 133 ml DI water, pH 5.5) for one hundred twenty minutes (120 min) at sixty degrees centigrade (60 °C). A 0.380 mL volume of a 2.5% (w/v) aqueous $NaCNBH_3$ solution was added to the one hundred milliliters (100 ml) PEG solution prior to adding the sample.

The construction was removed from the PEG solution and washed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1,000 ml of DI water, pH 9.0) for twenty minutes (20 min), and finally a rinse in DI water for fifteen minutes (15 min) followed by lyophilization of the entire construction to produce dry heparin bound to the ePTFE material.

In preparation for EtO sterilization, the lyophilized construction was placed and sealed in a Convertors® Self-Seal Pouch (Cardinal Health, McGaw Park, IL). Ethylene oxide sterilization was carried out under conditions of conditioning for one hour (1 hr), an EtO gas dwell time of one hour (1 hr), a set point temperature of fifty-five degree centigrade (55 °C), and an aeration time of twelve hours (12 hr).

Samples of the sterilized membrane (approx. 1 cm^2) with end-point attached heparin were cut and the immobilized heparin measured for anti-thrombin III binding activity using the above-described ATIII binding assay (Example 2). The results were expressed as picomoles of anti-thrombin III bound per unit of substrate surface area ($pmol/cm^2$).

The sample prepared as described in this example had a mean anti-thrombin III binding activity of 96 $pmol/cm^2$ (n=3). This example demonstrates a biocompatible organic composition can, in addition to the covalently bound end-point attached heparin, be covalently attached to the coating layer while maintaining significant heparin activity.

Example 13

This example demonstrates the covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the subsequent covalent

attachment of a biologically compatible organic composition (aldehyde activated polyethylene glycol, 5,000 molecular weight) to the covering, or coating, material. This composition was exposed to EtO sterilization and thereafter demonstrated significant biological heparin activity.

In this example, an ePTFE material in sheet form was obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration Media (GMM-406). This ePTFE material was provided with a heparin-containing coating using a process substantially equivalent to Example 2, however, the construction was stored in DI water after being coated rather than lyophilized.

The above-described construction coated with a covering material was exposed to an aldehyde activated PEG (5,000 molecular weight, Nanocs) solution (0.20 g PEG, 3.90 g NaCl dissolved in 133 ml DI water, pH 5.5) for one hundred twenty minutes (120 min) at sixty degrees centigrade (60 °C). A 0.380 mL volume of a 2.5% (w/v) aqueous $NaCNBH_3$ solution was added to the one hundred milliliters (100 ml) PEG solution prior to adding the sample.

The construction was removed from the PEG solution and washed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1,000 ml of DI water, pH 9.0) for twenty minutes (20 min), and finally a rinse in DI water for fifteen minutes (15 min) followed by lyophilization of the entire construction to produce dry heparin bound to the ePTFE material.

In preparation for EtO sterilization, the lyophilized construction was placed and sealed in a Convertors® Self-Seal Pouch (Cardinal Health, McGaw Park, IL). Ethylene oxide sterilization was carried out under conditions of conditioning for one hour (1 hr), an EtO gas dwell time of one hour (1 hr), a set point temperature of fifty-five degree centigrade (55 °C), and an aeration time of twelve hours (12 hr).

Samples of the sterilized membrane (approx. 1 cm^2) with end-point attached heparin were cut and the immobilized heparin measured for anti-thrombin III binding activity using the above-described ATIII binding assay (Example 2). The results were expressed as picomoles of anti-thrombin III bound per unit of substrate surface area ($pmol/cm^2$).

The sample prepared as described in this example had a mean anti-thrombin III binding activity of 64 $pmol/cm^2$ (n=3). This example demonstrates that a biocompatible organic composition can, in addition to the covalently bound end-point

attached heparin, be covalently attached to the coating layer while maintaining significant heparin activity.

Example 14

This example demonstrates the covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the subsequent covalent attachment of a biologically compatible organic composition (EDC activated USP heparin) to the covering material. This composition was exposed to EtO sterilization and thereafter demonstrated significant biological heparin activity.

In this example, an ePTFE material in sheet form was obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration Media (GMM-406). This ePTFE material was provided with a heparin-containing coating using a process substantially equivalent to Example 2, however, the construction was stored in DI water after being coated rather than lyophilized.

USP grade heparin was attached, or conjugated, to the PEI layer(s) already containing end point attached heparin by placing the construction in a USP grade heparin-containing sodium chloride salt solution (1.5 g USP heparin, 29.3 g NaCl dissolved in 1 L DI water, pH 3.9) for one hundred twenty minutes (120 min) at sixty degrees centigrade (60 °C). The constructions were transferred to a solution of 0.1 M MES [2-(N-morpholino)ethanesulfonic acid] BupH™ MES buffered saline (Pierce) , 1.5 g USP heparin, 29.3 g NaCl, 0.20 g *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC), and 0.13 g N-hydroxysulfosuccinimide (NHS) dissolved in 1 L DI water, at pH 5.5 for 4 hours (4 hr) at room temperature.

The construction was removed from the above-described solution and washed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1000 ml of DI water, pH 9.0) for twenty minutes (20 min), and finally a rinse in DI water for fifteen minutes (15 min) followed by lyophilization of the entire construction to produce dry heparin bound to the ePTFE material.

In preparation for EtO sterilization, the lyophilized construction was placed and sealed in a Convertors® Self-Seal Pouch (Cardinal Health, McGaw Park, IL). Ethylene oxide sterilization was carried out under conditions of conditioning for one

hour (1 hr), an EtO gas dwell time of one hour (1 hr), a set point temperature of fifty-five degree centigrade (55 °C), and an aeration time of twelve hours (12 hr).

Samples of the sterilized membrane (approx. 1 cm^2) with end-point attached heparin were cut and the immobilized heparin measured for anti-thrombin III binding activity using the above-described ATIII binding assay (Example 2). The results were expressed as picomoles of anti-thrombin III bound per unit of substrate surface area ($pmol/cm^2$).

The sample prepared as described in this example had a mean anti-thrombin III binding activity of 31 $pmol/cm^2$ (n=3). This example demonstrates that a biocompatible organic composition can, in addition to the covalently bound end-point attached heparin, be covalently attached to the coating layer while maintaining significant heparin activity.

Example 15

This example demonstrates covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by a secondary covalent attachment of the heparin to the coating layer. To achieve the secondary covalent attachment of the end-point attached heparin, the carboxylic acid groups are activated with EDC and reacted with the remaining primary amine groups present in the coating layer. This composition was exposed to EtO sterilization and thereafter demonstrated significant biological heparin activity.

In this example, an ePTFE material in sheet form was obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration Media (GMM-406). This ePTFE material was provided with a heparin-containing coating using a process substantially equivalent to Example 2, however, the construction was stored in DI water after being coated rather than lyophilized.

Membranes were transferred to a solution of 0.1 M MES [2-(N-morpholino)ethanesulfonic acid] BupH™ MES buffered saline (Pierce), 29.3 g NaCl, 0.20 g *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), and 0.13 g N-hydroxysulfosuccinimide (NHS) dissolved in 1 L DI water, at pH 5.5 for 4 hours (4 hr) at room temperature.

The construction was removed from the above-described solution and washed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric

acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1,000 ml of DI water, pH 9.0) for twenty minutes (20 min), and finally a rinse in DI water for fifteen minutes (15 min) followed by lyophilization of the entire construction to produce dry heparin bound to the ePTFE material.

In preparation for EtO sterilization, the lyophilized construction was placed and sealed in a Convertors® Self-Seal Pouch (Cardinal Health, McGaw Park, IL). Ethylene oxide sterilization was carried out under conditions of conditioning for one hour (1 hr), an EtO gas dwell time of one hour (1 hr), a set point temperature of fifty-five degree centigrade (55 °C), and an aeration time of twelve hours (12 hr).

Samples of the sterilized membrane (approx. 1 cm^2) with end-point attached heparin were cut and the immobilized heparin measured for anti-thrombin III binding activity using the above-described ATIII binding assay (Example 2). The results were expressed as picomoles of anti-thrombin III bound per unit of substrate surface area ($pmol/cm^2$).

The sample prepared as described in this example had a mean anti-thrombin III binding activity of 20 $pmol/cm^2$ (n=3). This example demonstrates that heparin can be further covalently attached to a coating layer, in addition to the covalent end-point attachment, while maintaining significant heparin activity following EtO sterilization.

Example 16

This example describes use of a peptide antibiotic agent as a biologically compatible organic composition in conjunction with biologically active heparin that is immobilized to a covered substrate material. The construction has greater than 5 $pmol/cm^2$ ATIII binding activity after mechanical compaction and expansion.

In this example, implantable medical devices in the form of endoluminal prostheses are heparinized as described in Example 3. Bacitracin is then applied using the conditions described in Example 8. The heparinized endoluminal prostheses are then mechanically compacted, mechanically expanded, rinsed, cut for testing, and assayed for ATIII binding as described in Example 5.

The sample treated with bacitracin and subsequently compacted and expanded has an anti-thrombin III binding activity of greater than 5 $pmol/cm^2$.

Example 17

This example describes the addition of a biologically compatible organic composition to biologically active heparin that is immobilized to a covered substrate material and previously compacted and expanded. A peptide antibiotic agent is selected as the biologically compatible organic composition. A construction treated in this way has significant heparin ATIII binding after compaction and expansion.

In this example, implantable medical devices in the form of endoluminal prostheses are heparinized and as described in Example 3 and mechanically compacted and expanded as described in Example 5. The endoluminal prosthesis is then treated with bacitracin and rinsed as described in Example 9. The heparinized endoluminal prostheses are then cut for testing and assayed for ATIII binding as described in Example 5.

A heparinized sample that is compacted and expanded, followed by the addition of bacitracin, has an anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 18

This example describes biologically active heparin immobilized to a covered substrate material. The immobilized biologically active heparin is admixed with a biologically compatible organic composition, mechanically compacted, mechanically expanded, and treated with a peptide antibiotic agent. A construction treated in this way has significant heparin ATIII binding after exposure to mechanical manipulation.

Endoluminal prosthesis are treated and tested as described in Example 17 with one exception; polyethylene glycol is admixed with the heparinized endoluminal prosthesis, as described in Example 10, prior to compaction and expansion.

A heparinized sample with an admixed biologically compatible organic composition that is compacted and expanded, followed by the addition of bacitracin, has an anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 19

This example demonstrates covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by covalent attachment of a biologically compatible organic composition (aldehyde activated dextran) to the

covering material. This composition is exposed to mechanical compaction and expansion and thereafter demonstrates significant biological heparin activity.

In this example, implantable medical devices in the form of endoluminal prostheses are heparinized as described in Example 3. Aldehyde activated dextran is immobilized to the covering layer as described in Example 11. The heparinized endoluminal prostheses are then mechanically compacted, mechanically expanded, cut for testing, and assayed for ATIII binding as described in Example 5.

The sample prepared as described in this example has anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 20

This example demonstrates the covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the subsequent covalent attachment of a biologically compatible organic composition (aldehyde activated polyethylene glycol, 1,000 molecular weight) to the covering material. This composition is exposed to mechanical compaction and expansion and thereafter demonstrates significant biological heparin activity.

In this example, implantable medical devices in the form of endoluminal prostheses are heparinized as described in Example 3. Aldehyde activated polyethylene glycol is immobilized to the covering layer as described in Example 12. The heparinized endoluminal prostheses are then compacted, expanded, cut for testing, and assayed for ATIII binding as described in Example 5.

The sample prepared as described in this example has anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 21

This example demonstrates the covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the subsequent covalent attachment of a biologically compatible organic composition (aldehyde activated polyethylene glycol, 5,000 molecular weight) to the covering material. This composition is exposed to mechanical compaction and expansion and thereafter demonstrates significant biological heparin activity.

In this example, implantable medical devices in the form of endoluminal prostheses are heparinized as described in Example 3. Aldehyde activated polyethylene glycol is immobilized to the covering layer as described in Example 13. The heparinized endoluminal prostheses are then compacted, expanded, cut for testing, and assayed for ATIII binding as described in Example 5.

The sample prepared as described in this example has anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 22

This example demonstrates covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the subsequent covalent attachment of a biologically compatible organic composition (EDC activated USP heparin) to the covering material. This composition is exposed to mechanical compaction and expansion and thereafter demonstrates significant biological heparin activity.

In this example, implantable medical devices in the form of endoluminal prostheses are heparinized as described in Example 3. USP Heparin is immobilized to the covering layer as described in Example 14. The heparinized endoluminal prostheses are then compacted, expanded, cut for testing, and assayed for ATIII binding as described in Example 5.

The sample prepared as described in this example has anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 23

This example demonstrates covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by a secondary covalent attachment of the heparin to reactive groups of the coating layer. To achieve the secondary covalent attachment of the end-point attached heparin, the carboxylic acid groups are activated with EDC and reacted with the remaining primary amine groups present in the coating layer. This composition is exposed to mechanical compaction and expansion. The construction thereafter demonstrates significant biological heparin activity.

In this example, implantable medical devices in the form of endoluminal prostheses are heparinized as described in Example 3. Further covalent attachment of the immobilized heparin to the covering layer is conducted as in Example 15. The heparinized endoluminal prostheses are then mechanically compacted, mechanically expanded, cut for testing, and assayed for ATIII binding as described in Example 5.

The samples prepared as described in this example have anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 24

This example demonstrates the covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the additional attachment of a biologically compatible organic composition via a labile bond to the covering material. The labile bond allows for local delivery of a therapeutic compound while the stably bound heparin retains significant ATIII binding activity following sterilization and mechanical compaction and expansion

In this example, implantable medical devices in the form of endoluminal prostheses are heparinized as described in Example 3. Additional aldehyde modified heparin is end point attached to the coating layer via a labile covalent bond by placing the construction in a heparin-containing sodium chloride salt solution (1.5 g aldehyde modified heparin, 29.3 g NaCl dissolved in 1 L DI water, pH 3.9) for one hundred twenty minutes (120 min) at sixty degrees centigrade (60 °C). It is important to note that the reducing agent, $NaCNBH_3$, is not added during this second conjugation of aldehyde modified heparin. The bond formed between primary amines and aldehydes, when left in the un-reduced state, is labile. The samples are then rinsed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1 L DI water, pH 9.0) for twenty minutes (20 min), and finally in DI water for fifteen minutes (15 min) followed by lyophilization of the entire construction to produce dry heparin bound to the ePTFE material.

The heparinized endoluminal prostheses are then compacted as described in Example 5 and sterilized as described in Example 3. They are then expanded, cut for testing, and assayed for ATIII binding as described in Example 5.

The samples prepared as described in this example have anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 25

This example demonstrates the covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the additional attachment of a biologically compatible organic composition via a labile bond to the covering material. The labile bond allows for local delivery of a therapeutic compound while the stably bound heparin retains significant ATIII binding activity following mechanical compaction and expansion.

In this example, implantable medical devices in the form of endoluminal prostheses are heparinized as described in Example 3. Additional aldehyde modified heparin is end point attached to the coating layer via a labile covalent bond by placing the construction in a heparin-containing sodium chloride salt solution (1.5 g aldehyde modified heparin, 29.3 g NaCl dissolved in 1 L DI water, pH 3.9) for one hundred twenty minutes (120 min) at sixty degrees centigrade (60 °C). It is important to note that the reducing agent, $NaCNBH_3$, is not added during this second conjugation of aldehyde modified heparin. The bond formed between primary amines and aldehydes, when left in the un-reduced state, is labile. The samples are then rinsed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1 L DI water, pH 9.0) for twenty minutes (20 min), and finally in DI water for fifteen minutes (15 min) followed by lyophilization of the entire construction to produce dry heparin bound to the ePTFE material.

The heparinized endoluminal prostheses are then compacted, expanded, cut for testing, and assayed for ATIII binding as described in Example 5.

The samples prepared as described in this example have anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 26

This example demonstrates the covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the additional attachment of a

biologically compatible organic composition via a labile bond to the covering material. The labile bond allows for local delivery of a therapeutic compound while the stably bound heparin retains significant ATIII binding activity following EtO sterilization.

In this example, an ePTFE material in sheet form is obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration Media (GMM-406) and provided with a heparin-containing coating using a process substantially equivalent to Example 2. Additional aldehyde modified heparin is end point attached to the coating layer via a labile covalent bond by placing the construction in a heparin-containing sodium chloride salt solution (1.5 g aldehyde modified heparin, 29.3 g NaCl dissolved in 1 L DI water, pH 3.9) for one hundred twenty minutes (120 min) at sixty degrees centigrade (60 °C). It is important to note that the reducing agent, $NaCNBH_3$, is not added during this second conjugation of aldehyde modified heparin. The bond formed between primary amines and aldehydes, when left in the un-reduced state, is labile. The samples are then rinsed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1 L DI water, pH 9.0) for twenty minutes (20 min), and finally in DI water for fifteen minutes (15 min) followed by lyophilization of the entire construction to produce dry heparin bound to the ePTFE material.

The heparinized material is then sterilized, cut for testing, and assayed for ATIII binding as described in Example 2.

The samples prepared as described in this example have anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 27

This example demonstrates the covalent attachment of biologically active heparin to a covering material placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the subsequent covalent attachment of a biologically compatible organic composition (aldehyde activated polyethylene glycol) to the covering material, with the final addition of a non-covalently admixed biologically compatible organic composition (Bacitracin). This composition is exposed to EtO sterilization and thereafter demonstrates significant biological heparin activity.

In this example, an ePTFE material in sheet form is obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration Media

(GMM-406) and provided with a heparin-containing coating using a process substantially equivalent to Example 2. Aldehyde activated polyethylene glycol (1,000 molecular weight) is covalently attached to the covering material as described in Example 12 and Bacitracin is then non-covalently admixed with this composition as described in Example 8. The composition is then sterilized and sampled as described in Example 8 and the immobilized heparin is measured for anti-thrombin III binding activity using the ATIII binding assay described in Example 2.

The samples prepared as described in this example have anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 28

This example demonstrates the covalent attachment of biologically active heparin to a covering material placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the subsequent covalent attachment of a biologically compatible organic composition (aldehyde activated polyethylene glycol) to the covering material, with the final addition of a non-covalently admixed biologically compatible organic composition (Bacitracin). This composition is exposed to mechanical compaction and expansion and thereafter demonstrates significant biological heparin activity.

In this example, implantable medical devices in the form of endoluminal prostheses are heparinized as described in Example 3. Aldehyde activated polyethylene glycol (1,000 molecular weight) is covalently attached to the covering material as described in Example 12 and Bacitracin is then non-covalently admixed with this composition as described in Example 8. The heparinized endoluminal prostheses are then mechanically compacted, mechanically expanded, cut for testing, rinsed, and assayed for ATIII binding as described in Example 5.

The sample prepared as described in this example has anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 29

This example demonstrates the covalent attachment of biologically active heparin to a covering material placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the subsequent covalent attachment of a biologically compatible organic composition (aldehyde activated dextran) to the covering

material, with the final addition of a non-covalently admixed biologically compatible organic composition (dexamethasone). This composition is exposed to EtO sterilization and thereafter demonstrates significant biological heparin activity.

In this example, an ePTFE material in sheet form is obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration Media (GMM-406) and provided with a heparin-containing coating using a process substantially equivalent to Example 2. Aldehyde activated dextran is covalently attached to the covering material as described in Example 11 and dexamethasone is then non-covalently admixed with this composition which is then sterilized and sampled as described in Example 2. The immobilized heparin is measured for anti-thrombin III binding activity using the ATIII binding assay described in Example 2.

The samples prepared as described in this example have anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 30

This example demonstrates the covalent attachment of biologically active heparin to a covering material placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the subsequent covalent attachment of a biologically compatible organic composition (aldehyde activated dextran) to the covering material, with the final addition of a non-covalently admixed biologically compatible organic composition (dexamethasone). This composition is exposed to mechanical compaction and expansion and thereafter demonstrates significant biological heparin activity.

In this example, implantable medical devices in the form of endoluminal prostheses are heparinized as described in Example 3. Aldehyde activated dextran is covalently attached to the covering material as described in Example 11 and dexamethasone is then non-covalently admixed with this composition as described in Example 2. The heparinized endoluminal prostheses are then mechanically compacted, mechanically expanded, cut for testing, rinsed, and assayed for ATIII binding as described in Example 5.

The sample prepared as described in this example has anti-thrombin III binding activity greater than 5 pmol/cm^2.

Example 31

This example demonstrates the covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the additional attachment of a biologically compatible organic composition (polyethylene glycol) via a labile bond to the covering material, with the final addition of a non-covalently admixed biologically compatible organic composition (dexamethasone). This composition is exposed to EtO sterilization and thereafter demonstrates significant biological heparin activity.

In this example, an ePTFE material in sheet form is obtained from W.L. Gore & Associates, Inc., Flagstaff, AZ under the tradename GORE™ Microfiltration Media (GMM-406) and provided with a heparin-containing coating using a process substantially equivalent to Example 2. Aldehyde modified polyethylene glycol (1,000 MW) is attached to the coating layer via a labile covalent bond using the process described in Example 12 excluding the addition of $NaCNBH_3$. It is important to note that the reducing agent, $NaCNBH_3$, is not added during the conjugation of aldehyde modified polyethylene glycol. The bond formed between primary amines and aldehydes, when left in the un-reduced state, is labile. The samples are then rinsed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1 L DI water, pH 9.0) for twenty minutes (20 min), and finally in DI water for fifteen minutes (15 min) followed by lyophilization of the entire construction to produce dry heparin and polyethylene glycol bound to the ePTFE material. Dexamethasone is then non-covalently admixed with this composition as described in Example 2. The heparinized material is then sterilized, sampled, and measured for anti-thrombin III binding activity using the ATIII binding assay described in Example 2.

The samples prepared as described in this example have anti-thrombin III binding activity greater than 5 $pmol/cm^2$.

Example 32

This example demonstrates the covalent attachment of biologically active heparin to a covering material, or coating layer, placed on an expanded polytetrafluoroethylene (ePTFE) material followed by the additional attachment of a biologically compatible organic composition (polyethylene glycol) via a labile bond to the covering material, with the final addition of a non-covalently admixed biologically

compatible organic composition (dexamethasone). This composition is exposed to mechanical compaction and expansion and thereafter demonstrates significant biological heparin activity.

In this example, implantable medical devices in the form of endoluminal prostheses are heparinized as described in Example 3. Aldehyde modified polyethylene glycol (1,000 MW) is attached to the coating layer via a labile covalent bond using the process described in Example 12 excluding the addition of $NaCNBH_3$. It is important to note that the reducing agent, $NaCNBH_3$, is not added during the conjugation of aldehyde modified polyethylene glycol. The bond formed between primary amines and aldehydes, when left in the un-reduced state, is labile. The samples are then rinsed in DI water for fifteen minutes (15 min), borate buffer solution (10.6 g boric acid, 2.7 g NaOH and 0.7 g NaCl dissolved in 1 L DI water, pH 9.0) for twenty minutes (20 min), and finally in DI water for fifteen minutes (15 min) followed by lyophilization of the entire construction to produce dry heparin and polyethylene glycol bound to the ePTFE material. Dexamethasone is then non-covalently admixed with this composition as described in Example 2. The heparinized endoluminal prostheses are then mechanically compacted, mechanically expanded, cut for testing, rinsed, and assayed for ATIII binding as described in Example 5.

The sample prepared as described in this example has anti-thrombin III binding activity greater than 5 pmol/cm^2.

What is claimed is:

1. A medical device comprising:

a substrate material;

a polymeric covering material attached to at least a portion of a surface of said substrate material, a plurality of biologically active entities having anti-thrombin III binding activity covalently attached to at least a portion of said polymeric covering material; and

a biologically compatible saccharide, polyethylene glycol and/or glycerol combined with said polymeric covering material, wherein biologically active entities have an anti-thrombin III binding activity of at least 5 picomoles anti-thrombin III per square centimeter ($pmol/cm^2$) substrate material following sterilization or compaction and expansion of said substrate material.

2. The medical device of claim 1 wherein said biologically compatible saccharide, polyethylene glycol and/or glycerol is non-covalently combined with said polymeric covering material.

3. The medical device of claim 1 wherein said biologically compatible saccharide, polyethylene glycol and/or glycerol is covalently combined with said polymeric covering material.

4. The medical device of claim 1 wherein said plurality of biologically active entities have an anti-thrombin III binding activity of at least 6 picomoles anti-thrombin III per square centimeter ($pmol/cm^2$) substrate material following compaction and expansion of said substrate material.

5. The medical device of claim 1 wherein said plurality of biologically active entities have an anti-thrombin III binding activity of at least 7 picomoles anti-thrombin III per square centimeter ($pmol/cm^2$) substrate material following compaction and expansion of said substrate material.

6. The medical device of claim 1 wherein said plurality of biologically active entities have an anti-thrombin III binding activity of at least 8 picomoles anti-thrombin III per square centimeter ($pmol/cm^2$) substrate material following compaction and expansion of said substrate material.

7. The medical device of claim 1 wherein said plurality of biologically active entities have an anti-thrombin III binding activity of at least 9 picomoles anti-thrombin III per square centimeter ($pmol/cm^2$) substrate material following compaction and expansion of said substrate material.

8. The medical device of claim 1 wherein said plurality of biologically active entities have an anti-thrombin III binding activity of at least 10 picomoles anti-thrombin III per square centimeter (pmol/cm^2) substrate material following compaction and expansion of said substrate material.

9. The medical device of claim 1 wherein said plurality of biologically active entities have an anti-thrombin III binding activity of at least 100 picomoles anti-thrombin III per square centimeter (pmol/cm^2) substrate material following compaction and expansion of said substrate material.

10. The medical device of claim 1 wherein at least a portion of said biologically compatible saccharide, polyethylene glycol and/or glycerol is released from said medical device in a 0.15M phosphate buffer solution having a temperature of about thirty- seven degrees centigrade and a neutral pH.

11. The medical device of claim 1 wherein said plurality of biologically active entities comprises a glycosaminoglycan.

12. The medical device of claim 11 wherein said plurality of biologically active entities comprises end-point attached heparin.

13. The medical device of claim 1 wherein said saccharide is a polysaccharide.

14. The medical device of claim 13 wherein said polysaccharide is a glycosaminoglycan.

15. The medical device of claim 14 wherein said polysaccharide is dextran.

16. The medical device of claim 14 wherein said polysaccharide is dextran sulfate.

17. The medical device of claim 1 wherein said polymeric covering material comprises multiple layers, wherein chemical components of at least one layer are cross-linked.

18. The medical device of claim 1 wherein said substrate material is polymeric in composition.

19. The medical device of claim 1 wherein said polymeric substrate material is fluoropolymeric in composition.

20. The medical device of claim 19 wherein said fluoropolymeric material is polytetrafluoroethylene.

21. The medical device of claim 1 wherein said substrate material is metallic in composition.

22. The medical device of claim 21 wherein said metallic composition is a nickel and titanium metal alloy.

23. The medical device of claim 1 wherein said polymeric covering material comprises at least one layer of polyethylene imine.

24. The medical device of any one of claims 1 through 23 further comprising a second biologically compatible composition admixed therewith prior to sterilization or compaction and expansion of said device.

25. The medical device of claim 24 wherein said second biologically compatible composition is an antiproliferative agent.

26. The medical device of claim 25 wherein said antiproliferative agent is dexamethasone.

27. The medical device of claim 24 wherein said second biologically compatible composition is an antimicrobial compound.

28. The medical device of claim 27 wherein said antimicrobial compound is bacitracin.

29. A medical device comprising:

a plurality of biological active entities having anti-thrombin III binding activity;

a substrate material for immobilizing the biological active entities present on at least a portion of a surface of said substrate material; and

a biologically compatible saccharide, polyethylene glycol and/or glycerol combined with the biological active entities,

wherein at least ninety percent of said anti-thrombin III binding activity is retained following compaction and expansion of said substrate material.

30. A sterilized medical device comprising:

a plurality of biological active entities having anti-thrombin III binding activity;

a substrate material for immobilizing the biological active entities present on at least a portion of a surface of said substrate material; and

a biologically compatible saccharide, polyethylene glycol and/or glycerol combined with the biological active entities,

wherein at least ninety percent of said anti-thrombin III binding activity is retained following sterilization of said chemical entities.

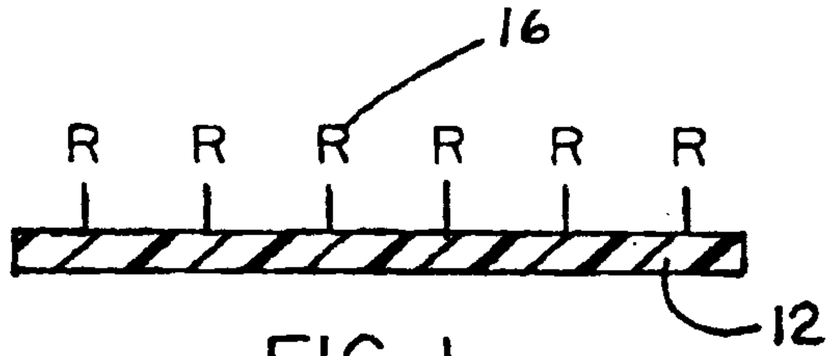


FIG. 1

PRIOR ART

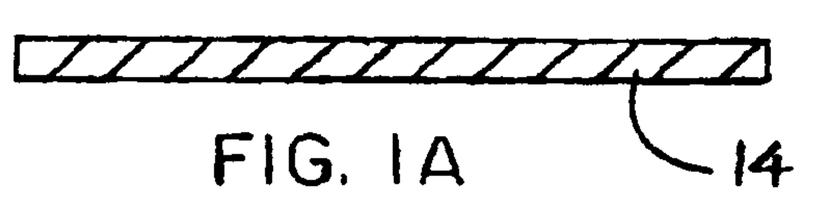


FIG. 1A

PRIOR ART

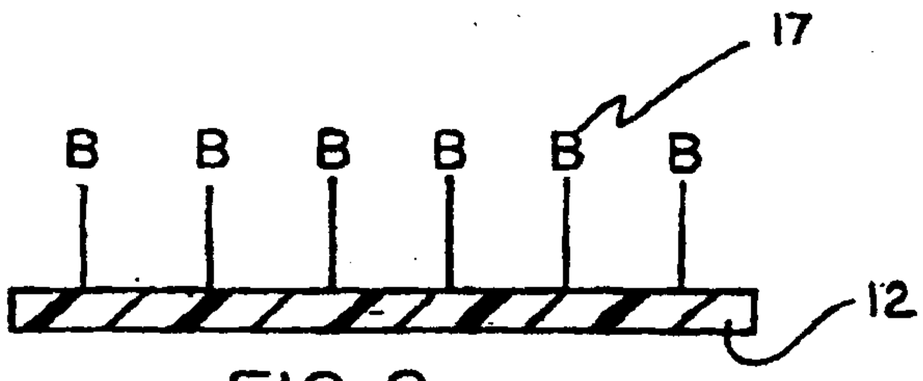


FIG. 2

PRIOR ART

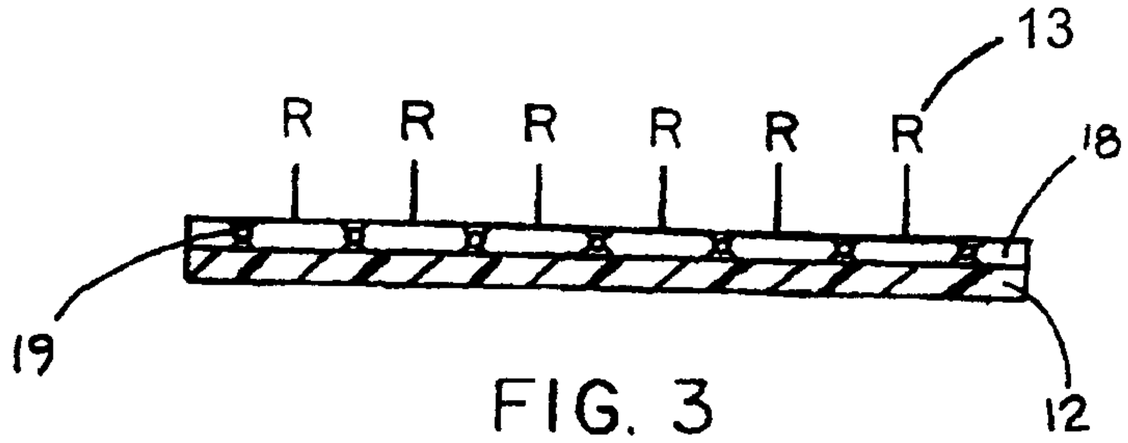


FIG. 3

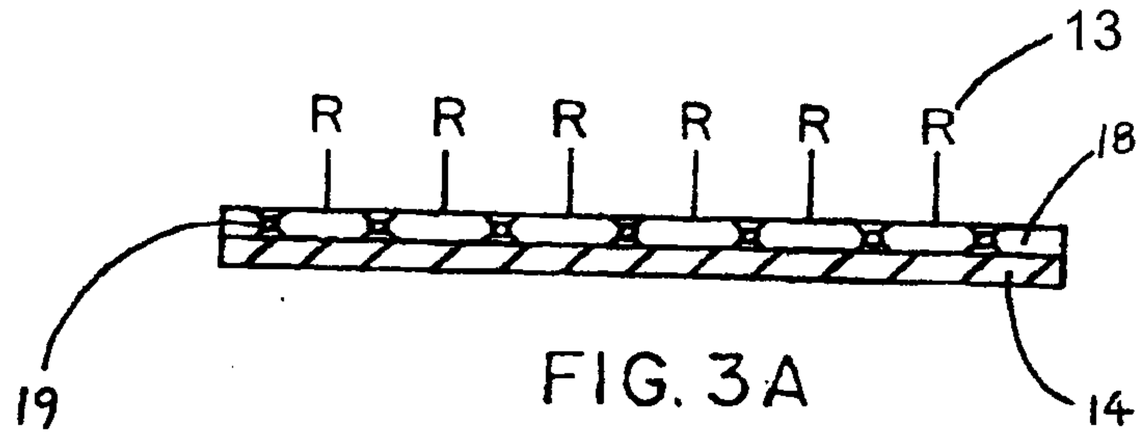


FIG. 3A

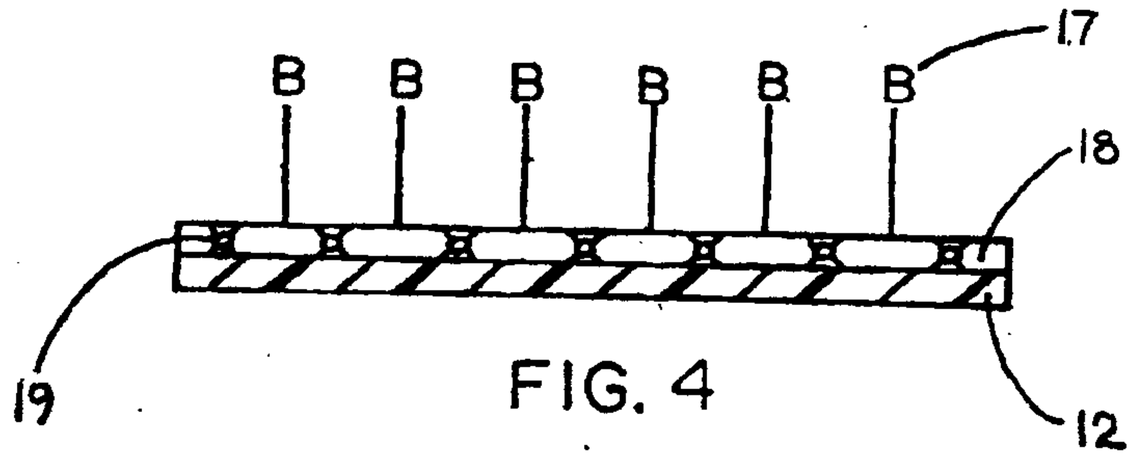


FIG. 4

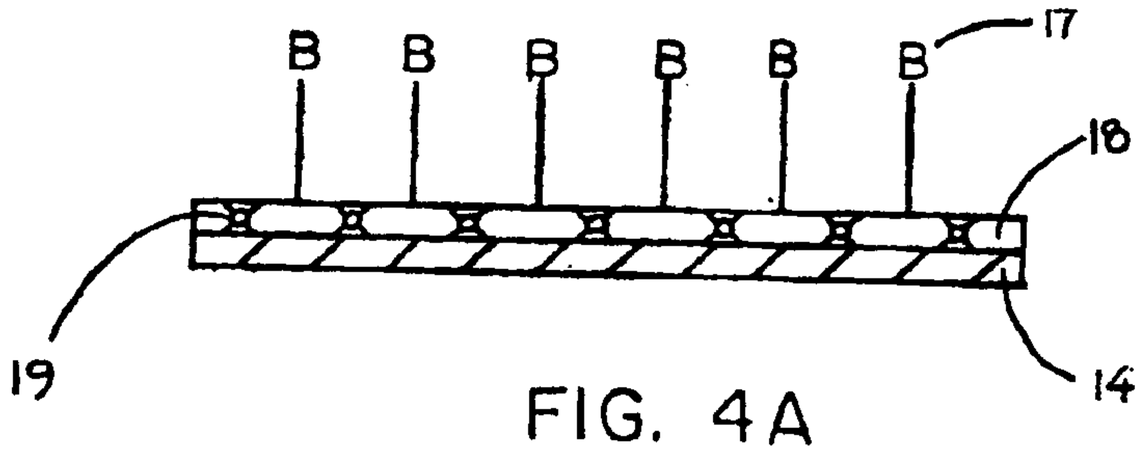


FIG. 4A

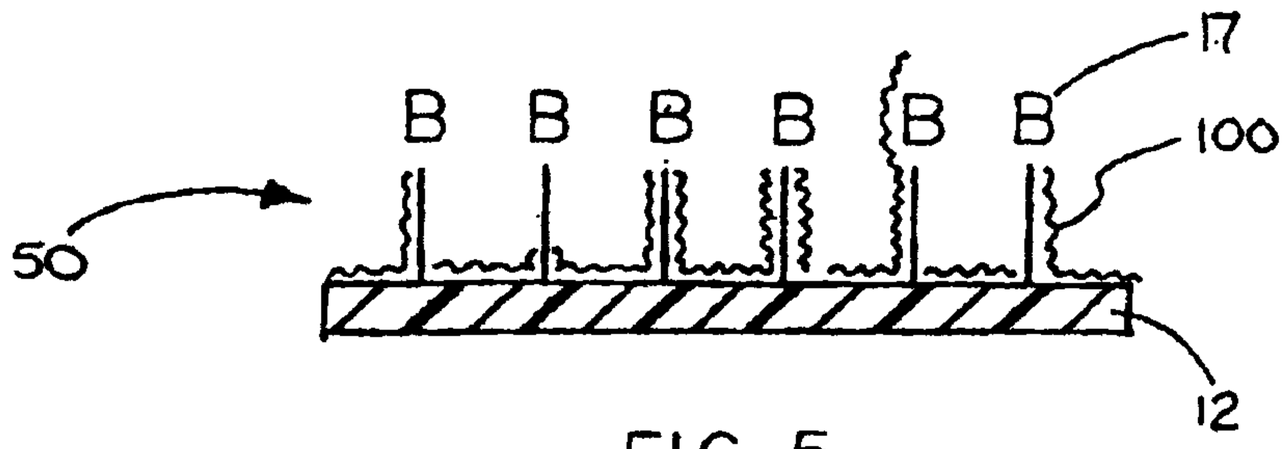


FIG. 5

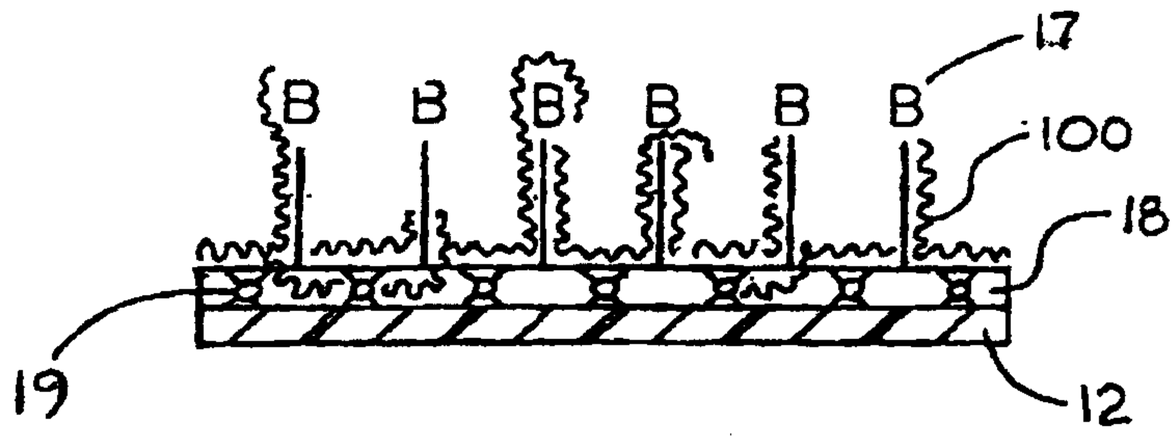


FIG. 6

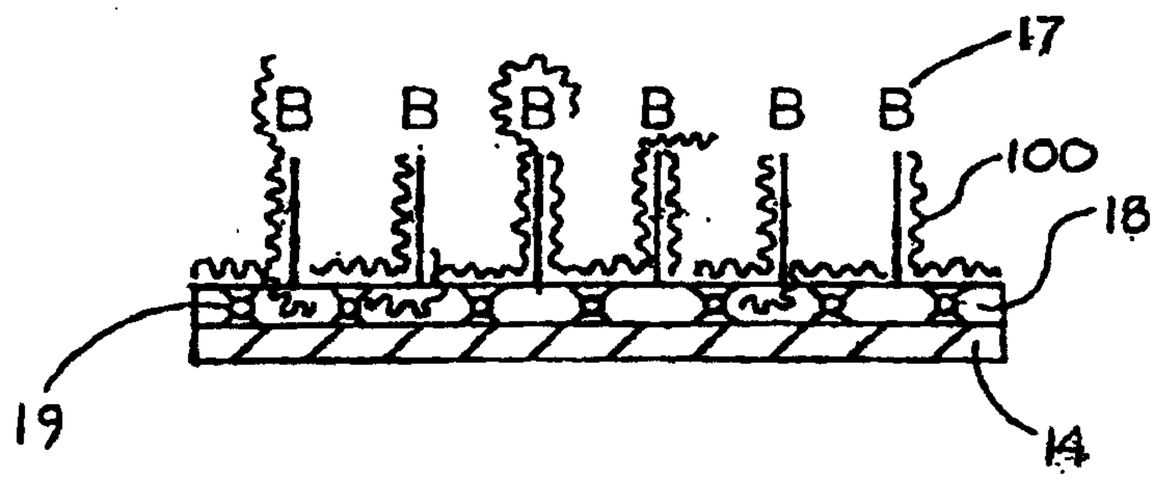


FIG. 6A

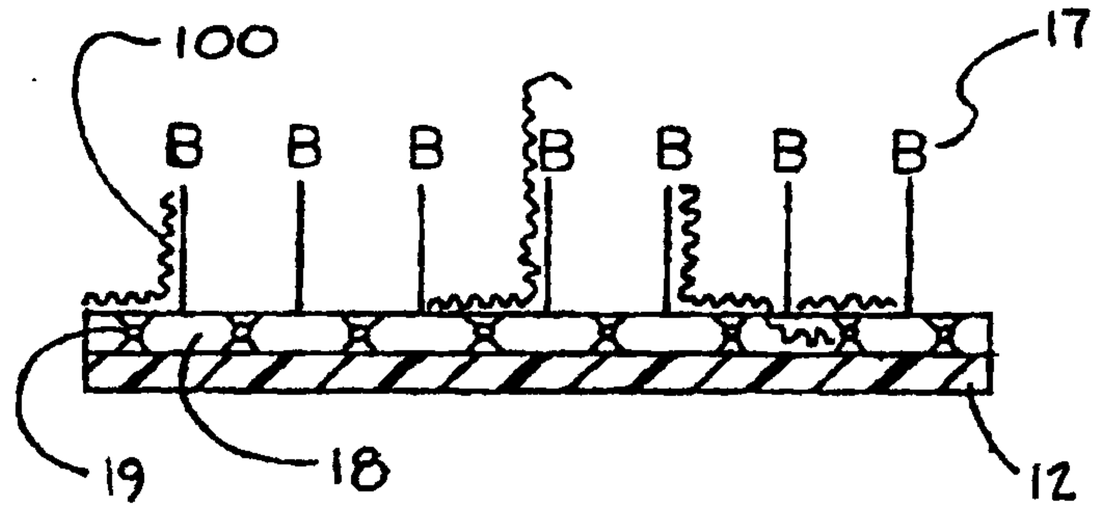


FIG. 6B

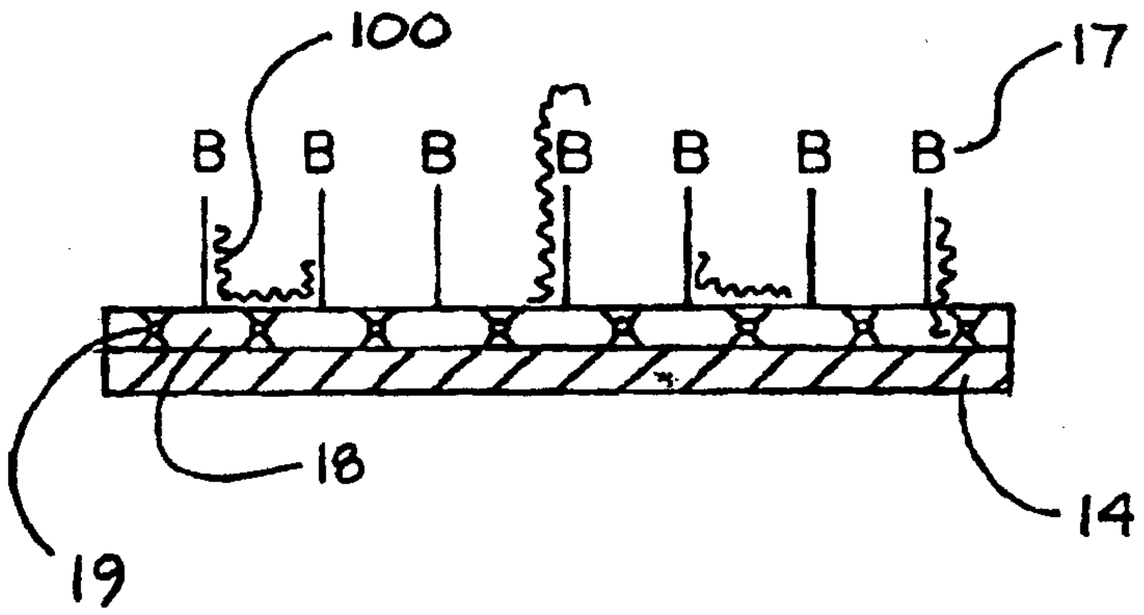


FIG. 6C

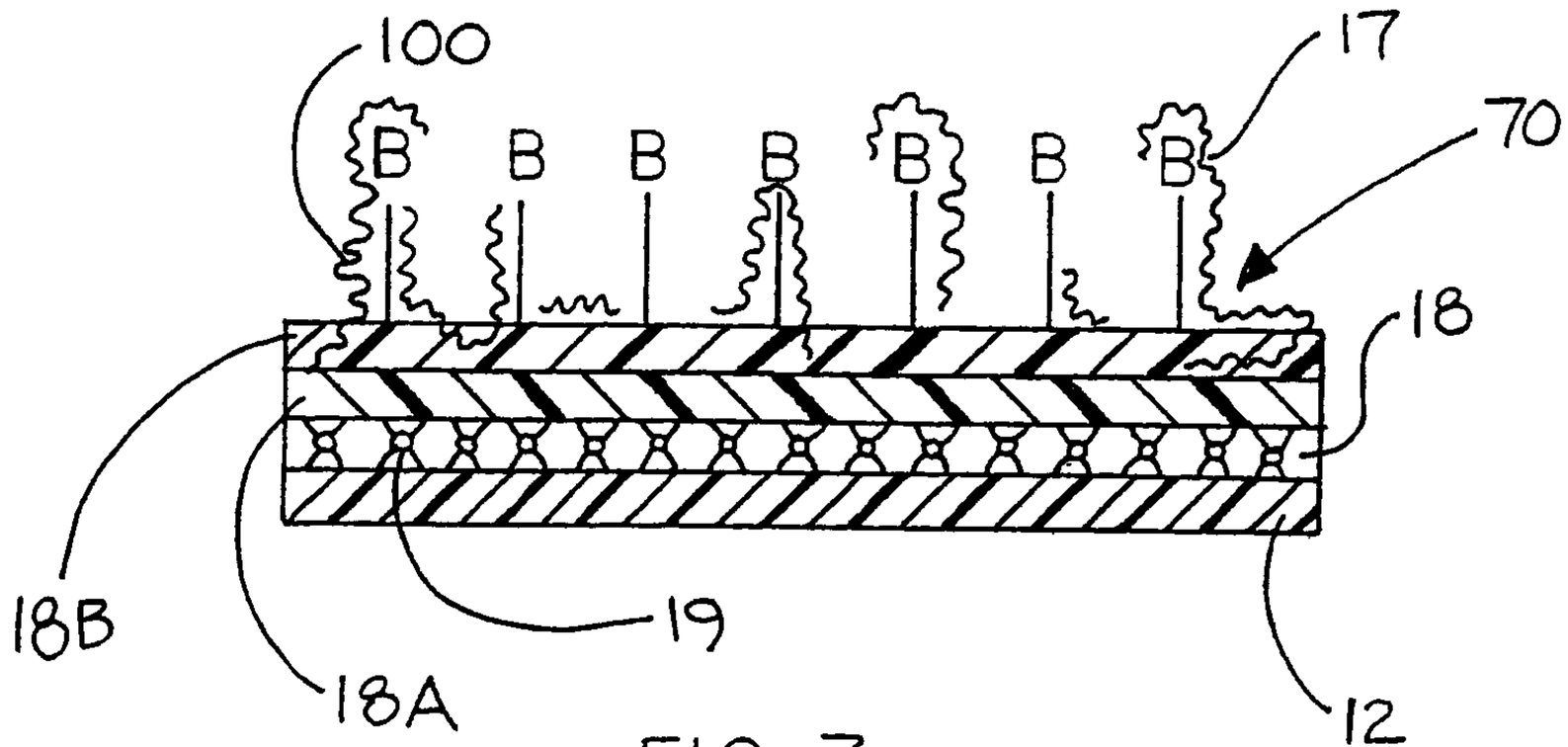

100
17
B
B
B
B
B
B
B
70
18
18B
18A
19
12

FIG. 7

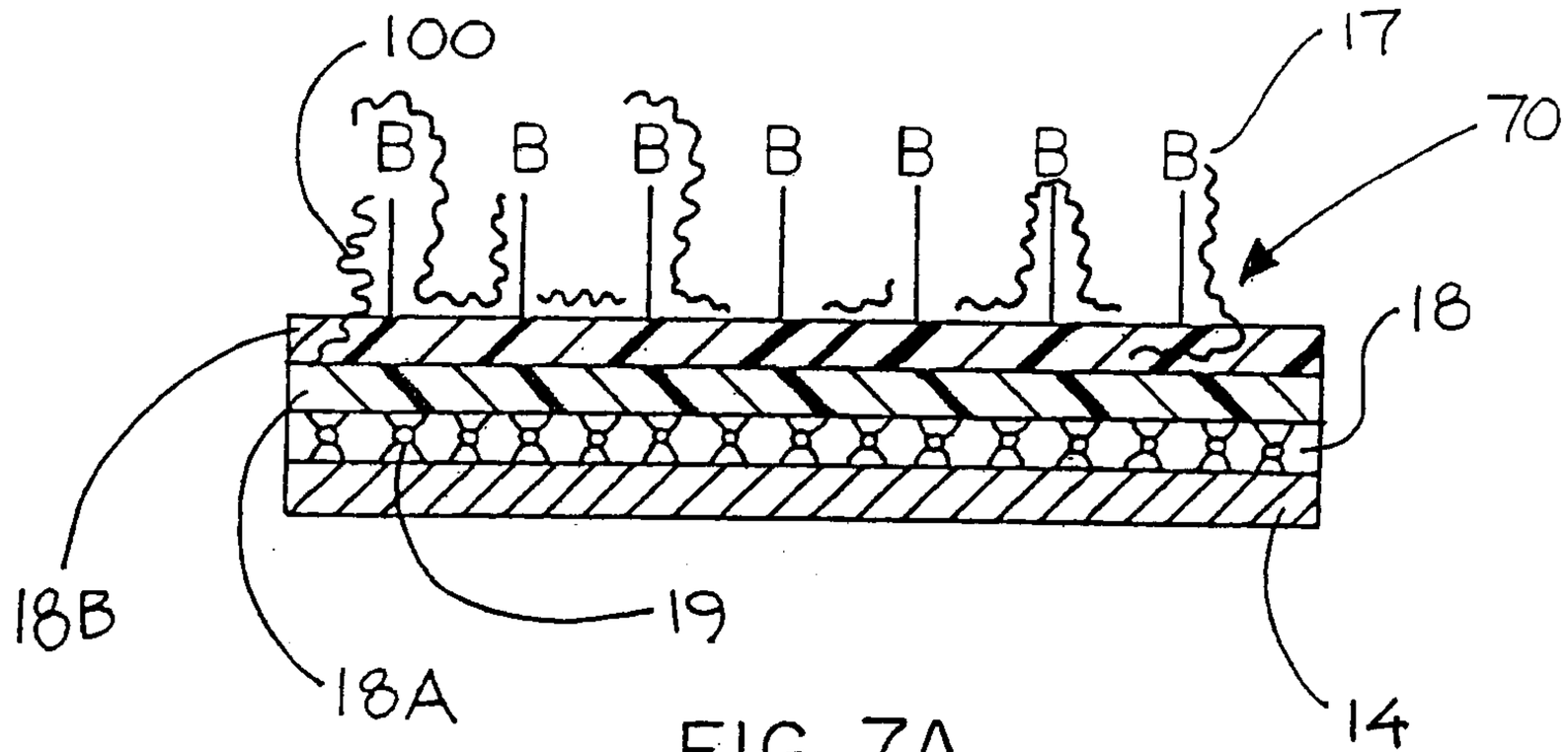

100
17
B
B
B
B
B
B
B
70
18
18B
18A
19
14

FIG. 7A

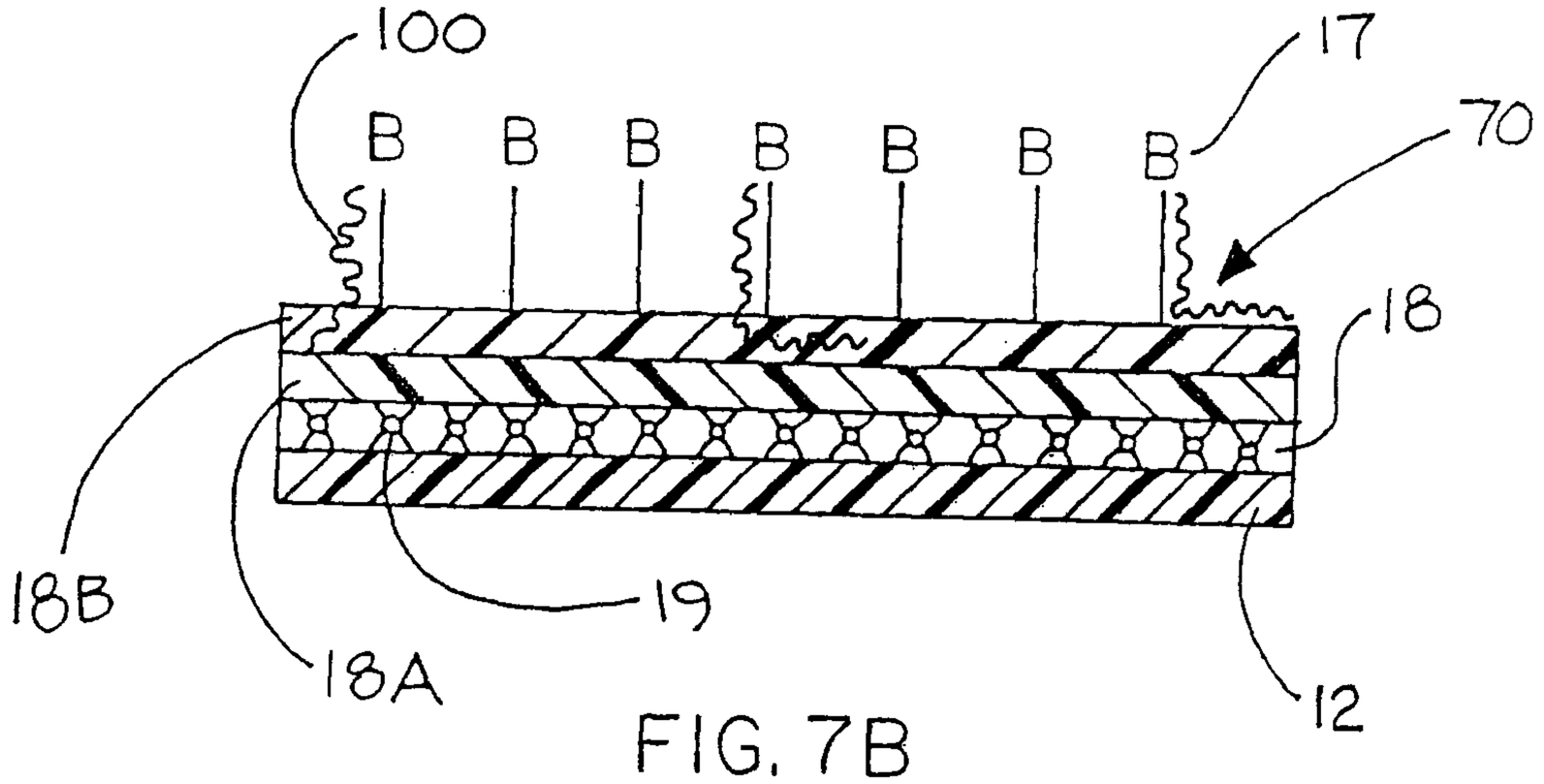

100
17
B
B
B
B
B
B
B
70
18
18B
18A
19
12

FIG. 7B

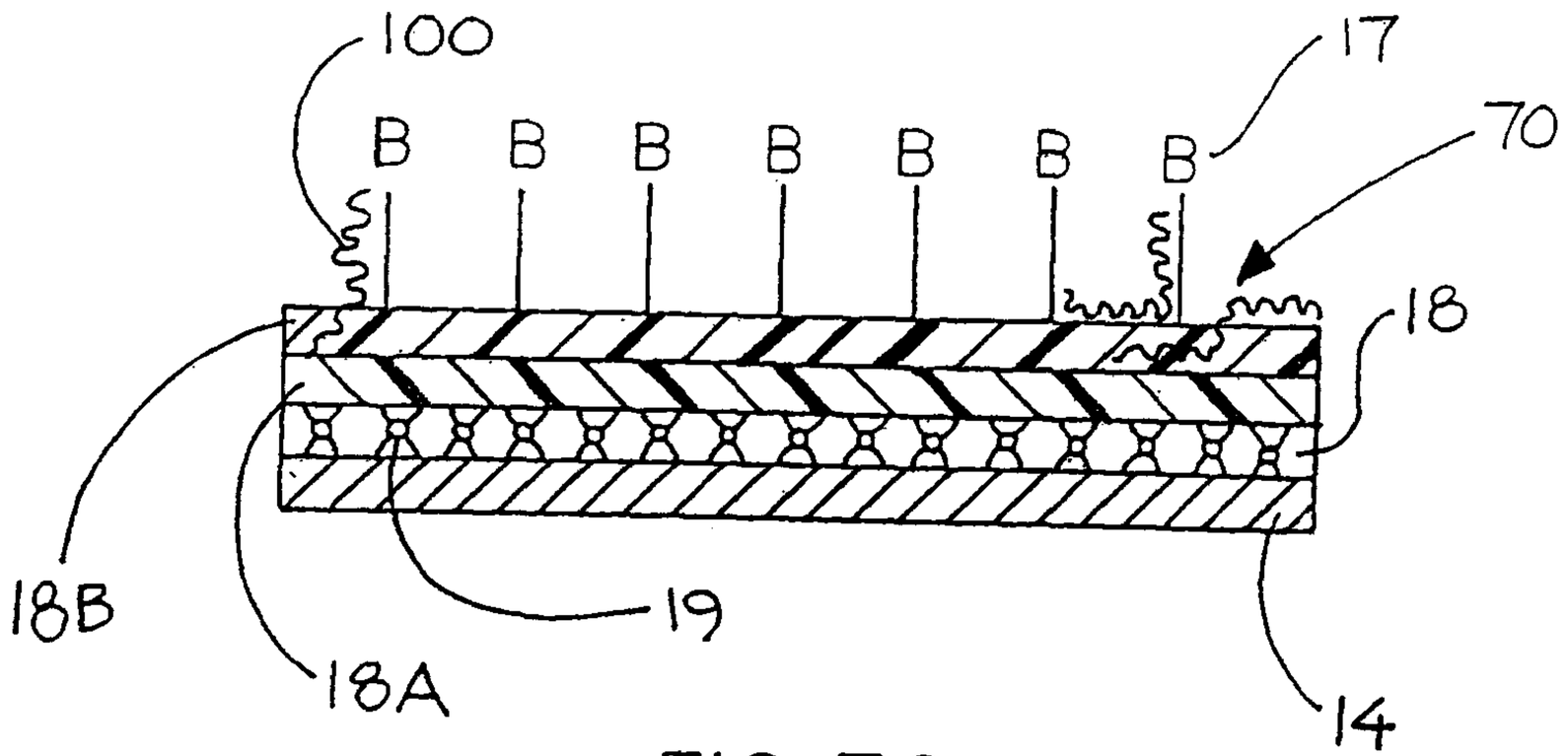

100
17
B
B
B
B
B
B
B
70
18
18B
18A
19
14

FIG. 7C

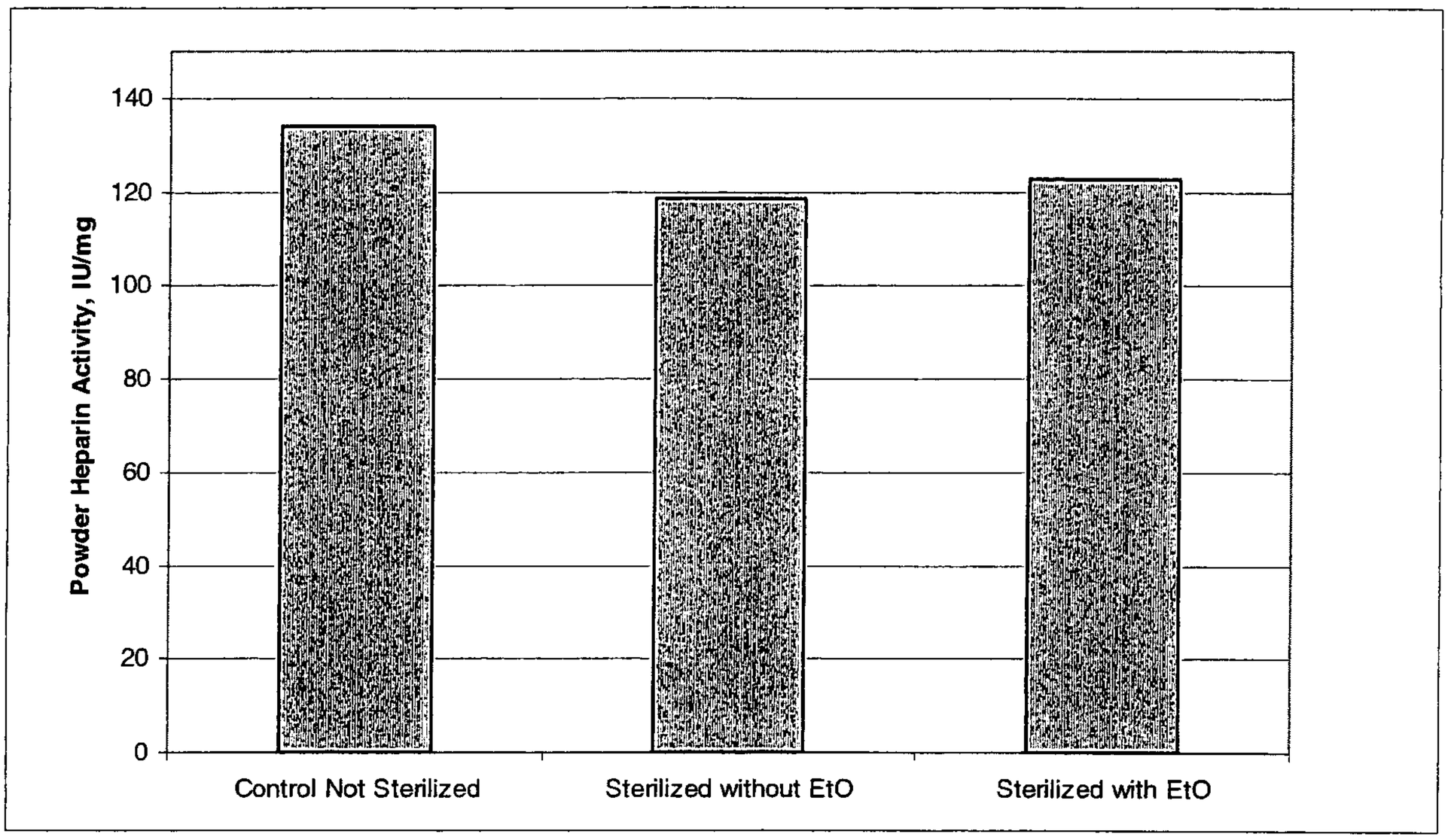

Powder Heparin Activity, IU/mg
140
120
100
80
60
40
20
0
Control Not Sterilized
Sterilized without EtO
Sterilized with EtO

Figure 8

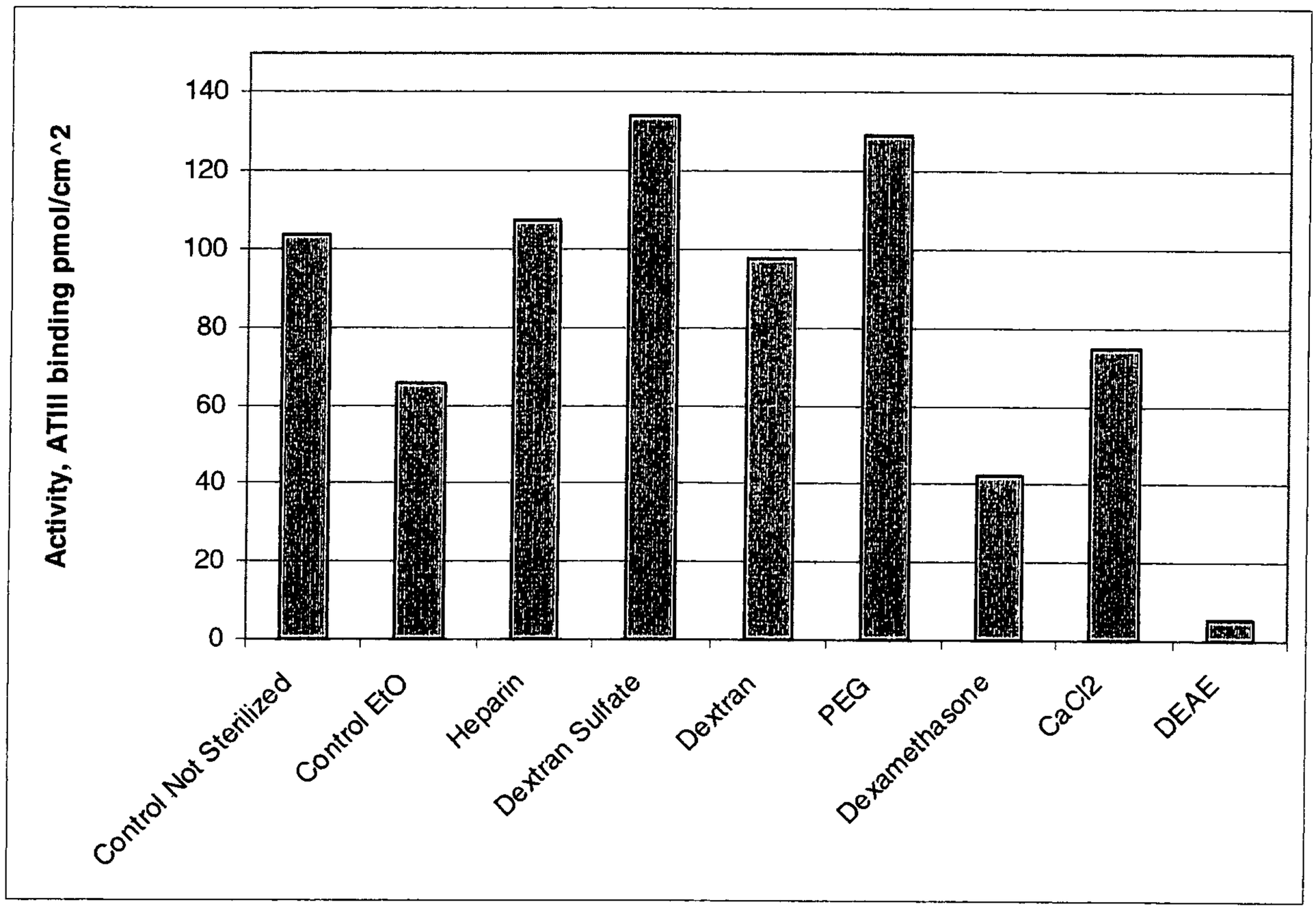

Activity, ATIII binding pmol/cm^2
140
120
100
80
60
40
20
0
Control Not Sterilized
Control EtO
Heparin
Dextran Sulfate
Dextran
PEG
Dexamethasone
CaCl2
DEAE

Figure 9

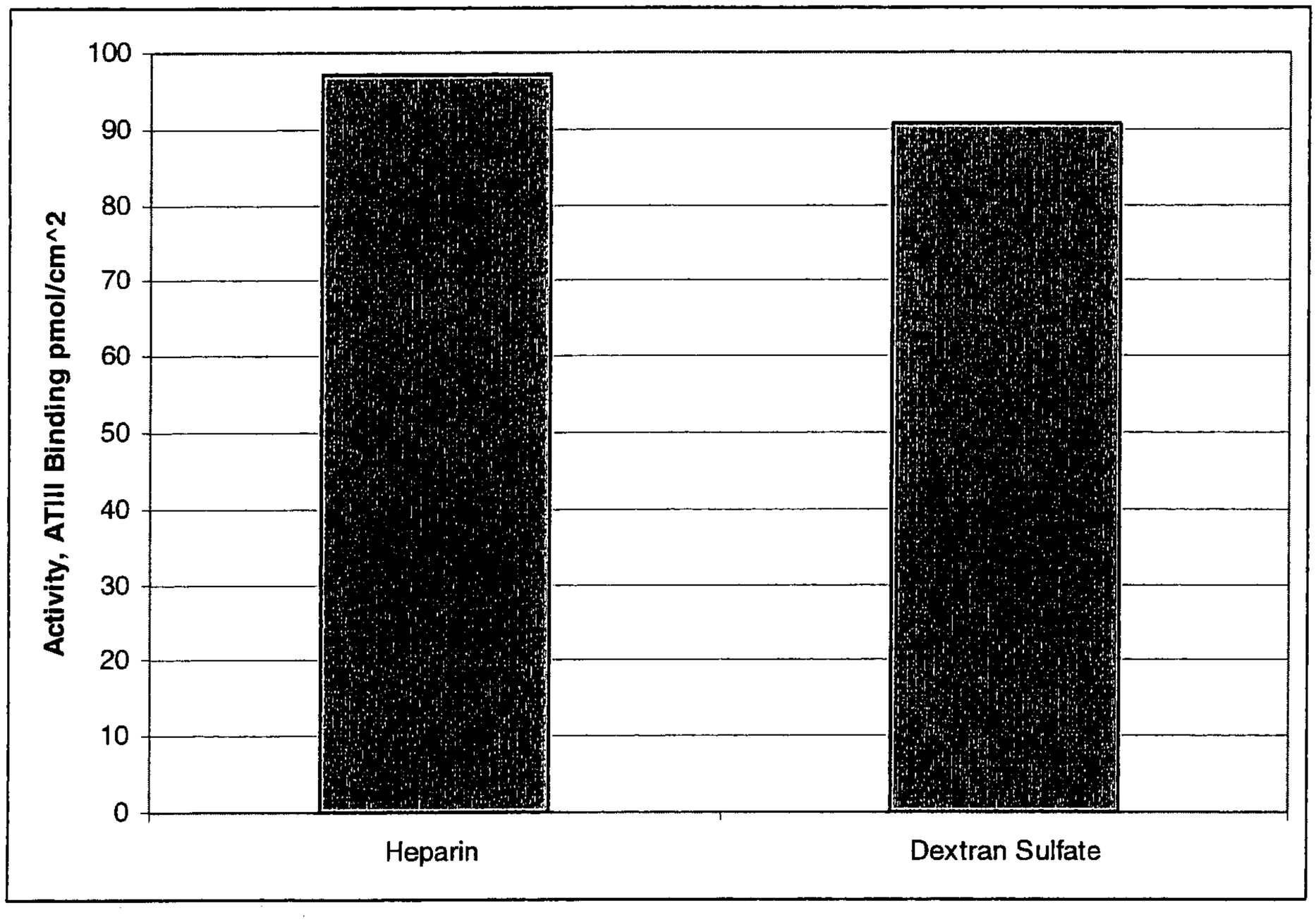

Activity, ATIII Binding pmol/cm^2
100
90
80
70
60
50
40
30
20
10
0
Heparin
Dextran Sulfate

Figure 10

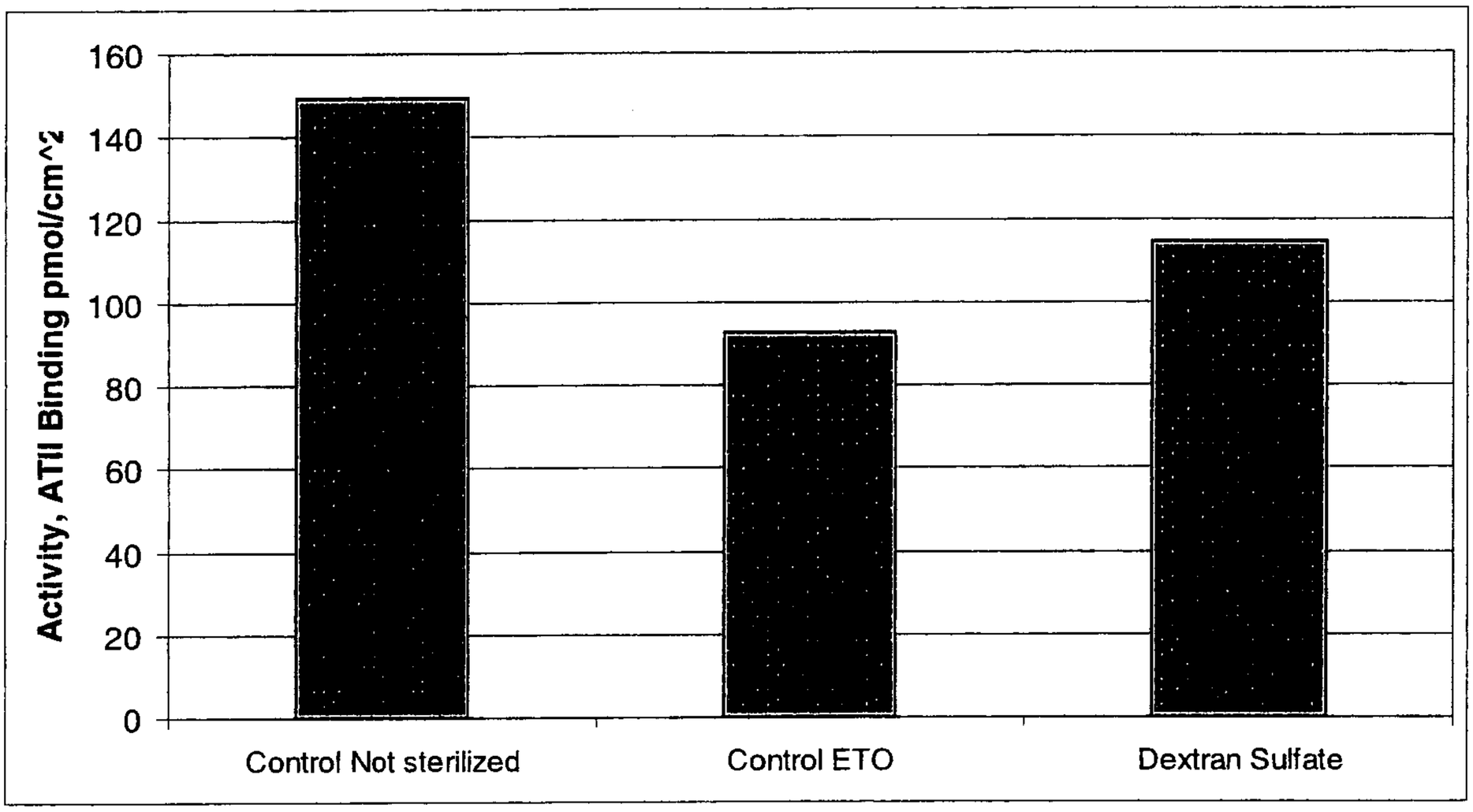

Activity, ATII Binding pmol/cm^2
160
140
120
100
80
60
40
20
0
Control Not sterilized
Control ETO
Dextran Sulfate

Figure 11

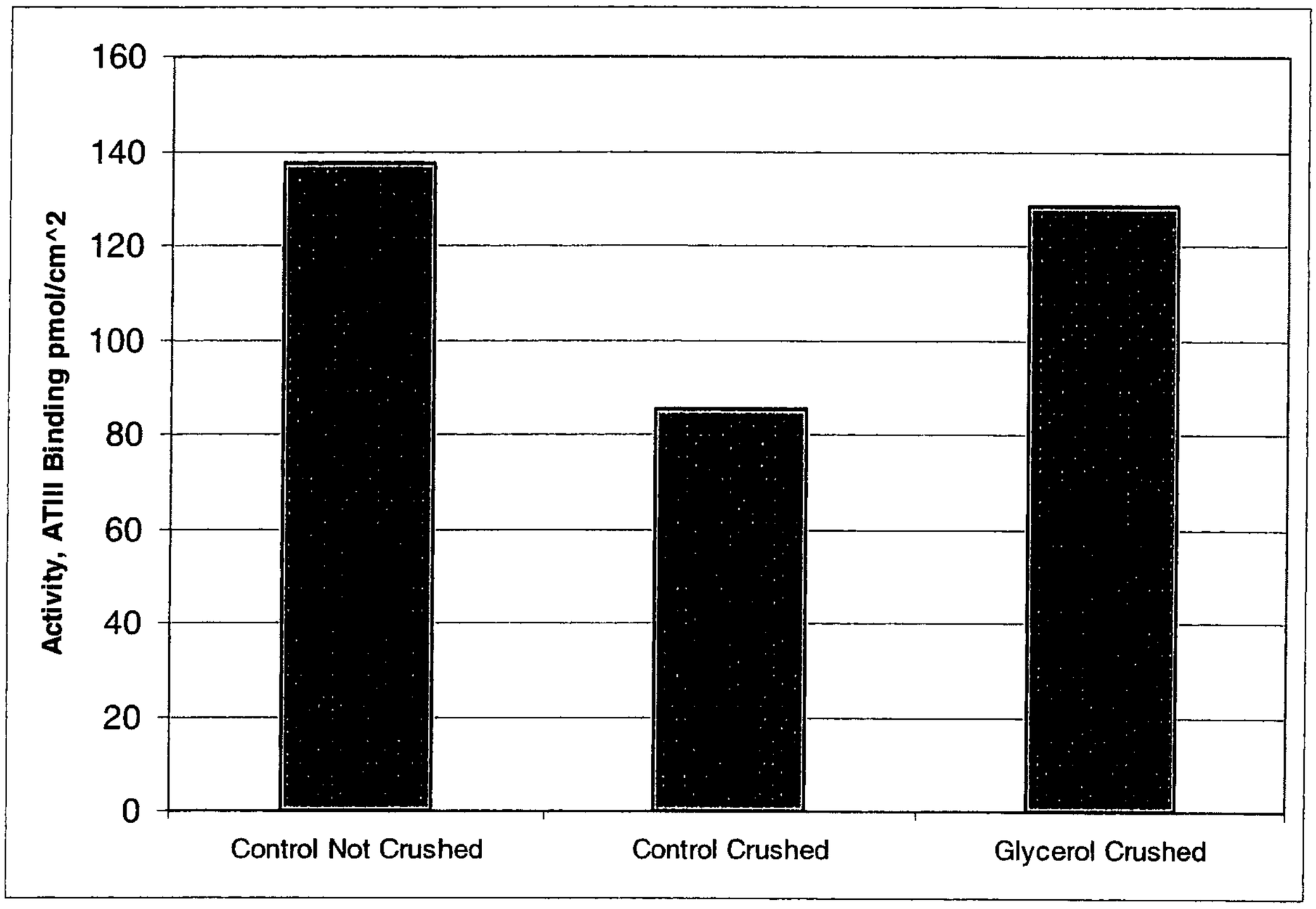

160
140
120
100
80
60
40
20
0
Activity, ATIII Binding pmol/cm^2
Control Not Crushed
Control Crushed
Glycerol Crushed

Figure 12

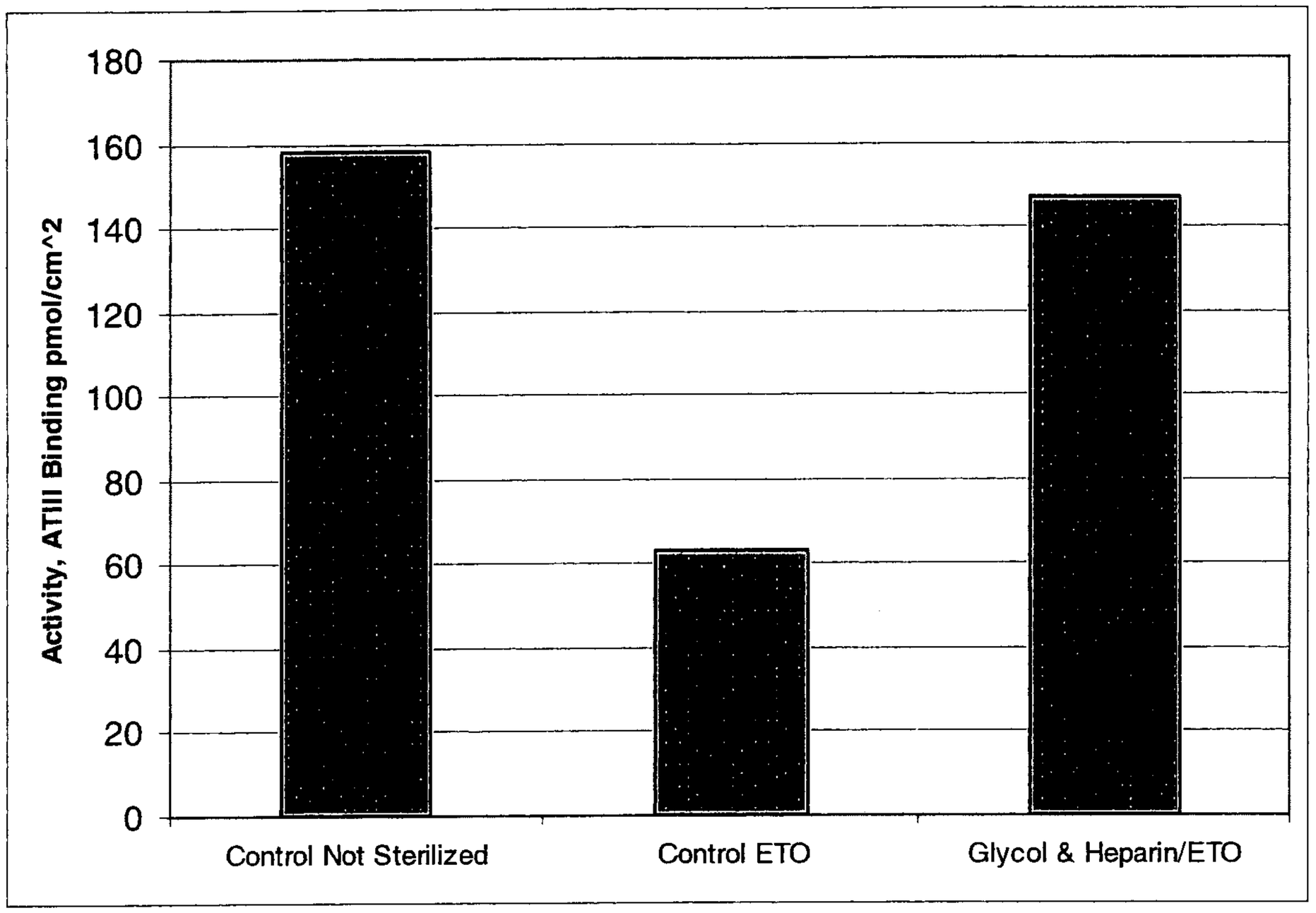

180
160
140
120
100
80
60
40
20
0
Activity, ATIII Binding pmol/cm^2
Control Not Sterilized
Control ETO
Glycol & Heparin/ETO

Figure 13

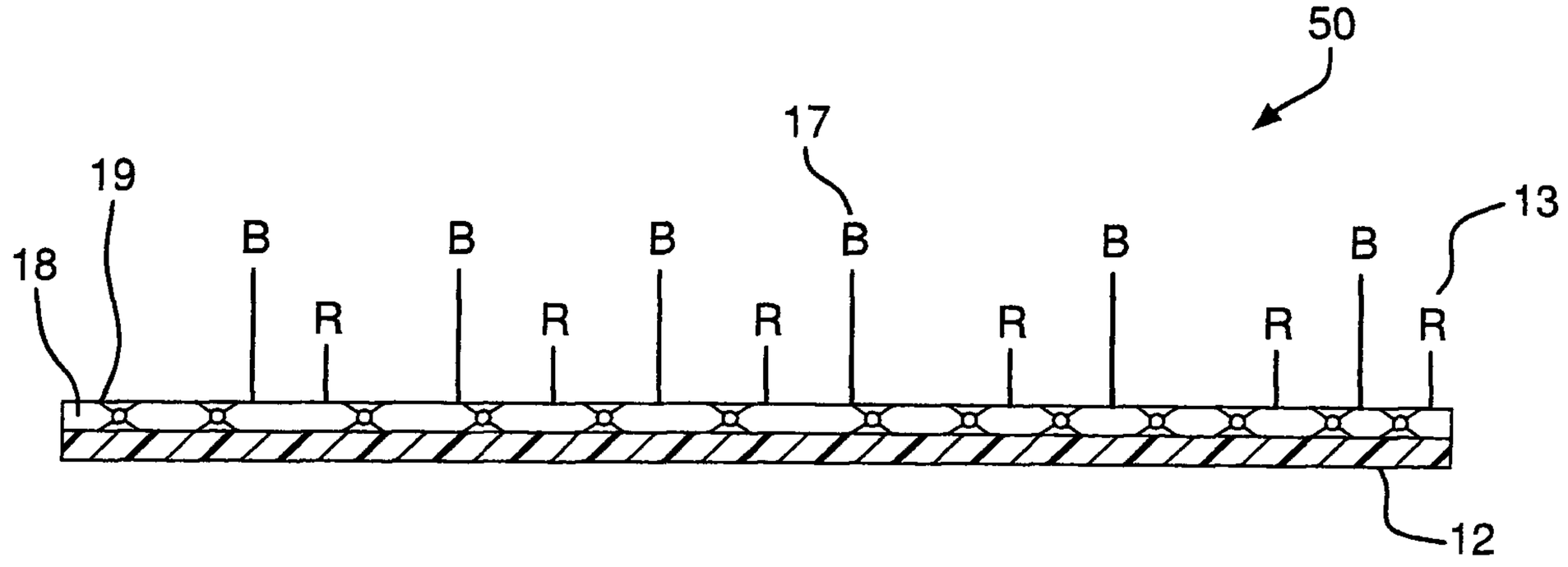

50
17
19
13
18
B
B
B
B
B
B
R
R
R
R
R
R
12

FIG. 14

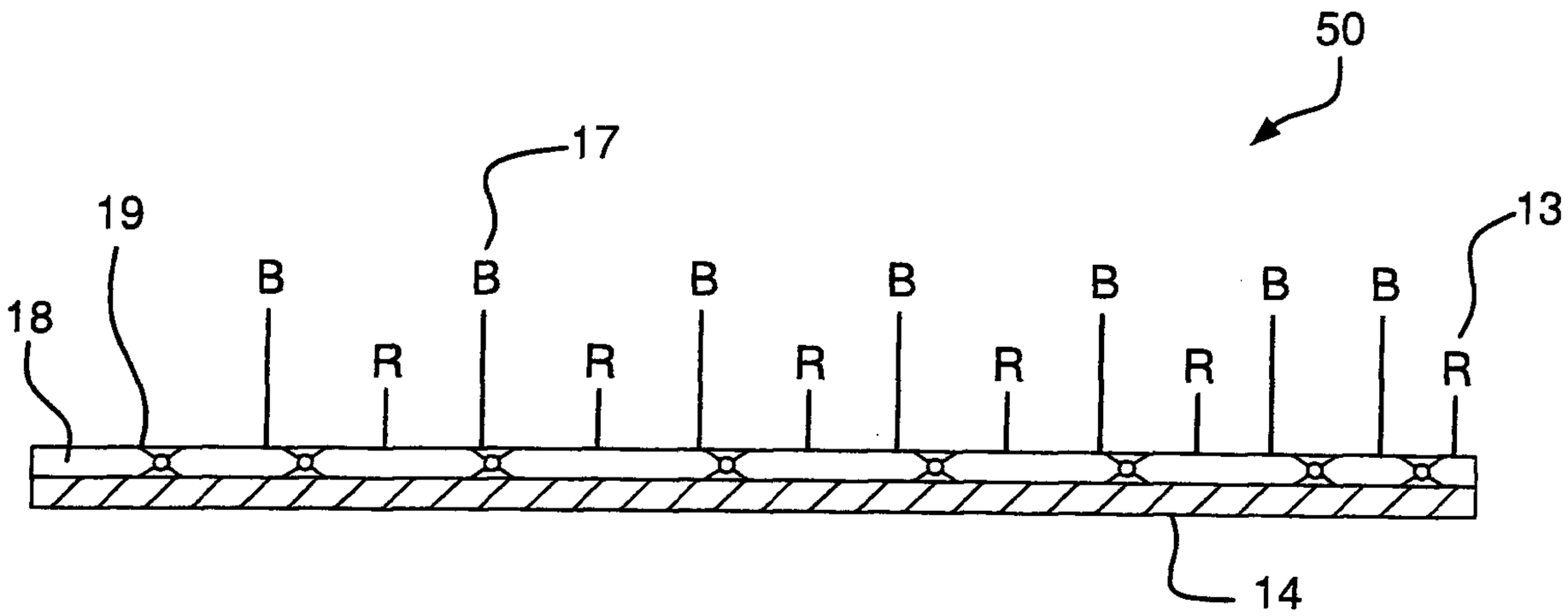

50
17
19
13
18
B
B
B
B
B
B
B
B
R
R
R
R
R
R
R
R
14

FIG. 15

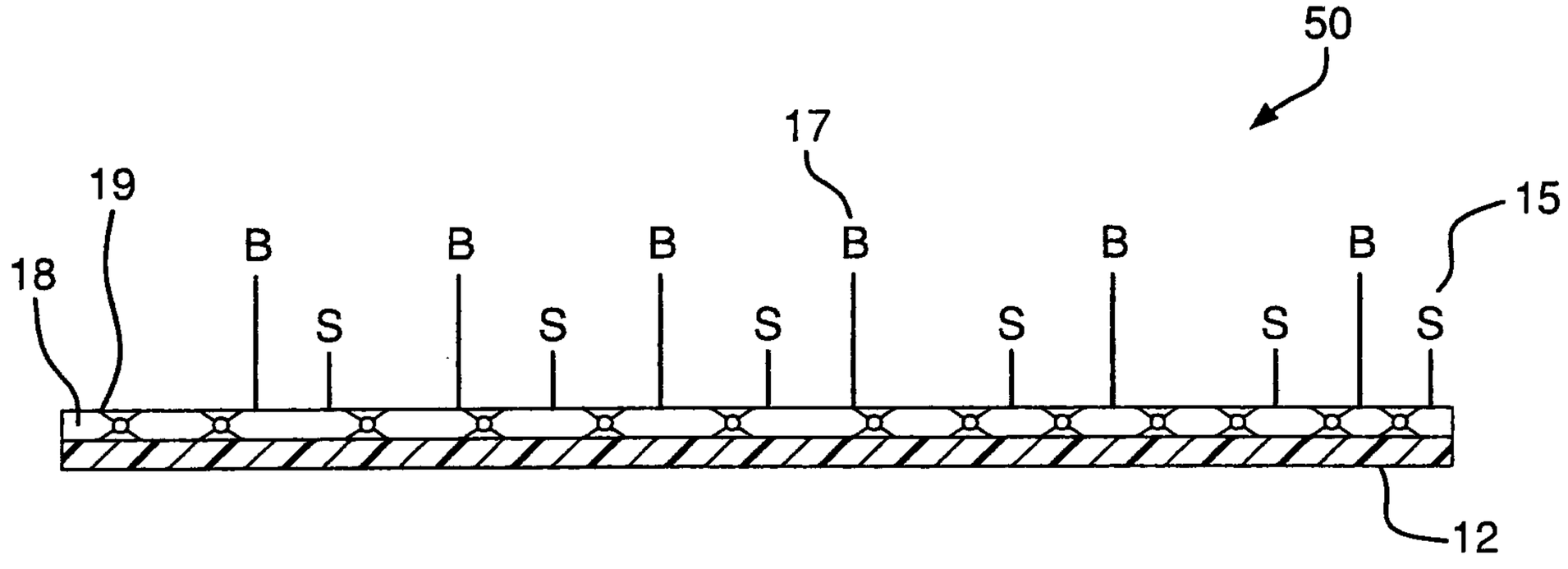


FIG. 16

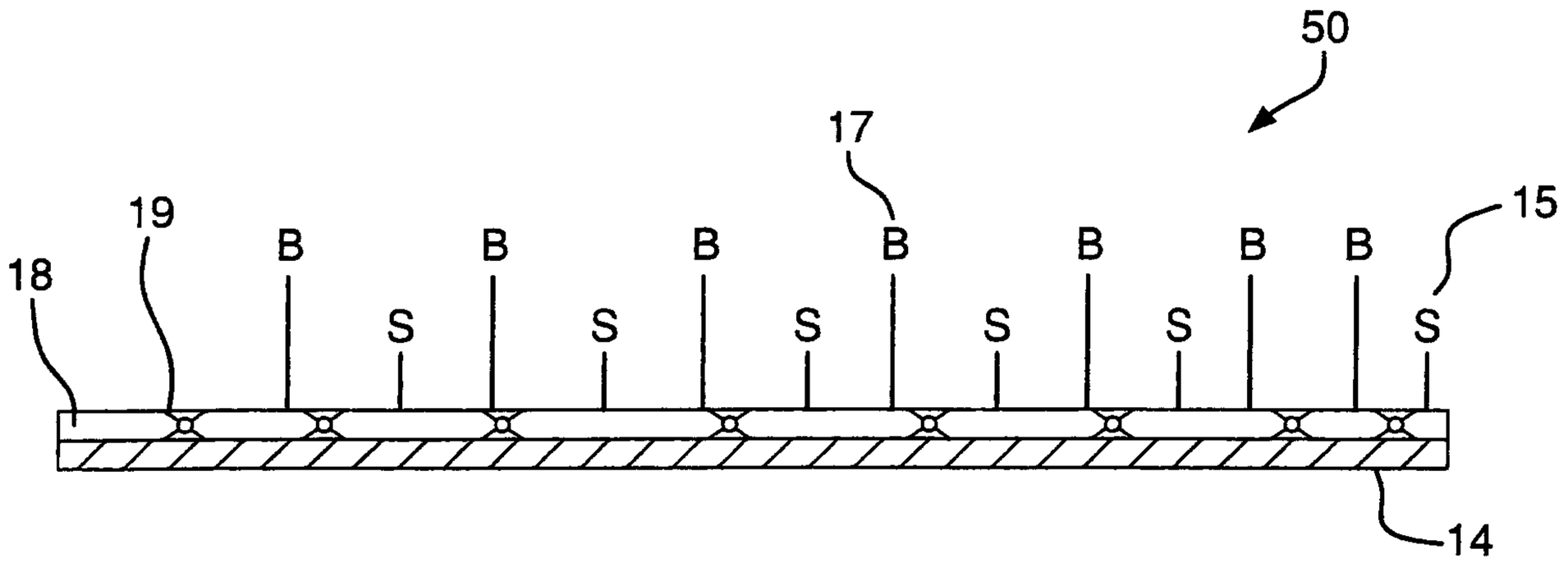


FIG. 17

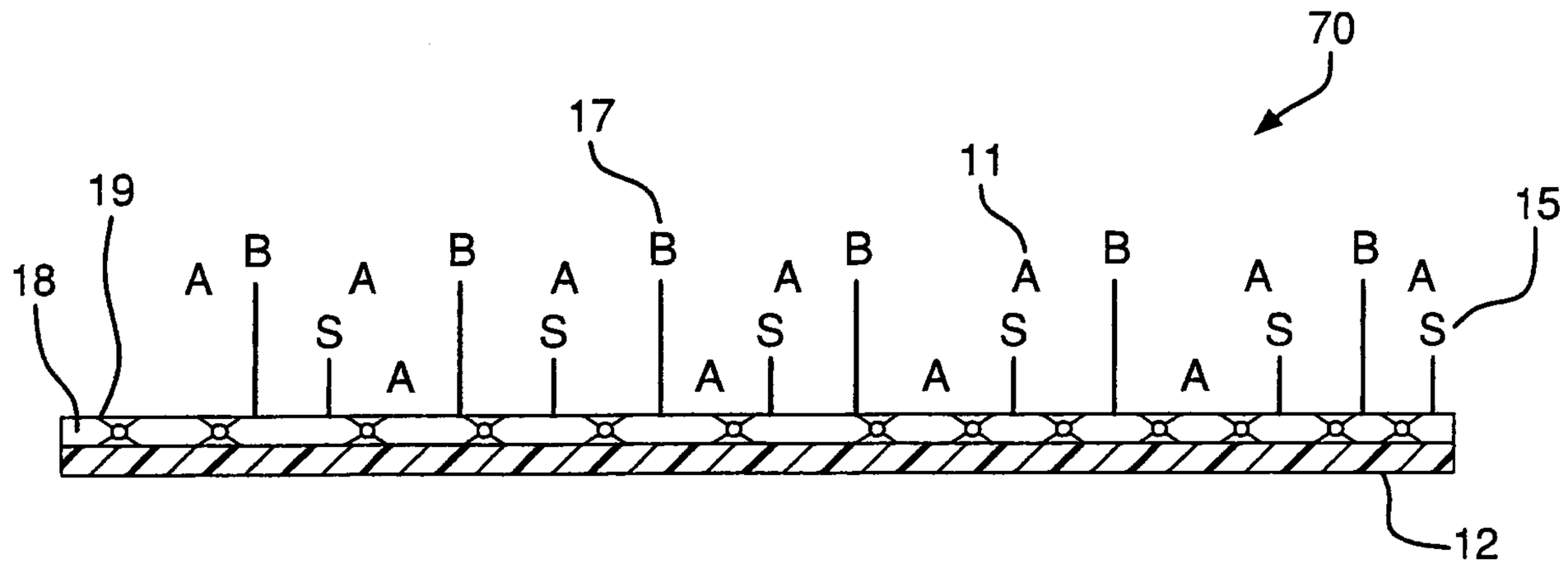


FIG. 18

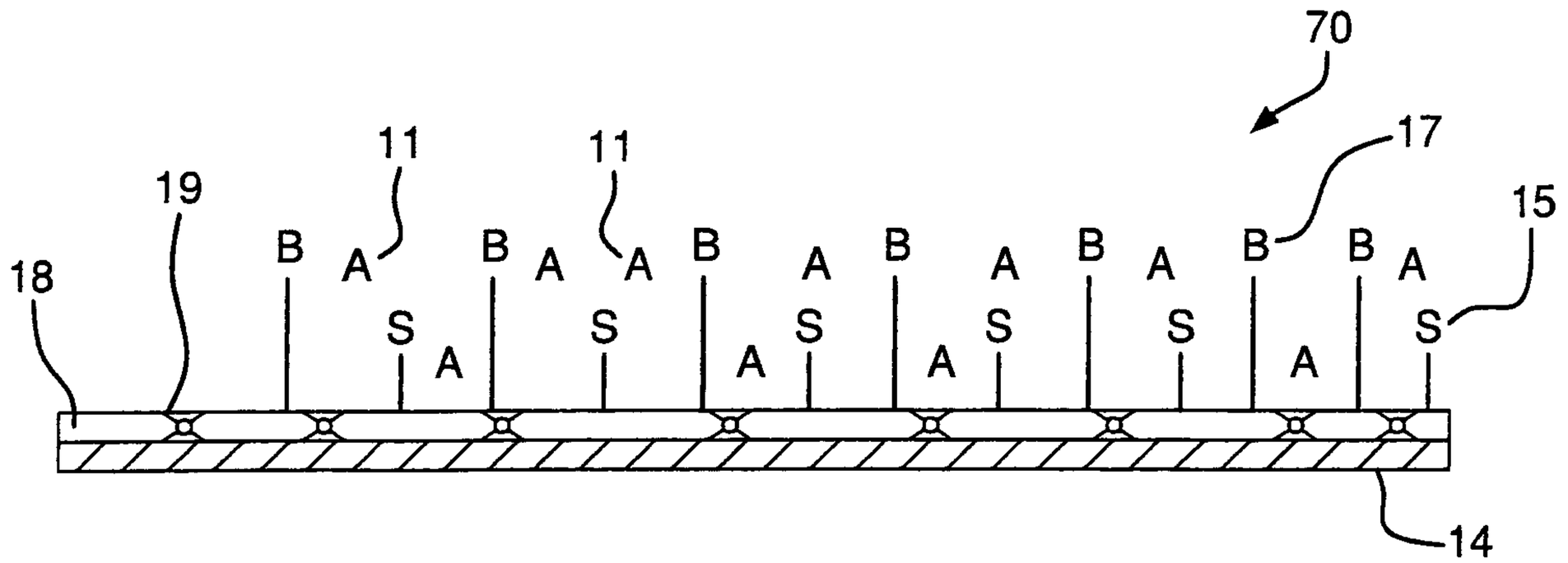


FIG. 19

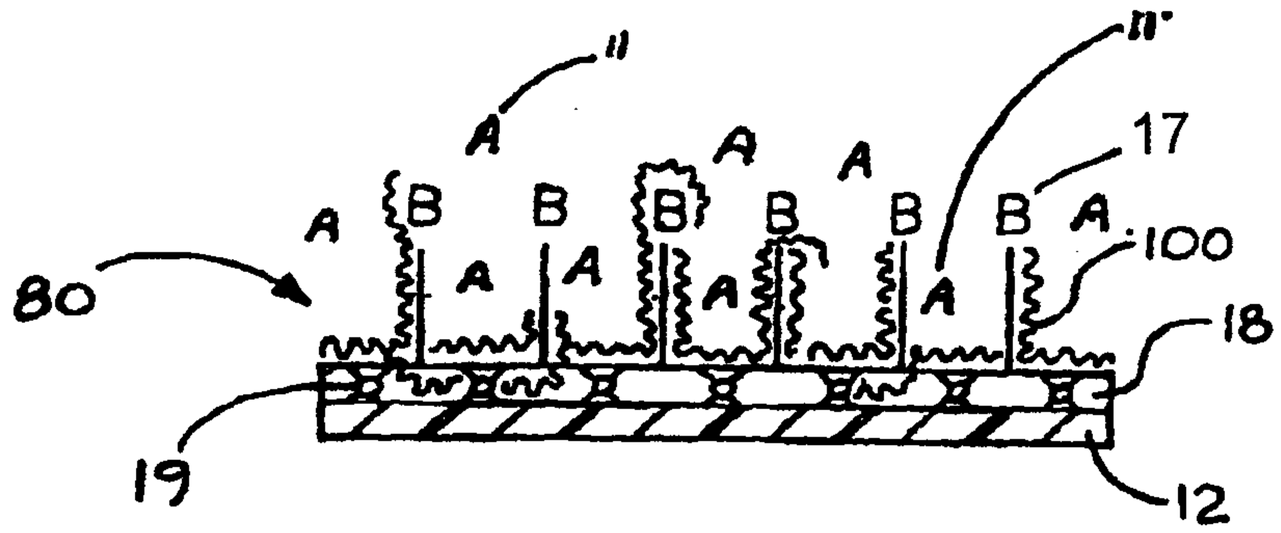

11
11'
A
A
A
17
A
B
B
B
B
B
B
A
A
A
A
A
A
100
80
18
19
12

FIG. 20

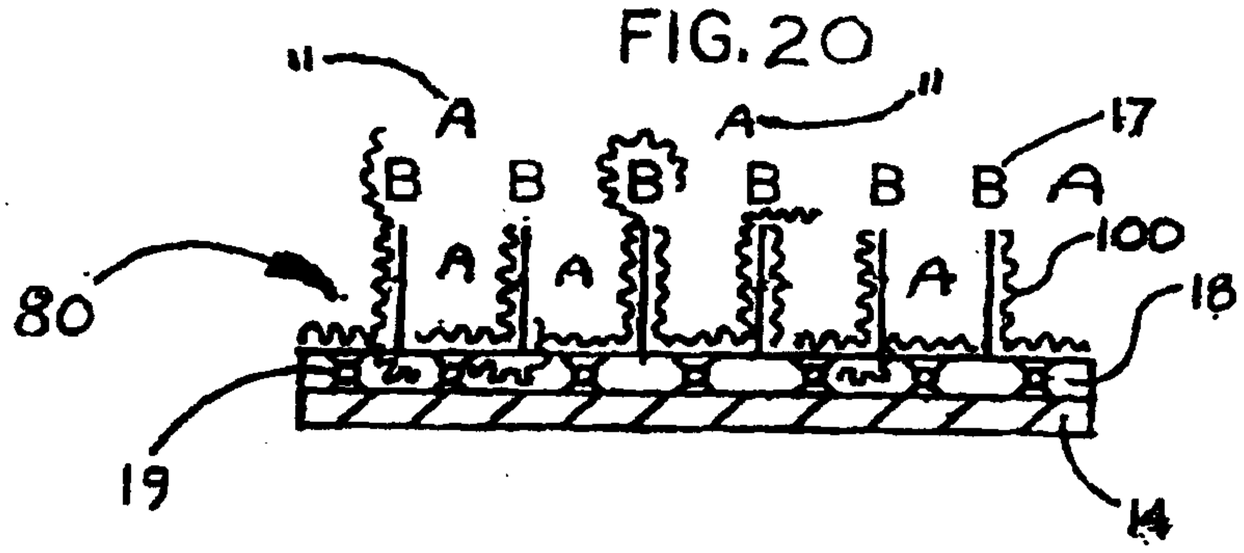
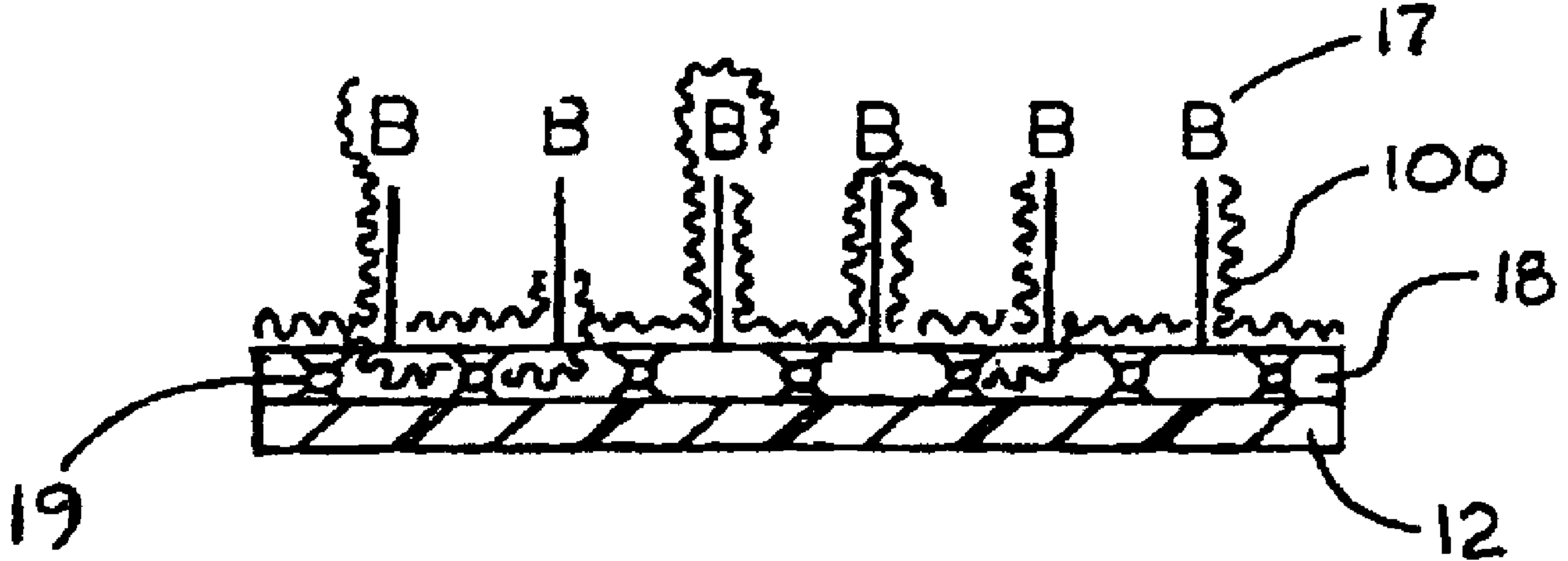

11
A
A
11'
17
B
B
B
B
B
B
A
A
A
A
A
100
80
18
19
14

FIG. 21


B
B
B
B
B
B
17
100
18
19
12