

Description

The present invention concerns surface-modified substrates and a method for their preparation.

It is known that chemical entities having a biological activity may be bound to the surface of a substrate if functional groups are made available on the substrate surface by surface modification. Such functional groups on the substrate surface may be charged for a ionic interaction or react covalently with functional groups on the chemical entity.

The preparation of support matrices for immobilized enzymes by adsorption of a polyamine on a substrate material and contacting the polyamine with a bifunctional reagent to cross-link and stabilize the polyamine, thus providing a surface with functional groups to which enzymes may be bound covalently, has been described in US Patent 4,268,423 (Rohrbach et al). The cross-linking agent used in this case is chosen from the group consisting of dialdehydes and diisocyanates.

In US Patent 4,229,838 (Mano et al) a vascular prothesis having antithrombogenic properties is prepared by adsorbing a particular polyamine viz polyethylene imine, to its porous surface, cross-linking this with a bifunctional agent such as a dialdehyde, and quarternising the remaining amino groups to achieve a positively charged surface on which negatively charged heparin may be adsorbed.

US Patent 4,565,740 (Gölander and Larsson) and EP-A-0 086 187 describe surface modified substrates prepared by adsorbing a complex of a polymeric cationic surfactant, carrying primary as well as secondary and/or tertiary amino nitrogens, preferably polyethylene imine or polyamides, and a dialdehyde which has 1-4 carbon atoms between the aldehyde groups, to the surface and eventually adsorbing several additional layers of polymeric anionic surfactants by means of intermediate layers of the complex. To a thus modified surface, a biologically active compound may be adsorbed by ionic binding or bound by covalent binding.

In general, it is desired that a surface modification should result in a high density of reactive functional groups. Further the reaction should be reproducible and easy to perform under normal reaction conditions, and the modified surface should be stable in the sense that no leakage from the layers of the surface modification should occur.

In practice it has been difficult to prepare surface modified substrates fleetling all these requirements in a satisfactory degree with the method according to the last mentioned prior art.

It has now been found that a surprisingly better surface modification may be achieved by adsorbing on the substrate surface a layer of a polyamine having a high average molecular weight.

The polyamine is stabilised by crosslinking with crotonaldehyde, which is a mono-aldehyde having a C-C double bond in conjugation with the aldehyde function. Thereafter a layer of anionic polysaccharide is adsorbed on the surface of the crosslinked polyamine, optionally followed by one or more further alternating layers of crosslinked polyamine and anionic polysaccharide. Finally there is adsorbed in the anionic polysaccharide layer, or on the outermost layer of anionic polysaccharide, a layer of non-crosslinked polyamine providing free primary amino groups.

The present invention also provides a surface modified substrate comprising:

- (i) a layer of a polyamine of high average molecular weight which has been crosslinked with crotonaldehyde absorbed on the surface of the substrate;
- (ii) a layer of an anionic polysaccharide absorbed on the surface of said crosslinked polyamine;
- (iii) optionally one or more additional alternating layers of said crosslinked polyamine (i) and said anionic polysaccharide (ii); and
- (iv) a layer of non-crosslinked polyamine providing free primary amino groups absorbed on the, or on the outermost, layer of anionic polysaccharide.

The method of the present invention may suitably be carried out by bringing the substrate into contact with an aqueous solution of the polyamine at pH 8-10, especially pH 9. The concentration of the initial polyamine solution will range from 1-10% by weight, especially 5% by weight, 1 ml of which may be diluted to a final volume of 500-2000 ml, especially 1000 ml. This final solution may also comprise from 100-1000 μ l, especially 340 μ l crotonaldehyde. Alternatively the substrate will be treated first with a solution of polyamine of said concentration and pH, and then with a solution of the crotonaldehyde of the said concentration and pH. The temperature is not critical, but it is preferred for the treatment to be at room temperature.

After rinsing with water, the substrate is treated with a solution of an anionic polysaccharide, containing from 10 mg to 500 mg, preferably 100 mg of the polysaccharide in a volume of 1000 ml. This step is executed at a temperature in the range of 40-70°C, preferably 55°C and pH 1-5, preferably pH 3.

After another rinsing with water, these first steps may be repeated one or several times and finally, after having adsorbed a layer of polysaccharide, the substrate is treated with a polyamine solution having a concentration 1-20 times, preferably 10 times that mentioned above, at the said temperature and pH.

The polyamine will preferably be a polymeric aliphatic amine, especially polyethylene imine, having a high average molecular weight, but any polyamine having a high average molecular weight and carrying free primary amino groups may be used.

The anionic polysaccharide will preferably be a sulfated polysaccharide, more preferably dextran sulfate.

The aminated surface may optionally be further stabilized by reduction with a suitable reducing agent such as sodium cyanoborohydride.

The modified surface according to present invention has free primary amino groups by which chemical entities may be bound either ionically or covalently.

Especially aldehyde containing chemical entities may be bound by formation of Schiff's bases, eventually followed by a stabilization reaction such as a reduction to convert the Schiff's bases to secondary amines.

According to present invention the following advantages may be achieved:

- A higher density of reactive amino functions resulting in a higher biological activity, when biologically active material is attached.
- A significantly lower concentration of cross-linking agent may be used, as compared to the prior art.
- A very stable final surface

The increased biological activity will appear from the following two examples showing the density of binding sites as will appear as the degree of heparinization achieved after the surface modification according to the present method and according to the prior art as represented by the working example of US Patent 4,565,740 (Gölander and Larsson) and EP-A-0 086 187:

a) comparing the results achieved when working with, according to present invention, low concentrations of cross-linking agent; and

b) comparing the results achieved by these two methods when working with higher concentrations according to the prior art of crosslinking agent:

Example 1

In these examples tubings of polyethylene (PE) were first etched with potassium permanganate in conc. sulfuric acid, whereafter the tubings were contacted with the following solutions:

1a) 1 ml 5% Polymin SN* and 340 µl crotonaldehyde in 1 l 0.17M borate buffer of pH 9

2a) 1 ml 5% Polymin SN* and 1.54 ml of a solution containing 25% by weight of glutaraldehyde in 1 l 0.17M borate buffer of pH 9

3) 0.1 g dextran sulphate* in 1l of a 0.15M sodium chloride solution, pH 3 at 55 °C

4) 10 ml 5% Polymin SN* in 1l water, pH 9

5) 0.25g heparin degraded with nitrous acid (as described in US Patent 4,613,665, (Larm)) and 0.025g sodium cyanoborohydride in 1 l 0.15 M sodium chloride solution of pH 3.9 at 55 °C

6) 0.17 M Borate buffer of pH 9

Polymin SN* is a polyethylene imine commercially available from BASF; "Polymin" is a registered trade mark. The dextran sulphate* used is commercially available from Pharmacia.

The PE-tubings were treated with the above solutions in the following order and rinsed with water between each step:

In the test according to the present invention:

1a, 3, 1a, 3, 4, 5 and 6. In the test according to the prior art:

2a, 3, 2a, 3, 4, 5 and 6.

The tubings were then rinsed with a solution of albumin for 24 hours and the following results were achieved for the heparin activity:

UPTAKE OF ANTITHROMBIN MEASURED AS THROMBIN INHIBITION IU/cm ²		
Crosslinking agent	before rinse	after albumin rinse
crotonaldehyde	27.1	20.5
glutaraldehyde	5.0	5.0

This shows clearly that a dramatically enhanced heparin activity is achieved by using the method according to the present invention with the crosslinking agent according to the present invention compared to a crosslinking agent of the prior art even though the crotonaldehyde was used at a comparatively low

concentration.

Example 2

The tubings were treated as in Example 1 except that the solutions 1a and 2a were replaced by 1b and 2b respectively, as follows:

1b) 1ml 5% Polymine SN* and 4,12ml crotonaldehyde in 1l 0.17M borate buffer of pH 9

2b) 1ml 5% Polymine SN* and 20ml glutaraldehyde (25%-solution) in 1l 0.17M borate buffer of pH 9

This gave the following results for the heparin activity:

UPTAKE OF ANTITHROMBIN MEASURED AS THROMBIN INHIBITION IU/cm ²		
Cross-linking agent	before rinse	after albumin rinse
crotonaldehyde	9.2	7.5
glutaraldehyde	4.7	4.2

These results indicate that a surprisingly better activity is also achieved when using the cross-linking agent of present invention at a concentration such as used in the methods of the prior art.

Further, as appears when comparing Example 1 with Example 2, the combination of the specific cross-linking agent of the present invention, namely crotonaldehyde, and the tenfold lower concentration according to the method of present invention gives a surprisingly higher activity when a biologically active substance is attached to the surface modified according to present invention.

To a surface treated according to present invention, chemical entities carrying functional groups that may react with amino groups without loss of biological activity may be bound. An example of such entities is heparin fragments having aldehyde groups, which are produced by degrading heparin with nitrous acid as described in US Patent 4,613,665 (Larm) for the preparation of heparinized surfaces.

Of course other chemical entities than heparin, having different biological activities and carrying groups that react with amino groups or which have been activated to react with amino groups, may be bound to the surface by the surface modification according to present invention.

Optionally the free primary amino groups on the surface which are achieved by the final layer of polyamine may be quarternised so that a negatively charged, biologically active compound may be ionically bonded.

As described in the prior art as represented by US Patent 4,565,740, the substrate may be of any material, such as metal especially stainless steel, aluminium, glass or a polymeric material, especially polyvinylchloride, polyurethane, silicon rubber, polytetrafluorethylene, polystyrene and polyolefins, which is commonly used for the production of medical articles, especially those intended for blood contact. Examples of such articles are tubes, catheters, oxygenators, filters, intra vascular probes, blood pumps or the like.

Claims

Claims for the following Contracting States : AT, BE, CH, LI, DE, DK, FR, GB, GR, IT, LU, NL, SE

1. A method for modifying the surface of a solid substrate, comprising the steps of:

(a) adsorbing on the surface of the substrate a polyamine of a high average molecular weight and crosslinking said polyamine with crotonaldehyde, said crotonaldehyde being added either simultaneously with or separately from said polyamine;

(b) then adsorbing on the surface of the crosslinked polyamine a layer of an anionic polysaccharide;

(c) optionally repeating steps (a) and (b) one or more times; and

(d) adsorbing on the anionic polysaccharide layer, or on the outermost layer of anionic polysaccharide, a layer of non-crosslinked polyamine providing free primary amino groups.

2. A method according to Claim 1, comprising the subsequent step of quarternizing said free primary amino groups.

3. A method according to Claim 1, wherein the substrate is a metal, glass or a polymeric material.

4. A method according to Claim 3, wherein the substrate is stainless steel or aluminium.



concentration.

Example 2

The tubings were treated as in Example 1 except that the solutions 1a and 2a were replaced by 1b and 2b respectively, as follows:

1b) 1ml 5% Polymin SN* and 4,12ml crotonaldehyde in 1l 0.17M borate buffer of pH 9

2b) 1ml 5% Polymin SN* and 20ml glutaraldehyde (25%-solution) in 1l 0.17M borate buffer of pH 9

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To a surface treated according to present invention, chemical entities carrying functional groups that may react with amino groups without loss of biological activity may be bound. An example of such entities is heparin fragments having aldehyde groups, which are produced by degrading heparin with nitrous acid as described in US Patent 4,613,665 (Larm) for the preparation of heparinized surfaces.

Of course other chemical entities than heparin, having different biological activities and carrying groups that react with amino groups or which have been activated to react with amino groups, may be bound to the surface by the surface modification according to present invention.

Optionally the free primary amino groups on the surface which are achieved by the final layer of polyamine may be quarternised so that a negatively charged, biologically active compound may be ionically bonded.

As described in the prior art as represented by US Patent 4,565,740, the substrate may be of any material, such as metal especially stainless steel, aluminium, glass or a polymeric material, especially polyvinylchloride, polyurethane, silicon rubber, polytetrafluorethylene, polystyrene and polyolefins, which is commonly used for the production of medical articles, especially those intended for blood contact. Examples of such articles are tubes, catheters, oxygenators, filters, intra vascular probes, blood pumps or the like.

Claims

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1. A method for modifying the surface of a solid substrate, comprising the steps of:

(a) adsorbing on the surface of the substrate a polyamine of a high average molecular weight and crosslinking said polyamine with crotonaldehyde, said crotonaldehyde being added either simultaneously with or separately from said polyamine;

(b) then adsorbing on the surface of the crosslinked polyamine a layer of an anionic polysaccharide;

(c) optionally repeating steps (a) and (b) one or more times; and

(d) adsorbing on the anionic polysaccharide layer, or on the outermost layer of anionic polysaccharide, a layer of non-crosslinked polyamine providing free primary amino groups.

2. A method according to Claim 1, comprising the subsequent step of quaternizing said free primary amino groups.

3. A method according to Claim 1, wherein the substrate is a metal, glass or a polymeric material.

4. A method according to Claim 3, wherein the substrate is stainless steel or aluminium.



5. A method according to Claim 3, wherein the substrate is selected from polyvinylchloride, polyurethane, silicon rubber, polytetrafluoroethylene, polystyrene and polyolefins.
6. A method according to any preceding claim, wherein the polysaccharide is dextran sulfate.
7. A method according to any preceding claim, wherein the polyamine is polyethylene imine.
8. A method according to Claim 1, or to any one of Claims 3-7 when appendant to Claim 1, comprising the subsequent step of binding a biologically active chemical entity carrying functional group reactive with said primary amino groups to the non-crosslinked polyamine layer.
9. A method according to Claim 8, wherein said chemical entity is heparin.
10. A method according to Claim 2, comprising the subsequent step of ionically bonding a negatively charged biologically active compound to the non-crosslinked, quaternized polyamine layer.
11. A surface modified substrate, comprising:
 - (i) a layer of a polyamine of high average molecular weight which has been crosslinked with crotonaldehyde absorbed on the surface of the substrate;
 - (ii) a layer of an anionic polysaccharide absorbed on the surface of said crosslinked polyamine;
 - (iii) optionally one or more additional alternating layers of said crosslinked polyamine (i) and said anionic polysaccharide (ii); and
 - (iv) a layer of non-crosslinked polyamine providing free primary amino groups absorbed on the, or on the outermost, layer of anionic polysaccharide.
12. A surface modified substrate according to Claim 11 and further comprising:
 - (v) a biologically active chemical entity covalently bound to said non-crosslinked polyamine.
13. A surface modified substrate according to Claim 12, wherein said biologically active chemical entity is heparin.
14. A modification of the invention defined in Claim 11, wherein said layer (iv) is a layer of non-crosslinked polyamine providing free amino groups which have been quaternized.
15. A surface modified substrate according to Claim 14, and further comprising:
 - (v) a biologically active compound ionically bound to the non-crosslinked, quaternized polyamine layer.

Claims for the following Contracting State : ES

1. A method for modifying the surface of a solid substrate, comprising the steps of:
 - (a) adsorbing on the surface of the substrate a polyamine of a high average molecular weight and crosslinking said polyamine with crotonaldehyde, said crotonaldehyde being added either simultaneously with or separately from said polyamine;
 - (b) then adsorbing on the surface of the crosslinked polyamine a layer of an anionic polysaccharide;
 - (c) optionally repeating steps (a) and (b) one or more times; and
 - (d) adsorbing on the anionic polysaccharide layer, or on the outermost layer of anionic polysaccharide, a layer of non-crosslinked polyamine providing free primary amino groups.
2. A method according to Claim 1, comprising the subsequent step of quaternizing said free primary amino groups.
3. A method according to Claim 1, wherein the substrate is a metal, glass or a polymeric material.
4. A method according to Claim 3, wherein the substrate is stainless steel or aluminium.
5. A method according to Claim 3, wherein the substrate is selected from polyvinylchloride, polyurethane, silicon rubber, polytetrafluoroethylene, polystyrene and polyolefins.



6. A method according to any preceding claim, wherein the polysaccharide is dextran sulfate.
7. A method according to any preceding claim, wherein the polyamine is polyethylene imine.
- 5 8. A method according to Claim 1, or to any one of Claims 3-7 when appendant to Claim 1, comprising the subsequent step of binding a biologically active chemical entity carrying functional group reactive with said primary amino groups to the non-crosslinked polyamine layer.
9. A method according to Claim 8, wherein said chemical entity is heparin.
- 10 10. A method according to Claim 2, comprising the subsequent step of ionically bonding a negatively charged biologically active compound to the non-crosslinked, quaternized polyamine layer.
11. A surface modified substrate, comprising:
 - 15 (i) a layer of a polyamine of high average molecular weight which has been crosslinked with crotonaldehyde absorbed on the surface of the substrate;
 - (ii) a layer of an anionic polysaccharide absorbed on the surface of said crosslinked polyamine;
 - (iii) optionally one or more additional alternating layers of said crosslinked polyamine (i) and said anionic polysaccharide (ii); and
 - 20 (iv) a layer of non-crosslinked polyamine providing free primary amino groups absorbed on the, or on the outermost, layer of anionic polysaccharide.
12. A modification of the invention defined in Claim 11, wherein said layer (iv) is a layer of non-crosslinked polyamine providing free amino groups which have been quaternized.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, LI, DE, DK, FR, GB, GR, IT, LU, NL, SE

1. Verfahren zum Modifizieren der Oberfläche eines festen Substrats mit den Verfahrensstufen:
 - 30 (a) Adsorbieren eines Polyamins hohen mittleren Molekulargewichts und Vernetzen des Polyamins mit Crotonaldehyd auf der Oberfläche des Substrats, wobei der Crotonaldehyd entweder gleichzeitig mit oder getrennt von dem Polyamin zugesetzt wird;
 - (b) nachfolgendes Adsorbieren einer Schicht eines anionischen Polysaccharids auf der Oberfläche des vernetzten Polyamins;
 - 35 (c) bedarfsweise Wiederholung der Stufen (a) und (b) ein- oder mehrmalig; und
 - (d) Adsorbieren einer Schicht nicht vernetzten Polyamins, das freie primäre Aminogruppen zur Verfügung stellt, auf der anionischen Polysaccharidschicht oder auf der äußersten anionischen Polysaccharidschicht.
- 40 2. Verfahren nach Anspruch 1, mit der weiteren Stufe der Quaternisierung der freien primären Aminogruppen.
3. Verfahren nach Anspruch 1, wobei das Substrat Metall, Glas und/oder ein polymeres Material ist.
- 45 4. Verfahren nach Anspruch 3, wobei das Substrat rostfreier Stahl oder Aluminium ist.
5. Verfahren nach Anspruch 3, wobei das Substrat Polyvinylchlorid, Polyurethan, Silikongummi, Polytetrafluorethylen, Polystyrol und/oder Polyolefin ist.
- 50 6. Verfahren nach einem der vorhergehenden Ansprüche, wobei das Polysaccharid Dextransulfat ist.
7. Verfahren nach einem der vorhergehenden Ansprüche, wobei das Polyamin Polyethylenimin ist.
8. Verfahren nach Anspruch 1 oder nach einem der Ansprüche 3 bis 7 unter Rückbeziehung auf Anspruch 1 mit dem weiteren Schritt der Verbindung einer biologisch aktiven chemischen Einheit mit funktionellen Gruppen, die mit den primären Aminogruppen reaktiv sind, mit der nicht vernetzten Polyaminschicht.



9. Verfahren nach Anspruch 8, wobei die chemische Einheit Heparin ist.
10. Verfahren nach Anspruch 2 mit der weiteren Stufe der ionischen Bindung einer negativ geladenen biologisch aktiven Verbindung mit der nicht vernetzten, quaternisierten Polyaminschicht.
- 5 11. Oberflächenmodifiziertes Substrat mit
 - (i) einer Schicht eines Polyamins hohen mittleren Molekulargewichts, das mit auf der Oberfläche des Substrats absorbiertem Crotonaldehyd vernetzt wurde;
 - (ii) einer Schicht eines auf der Oberfläche des vernetzten Polyamins absorbierten anionischen Polysaccharids;
 - 10 (iii) bedarfsweise zwei oder mehr zusätzlichen wechselnden Schichten des vernetzten Polyamins (i) und des anionischen Polysaccharids (ii); und
 - (iv) einer Schicht nicht vernetzten Polyamins, das freie primäre Aminogruppen zur Verfügung stellt, die auf der oder auf der äußersten Schicht anionischem Polysaccharids absorbiert ist.
- 15 12. Substrat nach Anspruch 11, das des weiteren
 - (v) eine biologisch aktive chemische Einheit enthält, die an das nicht vernetzte Polyamin kovalent gebunden ist.
- 20 13. Substrat nach Anspruch 12, wobei die biologisch aktive chemische Einheit Heparin ist.
14. Substrat nach Anspruch 11, wobei die Schicht (iv) eine Schicht eines nicht vernetzten Polyamins mit freien Aminogruppen ist, die quaternisiert wurden.
- 25 15. Substrat nach Anspruch 14, das zusätzlich
 - (v) eine biologisch aktive Verbindung aufweist, die ionisch mit der nicht vernetzten quaternisierten Polyaminschicht verbunden ist.

Patentansprüche für folgenden Vertragsstaat : ES

- 30 1. Verfahren zum Modifizieren der Oberfläche eines festen Substrats mit den Verfahrensstufen:
 - (a) Adsorbieren eines Polyamins hohen mittleren Molekulargewichts und Vernetzen des Polyamins mit Crotonaldehyd auf der Oberfläche des Substrats, wobei der Crotonaldehyd entweder gleichzeitig mit oder getrennt von dem Polyamin zugesetzt wird;
 - 35 (b) nachfolgendes Adsorbieren einer Schicht eines anionischen Polysaccharids auf der Oberfläche des vernetzten Polyamins;
 - (c) bedarfsweise Wiederholung der Stufen (a) und (b) ein- oder mehrmalig; und
 - (d) Adsorbieren einer Schicht nicht vernetzten Polyamins, das freie primäre Aminogruppen zur Verfügung stellt, auf der anionischen Polysaccharidschicht oder auf der äußersten anionischen Polysaccharidschicht.
 - 40
2. Verfahren nach Anspruch 1, mit der weiteren Stufe der Quaternisierung der freien primären Aminogruppen.
- 45 3. Verfahren nach Anspruch 1, wobei das Substrat Metall, Glas und/oder ein polymeres Material ist.
4. Verfahren nach Anspruch 3, wobei das Substrat rostfreier Stahl oder Aluminium ist.
5. Verfahren nach Anspruch 3, wobei das Substrat Polyvinylchlorid, Polyurethan, Silikongummi, Polytetrafluorethylen, Polystyrol und/oder Polyolefin ist.
- 50 6. Verfahren nach einem der vorhergehenden Ansprüche, wobei das Polysaccharid Dextransulfat ist.
7. Verfahren nach einem der vorhergehenden Ansprüche, wobei das Polyamin Polyethylenimin ist.
- 55 8. Verfahren nach Anspruch 1 oder nach einem der Ansprüche 3 bis 7 unter Rückbeziehung auf Anspruch 1 mit dem weiteren Schritt der Verbindung einer biologisch aktiven chemischen Einheit mit funktionellen Gruppen, die mit den primären Aminogruppen reaktiv sind, mit der nicht vernetzten Polyamin-



schicht.

9. Verfahren nach Anspruch 8, wobei die chemische Einheit Heparin ist.

10. Verfahren nach Anspruch 2 mit der weiteren Stufe der ionischen Bindung einer negativ geladenen biologisch aktiven Verbindung mit der nicht vernetzten, quaternisierten Polyaminschicht.

11. Oberflächenmodifiziertes Substrat mit

- (i) einer Schicht eines Polyamins hohen mittleren Molekulargewichts, das mit auf der Oberfläche des Substrats absorbiertem Crotonaldehyd vernetzt wurde;
- (ii) einer Schicht eines auf der Oberfläche des vernetzten Polyamins absorbierten anionischen Polysaccharids;
- (iii) bedarfsweise zwei oder mehr zusätzlichen wechselnden Schichten des vernetzten Polyamins (i) und des anionischen Polysaccharids (ii); und
- (iv) einer Schicht nicht vernetzten Polyamins, das freie primäre Aminogruppen zur Verfügung stellt, die auf der oder auf der äußersten Schicht anionischem Polysaccharids absorbiert ist.

12. Substrat nach Anspruch 11, wobei die Schicht (iv) eine Schicht eines nicht vernetzten Polyamins mit freien Aminogruppen ist, die quaternisiert wurden.

Revendications

Revendications pour les Etats contractants suivants : AT, BE, CH, LI, DE, DK, FR, GB, GR, IT, LU, NL, SE

1. Procédé pour modifier la surface d'un substrat solide, ce procédé comprenant les étapes consistant à :
 - (a) provoquer l'adsorption, sur la surface du substrat, d'une polyamine ayant un poids moléculaire moyen élevé et provoquer la réticulation de ladite polyamine à l'aide de crotonaldéhyde, ledit crotonaldéhyde étant ajouté en même temps que ladite polyamine ou bien séparément de ladite polyamine ;
 - (b) puis provoquer l'adsorption, sur la surface de la polyamine réticulée, d'une couche d'un polysaccharide anionique ;
 - (c) éventuellement répéter les étapes (a) et (b) une ou plusieurs fois ; et
 - (d) provoquer l'adsorption, sur la couche du polysaccharide anionique, ou sur la couche la plus externe du polysaccharide anionique, d'une couche de polyamine non réticulée fournissant des groupes amino primaires libres.
2. Procédé selon la revendication 1, comprenant l'étape subséquente consistant à quaterniser lesdits groupes amino primaire libres.
3. Procédé selon la revendication 1, dans lequel le substrat est un métal, du verre ou une matière polymère.
4. Procédé selon la revendication 3, dans lequel le substrat est de l'acier inoxydable ou de l'aluminium.
5. Procédé selon la revendication 3, dans lequel le substrat est choisi parmi du poly(chlorure de vinyle), du polyuréthane, du caoutchouc de silicone, du polytétrafluoréthylène, du polystyrène et des polyoléfin.
6. Procédé selon l'une quelconque des revendications précédentes, dans lequel le polysaccharide est du sulfate de dextrane.
7. Procédé selon l'une quelconque des revendications précédentes, dans lequel la polyamine est de la polyéthylène imine.
8. Procédé selon la revendication 1, ou selon l'une quelconque des revendications 3 à 7 associée à la revendication 1, comprenant l'étape subséquente consistant à lier ou fixer, sur la couche de polyamine non réticulée, une entité chimique biologiquement active portant des groupes fonctionnels capables de réagir avec lesdits groupes amino primaires.



9. Procédé selon la revendication 8, dans lequel ladite entité chimique est de l'héparine.
10. Procédé selon la revendication 2, comprenant l'étape subséquente consistant à lier ou fixer ioniquement, sur la couche de polyamine quaternisée et non réticulée, un composé biologiquement actif et négativement chargé.
11. Substrat à surface modifiée (ou modifié en surface), comprenant :
 - (i) une couche d'une polyamine à poids moléculaire moyen élevé, qui a été réticulée à l'aide de crotonaldéhyde, couche adsorbée à la surface du substrat ;
 - (ii) une couche d'un polysaccharide anionique adsorbé à la surface de ladite polyamine réticulée ;
 - (iii) éventuellement une ou plusieurs couches alternées supplémentaires de ladite polyamine réticulée (i) et dudit polysaccharide anionique (ii) ; et
 - (iv) une couche de polyamine non réticulée, fournissant des groupes amino primaires libres, couche adsorbée sur la couche, ou sur la couche la plus extérieure, du polysaccharide anionique.
12. Substrat à surface modifiée (ou modifié en surface), selon la revendication 11 et comprenant en outre :
 - (v) une entité chimique, biologiquement active, fixée par covalence sur ladite polyamine non réticulée.
13. Substrat modifié en surface selon la revendication 12, dans lequel ladite entité chimique biologiquement active est l'héparine.
14. Variante ou modification de l'invention définie à la revendication 11, dans laquelle ladite couche (iv) est une couche de polyamine non réticulée fournissant des groupes amino libres qui ont été quaternisés.
15. Substrat modifié en surface (ou à surface modifiée) selon la revendication 14, comprenant en outre :
 - (v) un composé biologiquement actif, ioniquement fixé sur la couche de polyamine quaternisée et non réticulée.

30 Revendications pour l'Etat contractant suivant : ES

1. Procédé pour modifier la surface d'un substrat solide, ce procédé comprenant les étapes consistant à :
 - (a) provoquer l'adsorption, sur la surface du substrat, d'une polyamine ayant un poids moléculaire moyen élevé et provoquer la réticulation de ladite polyamine à l'aide de crotonaldéhyde, ledit crotonaldéhyde étant ajouté en même temps que ladite polyamine ou bien séparément de ladite polyamine ;
 - (b) puis provoquer l'adsorption, sur la surface de la polyamine réticulée, d'une couche d'un polysaccharide anionique ;
 - (c) éventuellement répéter les étapes (a) et (b) une ou plusieurs fois ; et
 - (d) provoquer l'adsorption, sur la couche du polysaccharide anionique, ou sur la couche la plus externe du polysaccharide anionique, d'une couche de polyamine non réticulée fournissant des groupes amino primaires libres.
2. Procédé selon la revendication 1, comprenant l'étape subséquente consistant à quaterniser lesdits groupes amino primaire libres.
3. Procédé selon la revendication 1, dans lequel le substrat est un métal, du verre ou une matière polymère.
4. Procédé selon la revendication 3, dans lequel le substrat est de l'acier inoxydable ou de l'aluminium.
5. Procédé selon la revendication 3, dans lequel le substrat est choisi parmi du poly(chlorure de vinyle), du polyuréthane, du caoutchouc de silicone, du polytétrafluoréthylène, du polystyrène et des polyoléfines.
6. Procédé selon l'une quelconque des revendications précédentes, dans lequel le polysaccharide est du sulfate de dextrane.



7. Procédé selon l'une quelconque des revendications précédentes, dans lequel la polyamine est de la polyéthylène imine.
- 5 8. Procédé selon la revendication 1, ou selon l'une quelconque des revendications 3 à 7 associée à la revendication 1, comprenant l'étape subséquente consistant à lier ou fixer, sur la couche de polyamine non réticulée, une entité chimique biologiquement active portant des groupes fonctionnels capables de réagir avec lesdits groupes amino primaires.
9. Procédé selon la revendication 8, dans lequel ladite entité chimique est de l'héparine.
- 10 10. Procédé selon la revendication 2, comprenant l'étape subséquente consistant à lier ou fixer ioniquement, sur la couche de polyamine quaternisée et non réticulée, un composé biologiquement actif et négativement chargé.
- 15 11. Substrat à surface modifiée (ou modifié en surface), comprenant :
- (i) une couche d'une polyamine à poids moléculaire moyen élevé, qui a été réticulée à l'aide de crotonaldéhyde, couche adsorbée à la surface du substrat ;
- (ii) une couche d'un polysaccharide anionique adsorbé à la surface de ladite polyamine réticulée ;
- (iii) éventuellement une ou plusieurs couches alternées supplémentaires de ladite polyamine réticulée (i) et dudit polysaccharide anionique (ii) ; et
- 20 (iv) une couche de polyamine non réticulée, fournissant des groupes amino primaires libres, couche adsorbée sur la couche, ou sur la couche la plus extérieure, du polysaccharide anionique.
12. Modification ou variante de l'invention définie à la revendication 11, dans laquelle ladite couche (iv) est
- 25 une couche de polyamine non réticulée fournissant des groupes amino libres qui ont été quaternisés.
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