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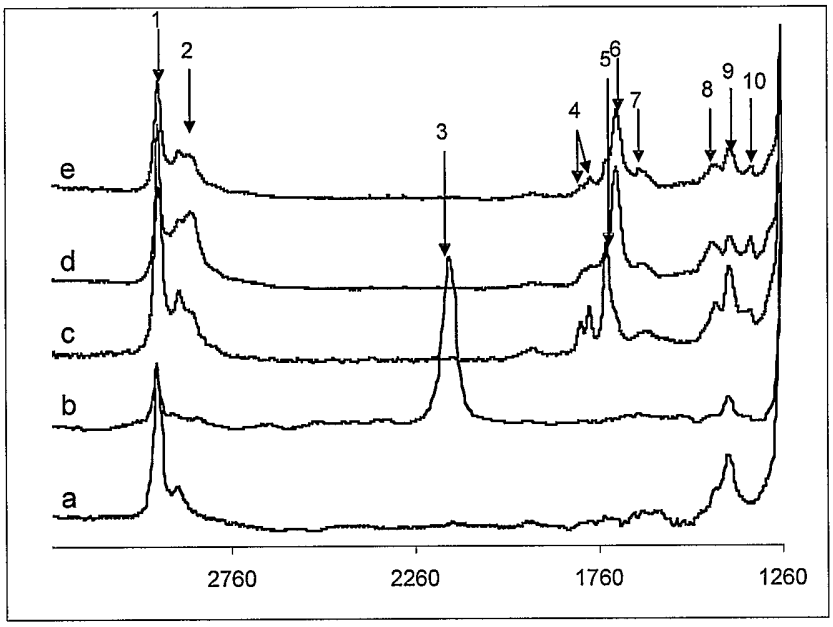
(54) Title: BIOLOGICAL MOLECULE-REACTIVE HYDROPHILIC SILICONE SURFACE



(57) Abstract: A silicone polymer having a modified surface is described, wherein said modification consists of a covalently attached water soluble polymer bearing an activating group, wherein said activating group reacts with reactive functionalities on one or more biological molecules so that said one or more biological molecules become covalently attached to said silicone polymer. The modified silicones are reacted with biological molecules to make them more biocompatible for use in biodiagnostic, biosensor or bioaffinity applications, or for coatings for in vivo transplantation or for liners exposed to biological broths.

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Title: Biological Molecule-Reactive Hydrophilic Silicone Surface

FIELD OF THE INVENTION

The present invention relates to modified silicone materials, specifically silicone materials that have been modified so that they are biocompatible, as well as to methods of making such materials.

BACKGROUND OF THE INVENTION

When synthetic biomaterials are implanted, they are met with a complex and aggressive biological system that ultimately passivates the material or creates a fibrotic capsule, essentially walling the material off from the system with which it was to interact. Various synthetic strategies have made impressive inroads to the problems of preparing compatible biomaterials ([1]). One promising approach exploits the plasma polymerization of hydrophilic monomers such as alkylamines or tetraglyme onto an existing polymer surface ([2,3,4]). However, likely the most general and powerful methods ([5]) involve the formation of layers of hydrophilic polymers, of which oligo- ([6,7,8]) and poly(ethylene oxide)([9,10,11,12]) are exemplary, on the surface. The polymers either bloom from polymer blends to an aqueous interface, or are covalently grafted onto an activated polymer surface ([13,14]). While promising, it is clear that more biocompatible surfaces can be produced when constituents of the local biology are harnessed to "bioactivate" the surface ([15]), either alone or in combination with hydrophilic polymers. Such approaches include modification with amino acids, cell adhesion peptides, growth factors, and (glyco)proteins. These materials are generally tethered at multiple sites, reducing the mobility of the linking chain. The specific spacing of the tethered biomolecules from the polymer interface is not normally controllable.

Silicone polymers offer many advantages as biocompatible supports, including their very high oxygen transmissibility and the ease with which a variety of different substrates can be conformally coated using several different crosslinking processes. Silicones possess, however, an extremely high surface hydrophobicity to which biomolecules readily adhere ([16,17]) generally resulting, in the case of proteins, in the subsequent mediation of biological reactions ([15]).

Polyethylene glycol (PEO), a water soluble, nontoxic, and nonimmunogenic polymer, has been widely shown to improve the biological compatibility of materials.

The presence of a layer of PEO on a biomaterial surface is accompanied by reductions in protein adsorption, and cell and bacterial adhesion ([18,19,20,,21]). While silicones do not normally possess appropriate surface functional groups that could be used to tether passivating polymers such as PEO, several approaches have been developed to introduce organic functionalities on silicone surfaces including the use of a mercury lamp to create radicals ([22]) and oxidation by an O_2-based plasma to give alcohols and more highly oxidized species ([23]). Alternative methods exploit plasma polymerization of various molecules to generate a functional surface for subsequent modification ([24,25,26]). However, these methods require several synthetic steps, are not always reproducible and often result in incomplete surface coverage with the functional molecule of interest ([27]).

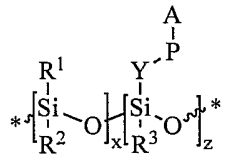
The remains a need for an efficient and general method to introduce functionalities onto silicone surfaces that will render these materials biocompatible.

SUMMARY OF THE INVENTION

The present inventors have developed a flexible, asymmetric linker that provides a facile route to convert hydrophobic silicones into activated ester-terminated, PEO-modified surfaces. These surfaces react effectively with nucleophiles, such as amines and alcohols, and thus serve as key intermediates in the preparation of saccharide-, peptide-, nucleotide-modified and analogous surfaces. High density films of biomolecules, including the peptides, RGD and YIGSR, proteins (epidermal growth factor (EGF), albumin, fibrinogen, mucin and lysozyme) and the glycoprotein heparin, have been prepared on silicone. The resulting surfaces are thus tailored to be selectively repellent or adherent to biomolecules and, as a result, biocompatible in a variety of applications.

Accordingly, the present invention relates to a silicone polymer having a modified surface wherein said modification consists of a covalently attached water soluble polymer bearing an activating group, wherein said activating group reacts with reactive functionalities on one or more biological molecules so that said one or more biological molecules become covalently attached to said silicone polymer.

The present invention further relates to a silicone polymer having the general Formula I:



I

wherein

x is an integer between, and including, 1- 20000;

z is an integer between, and including, 1 and 1000;

R^1, R^2 and R^3 are each, independent of one another, selected from H, C_{1-30}alkyl, C_{2-30}alkenyl, C_{2-30}alkynyl and aryl, with the latter four groups being unsubstituted or substituted with one or more groups independently selected from halo, OH, NH_2, NHC_{1-6}alkyl, $N(C_{1-6}alkyl)(C_{1-6}alkyl)$, OC_{1-6}alkyl and halo-substituted C_{1-6}alkyl;

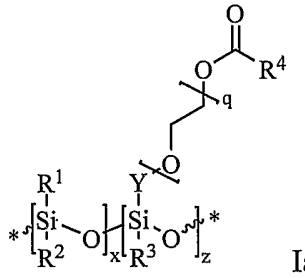
Y is a linker group;

P is a water soluble polymer; and

A is an activating group wherein said activating group reacts with reactive functionalities on one or more biological molecules so that said one or more biological molecules become covalently attached to said silicone polymer.

The polymer of Formula I may also be tethered to another polymer using, for example, the substituents on R^1, R^2 and/or R^3, or through crosslinking reactions known to those skilled in the art, or may be the result of the formation of an interpenetrating network. The polymer of Formula I may also be an elastomer, in which R^1, R^2 and/or R^3 forms a bridge to an adjacent polymeric chain.

In an embodiment of the invention, the water soluble polymer, P, is polyethylene oxide, and the activating group is an activated carboxylic acid. Accordingly, the present invention further relates to a silicone polymer having the general Formula Ia:



Ia

wherein

x is an integer between, and including, 1- 20000;

z is an integer between, and including, 1 and 1000;

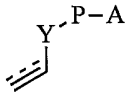
R^1, R^2 and R^3 are each, independent of one another, selected from H, C_{1-30}alkyl, C_{2-30}alkenyl, C_{2-30}alkynyl and aryl, with the latter four groups being unsubstituted or substituted with one or more groups independently selected from halo, OH, NH_2, NHC_{1-6}alkyl, $N(C_{1-6}alkyl)(C_{1-6}alkyl)$, OC_{1-6}alkyl and halo-substituted C_{1-6}alkyl;

Y is a linker group;

q is an integer between, and including, 1-225; and

R^4 is an activating group which activates the adjacent carbonyl group so that nucleophilic functionalities on one or more biological molecules will react therewith and said one or more biological molecules become covalently attached to said silicone polymer.

Also included within the scope of the present invention is a compound of Formula II:



II

wherein

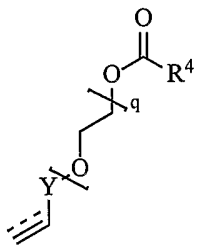
P is a water soluble polymer;

Y is a linker group;

≡ represents a double or triple bond; and

A is an activating group wherein said activating group reacts with reactive functionalities on one or more biological molecules.

Also included within the scope of the present invention is a compound of Formula IIa



IIa

wherein

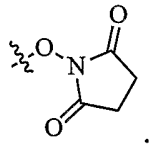
≡ represents a double or triple bond; and

Y is a linker group;

q is an integer between, and including, 1-225; and

R^4 is an activating group which activates the adjacent carbonyl group so that nucleophilic functionalities on one or more biological molecules will react therewith and said one or more biological molecules become covalently attached to said silicone polymer.

In an embodiment of the invention, R^4 is an *N*-hydroxysuccinimidyl (NHS) group:



The compounds of Formula II, may be reacted with silicone materials bearing Si-H surface functional groups, using standard hydrosilylation conditions, to provide compounds of Formula I.

The compounds of Formula I may then be reacted with reactive functionalities, for example nucleophilic functionalities, on any biological molecule to provide silicone surfaces that are biocompatible for a variety of applications.

Accordingly, the present invention further includes a method of preparing a biocompatible silicone material comprising reacting compounds of Formula I, as defined above, with one or more biological molecules bearing reactive functionalities, so that the one or more biological molecules becomes covalently attached to said compounds of Formula I.

The present invention also provides methods of using the biocompatible silicone materials in biodiagnostic, biosensor and bioaffinity applications, as well as for coatings, for example, for *in vivo* bioimplantation and for reactors liners exposed to biological broths, such as fermentors.

The present invention relates to a simple two step procedure to modify the biocompatibility of any silicone material. The silicone materials represented by Formula I are generic in that they will react with any reactive functionality, in particular alcohols and amines, making the surface readily amenable to modification

by biomolecules. The density of groups attached to the silicone material can be varied as can the nature of groups to facilitate rejection or attraction of available biomolecules. The polymers of Formula I have a well defined structure, that has been fully characterized. The biomolecule-modified silicone materials made from the polymers of Formula I can be any surface, including flat sheets, solid objects, coated objects and even surfaces having complicated shapes.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will now be described in greater detail with reference to the following drawings in which:

Figure 1 shows FT-IR spectra of: (a) PDMS; (b) Si-H modified PDMS; (c) succinimidyl carbonate PEG-modified PDMS surfaces **3**, (d) RGD-modified **9**, and (e) YIGSR-modified **10** PDMS surfaces, respectively.

Figure 2 shows survey XPS spectra: (a) unmodified PDMS; (b) succinimidyl carbonate PEG- **3**, (c) RGDS-PEG **9**, and, (d) YIGSR PEG modified-PDMS **10** surfaces.

Figure 3 is a bar graph showing the water contact angle of control, RGDS **9** and YIGSR **10** modified surfaces.

Figure 4 is a bar graph showing contact angle data for heparinized silicone surfaces.

Figure 5 is a bar graph showing binding EGF to the surfaces: PDMS = control, PDMS-PEO = modified surface as disclosed below.

Figure 6 shows A: Adsorption of albumin on the control and **2** giving **5** before and after washing with SDS. B: Adsorption of fibrinogen onto albumin coated control or onto **5** giving **6**. C: Surfaces coated with albumin, then fibrinogen, and then washed with SDS.

Figure 7 shows A: Adsorption of lysozyme onto various silicone surfaces before and after exposure to SDS.

Figure 8 shows adsorption of plasminogen from plasma.

Figure 9 shows the ability of thrombin to process *N-p*-tosyl-gly-pro-arg *p*-nitroanilide under various conditions.

Figure 10 shows Human Corneal Epithelial Cells (HCEC) grown on control, RGDS **4** and YIGSR **5** modified surface (7 days).

Figure 11 shows the NMR assignments of **2**.

Figure 12 shows a calibration curve for measuring total heparin density.

Figure 13 shows the growth of human corneal epithelial cells on A: control (silicone), B: PEO-modified silicone, C: EGF-coated silicone or D: **4.**

Figure 14 shows thrombin inactivation by AT bound to heparin surface **13** and versus AT directly bound to **3**.

DETAILED DESCRIPTION OF THE INVENTION

Silicone surfaces have been modified with a flexible, asymmetric linker which provides materials with activated ester-terminated, PEO-modified surfaces. These surfaces react effectively with reactive functionalities, such as amines and alcohols, and thus serve as key intermediates in the preparation of saccharide-, peptide-, nucleotide-modified and analogous surfaces. The resulting surfaces may be tailored to be selectively repellent or adherent to biomolecules and, as a result, biocompatible in a variety of applications.

Accordingly, the present invention relates to a silicone polymer having a modified surface wherein said modification consists of a covalently attached water soluble polymer bearing an activating group, wherein said activating group reacts with reactive functionalities on one or more biological molecules so that said one or more biological molecules become covalently attached to said silicone polymer.

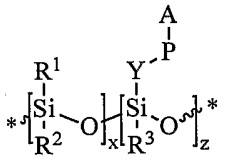
In embodiments of the invention, the silicon polymer may be tethered to another polymer through crosslinking or be part an interpenetrating network or be an elastomeric species by forming bridges with adjacent polymer chains.

In an embodiment of the invention, the water soluble polymer is, selected from any such compound and includes, but is not limited to, polyethers, for example, polyethylene oxide (PEO), polyethylene glycol (PEG), amino-terminated polyethylene glycol (PEG-NH_2), polypropylene glycol (PPG), polypropylene oxide (PPO), polypropylene glycol bis(2-amino-propyl ether) (PPG-NH_2); polyalcohols, for example, polyvinyl alcohol; polysaccharides, e.g. dextran and related compounds; poly(vinyl pyridine); polyacids, for example, poly(acrylic acid); polyacrylamides e.g.

poly(*N*-isopropylacrylamide) (polyNIPAM); and polyallylamine (PAM). In a further embodiment of the invention, the water soluble polymer is PEO, or a modified PEO. In a further embodiment of the invention, the PEO has a molecular weight of up to about 2000 g/mol, more specifically up to about 1000 g/mol. By "water soluble" it is meant that the polymer is capable of being formed into an aqueous solution having a suitable concentration. It should be noted that the terms "oxide" (as in polyethylene oxide) and "glycol" (as in polyethylene glycol) may be used interchangeably and the use of one term over the other is not meant to be limiting in any way.

The activating group on the water soluble polymer and the reactive functionalities on the biological molecule are designed so that they are complementary and will react with each other to form a covalent linkage. For example, when the activating group is an activated carboxylic acid, the reactive functionalities on the biological molecule would comprise a nucleophile, for example an amine, alcohol or thiol.

The present invention further relates to a silicone polymer having the general Formula I:



I

wherein

x is an integer between, and including, 1- 20000;

z is an integer between, and including, 1 and 1000;

R^1, R^2 and R^3 are each, independent of one another, selected from H, C_{1-30}alkyl, C_{2-30}alkenyl, C_{2-30}alkynyl and aryl, with the latter four groups being unsubstituted or substituted with one or more groups independently selected from halo, OH, NH_2, NHC_{1-6}alkyl, $N(C_{1-6}alkyl)(C_{1-6}alkyl)$, OC_{1-6}alkyl and halo-substituted C_{1-6}alkyl;

Y is a linker group;

P is a water soluble polymer; and

A is an activating group wherein said activating group reacts with reactive functionalities on one or more biological molecules so that said one or more biological molecules become covalently attached to said silicone polymer.

The polymers of Formula I may also be tethered to another polymer using, for example, the substituents on R^1, R^2 and/or R^3, or through crosslinking, or may be the result of the formation of an interpenetrating network. The polymer of Formula I may also be an elastomer, in which R^1, R^2 and/or R^3 forms a bridge to an adjacent polymeric chain. Reactions to effect the formation of such co-polymers and elastomers are known to those skilled in the art.

The polymers of Formula I include those in which x is an integer between, and including, 1- 20000. In an embodiment of the invention x is an integer between and including, 5 - 600, suitably 10 - 600.

The polymers of Formula I include those in which z is an integer between, and including, 1- 1000. In an embodiment of the invention z is an integer between and including, 1 – 60.

The term "halo" as used herein means halogen and includes chloro, fluoro, bromo and iodo. In an embodiment of the invention, halo is fluoro.

The term "$C_{1\text{-}n}$alkyl" as used herein means straight and/or branched chain, saturated alkyl radicals containing from one to n carbon atoms and includes (depending on the identity of n) methyl, ethyl, propyl, isopropyl, n-butyl, s-butyl, isobutyl, t-butyl, 2,2-dimethylbutyl, n-pentyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, n-hexyl and the like.

The term "$C_{1\text{-}n}$alkenyl" as used herein means straight and/or branched chain, unsaturated alkyl radicals containing from one to n carbon atoms and one or more, suitably one or two, double bonds, and includes (depending on the identity of n) vinyl, allyl, 2-methylprop-1-enyl, but-1-enyl, but-2-enyl, but-3-enyl, 2-methylbut-1-enyl, 2-methylpent-1-enyl, 4-methylpent-1-enyl, 4-methylpent-2-enyl, 2-methylpent-2-enyl, 4-methylpenta-1,3-dienyl, hexen-1-yl and the like.

The term "$C_{1\text{-}n}$alkynyl" as used herein means straight and/or branched chain, unsaturated alkyl radicals containing from one to n carbon atoms and one or more, suitably one or two, triple bonds, and includes (depending on the identity of n) ethynyl, propargyl, 1-propynyl, 1-octynyl, and the like.

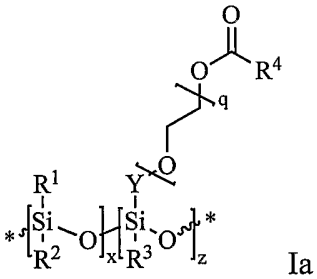
The term "halo-substituted C_{1-n}alkyl" as used herein means a C_{1-n}alkyl group substituted with one or more halo, in particular 1 or more fluoro, and includes CF_3, CF_2CF_3, CH_2CF_3, and the like.

The term "aryl" as used herein means a monocyclic or bicyclic carbocyclic ring system containing one or two aromatic rings and from 6 to 14 carbon atoms and includes phenyl, naphthyl, anthraceneyl, 1,2-dihydronaphthyl, 1,2,3,4-tetrahydronaphthyl, fluorenyl, indanyl, indenyl and the like.

In the polymers of Formula I, R^1, R^2 and R^3 are each, independent of one another, selected from H, C_{1-30}alkyl, C_{2-30}alkenyl, C_{2-30}alkynyl and aryl, with the latter four groups being unsubstituted or substituted with one or more groups independently selected from halo, OH, NH_2, NHC_{1-6}alkyl, $N(C_{1-6}alkyl)(C_{1-6}alkyl)$, OC_{1-6}alkyl and halo-substituted C_{1-6}alkyl. In an embodiment of the invention, R^1, R^2 and R^3 are each, independent of one another, selected from H, C_{1-10}alkyl, C_{2-10}alkenyl, C_{2-10}alkynyl and aryl, with the latter four groups being unsubstituted or substituted with one or more groups independently selected from halo, OH, NH_2, NHC_{1-4}alkyl, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, OC_{1-4}alkyl and halo-substituted C_{1-4}alkyl. In a further embodiment of the invention, R^1, R^2 and R^3 are each, independent of one another, selected from H, C_{1-4}alkyl, C_{2-4}alkenyl, C_{2-4}alkynyl and phenyl, with the latter four groups being unsubstituted or substituted with one or more groups independently selected from F, Cl, OH, NH_2, $NHCH_3$, $N(CH_3)_2$, OCH_3 and CF_3. In a still further embodiment of the invention, R^1, R^2 and R^3 are each, independent of one another, selected from H, C_{1-4}alkyl, C_{2-4}alkenyl and C_{2-4}alkynyl. In even further embodiments of the invention R^1, R^2 and R^3 are each, CH_3.

The linker group, Y, may be any suitable bivalent group. In an embodiment of the invention Y comprises at least one CH_2 group between the silicon atom and the polymer, P. In a further embodiment of the invention, Y is $-(CH_2)_t-$, wherein t is an integer between and including 1 and 30, suitably between 1 and 10, more suitably 3.

In an embodiment of the invention, the water soluble polymer, P, is polyethylene oxide, and the activating group is an activated carboxylic acid. Accordingly, the present invention further relates to a silicone polymer having the general Formula Ia:



Ia

wherein

x is an integer between, and including, 1- 20000;

z is an integer between, and including, 1 and 1000;

R^1, R^2 and R^3 are each, independent of one another, selected from H, C_{1-30}alkyl, C_{2-30}alkenyl, C_{2-30}alkynyl and aryl, with the latter four groups being unsubstituted or substituted with one or more groups independently selected from halo, OH, NH_2, NHC_{1-6}alkyl, $N(C_{1-6}alkyl)(C_{1-6}alkyl)$, OC_{1-6}alkyl and halo-substituted C_{1-6}alkyl;

Y is a linker group;

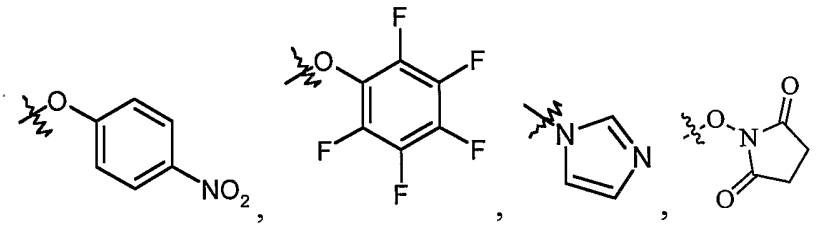
q is an integer between, and including, 1-225; and

R^4 is an activating group which activates the adjacent carbonyl group so that nucleophilic functionalities on one or more biological molecules will react therewith and said one or more biological molecules become covalently attached to said silicone polymer.

In the compounds of Formula Ia, q is an integer between and including 1 and 225. In an embodiment of the invention, q is an integer between and including, 2 and 100, specifically between 4 and 11.

In the polymers of Formula I, A may be any suitable functional group with complementary reactivity to functional groups on the biological molecule. In an embodiment of the invention A is an electrophilic functional group that reacts with nucleophilic functional groups on the biological molecule. A person skilled in the art would appreciate that there are many functional groups that are capable of reacting with nucleophiles, such as amines, alcohols and thiols, in biological molecules to form a covalent linkage between the biological molecule and the polymer. In an embodiment of the invention, A and $-C(O)-R^4$, in Formulae I and Ia, respectively, form an activating group that is used in peptide synthesis, for example a carbodiimide,

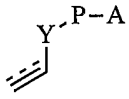
an anhydride, an activated ester or an azide, In an embodiment of the invention, R^4 is selected from *p*-nitrophenyl (i), perfluorophenyl (ii), imidazolyl (iii) or related *N*-heterocycles and *N*-hydroxysuccinimidyl (iv) (NHS):



(i) (ii) (iii) (iv)

In further embodiments of the invention, R^4 is NHS.

Also included within the scope of the present invention is a compound of Formula II:



II

wherein

P is a water soluble polymer;

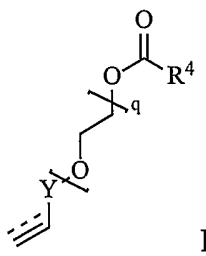
Y is a linker group;

≡ represents a double or triple bond; and

A is an activating group wherein said activating group reacts with reactive functionalities on one or more biological molecules.

In an embodiment of the invention, ≡ represents a double bond.

Further, the present invention also includes a compound of Formula IIa



IIa

wherein

≡ represents a double or triple bond; and

Y is a linker group;

q is an integer between, and including, 1-225; and

R^4 is an activating group which activates the adjacent carbonyl group so that nucleophilic functionalities on one or more biological molecules will react therewith and said one or more biological molecules become covalently attached to said silicone polymer.

The term "biological molecule" as used herein refers to any molecule known to be found in biological systems and includes, amino acids, proteins, peptides, nucleic acids (including DNA and RNA), saccharides, polysaccharides and the like. Biological molecules include those which are naturally occurring as well as those which have been modified using known techniques.

The term "biocompatible" as used herein means that the material either stabilizes proteins and/or other biomolecules against denaturation or does not facilitate denaturation. The term "biocompatible" also means compatible with *in vivo* use, in particular in animal subjects, including humans.

The "nucleophilic functionalities" on the biomolecule may be any nucleophilic group, for example, an amine (NH_2), hydroxy (OH) or thiol (SH) group. In an embodiment of the invention, the "nucleophilic functionality" is an amine (NH_2) or hydroxy (OH) group.

The compounds of Formula II, may be reacted with silicone materials bearing Si-H surface functional groups, using standard hydrosilylation conditions, to provide compounds of Formula I.

The compounds of Formula I may then be reacted with reactive functionalities on any biological molecule to provide silicone surfaces that are biocompatible for a variety of applications.

Accordingly, the present invention further includes a method of preparing a biocompatible silicone material comprising reacting compounds of Formula I, as defined above, with one or more biological molecules bearing reactive functionalities, so that the one or more biological molecules becomes covalently attached to said compounds of Formula I.

Also included within the scope of the present invention are biocompatible silicone materials prepared using this method.

Hydrosilylationconditions, for example, typically include reacting a Si-H modified silicone with a compound comprising a double or triple bond in the presence of a platinum catalyst, for example platinum-divinyltetramethyldisiloxane complex or Karstedt's catalyst, in a solvent at ambient temperatures.

Si-H modified silicones are well known in the art and are commercially available. An example of a Si-H modified silicone is DC1107 $(MeHSiO)_n$ available from Dow Corning.

The compounds of Formula I may be reacted with the one or more biological molecules bearing reactive functionalities under standard conditions known to those skilled in the art. For example, when the reactive functionality is a nucleophile on a protein or peptide, the reaction may be carried out in a buffered solution, for example a buffer at pH of about 5-9.5, suitably at about 7-8.5.

The immobilization of amino acids, peptides, proteins, sugars, polysaccharides; nucleosides, nucleotides (RNA, DNA), etc., and modified versions thereof, is a commonly exploited strategy to change the chemistry of a surface. The modified surfaces may then be used for biodiagnostic, biosensor, bioaffinity, and related applications. They may also be used to change the nature of subsequent deposition of biomolecules so that *in vivo* applications such as antithrombogenic coatings on stents, shunts and catheters or nonfouling contact lens surfaces can be achieved. Less complex, but equally important applications include non-fouling surfaces on membranes or in vessels used for fermentation. Silicones are also extremely useful as coating materials (conformal coatings are easy to prepare from silicones).

Biomaterials destined for implantation generally should not be recognized as a foreign body. If they are recognized as foreign at all, the interactions with the body must be extremely weak. One of the first events that takes place after implantation is the adsorption of proteins on the substrate surface, which initiates a cascade of biological events, generally to the detriment of the biomaterial. Minimizing this behaviour, and particularly any subsequent changes in protein structure (denaturing) after deposition is one of the main challenges which remain in bioimplantable materials. Silicone materials modified with PEO are demonstrably excellent at repelling a series of proteins. By contrast, the silicone materials of the present invention are readily surface modified with amino acids, peptides, proteins or

carbohydrates. These tethered biomolecules retain their bioactivity and further interact with other biomolecules in the environment. Thus, the surfaces of the present invention will be useful for *in vivo* implantation and for liners exposed to biological broths (e.g., fermentation, drug delivery systems, etc.). In addition to implantation, there will be other applications in coatings.

According, the present invention relates to a method of coating a surface to modulate biocompatability comprising applying silicone material of Formula I, as defined above, to said surface.

The term "modulate" as used herein means to increase or decrease or otherwise change a function or activity in the presence of a substance, compared to otherwise same conditions in the absence of the substance.

The present invention also provides methods of using the biocompatible silicone materials in biodiagnostic, biosensor and bioaffinity applications, in addition to coatings, for example, for *in vivo* transplantation and for liners exposed to biological broths.

While the following Examples illustrate the invention in further detail, it will be appreciated that the invention is not limited to the specific Examples.

EXAMPLES

Materials and Methods

(a) Reagents

Poly(ethylene glycol) monoallylether (average MW 500) was obtained as a gift from JuTian Chemical Co. (Nanjing, China). It was dried by azeotropic distillation with toluene before use. *N,N'*-Disuccinimidyl carbonate, *o*-xylene (97%, anhydrous), triethylamine (99%), acetonitrile (99%, anhydrous), Karstedt's Pt catalyst (2-3 wt% in xylene, $[(Pt)_2(H_2C{=}CH\text{-}SiMe_2OSiMe_2CH{=}CH_2)_3]$), 2-mercaptoethanol, CF_3SO_3H were purchased from Aldrich Chemical Co. Sylgard 184 (a platinum cured silicone rubber $H_2C{=}CH$-Silicone + HSi-silicone $\rightarrow$ Silicone-CH_2CH_2Si-silicone) and DC1107 $(MeHSiO)_n$ were purchased from Dow Corning (Midland, MI). Human serum albumin (HSA), Tyr-Ile-Gly-Ser-Arg (YIGSR), Arg-Gly-Asp-Ser (RGDS) and Sephadex G-25 columns were obtained from Sigma. Epidermal growth factor (EGF) was obtained from RDI. Fibrinogen was obtained from Enyzme Research Laboratories. Toluene was dried by refluxing over Na prior to distillation, and MeOH was dried by refluxing over Mg and was distilled before use.

(b) Materials Characterization

^{1}H and ^{13}C NMR spectra were recorded at 30 °C on a Bruker AC-200 spectrometer (at 200 MHz and 50.3 MHz for ^{1}H and ^{13}C, respectively).

Attenuated Total Reflection Fourier Transform IR Spectroscopy (ATR-FTIR) measurements were carried out on a Bruker VECTOR 22 Fourier transform infrared spectrometer (Bruker Instruments, Billerica, MA) equipped with Harrick ATR accessory MUP with GeS crystal; 200 scans were collected for each sample.

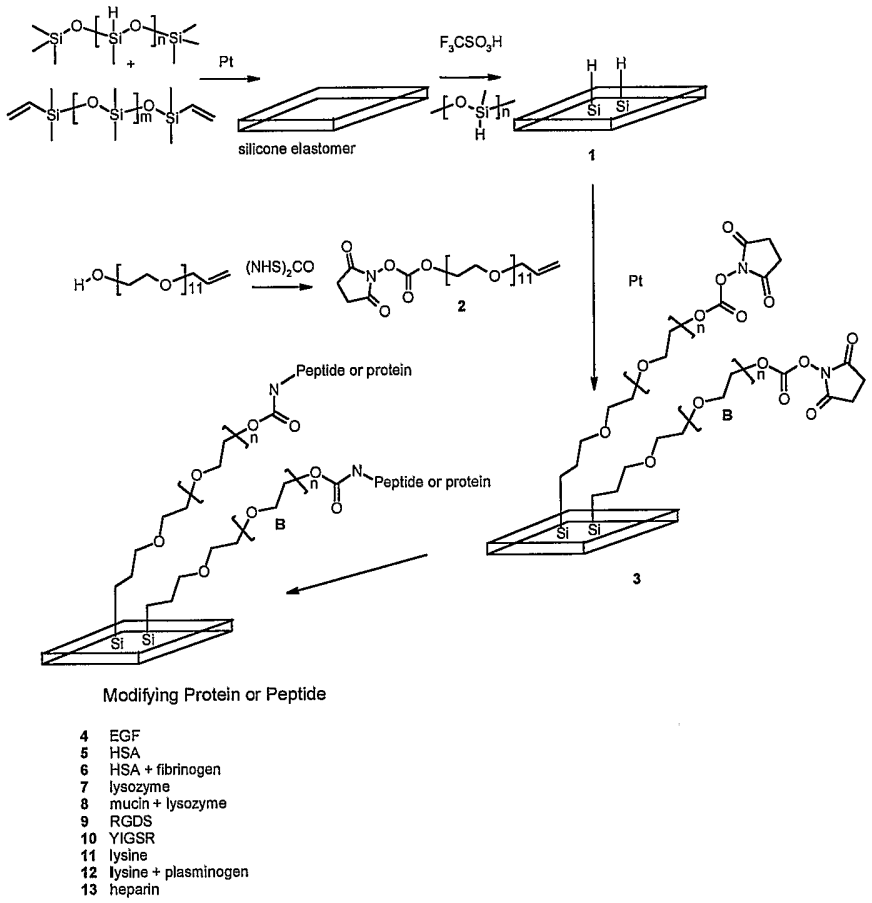
Electrospray mass spectra (ESI-MS) were recorded on a Micromass Quattro LC, triple quadruple MS.

1.2 Materials Characterization

Water contact angle Advancing and receding sessile drop contact angles were measured on PEO grafted surfaces using a Ramé Hart NRL C.A. goniometer (Mountain Lakes, NJ). Milli-Q water (18 MΩ/cm) was used with a drop volume of approximately 0.02 mL. Results are presented as an average of 18 measurements or more on at least three different surfaces. Contact angles were also measured using the captive bubble method, where an air bubble was injected from a syringe onto an inverted sample surface immersed into Milli-Q water. Results are presented as the average of at least 10 measurements on three different surfaces.

X-ray photoelectron spectroscopy (XPS) was performed at Surface Interface Ontario, University of Toronto using a Leybold Max 200 X-ray photoelectron spectrometer with a MgK-α non-monochromatic X-ray source.

Scheme 1



In the following examples refer to Scheme I for the structures corresponding to the compound numbers.

Example 1: Preparation of *N*-succinimidyl carbonate PEG grafted PDMS surfaces

(a) Synthesis of α-allyl-ω-N-succinimidyl carbonate-poly(ethylene glycol), 2

To a solution of poly(ethylene glycol) monoallylether (2.0 g, 4.0 mmol) and triethylamine (1.62 g, 16 mmol) in CH_3CN (10 mL) was added *N,N'*-disuccinimidyl carbonate (4.1 g, 16 mmol). The mixture was allowed to stir at room temperature over 10 h under N_2. After removal of the solvent in vacuo, the residue was dissolved in dry toluene (25 mL) and the solution was cooled to 0 °C. A pale brown precipitate was filtered off. The toluene was removed under reduced pressure. This procedure was repeated 3 times. The resultant compound **2** was a yellow oil (1.2 g, 60% yield). IR (neat): 1739 (NC=*O*), 1788 (OC=*O*). ^{1}H NMR (200.2 MHz, $CDCl_3$, Figure 10): δ 2.78 (s, 4H, O=CCH_2CH_2C=O), 3.57 (bs, 40H, PEG's OCH_2), 3.72 (bs, 2H,

$OCH_2CH_2OC=O$), 3.95 (d, 2H, J=5.6Hz, $CH_2=CHCH_2O$), 4.39 (m, 2H, $OCH_2CH_2OC=O$), 5.20 (m, 2H, $CH_2=CHCH_2O$), 5.82 (m, 1H, $CH_2=CHCH_2O$) ppm. ^{13}C NMR (50.3 MHz, $CDCl_3$): δ 25.2 ($O=CCH_2CH_2C=O$), 68.1 ($OCH_2CH_2OC=O$), 69.2 ($O=COCH_2CH_2O$), 70.4 (PEG's OCH_2), 72.0 ($CH_2=CHCH_2O$), 117.0 ($CH_2=CHCH_2O$), 134.5 ($CH_2=CHCH_2O$), 151.4 ($OC=OO$), 168.5 ($NC=OCH_2$) ppm. MS (ESI): m/z = 745.6 ($M+NH_4^+$, n=12, 100).

(b) Elastomer preparation

Silicone elastomers were prepared according to the procedure provided by Dow Corning. Sylgard 184 PDMS pre-polymer and catalyst was mixed thoroughly with its cross-linker in a 10:1 ratio (w/w) in a plate mold and degassed under vacuum. Films were allowed to cure at room temperature for 48 h. After curing, the silicone elastomer films were punched into disks, approximately 5 mm in diameter and 0.5 mm thick. The disks were washed with hexane and then dried under vacuum for further use.

(c) Si-H Surface Functionalization 1

For Si-H functionalization of the surface, 20 silicone elastomer disks were immersed in a mixture of DC1107 (3 mL) and methanol (5 mL). To this was added F_3CSO_3H (0.02 mL, 0.26 mmol). After stirring at room temperature for 30 min, the functionalized surfaces were rinsed with methanol and hexane, and dried under vacuum (for surface characterization, see below).

(d) Addition of PEG derivative: 3

Si-H modified silicone surfaces **1** were incubated in a solution of 2-methoxyethyl ether solvent and **2** (80:20 wt%:wt%, 3 mL). Pt-catalyst (platinum-divinyltetramethyldisiloxane complex, 1 drop) was added and the mixture was stirred for 15 h at room temperature. Following modification, the PEG modified surfaces **3** were washed thoroughly with dry acetone and dried under vacuum.

Example 2: Characterization of NHS and modified surfaces

ATR-FTIR

As described above, *N,N'*-disuccinimidyl carbonate was used to activate the hydroxy-terminal of α-allyl-ω-polyethylene glycol. The desired compound **2** was obtained as determined by ^1H NMR, with the resonance of the $-CH_2-CH_2-$ on the NHS (2.78 ppm) being diagnostic. Two types of C=O were observed on the NHS-activated termini, and the O-C(O)-O linkage were detected by ^{13}C NMR (168.8 ppm and 151.7

ppm, respectively). Assignment of the FT-IR spectrum of the NHS-activated PEO is outlined in Table 1. The band at 1739 cm^{-1}, representing the C=O stretch of the NHS group, can be used to further diagnose the succinimidyl carbonate PEG grafting process.

H-Si functionalized silicone surfaces **1** were obtained by acid-catalyzed equilibration of a silicone elastomer in the presence of $(MeHSiO)_n$ as noted above The ATR-FTIR spectra of the resulting surfaces exhibited a band at 2166 cm^{-1} due to the Si-H stretch. The succinimidyl carbonate PEO was grafted onto the silicone rubber surfaces via a hydrosilylation reaction with the H-Si groups. In the FTIR spectrum of the succinimidyl carbonate PEG grafted surfaces **3**, the band at 2166 cm^{-1} due to H-Si was no longer visible following the reaction. There were two C=O stretches at 1741 and 1789 cm^{-1}, respectively, that were assigned to the C=O groups at the succinimidyl carbonate termini, and the O-C(O)-O linkage, which was also present in the starting material. The PEO CH_2 scissoring band at 1454 cm^{-1}, the antisymmetric stretch mode of the CH_2-O-CH_2 chain at 1351 cm^{-1}, and the symmetric stretch mode of the CH_2-O-CH_2 chain at 1258 cm^{-1} indicated the presence of PEO chains at the resulting surface.

XPS

NHS-PEO binding to the surface **3** was further by the presence of an N1s signal in the XPS survey scan due to the amine groups in the NSC-PEO polymer. The C1s high resolution spectrum shows a distinct peak at 286.4 eV which corresponds to the C-C-O bond in PEO repeat unit.

Example 3: Conjugation of various molecules to the NHS-modified surface

(a) Peptide Conjugation

The covalent conjugation of peptide to the functionalized surfaces was carried out in a phosphate buffered saline (PBS) buffer solution (pH 7.5). The *N*-succinimidyl carbonate PEG grafted surfaces **3** were immersed in PBS buffer containing the peptide RGDS or YIGSR, (10 μg/mL) for 12 h to give **9** or **10**, respectively. After rinsing three times with PBS for 10 min, for a total of 30 min, the surfaces were dried under vacuum.

(b) Characterization

The IR spectra of modified surfaces **3**, **9** and **10**, respectively, are shown in Figure 1. Distinct bands at 1652 cm^{-1} and 1656 cm^{-1} (Table 1,) due to amide I, were observed on both the RGDS- and YIGSR-modified surfaces: the C=O stretch mode at 1741 cm^{-}

[1], due to the NHS group, disappeared in both cases following modification. These spectral changes indicated the coupling of the succinimidyl carbonate PEG to the peptides. Peptide immobilization was further demonstrated by an increase in the XPS N1s signal to 1.9 and 2.7% for RGDS and YIGSR, respectively, due to the amine groups in the peptides and a decrease in the Si2p signal as shown in Table 2. Note that the starting succinimidyl carbonate PEO-modified PDMS **3** showed very weak nitrogen peak due to the single nitrogen in NHS. The nitrogen intensity, post modification, was much higher (Figure 2).

(c) EGF Conjugation **4**

The covalent conjugation of EGF to the functionalized surfaces was carried out in a phosphate buffered saline (PBS) solution (pH 7.4). EGF was first labeled with ^{125}I (ICN Pharmaceuticals, Irvine CA) using the iodogen method. The *N*-succinimidyl carbonate PEG grafted surface **3** was immersed in a PBS buffer (pH 7.4) containing radiolabeled EGF (10 μg/mL) for 2 and 24 h, rinsed three times with PBS for 10 minutes each, (30 minutes total), wicked onto filter paper to remove residual adherent buffer, transferred to clean tubes, and their radioactivity determined by counting using a gamma counter. Radioactivity counts were converted to surface protein concentrations. One milliliter of a 2% sodium dodecyl sulfate (SDS) solution was then added to each tube and left at room temperature for 4 h and overnight at 4 °C. After three PBS rinses, the surfaces were transferred to clean tubes and radioactivity measured to determine the levels of EGF remaining after the SDS treatment, which indicated that the growth factor was covalently immobilized to the surface.

(d) Human serum albumin conjugation **5**

Human serum albumin was labeled with ^{125}I (ICN Pharmaceuticals, Irvine CA) using the ICl method. The labeled protein was passed through an AG 1-X4 column (Bio-Rad Laboratories, Hercules, CA, USA) to remove any free iodide. For measurement of non-specific adsorption of protein from buffer and covalent coupling of albumin to the surfaces, a mixture of labeled and unlabeled protein (1:20) at a total concentration of 1 mg/mL was prepared. NHS-PEO modified surfaces **3** were incubated with albumin for 2 h at room temperature, rinsed three times with PBS for 10 min, (250 μL per rinse per disk, 30 minutes total), wicked onto filter paper to remove residual adherent buffer, transferred to clean tubes, and the radioactivity determined by counting using a gamma counter. Radioactivity was converted to the protein amounts

bound to the surfaces. To confirm covalent attachment, one milliliter of a 2% sodium dodecyl sulfate (SDS) solution was then added to each tube and left overnight. After three 10 min rinses (250 μL per rinse), the surfaces were transferred to clean tubes and activity measured again to determine the levels of protein remaining after the SDS treatment.

A summary of adsorption or covalent grafting of albumin is shown in Figure 6. The albumin concentration was 0.226μg/cm^2 on the control silicone surfaces. Surfaces modified with the *N*-succinimidyl carbonate PEG **3** had less albumin, with a surface density of 0.179μg/cm^2; however this albumin is believed to be covalently bound. After both surfaces were treated with SDS solution for 24h, the albumin concentration on the control surface decreased to 0.056μg/cm^2 while albumin concentration on NHS-PEG modified surfaces remained almost unchanged at 93% of the original value (0.168μg/cm^2). This observation is consistent with the covalent binding of most of the albumin to the silicone through PEG spacers. Figure 6 shows the results of fibrinogen adsorption on the albumin pretreated surfaces.

(e) Fibrinogen adsorption **6**

Fibrinogen was labeled with ^{131}I (ICN Pharmaceuticals, Irvine CA) using the ICl method. The labeled protein was passed through an AG 1-X4 column (Bio-Rad Laboratories, Hercules, CA, USA) to remove any free iodide. The untreated control (PDMS elastomer) surface, ^{125}I-albumin pretreated control surface and ^{125}I-albumin pretreated NHS-PEG modified surfaces **5** were incubated in PBS solution containing the radiolabelled fibrinogen at a concentration of 1 mg/mL for 2h. The fibrinogen amounts on various surfaces were determined radioactively as described above (Figure 6).

(f) Conjugation of mucin **8**

NHS surfaces **3** were incubated in 5 mg/mL solution of mucin from bovine (submaxillary glands, Type I-S, Sigma) in PBS buffer (pH=8.0) for 6 h. Surfaces were subsequently rinsed three times with fresh PBS.

(g) Conjugation of lysozyme **7**

Lysozyme adsorption to various surfaces was carried out in a phosphate buffered saline (PBS, pH 7.4). Lysozyme was labeled with ^{125}I (ICN Pharmaceuticals, Irvine CA) using the ICl method. The *N*-succinimidyl carbonate PEG grafted surface **3**, PEG350 grafted surface, mucin modified surface and control surface, respectively,

were immersed in a PBS buffer (pH 7.4) containing (unlabeled : radiolabeled=9:1) lysozyme (1mg/mL) for 3 h, rinsed three times with PBS for 10 minutes each,(30 minutes total), wicked onto filter paper to remove residual adherent buffer, transferred to clean tubes, and their radioactivity determined by counting using a gamma counter. Radioactivity counts were converted to surface protein concentrations. One mL of a 2% sodium dodecyl sulfate (SDS) solution was then added to each tube and left at room temperature for 4 h and overnight at 4 °C. After three PBS rinses, the surfaces were transferred to clean tubes and the radioactivity was measured to determine the levels of lysozyme remaining after the SDS treatment (Figure7).

(h) Lysine surface ***11***

Surface **3** was incubated in solution of H-Lys(Fmoc)-OH (Chem-Impex International.Inc., 1 mg/mL) in hexafluoroisopropanol (HFIP, Aldrich) for 6 h. After rinsing three times with HFIP and then incubated in piperidine (Aldrich, 20% in DMF) for 2 h. Surfaces were washed with PBS buffer (10 mL) 3 times (1h/wash).

(i) Plasminogen adsorption from plasma

Plasminogen was radiolabeled with Na ^{125}I (ICN, Irvine, California), using the ICl method. Labeled plasminogen was added to pooled acid citrate dextrose human plasma as a tracer and then exposed to control surface, surface **3** and lysine grafted surface **11**, respectively, for 3h at room temperature. Surfaces were rinsed three times with fresh PBS prior to γ counting.

(j) Heparin Conjugation **13**

NHS surfaces **3** were incubated in 10 mg/mL solution of heparin (Sigma Aldrich) in PBS buffer (pH=8.0) for 6 h. Surfaces were subsequently rinsed three times with fresh PBS. The density of heparin on the NHS surface **13** is 0.68 μg/cm^2 as shown by the calibration curve (see next section). More than 90% of this heparin was active as determined by a hepanorm standard assay.

A series of heparin standard solutions with concentrations varying from 0 to 20 μg/mL were prepared by diluting a stock solution. The stock solution was obtained by dissolving 10 mg heparin in an aqueous 0.2 wt% NaCl solution.

Toluidine blue (Sigma-Aldrich Canada, 50 mg) was dissolved in HCl (1mL, 0.01 N solution), in which 0.2 wt% NaCl had been previously added and dissolved. The 50 mg/mL toluidine blue solution was diluted to a 0.005 mg/mL (0.0005%) toluidine blue solution with deionized water. The solution (1.0 mL) was added to a 5 mL tube,

then 0.1 mL of the above heparin standard solution was added. The mixed solution was vortexed by a Vortex mixer for 30 s. *n*-Hexane (Aldrich-Sigma Canada)1 mL was added and the solution was vigorously mixed for 30s, and then allowed to separate into 2 phases over 5 min. The heparin-toluidine blue complex was extracted into the upper transparent organic layer. After the organic layer was removed, the absorbance of the aqueous layer at 631nm was measured on a Beckman DU640UV/VIS spectrophotometer. A linear standard calibration curve was obtained by plotting absorbance at 631 nm versus concentration of heparin in the aqueous NaCl solution (Figure 12). The amount of heparin immobilized on the polymer surfaces was determined by this calibration curve. The activity of the heparin on the surface was determined using a hepanorm assay, based on the interaction of Factor Xa with heparin. Heparinized surfaces and standard solutions were incubated with hepanorm, antithrombin III in PBS buffer and the activity of the solutions and the heparinized surfaces determined.

Prior to testing, polymer samples were incubated in 0.05 M Tris-buffered saline (TBS) with pH7.4 at room temperature overnight to hydrate the surfaces. For each experiment, 0.1 mL of 0.2% NaCl solution and 1.0 mL of 0.005 mg/mL (0.0005%) toluidine blue solution were mixed in a 5 mL polypropylene test tube. The heparin-modified (polymer) surfaces **13** with 0.77 cm^2 area were immersed in the solution, which was vortexed for 30 s. Then, 1 mL *n*-hexane was added and well shaken. The mixture was allowed to phase-separate for 5 min after removal of the surfaces. As above, the upper organic layer was removed and the absorbance of the aqueous layer at 631nm was investigated on a Beckman DU640 UV/VIS spectrophotometer. The density of total heparin immobilized on the surfaces was calculated from the above calibration curve. For each surface, the heparin density was expressed by mass per unit surface area ($\mu g/cm^2$)

(k) Thromboresistant properties

Platelin® was obtained from Organon Teknila Corp., Durham, NC, USA (No. 35501). TBS/Ca^{2+}/Platelin® (0.1M $CaCl_2$ with a 1:10 dilution of platelin) buffer solution was made by dissolving $CaCl_2$ (1.11 g) and 4 standard vials of Platelin® in 10 mL of Milli-Q water (10 mL). The volume was then brought to 100 mL with TBS (0.05 M, pH=7.4). Thrombin substrate *N*-*p*-tosyl-gly-pro-arg *p*-nitroanilide (Sigma-

Aldrich) (5 mg) was dissolved in TBS (10 mL) to give a solution with a final concentration of 0.5 mg/mL.

In order to passivate the walls of the 96-wells microtitration plate, the wells were exposed to human serum albumin in TBS (10 mg/mL) overnight at 4 °C. The albumin solution was then withdrawn from the wells and the wells were aspirated and washed three times with fresh TBS (0.3 mL/well/time) before adding the unmodified and heparin modified silicone surfaces. The heparin-modified surface was incubated in antithrombin TBS buffer solution (0.25 mg/mL) for 30 minutes before testing.

The disks were placed vertically in the wells and 10% diluted pooled human citrated plasma (200 μL) was added to the wells. After the plate was warmed to 37 °C, TBS/Ca^{2+}/platelin buffer solution (20 μL) and thrombin substrate (30 μL of 0.5mg/mL) were added. The release of *p*-nitroaniline by thrombin was measured as a function of time by recording the optical density at 405 nm and 37 °C using a UV-Vis plate reader (Figure 9).

(l) Cell culture on peptide modified surfaces

Surfaces (~5 mm disks) **9** or **10** as well as controls were washed three times with PBS supplemented with antibiotics (penicillin, streptomycin and gentamycin) and subsequently stored overnight at 4 °C in Keratinocyte Serum Free Medium (KSFM, Invitrogen, Grand Island NY) medium containing antibiotics. Under sterile conditions, the surfaces were transferred to a 24 well plate and plated with human corneal epithelial cells (HCECs, 10^4 cells per well) in KSFM supplemented with penicillin, streptomycin, gentamycin and EGF. The cells were cultured at 37 °C in 5% CO_2. Samples were imaged at 24, 48, 72 and 96 h. All images were taken at 100x magnification (Figure 10).

Discussion for Examples 1-3

Unlike most polymers, silicones can be readily formed, and degraded, under thermodynamic control ([28]). Thus, treatment of monomers and/or polymers with endcapping molecules in the presence of acid or base, allows the preparation of homo- or copolymers of various molecular weights. By carefully controlling the swelling conditions, using relatively poor solvents for silicone such as methanol, it was possible to preferentially introduce Si-H surface functional groups to a variety of pre-cured silicone elastomers giving **1** by a redistribution polymerization with triflic acid ([29]), as readily shown by the characteristic strong IR absorption at 2166 cm^{-1}

(Figure 1, Table 1). This group underwent efficient hydrosilylation with a series of olefins, including allyl-PEO and, more importantly[13], allyl-PEO-NHS **2**, prepared by the reaction of allyl-PEO-OH with bis-*N*-hydroxysuccinimidyl carbonate (Scheme 1) to give a high density, reactive NHS surface **3**. Surface properties (ATR-IR, XPS, Figure 1, Figure 2) were determined using traditional methods (see Experimental Section).

The NHS group was chosen as the functional group to link surface **3** to biomolecules because it is mild, selective for amines over alcohols, and reacts with both groups much faster than with water. A series of proteins, peptides and amino acids including epidermal growth factor (EGF, **4**), human serum albumin (HSA, **5**) plus fibrinogen (**6**), lysozyme (**7**), mucin plus lysozyme (**8**), the cell adhesion peptides Arg-Gly-Asp-Ser (RGDS, **9**) and Tyr-Ile-Gly-Ser-Arg (YIGSR, **10**), lysine (**11**), lysine plus plasminogen (**12**) and heparin (**13**) were bonded to the modified silicone **3** in phosphate buffered saline (PBS) solutions (pH=8.0). The resulting surfaces were characterized by the techniques mentioned above (Figure 1, Figure 2, Figure 3, Figure 4, Table 2). The total quantities of the linked and adsorbed peptides or proteins were determined by radioactivity assays before and after exhaustively washing the modified surface with sodium dodecyl sulfate (SDS). This method also provided a minimum estimate of the total graft density of the surface. For example, after 24 hours of reaction with EGF, the resulting surface **4** exhibited a surface concentration of 190 ng/cm^2 (*ca.* 0.2 EGF molecules/nm^2): After washing, the control surface showed 26 ng/cm^2 while the EGF-g-PEO surface was essentially unchanged (Figure 5). This surface concentration is comparable to the high densities found on model SAM-modified gold surfaces ([30]). Since the molecular weight of EGF is *ca.* 6000, the graft density of 0.2/nm^2 is presumably an underestimation of available Si-H groups and of the PEO density; some of the active NHS groups on the surface will likely be sterically blocked by covalently linked protein. Lysozyme (MW *ca.* 14000) was analogously grafted to the surface. After extensive washing with SDS, 402 ng/cm^2 (0.15 molecules/nm^2) remained, giving an even more efficient surface coverage than with EGF. Similarly, heparin was found on the surface with a graft density of 0.68 μg/cm^2 (see below).

In order to assay the bioactivity of the grafted EGF, the surface **4** was cultured with human corneal epithelial cells in the absence of serum. However, unlike other studies

in which various proteins including EGF and a bovine pituitary extract are added back to the medium, the cells were cultured in medium with antibiotics only, eliminating any potential exogenous effects. Patches of cell growth were clearly evident on the EGF modified surfaces; there were no cells adherent on either the bare PDMS or on the PEO modified PDMS surfaces, demonstrating that the EGF attached to the surfaces was active and able to stimulate cell proliferation and extracellular matrix production (Figure 13).

Albumin, the most abundant protein in blood, can be used to passivate implanted synthetic surfaces ([31]). Less protein was initially found on the NHS-modified surface **5** than on the control (0.22 vs 0.18 $\mu g/cm^2$, Figure 6A). However, the control surface was mostly washed free of the ^{125}I-labeled protein with SDS (0.05 $\mu g/cm^2$ remained), while 0.17 $\mu g/cm^2$ remained on the functionalized surface. This data is consistent with initial protein physisorption that was converted to chemisorption **5**, before the protein can migrate across the NHS surface to form a monolayer. That is, the albumin binds on contact, leaving a non-coherent film and accessible interstitial areas. Attempts to form a coherent albumin film prior to covalent linkage, by controlling the rate of surface binding, have not so far been successful.

The ability to displace the albumin by ^{131}I-labeled fibrinogen, from control and **5** surfaces, respectively, was examined. Fibrinogen adsorbed effectively to the control surface, without accompanying loss of the albumin already present there, whereas much less fibrinogen was able to contact and bind to the albumin passivated NHS surface Figure 6B to give **6** (control 0.58 ng/cm^2 vs **6** 0.045 ng/cm^2) both before and after washing with SDS Figure 6C. This is consistent with a control surface in which albumin can be "nudged aside" by fibrinogen, but a covalently linked surface **5** in which only a few interstitial spaces are sufficiently large to accommodate the large fibrinogen molecule (340 kD) giving **6**.

Lysozyme, one of the proteins responsible for ophthalmic disinfection, was exposed to a variety of modified silicones. Significantly more lysozyme was adsorbed to the pre-grafted mucin **8** and NHS-surfaces **3** (0.173 molecules/nm^2) than the control or simple PEO surfaces ([13,32,33])(Figure 7). The natural surface with which lysozyme interacts in the eye is mucin ([34]). Thus, this modified polymer may prove useful as a model system to examine surface fouling by lysozyme in ophthalmic applications.

Analogous chemistry may be used to prepare a lysine rich surface. Exposure of Fmoc-protected lysine (the ε amine group was protected by Fmoc) to **3** followed by deprotection with piperidine led to the amino acid (containing a free ε amine group) - modified surface **11**. It is now established that lysine rich surfaces are particularly attractive to plasminogen, which both recognizes and binds the amino acid ([35,36,37]). It was demonstrated that **3** is a generic surface to which amines will bind. However, as shown in Figure 8, plasminogen adsorbs only marginally more effectively to **3**, (giving **12**), than to the control silicone. By contrast, a ten fold increase in plasminogen adsorption is found on the lysine surface. Previous reports([35]) showed that surface-bond plasminogen was readily converted to plasmin in the presence of tissue plasminogen activator(t-PA), and enzymatic activity increased with increasing surface density of ε-amine free lysine. The disclosed surface design exploits surface bound lysine through PEO as spacer that can repel non-specific proteins and selectively bind plasminogen. It furthermore demonstrates that binding via interaction with lysine is far more effective (four fold) than the formation of a covalent linkage with the surface.

Previous work has demonstrated that surfaces with high densities of conjugated lysine, prepared using an industrially developed process, are able to lyse incipient clots ([34,35,36]). In the current work, a similar assay was performed with lysinated surfaces **12**. The activity of the plasmin (plasminogen was activated by t-PA) on these surfaces was clear. There was little or no clot formation based on the assay parameters used (increase in optical density) suggesting that these surfaces are highly non-thrombogenic. Current experiments are examining whether **12** is acting as an inhibitor of clot formation and/or whether the surface is simply extremely efficient at clot lysis. Irrespective, the surface shows a lower degree of clot formation than those previously described ([34,35,36]).

Heparin, a highly sulfonated, anionic polysaccharide that is a well known antithrombotic agent, was analogously grafted with high density and high activity (0.68 $\mu g/cm^2$, ~90%) to the **3** surface giving **13**. The surface was subsequently exposed to thrombin, via interaction of $CaCl_2$ with plasma, in the presence of the chromogenic substrate *N-p*-tosyl-gly-pro-arg *p*-nitroanilide (the generation of thrombin would normally be expected in a relatively static system such as that used). Release of the *p*-nitroaniline hydrolysis product was followed over 3 hours (Figure 9).

Although nitroaniline was formed in the presence of **13**, it did so at a significantly lower rate and with a significantly longer half life than the case observed with plasma alone, or in the presence of the control silicone surface or the NHS-PEO-modified surface **3**. The heparinized surface is demonstrably less thrombogenic than the other surfaces examined or those described in previous reports ([38]).More interesting was the observation that antithrombin **3** complexed to the heparin surface **13** giving **14** silicone surface was far more efficient at inhibiting thrombin than AT3 directly bound to the surface (Figure 14).

An important consideration for any biomedical surface is the degree to which it is accepted by the local biological environment. Human corneal epithelial cells (HCEC) were cultured on NHS surfaces modified with the cell adhesion peptides RGDS and YIGSR, **9** and **10**, respectively, under serum free conditions. As shown in Figure 10, cells readily adhere to, spread and mitose on the peptide modified surfaces **9** and **10** to give confluent monolayers in less than 96 hours. Significantly lower levels of confluence were observed on both the control and PEO modified surfaces, respectively: even with highly biocompatible PEO-modified silicone surfaces ([13]), confluent layers of corneal cells have previously been not possible to achieve.

Several research groups have previously examined methods to generically graft biomolecules to surfaces. For example, NHS surfaces were prepared as self assembled monolayers on gold surfaces ([29]). However, these surfaces are not readily adaptable to complex devices themselves, or to coatings on devices comprised of other polymers. The surfaces described herein were shown to bind comparable or higher levels of biomolecules even when compared to model gold systems.

It is extremely easy to form complex shapes or to conformally coat a variety of substrates with silicones. The surface modifications described in the present work are amenable to any silicone elastomer, irrespective of cure chemistry. This process offers advantages over previous methods because activating groups, such as NHS groups, can be introduced to the surface in high density; this is facilitated by the absence of water, such that PEO swelling is reduced ([9]). The activating groups, for example the NHS groups, provide a generic route to graft biomolecules to the surfaces.

The generic nature of these modified silicone surfaces is amply demonstrated by the wide variety of biomolecules that can be readily grafted to them, and the maintenance of their bioreactivity after modification, which compatibilizes the surface.

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Where a term in the present application is found to be defined differently in a document incorporated herein by reference, the definition provided herein is to serve as the definition for the term.

Table 1. Assignment of FT-IR spectra of succinimidyl carbonate PEG and modified surfaces (see Figure 1 for spectra).

Peak	Wavenumber (cm^{-1})					Assignment
	NHS-PEO	H-Si surface	NHS-PEO surface	RGD surface	YIGSR surface	
1	2965	2966	2961	2961	2961	C-H stretch
2	2868		2873	2874	2874	Glycol CH_2 stretch
3	—	2166	—	—	—	Si-H stretch
4	1788	—	1789			C=O (on O-C(O)-O linkage) stretch
5	1740	—	1741	—	—	C=O (on NHS group) stretch
6	1717	—	1715	1713	1713	
7	—	—	—	1652	1656	Amide I stretch
8	1454		1456	1449	1453	Glycol CH_2 scissoring
9	1410	1410	1411	1411	1409	
10	1351		1351	1348	1350	Glycol (OCH_2-CH_2) chain antisymmetric stretch

[a] Refers to peak numbers in Figure 1

Table 2. XPS Surface Analysis of Unmodified PDMS, succinimidyl carbonate PEO modified **3**, RGDS-PEO modified **9** and YIGSR-PEO modified **10** PDMS surfaces

Elemental	Control PDMS	NHS-PEO	RGDS-PEO	YIGSR-PEO
C	46	52.2	54.6	55.4
N	0	0.9	1.9	2.7
O	26.5	26.3	25.2	26.5
Si	27.4	20.6	18.4	15.5

FULL CITATIONS FOR DOCUMENTS REFERRED TO IN THE SPECIFICATION

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WHAT IS CLAIMED IS:

1. A silicone polymer having a modified surface wherein said modification consists of a covalently attached water soluble polymer bearing an activating group, wherein said activating group reacts with reactive functionalities on one or more biological molecules so that said one or more biological molecules become covalently attached to said silicone polymer.

2. The silicone polymer according to claim 1, wherein the water soluble polymer is selected from polyethers, polyalcohols, polysaccharides, poly(vinyl pyridine), polyacids, polyacrylamides and polyallylamine (PAM).

3. The silicone polymer according to claim 2, wherein the water soluble polymer is selected from polyethylene oxide (PEO), polyethylene glycol (PEG), amino-terminated polyethylene glycol (PEG-NH_2), polypropylene glycol (PPG), polypropylene oxide (PPO), polypropylene glycol bis(2-amino-propyl ether) (PPG-NH_2), polyvinyl alcohol, dextran, poly(vinyl pyridine), poly(acrylic acid), poly(*N*-isopropylacrylamide); (polyNIPAM) and polyallylamine (PAM).

4. The silicone polymer according to claim 3, wherein the water soluble polymer is PEO.

5. The silicone polymer according to claim 4, wherein the PEO has a molecular weight of up to about 2000 g/mol.

6. The silicone polymer according to claim 4, wherein the PEO has a molecular weight of up to about 1000 g/mol.

7. The silicone polymer according to any one of claims 1-6, wherein the activating group is an activating group used in peptide synthesis and the reactive functionalities on the biological molecule comprises a nucleophile.

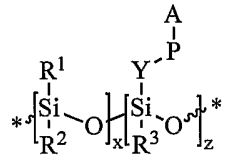
8. The silicone polymer according to claim 7, wherein the activating group is selected from a carbodiimide, an anhydride, an activated ester and an azide,

9. The silicone polymer according to claim 7 or 8, wherein the nucleophile is an amine, alcohol or thiol.

10. The silicone polymer according to claim 9, wherein the nucleophile is an amine or alcohol.

11. The silicone polymer according to any one of claims 1-10 which is tethered to another polymer through crosslinking or which is part an interpenetrating network or which is an elastomeric species formed by bridging with adjacent polymer chains.

12. The silicone polymer according to claim 1, having the general Formula I:



I

wherein

x is an integer between, and including, 1- 20000;

z is an integer between, and including, 1 and 1000;

R^1, R^2 and R^3 are each, independent of one another, selected from H, C_{1-30}alkyl, C_{2-30}alkenyl, C_{2-30}alkynyl and aryl, with the latter four groups being unsubstituted or substituted with one or more groups independently selected from halo, OH, NH_2, NHC_{1-6}alkyl, $N(C_{1-6}alkyl)(C_{1-6}alkyl)$, OC_{1-6}alkyl and halo-substituted C_{1-6}alkyl;

Y is a linker group;

P is a water soluble polymer; and

A is an activating group wherein said activating group reacts with reactive functionalities on one or more biological molecules so that said one or more biological molecules become covalently attached to said silicone polymer.

13. The polymer according to claim 12, which is tethered to another polymer using the substituents on R^1, R^2 and/or R^3, or through crosslinking, or which is part an interpenetrating network.

14. The polymer according to claim 12 or 13, wherein x is an integer between and including, 5 - 600.

15. The polymer according to any one of claims 12-14, wherein z is an integer between and including, 1 – 60.

16. The polymer according to any one of claims 12-15, wherein R^1, R^2 and R^3 are each, independent of one another, selected from H, C_{1-10}alkyl, C_{2-10}alkenyl, C_{2-10}alkynyl and aryl, with the latter four groups being unsubstituted or substituted with one or more groups independently selected from halo, OH, NH_2, NHC_{1-4}alkyl, $N(C_{1-4}alkyl)(C_{1-4}alkyl)$, OC_{1-4}alkyl and halo-substituted C_{1-4}alkyl.

17. The polymer according to claim 16, wherein R^1, R^2 and R^3 are each, independent of one another, selected from H, C_{1-4}alkyl, C_{2-4}alkenyl, C_{2-4}alkynyl and phenyl, with the latter four groups being unsubstituted or substituted with one or more groups independently selected from Cl, F, OH, NH_2, $NHCH_3$, $N(CH_3)_2$, OCH_3 and CF_3.

18. The polymer according to claim 17, wherein R^1, R^2 and R^3 are each, independent of one another, selected from H, C_{1-4}alkyl, C_{2-4}alkenyl and C_{2-4}alkynyl.

19. The polymer according to claim 18, wherein R^1, R^2 and R^3 are each CH_3.

20. The polymer according to any one of claims 12-19, wherein Y comprises at least one CH_2 group between the silicon atom and the polymer, P.

21. The polymer according to claim 20, wherein Y is $-(CH_2)_t-$, and t is an integer between and including 1 and 30.

22. The polymer according to claim 21, wherein t is an integer between, and including, 1 and 10.

23. The polymer according to claim 22, wherein t is 3.

24. The polymer according to any one of claims 12-23, wherein P is selected from polyethers, polyalcohols, polysaccharides, poly(vinyl pyridine), polyacids, polyacrylamides and polyallylamine (PAM).

25. The polymer according to claim 24, wherein P is selected from polyethylene oxide (PEO), polyethylene glycol (PEG), amino-terminated polyethylene glycol (PEG-NH_2), polypropylene glycol (PPG), polypropylene oxide (PPO), polypropylene glycol bis(2-amino-propyl ether) (PPG-NH_2), polyvinyl alcohol, dextran, poly(vinyl pyridine), poly(acrylic acid), poly(*N*-isopropylacrylamide); (polyNIPAM) and polyallylamine (PAM).

26. The polymer according to claim 25, wherein P is PEO.

27. The polymer according to claim 26, wherein the PEO has a molecular weight of up to about 2000 g/mol.

28. The polymer according to claim 27, wherein the PEO has a molecular weight of up to about 1000 g/mol.

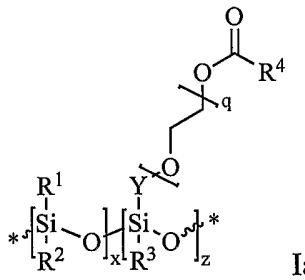
29. The polymer according to any one of claims 12-28, wherein A is an activating group used in peptide synthesis and the reactive functionalities on the biological molecule comprise a nucleophile.

30. The polymer according to claim 29, wherein A is selected from a carbodiimide, an anhydride, an activated ester and an azide,

31. The polymer according to claim 29 or 30, wherein the nucleophile is an amine, alcohol or thiol.

32. The polymer according to claim 31, wherein the nucleophile is an amine or alcohol.

33. The polymer according to claim 12, having the Formula Ia:



Ia

wherein

x is an integer between, and including, 1- 20000;

z is an integer between, and including, 1 and 1000;

R^1, R^2 and R^3 are each, independent of one another, selected from H, C_{1-30}alkyl, C_{2-30}alkenyl, C_{2-30}alkynyl and aryl, with the latter four groups being unsubstituted or substituted with one or more groups independently selected from halo, OH, NH_2, NHC_{1-6}alkyl, $N(C_{1-6}alkyl)(C_{1-6}alkyl)$, OC_{1-6}alkyl and halo-substituted C_{1-6}alkyl;

Y is a linker group;

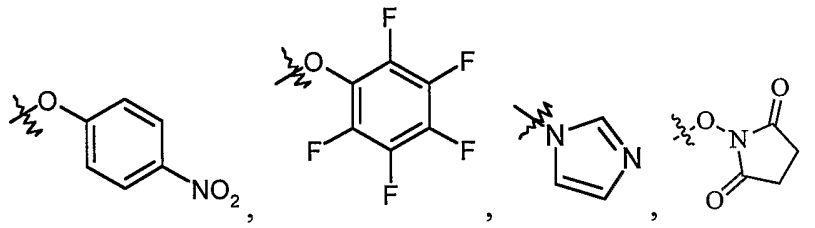
q is an integer between, and including, 1-225; and

R^4 is an activating group which activates the adjacent carbonyl group so that nucleophilic functionalities on one or more biological molecules will react therewith and said one or more biological molecules become covalently attached to said silicone polymer.

34. The polymer according to claim 33, wherein q is an integer between, and including, 2 and 100,.

35. The polymer according to claim 34, wherein q is an integer between, and including, 4 and 11.

36. The polymer according to any one of claims 33-35, wherein R^4 is selected from *p*-nitrophenyl (i), perfluorophenyl (ii), imidazolyl (iii) or related *N*-heterocycles and *N*-hydroxysuccinimidyl (iv) (NHS):



(i) (ii) (iii) (iv)

37. The polymer according to claim 36, wherein R^4 is NHS.

38. A method of preparing a biocompatible silicone material comprising reacting polymers according to any one of claims 1-37 with one or more biological molecules bearing reactive functionalities, so that the one or more biological molecules becomes covalently attached to said polymers.

39. The method according to claim 21-38, wherein the reactive functionality is a nucleophile and the reaction is carried out in a buffer at a pH of about 5-9.5.

40. The method according to claim 38 or 39, wherein the one or more biological molecules comprises amino acids, proteins or peptides.

41. The method according to claim 40, wherein the one or more biological molecules is selected from growth factors, human serum albumin, human serum albumin plus fibrinogen, lysozyme, mucin plus lysozyme, a cell adhesion peptide, lysine, lysine plus plasminogen and heparin.

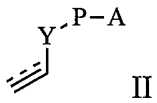
42. A biocompatible silicone material prepared using the method according to any

one of claims 38-41.

43. A method of coating a surface to modulate biocompatability comprising applying silicone material according to claim 42 to said surface.

44. The method according to claim 43, wherein the coated surface is used in biodiagnostic, biosensor or bioaffinity applications, or for coatings for *in vivo* transplantation or for liners exposed to biological broths.

45. A method of preparing a compound of Formula I according to any one of claims 1-37 comprising reacting a compound of Formula II:



II

wherein

P is a water soluble polymer;

Y is a linker group;

≡ represents a double or triple bond; and

A is an activating group wherein said activating group reacts with reactive functionalities on one or more biological molecules,

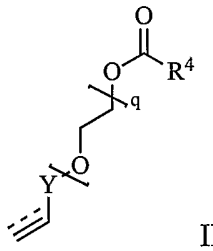
with a silicone material bearing reactive Si-H functional groups under hydrosilylation conditions.

46. The method according to claim 45, wherein the hydrosilylationinclude in the presence of a platinum catalyst and in a solvent at ambient temperatures.

47. The method according to claim 46, wherein the platinum catalyst is platinum-divinyltetramethyldisiloxane complex (Karstedt's catalyst).

48. The method according to any one of claims 45-47, wherein ≡ represents a double bond.

49. The method according to any one of claims 45-47, wherein the compound of Formula II has the Formula IIa:



IIa

wherein

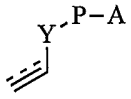
represents a double or triple bond; and

Y is a linker group;

q is an integer between, and including, 1-225; and

R^4 is an activating group which activates the adjacent carbonyl group so that nucleophilic functionalities on one or more biological molecules will react therewith and said one or more biological molecules become covalently attached to said silicone polymer.

50. A compound of Formula II:



II

wherein

P is a water soluble polymer;

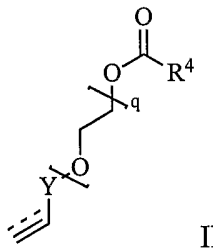
Y is a linker group;

represents a double or triple bond; and

A is an activating group wherein said activating group reacts with reactive functionalities on one or more biological molecules.

51. The compound according to claim 50, wherein represents a double bond.

52. The compound according to claim 50 or 51, having the Formula IIa:



IIa

wherein

≡ represents a double or triple bond; and

Y is a linker group;

q is an integer between, and including, 1-225; and

R^4 is an activating group which activates the adjacent carbonyl group so that nucleophilic functionalities on one or more biological molecules will react therewith and said one or more biological molecules become covalently attached to said silicone polymer.

Figure 1

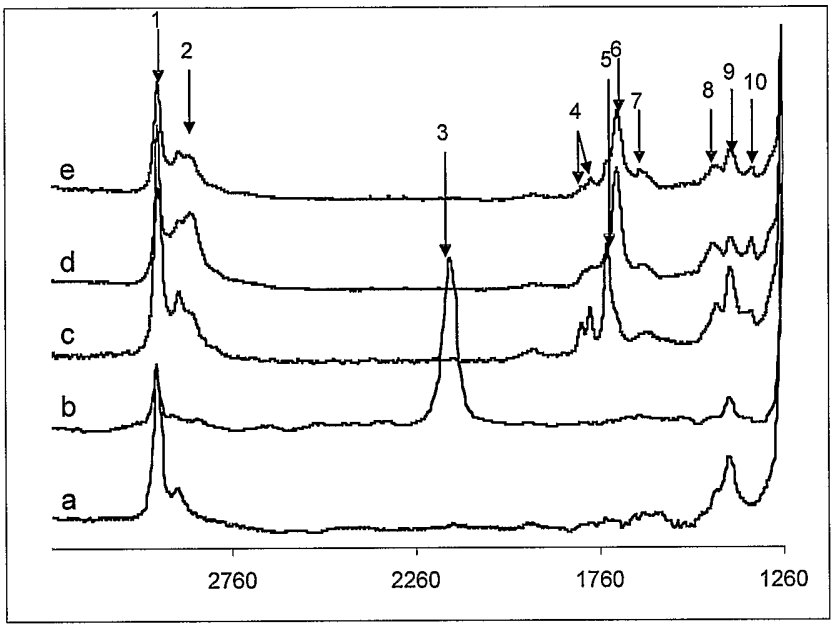


Figure 2

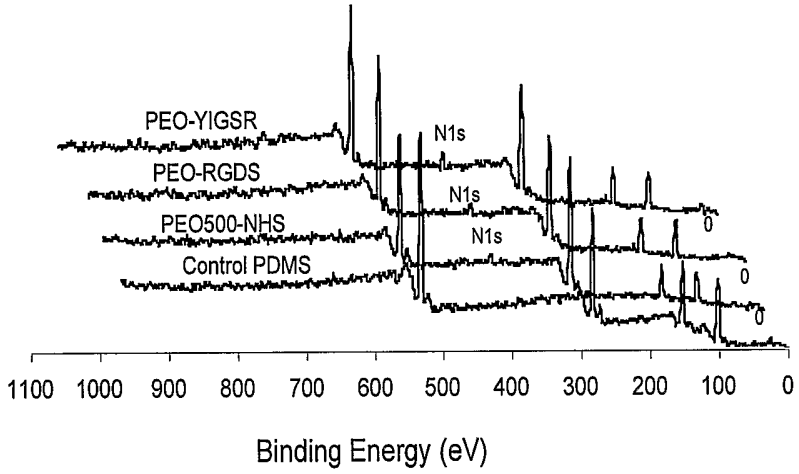

PEO-YIGSR
N1s
PEO-RGDS
N1s
PEO500-NHS
N1s
Control PDMS
0
0
0
1100 1000 900 800 700 600 500 400 300 200 100 0
Binding Energy (eV)

Figure 3

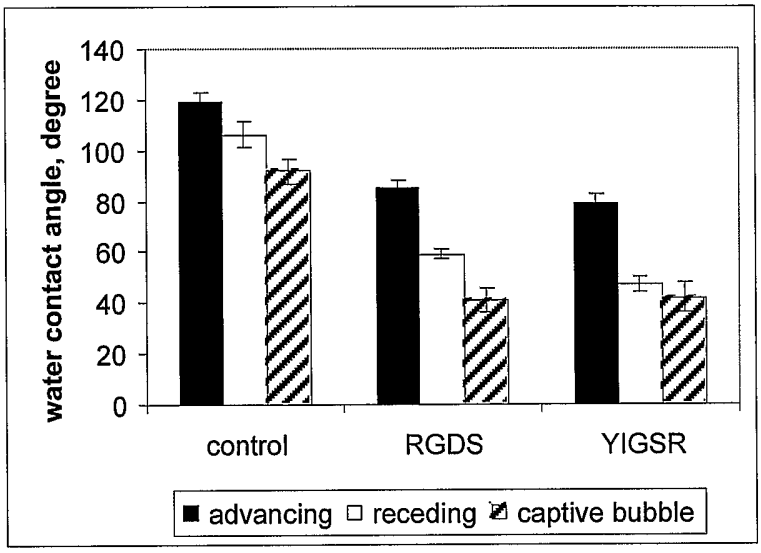

water contact angle, degree
140
120
100
80
60
40
20
0
control
RGDS
YIGSR
advancing
receding
captive bubble

Figure 4

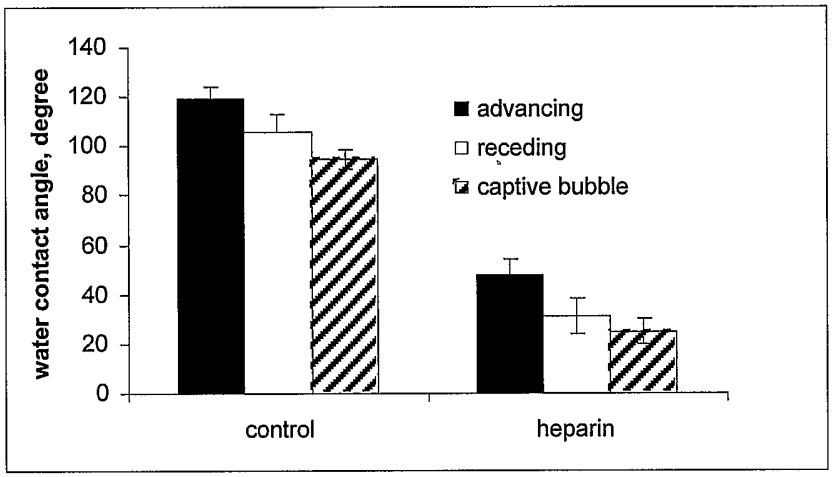

water contact angle, degree
140
120
100
80
60
40
20
0
advancing
receding
captive bubble
control
heparin

Figure 5

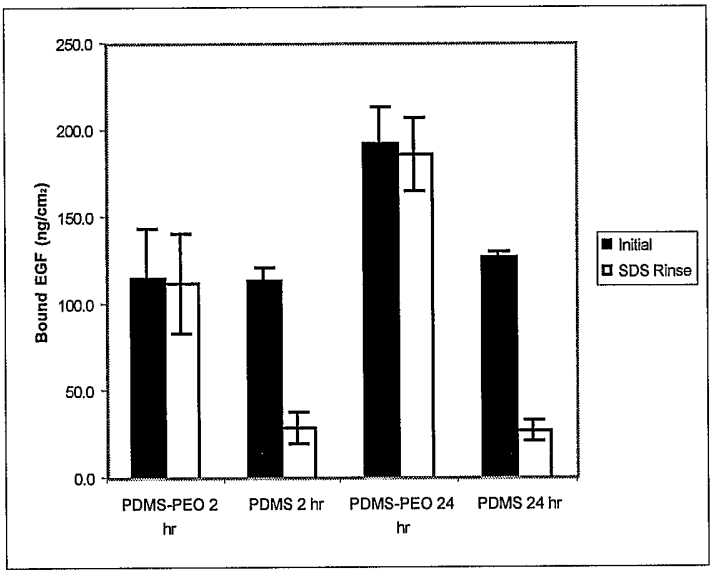
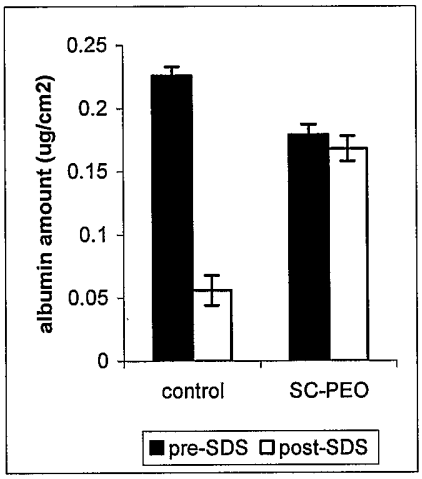
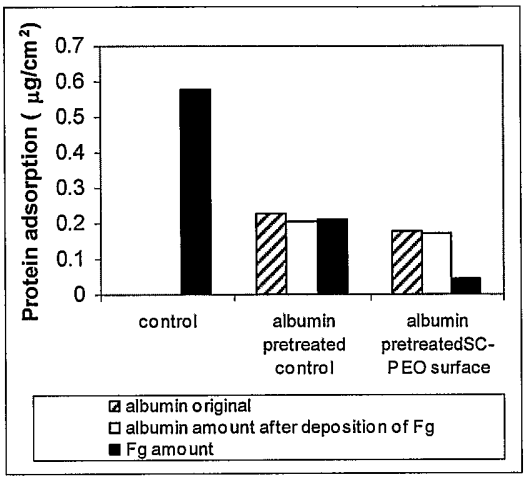

250.0
200.0
150.0
100.0
50.0
0.0
Bound EGF (ng/cm2)
PDMS-PEO 2 hr
PDMS 2 hr
PDMS-PEO 24 hr
PDMS 24 hr
Initial
SDS Rinse

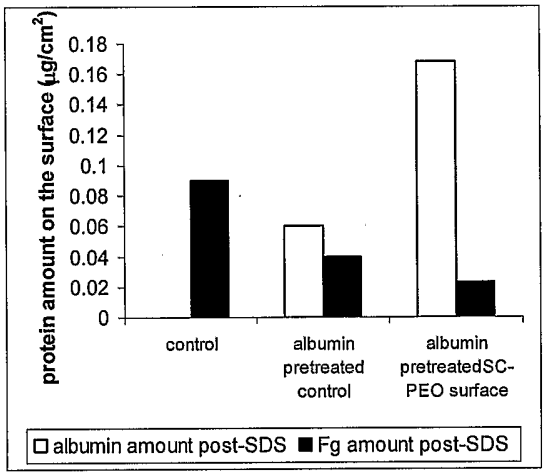
Figure 6


albumin amount (ug/cm2)
0.25
0.2
0.15
0.1
0.05
0
control
SC-PEO
pre-SDS
post-SDS

A

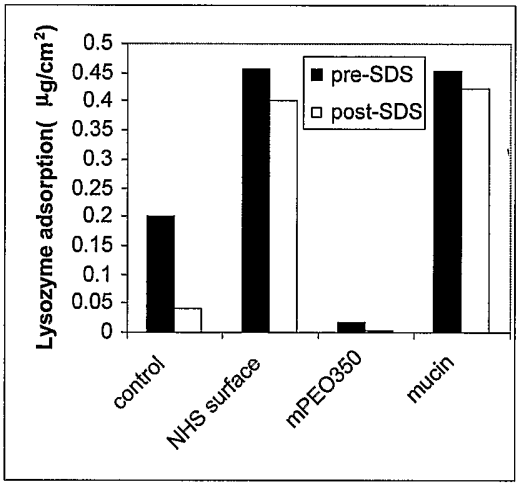

Protein adsorption (µg/cm²)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
control
albumin pretreated control
albumin pretreatedSC-PEO surface
albumin original
albumin amount after deposition of Fg
Fg amount

B

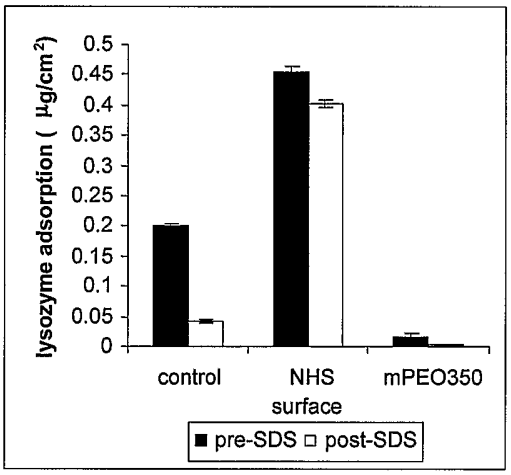

protein amount on the surface (µg/cm²)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
control
albumin pretreated control
albumin pretreatedSC-PEO surface
albumin amount post-SDS
Fg amount post-SDS

C

Figure 7


Lysozyme adsorption(μg/cm²)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
pre-SDS
post-SDS
control
NHS surface
mPEO350
mucin

A


lysozyme adsorption (μg/cm²)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
control
NHS surface
mPEO350
pre-SDS post-SDS

B

Figure 8

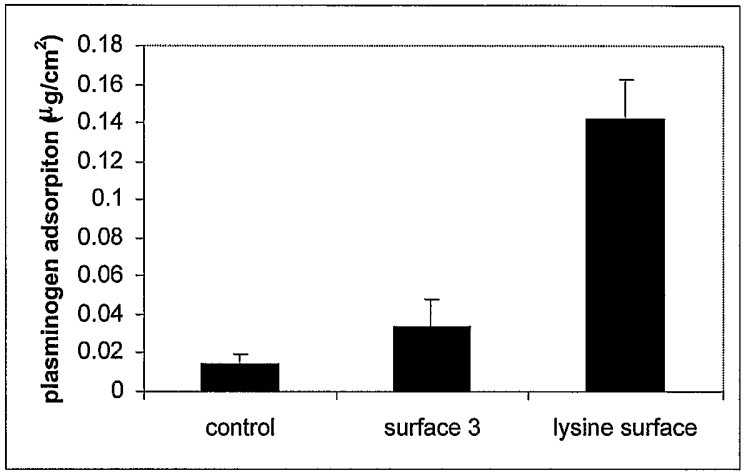

plasminogen adsorpiton (µg/cm²)
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
control
surface 3
lysine surface

Figure 9

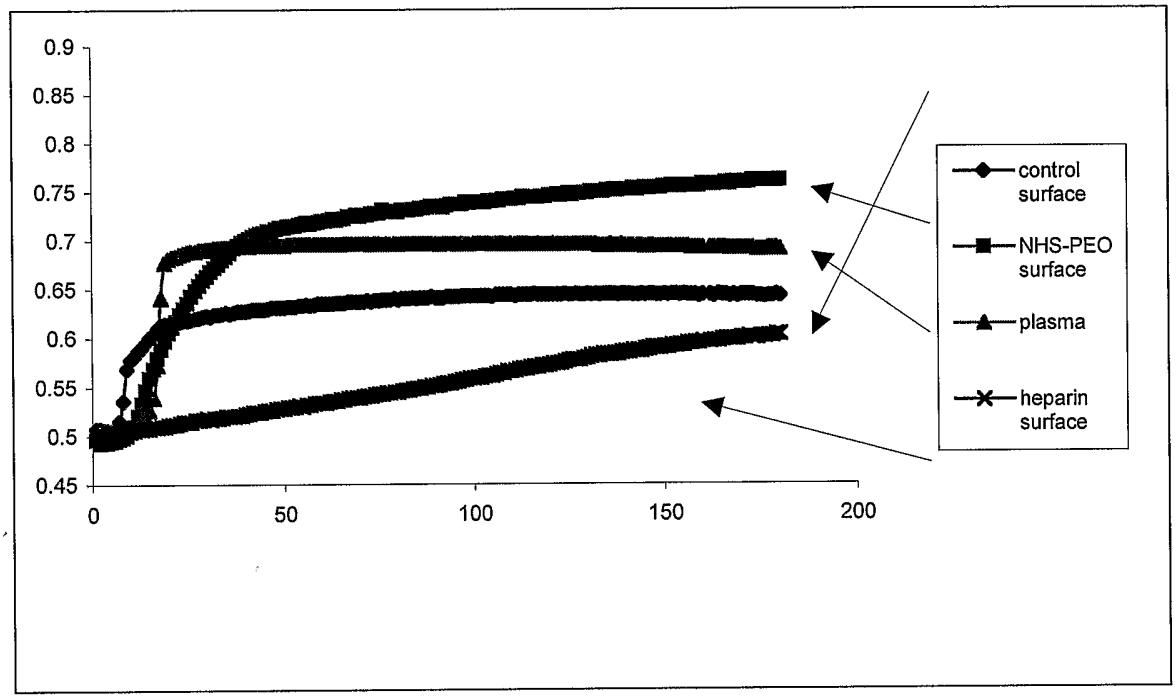

0.9
0.85
0.8
0.75
0.7
0.65
0.6
0.55
0.5
0.45
0
50
100
150
200
control surface
NHS-PEO surface
plasma
heparin surface

Figure 10

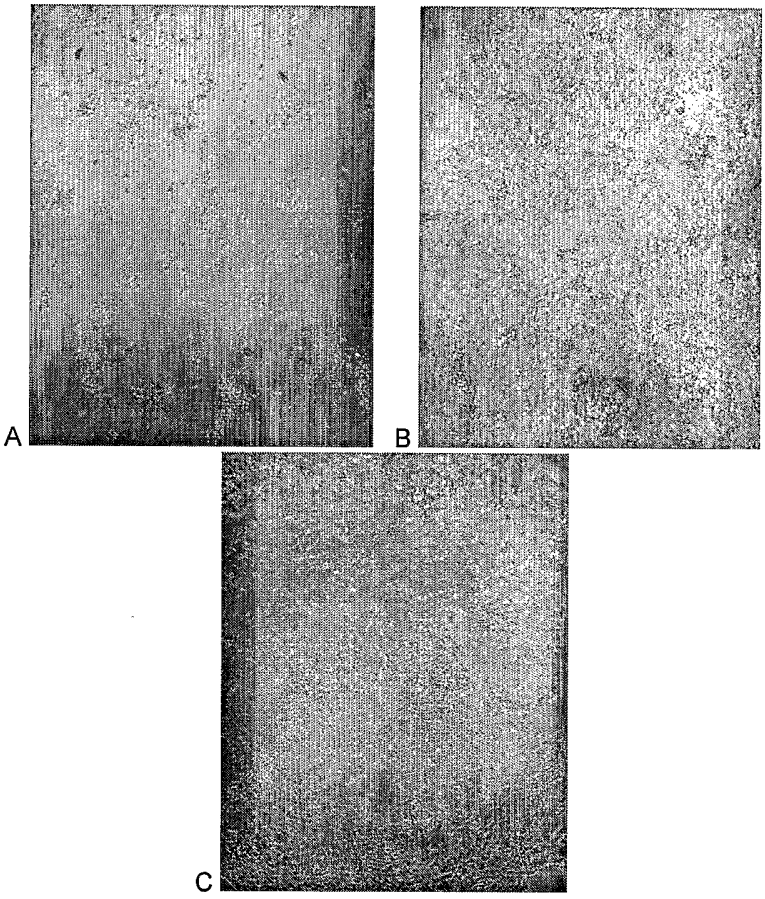
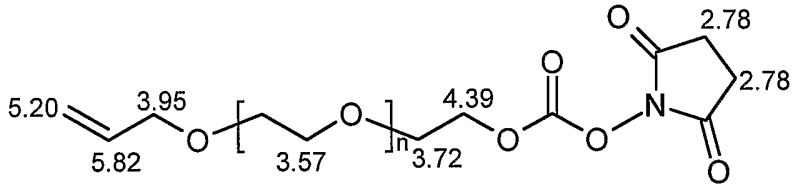


Figure 11

1H NMR assignment



13C NMR assignment

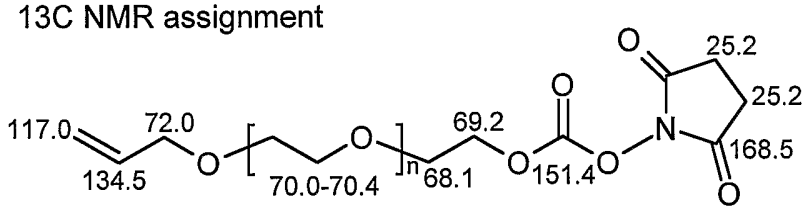


Figure 12

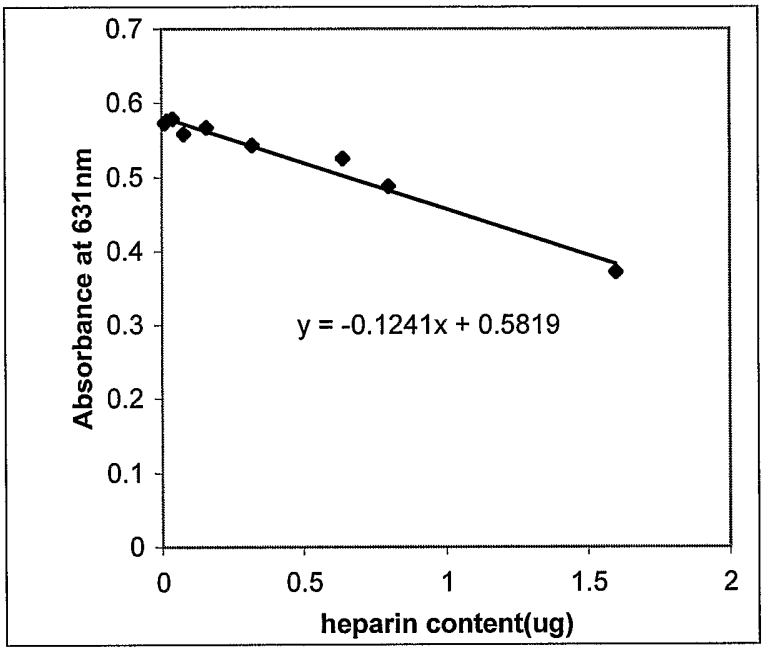

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Absorbance at 631nm
y = -0.1241x + 0.5819
0
0.5
1
1.5
2
heparin content(ug)

Figure 13

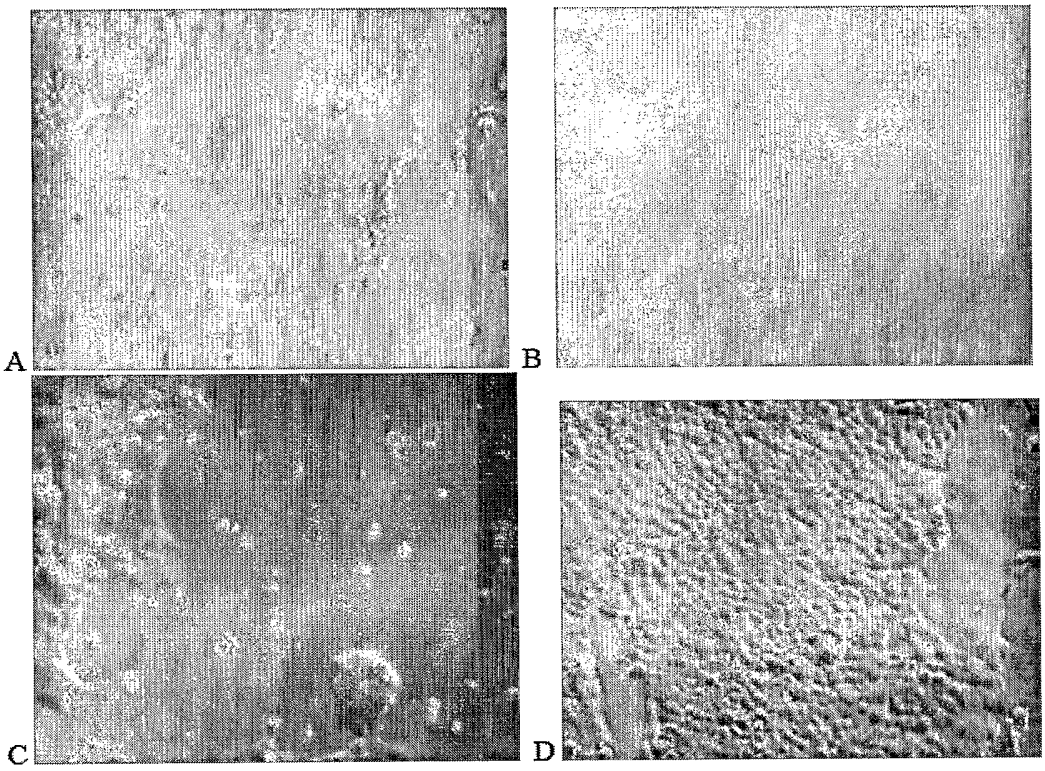
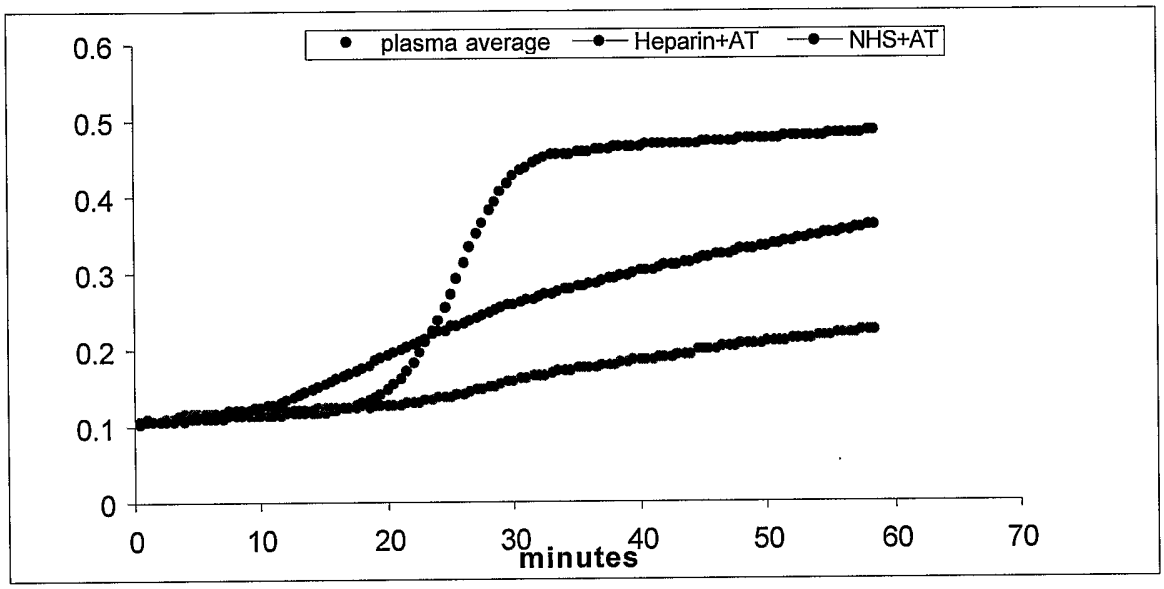


Figure 14


plasma average
Heparin+AT
NHS+AT
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60
70
minutes

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2005/000739

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7): C08G 77/42, C08G 77/38, C08G 77/46, C08G 65/336, C08G 65/332, C07C 69/02, C07D 207/46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(7): C08G-77/46; C08G-65/332

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Delphion (Derwent); CPD

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages			Relevant to claim No.
X	CA 1,261,528	26-09-1989	Ona et al.	1-6, 12, 14-28, 45-48, 50, 51
X A	Poly(Ethylene Glycol) Chemistry, Harris (Ed.), 1992 (Plenum Press, New York) see chapter 18.4 see chapter 19.4.4			1-6, 38, 40-44
X	US 5,455,040	03-10-1995	Marchant	1-52
A	CA 2,492,775 see especially pp 19-20	12-02-2004	Bailon et al.	
A	CA 2,393,638	28-06-2001	Kozlowski	
A	US 6,372,874	16-04-2002	Cameron	
A	US 6,346,583	12-02-2002	Kilgour et al.	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
19 July 2005 (19-07-2005)

Date of mailing of the international search report
22 September 2005 (22-09-2005)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 001(819)953-2476

Authorized officer
Chris Evans (819) 934-2323

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages			Relevant to claim No.
A	*Biomaterials*, 25, pp 2273-2282, 18-11-2003		Chen et al.	
A, P	*Biomaterials*, 26, pp 2391-2399, 23-09-2004		Chen et al.	
X	CA 913,092	24-10-1972	Little	50, 51

INTERNATIONAL SEARCH REPORT

ernational application No.
'T/CA2005/000739

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [ ] Claim Nos. :
because they relate to subject matter not required to be searched by this Authority, namely :

2. [X] Claim Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

See Extra Sheet

3. [ ] Claim Nos. :
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest
[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
[ ] No protest accompanied the payment of additional search fees.

Continuation of Box No. II:

The intended scope of the claims is obscured by applicant's choice of language. Consequently, it is not explicitly clear whether applicant intended to claim surface-functionalised silicone elastomers or silicone graft-copolymers. Further, it is not clear what scope should be given to the "activating group" in view of the broad definition taught for "biological molecules" in the description (see page 13, N.B. - the scope of the modifications is not restricted).

The scope of the search was therefore largely restricted to polyethylene oxide-substituted silicone polymers, comprising PEO chains terminated by a nucleophile-reactive group. The search was consequently performed in IPC subgroup C08G-77/46.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/CA2005/000739

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
CA 1,261,528	26-09-1989	EP0193402 A2	03-09-1986
		JP1850506 C	21-06-1994
		US4684709 A	04-08-1987
US 5,455,040	03-10-1995	AU5613194 A	08-06-1994
		WO9410938 A1	26-05-1994
CA 2,492,775	12-02-2004	AU2003258518 A1	23-02-2004
		BR0313212 A	28-06-2005
		EP1526872 A1	04-05-2005
		US2004106747 A1	03-06-2004
		WO2004012773 A1	12-02-2004
CA 2,393,638	28-06-2001	AU781729 B2	09-06-2005
		EP1259563 A2	27-11-2002
		JP2003518151 T	03-06-2003
		MXPA02006215 A	15-10-2003
		US6376604 B2	23-04-2002
		US6624246 B2	23-09-2003
		US6710125 B2	23-03-2004
		US2004157991 A1	12-08-2004
		WO0145796 A2	28-06-2001
US 6,372,874	16-04-2002	DE69918645 D1	19-08-2004
		EP1006140 A2	07-06-2000
		JP2000234025 A	29-08-2000
US 6,346,583	12-02-2002	EP1230292 A1	14-08-2002
		JP2003507551 T	25-02-2003
		US6545085 B2	08-04-2003
		US6548629 B2	15-04-2003
		US6797742 B2	28-09-2004
		WO0114458 A1	01-03-2001
CA 913,092	none		