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(54) **Title:** MULTI-FUNCTIONAL POLYMERIC LAYERS AND ITS USES

(57) **Abstract:** The present invention relates to a process of preparing a polymer-coated anti-biofouling solid substrate comprising a covalently-bondable functional group, comprising (a) preparing a polymer comprising a surface- anchoring part, a protein-repellent part and a reactive part by reacting (i) a first monomer comprising a surface-anchoring part that can be bound onto the surface of the solid substrate, (ii) a second monomer comprising an anti- biofouling polymer, and (iii) a third monomer comprising a covalently-bondable functional group; and (b) coating the polymer onto the surface of the solid substrate.

【DESCRIPTION】

【Invention Title】

MULTI-FUNCTIONAL POLYMERIC LAYERS AND ITS USES

【Technical Field】

<1> The present invention relates to a process of preparing a solid substrate, a solid substrate, a biochip or biosensor and a novel polymer useful in coating the solid substrate.

【Background Art】

<2> There has been an increasing interest in generating patterns of biologically active molecules (e.g., biotin, DNAs, saccharides, peptides and proteins)[1] and cells on solid substrates[2] because of their potential applications as biosensors[3] or assay systems for high-throughput screening.[4] Of particular interest is the construction of protein microarrays on SiO_2-based substrates in that they have been currently used for the study of proteomes or diagnose of diseases.[5] In general, two functionalities are needed for successful construction of protein arrays: (i) a bio-active surface to immobilize probe proteins and (ii) a bio-inert (anti-biofouling) one to minimize nonspecific adsorption of unwanted proteins that results in a poor signal-to-noise ratio (S/N ratio).[6] Conventional strategies for the purpose rely on self-assembled monolayers (SAMs) formed by monovalent compounds comprising a surface reactive group (i.e., trimethoxy or trichloro silane) at one end and a functional group at the other end (i.e., amine, alcohol, oligoethylene glycol).[7] However, unlike straightforward formation of SAMs on a gold surface,[8] the preparation of those on SiO_2 substrates using monovalent silicon compounds often generates many defects as well as aggregates (polymerized siloxanes) on the surface, resulting in poor reproducibility.[9] Therefore, it would be beneficial to develop a new platform for specific immobilization of biomolecules onto SiO_2 substrates

while preventing their nonspecific adsorption.

<3> The above information disclosed in this Background section is only for enhancement of understanding of the background of the invention and therefore it may contain information that does not form the prior art that is already known in this country to a person of ordinary skill in the art.

【Disclosure】

【Technical Problem】

<4> The present invention has been made in an effort to solve the above-described problems associated with prior art. The present inventors have exerted extensive researches to improve the technique of coating substrate surface, and particularly to provide biosensors and biochips superior in analytic performance and reproducibility by efficiently immobilizing probe proteins onto substrate surface, while minimizing nonspecific adsorption of unwanted proteins and remarkably improving a signal-to-noise ratio. As a result, a polymer has been prepared by using three kinds of monomers with a surface-anchoring, an anti-biofouling and a reactive part, respectively. Further, a bio-active substrate coated with the polymer has been ascertained to have superior properties. The present invention has thus been completed on the basis of the aforementioned findings.

【Technical Solution】

<5> In one aspect, the present invention provides a process of preparing a polymer-coated anti-biofouling solid substrate comprising a covalently-bondable functional group.

<6> In another aspect, the present invention provides a dual functional solid substrate comprising a surface-anchoring part, an anti-biofouling part and a reactive part.

<7> In still another aspect, the present invention provides a biochip or biosensor comprising a solid substrate herein.

<8> In a further aspect, the present invention provides a novel polymer useful in the coating of a solid substrate.

<9> Other aspects of the invention are discussed infra.

【Advantageous Effects】

<11> The present invention relates to the use of triply functional polymer comprising a surface-anchoring, an anti-biofouling and a reactive part. According to the present invention, polymeric self-assembled monolayers can be stably coated onto solid substrate, while remarkably improving signal-to-noise ratio due to the superior anti-biofouling property. Further, desired biomolecules are efficiently immobilized onto solid substrate to give stable micropatterns through functional groups of a polymer herein. Moreover, bioreactivivity and anti-biofouling property can be tailored by simply changing initial feed ratios of each monomer in the synthesis of a polymer herein. In the present invention, pSAMs are also efficiently formed on solid substrate surface regardless of the kind of solid substrate. As a result, the present invention provides biosensors or biochips with a remarkably increased signal-to-noise ratio.

【Description of Drawings】

<12> The above and other features of the present invention will now be described in detail with reference to certain exemplary embodiments thereof illustrated the accompanying drawings which are given hereinbelow by way of illustration only, and thus are not limitative of the present invention, and wherein:

<13> Figure 1 schematically represents the specific immobilization of biomolecules onto anti-biofouling pSAMs of the present invention;

<14> Figure 2 is ^{1}H NMR spectra of poly-1, poly-2 and poly-3 of the present invention;

<15> Figure 3 is the high resolution C(1s) X-ray photoelectron spectra of the pSAM of poly-1 formed on Si/SiO2 wafers;

<16> Figure 4 is fluorescence microscope images of TRITC-labeled streptavidin patterns prepared following μCP of biotin-EO-amine on the pSAMs of poly-1 using 50 × 50 μm and (b) 25 × 25 μm of circle patterned stamp;

<17> Figure 5 is fluorescence microscope images of TRITC-labeled streptavidin patterns on the each pSAMs (poly-1, poly-2, and poly-3) prepared

following μCP using 50 × 50 μm of circle patterned stamp;

<18> Figure 6 is high-resolution C(1s) X-ray photoelectron spectra of the pSAMs (poly-4, poly-5 and poly-6) formed on polystyrene substrate;

<19> Figure 7 is fluorescence microscope images of TRITC-labeled streptavidin patterns prepared following μCP of biotin-amine on the polymer-coated plastic surface using 50 x 50 μm of circle patterned stamp;

<20> Figure 8 schematically represents a 96-well plate coated with streptavidin by using polymer for detecting biotin-conjugated mouse anti-rabbit IgG;

<21> Figure 9 is results of ELISA detection of adsorbed captured biotinylated mouse IgG by automated ELISA reader. Immobilized biotinylated mouse IgG was detected by HRP conjugated anti-mouse IgG;

<22> Figure 10 schematically represents an orientation of anti-BSA by using protein A on polymer-coated plastic surface; and

<23> Figure 11 is fluorescence microscope images of FITC-labeled BSA patterns prepared following μCP of protein A and adding anti-BSA on the polymer-coated plastic surface using 50 x 50 μm of circle patterned stamp.

<24> It should be understood that the appended drawings are not necessarily to scale, presenting a somewhat simplified representation of various preferred features illustrative of the basic principles of the invention. The specific design features of the present invention as disclosed herein, including, for example, specific dimensions, orientations, locations, and shapes will be determined in part by the particular intended application and use environment.

【Best Mode】

<25> In one aspect, the present invention the present invention provides a process of a polymer-coated anti-biofouling solid substrate comprising a covalently-bondable functional group, which comprises the steps of:

<26> (a) preparing a polymer comprising a surface-anchoring part, a protein-repellent part and a reactive part by reacting (i) a first monomer comprising a surface-anchoring part that can be bound onto the surface of the solid

substrate, (ii) a second monomer comprising an anti-biofouling polymer, and (iii) a third monomer comprising a covalently-bondable functional group; and

<27> (b) coating the polymer onto the surface of the solid substrate.

<28>

<29> In another aspect, the present invention provides a dual functional solid substrate comprising a surface-anchoring part, an anti-biofouling part and a reactive part, the solid substrate being coated with a polymer prepared by the polymerization of the following monomers:

<30> (i) a first monomer comprising a surface-anchoring part that can be bound onto the surface of the solid substrate,

<31> (ii) a second monomer comprising an anti-biofouling polymer, and

<32> (iii) a third monomer comprising a covalently-bondable functional group.

<33> The present inventors have exerted extensive researches to improve the technique of coating substrate surface, and particularly to provide biosensors and biochips superior in analytic performance and reproducibility by efficiently immobilizing probe proteins onto substrate surface, while minimizing nonspecific adsorption of unwanted proteins and remarkably improving a signal-to-noise ratio. As a result, a polymer has been prepared by using three kinds of monomers with a surface-anchoring, an anti-biofouling and a reactive part, respectively. Further, a bio-active substrate coated with the polymer has been ascertained to have superior properties.

<34> Biomolecules (e.g., proteins, peptides, nucleotides and saccharides) or chemicals are immobilized onto the surface of solid substrate and used for preparing biosensors or biochips. The present invention discloses a technique of immobilizing biomolecules onto the substrate surface. In an embodiment of the present invention, the bio-active polymers are coated onto substrate surface.

<35> Polymer of the present invention is prepared by using three kinds of monomers. Each of the monomers comprises (i) a surface-anchoring part, (ii) an anti-biofouling part and (iii) a reactive part, respectively. These monomers enable to achieve dual functionality of the present invention, i.e.

anti-biofouling and bio-reactive properties.

<36> Among these monomers, a first monomer comprises a surface-anchoring part that can be bound onto the surface of solid substrate.

<37> Examples of a first monomer herein include, but are not limited to, acrylic acid, acrylonitrile, allylamine, methacrylic acid, alkyl acrylate, alkyl methacrylate, butadiene, carbomethylsilane, (carbonate)urethane, polydimethylsiloxane acrylate, polydimethylsiloxane methacrylate, ethylene, ethylene glycol, propylene glycol, (ether) urethane, urethane, vinyl chloride, vinyl alcohol, maleic anhydride, cellulose chloride, vinyl alcohol, cellulose nitrate, carboxymethyl cellulose, dextran, dextran sulfate, propylene, ester, carbonate, ether, butene, maleic acid, fluoropolymer monomer unit, unsaturated polymer monomer, isoprene, melamine, sulfone, biological polymer monomer unit, protein, gelatin, protein, gelatin, elastin, butyl methacrylate, butyl methacrylate, hydroxyethyl methacrylate, polyethylene glycol dimethacrylate, polypropylene glycol diglycidal ether, polypropylene glycol diglycidyl ether, N-acryloxysuccinimide, glycidyl methacrylate, hexamethylene diisocyanate, acrolein, glycrol monomethacrylate, heparin methacrylate, methacryloylethyl phosphorylcholine, butyl acrylate, polyethylene glycol monomethacrylate, isobutyl methacrylate, cyclohexyl methacrylate, ethyl acrylate, 2-hydroxyethyl acrylate, 2-ethylhexyl methacrylate, ethyl methacrylate, methyl acrylate, hexadecyl methacrylate, octadecyl methacrylate, styrene, methyl styrene, vinyl toluene, *tert*-butyl acrylate, n-vinyl pyrrolidone, glycolide, lactide, butyrolactone, caprolactone, hydroxyalkanoate, 3-hydroxybutyrate, 4-hydroxybutyrate, 3-hydroxyvalerate and 3-hydroxyhexanoate.

<38> Preferable examples of a first monomer herein include, but are not limited to, acrylate- or methacrylate-based monomers, such as methacrylic acid, acrylic acid, alkyl methacrylate (e.g., methyl methacrylate, ethyl methacrylate, butyl methacrylate, isobutyl methacrylate, hexadecyl methacrylate, octadecyl methacrylate and cyclohexyl methacrylate), alkyl acrylate (e.g., methyl acrylate, ethyl acrylate and *tert*-butyl acrylate),

aryl acrylate (e.g., benzyl acrylate), aryl methacrylate (e.g., benzyl methacrylate), 2-ethylhexyl methacrylate, lauryl methacrylate, hydroxylethyl methacrylate, PEG acrylate, PEG methacrylate, hydroxypropyl methacrylate, hydroxypropylmethacrylamide, 3-trimethylsilylpropyl methacrylate, 3-trimethoxysilylpropyl methacrylate, acrylonitrile, polydimethylsiloxane acrylate, polydimethyl siloxane methacrylate, glycidyl methacrylate, glycrol monomethacrylate, heparin methacrylate, polyethylene glycol monomethacrylate, 2-hydroxyethyl acrylate and 2-ethylhexyl methacrylate.

<39> In a preferred embodiment of the present invention, a first monomer herein comprises a substituted or unsubstituted silane group, a substituted or unsubstituted alkyl group or a substituted or unsubstituted aryl group as a surface-anchoring part.

<40> A substituted or unsubstituted silane group of a first monomer herein forms a covalent bond with a hydroxyl group of a solid substrate (e.g., Si/SiO_2 wafer), thereby anchoring polymeric layer to the solid substrate.

<41> A first monomer can comprise unsubstituted silane groups (SiH_4), which anchors onto solid substrate surface. Preferably, a first monomer comprises substituted silane groups, which also anchors onto solid substrate surface.

<42> Examples of substituted silane group of a first monomer as a surface-anchoring part include, but are not limited to, silane groups substituted with preferably alkoxy group (i.e., alkoxysilyl group) and silane groups substituted with more preferably C_1-C_6 alkoxy group. As used herein, the term "alkoxy" group refers to -O-alkyl group. As used herein, the term "alkoxysilyl" group means alkoxy-substituted silyl group, preferably trimethoxysilyl and triethoxysilyl group, and more preferably trimethoxysilyl group.

<43> A first monomer can also comprise hydroxy-substituted silane group (i.e., hydroxysilyl group). As used herein, the term "hydroxysilyl" group refers to hydroxy-substituted silyl group, preferably trihydroxy silyl group.

<44> In a preferable embodiment of the present invention, a first monomer can

comprises substituted or unsubstituted alkyl group. This alkyl group renders a first monomer hydrophobic and allows the polymer to anchor onto solid substrate (e.g., polystyrene substrate having hydrophobic surface) via a hydrophobic interaction and/or a dipole interaction.

<45> Preferable examples of the alkyl group of a first monomer include, but are not limited to, C_{6-30} alkyl group, more preferably C_{8-30} alkyl group. As used herein, the term "alkyl" group is intended to encompass linear or branched saturated hydrocarbon group with a particular number of carbons. The alkyl group can be substituted with preferably F, Cl or Br, more preferably F.

<46> In a preferred embodiment of the present invention, a first monomer can comprises substituted or unsubstituted aryl group. This aryl group allows polymer to anchor onto solid substrate (e.g., polystyrene substrate with hydrophobic surface) via p-p interaction. As used herein, the term "aryl" group means a fully or partially unsaturated substituted or unsubstituted monocyclic or polycyclic hydrocarbon cycle, preferably monoaryl or biaryl group. Monoaryl and biaryl groups are preferred to have 5-6 carbons and 9-10 carbons, respectively. Mos preferable example of the aryl group include substituted or unsubstituted phenyl group. Monoaryl (e.g., phenyl) group can be substituted at various sites with various substituents, preferably halo, hydroxy, nitro, cyano, C_1-C_4 substituted or unsubstituted linear or branched alkyl, C_1-C_4 linear or branched alkoxy, alkyl substituted sulfanyl, phenoxy, C_3-C_6 cycloheteroalkyl or substituted or unsubstituted amino group.

<47> A second monomer herein has a similar chemical structure to the aforementioned first monomer. Preferable examples of a second monomer include, but are not limited to, methacrylate- or acrylate-based monomer as explained above.

<48> A second monomer comprises a polymer that show an anti-biofouling property. Examples of an anti-biofouling polymer herein include, but are not limited to, polyethylene glycol (PEG), polyalkylene oxide (e.g.,

polyoxyethylene, polyoxypropylene or copolymer thereof such as polyethylene oxide-polypropylene oxide-polyethylene oxide copolymer), polyphenylene oxide, a copolymer of PEG and polyalkylene oxide, poly(methoxyethyl methacrylate), poly(methacryloyl phosphatidylcholine), perfluoropolyether, dextran and polyvinylpyrrolidone, more preferably PEG, polyalkylene oxide or a copolymer of PEG and polyalkylene oxide, most preferably PEG.

<49> A third monomer herein has a similar chemical structure to the aforementioned first monomer. Preferable example of a second monomer include, but are not limited to, methacrylate- or acrylate-based monomer as explained above.

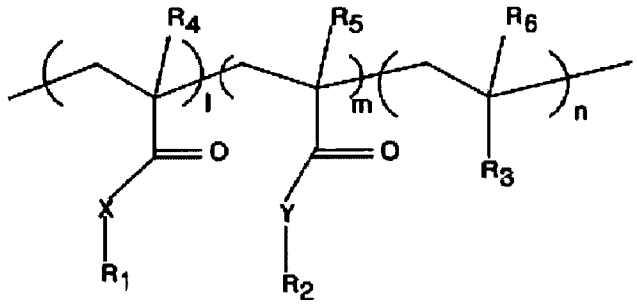
<50> A third monomer comprises a functional group. This functional group can react with proteins, peptides, nucleotides (DNA or RNA), saccharides or chemicals and form covalent bonds. Accordingly, various functional groups can be the functional group of a third monomer. Examples of a functional group in a third monomer include, but are not limited to, aldehyde; epoxy; haloalkyl; primary amine; thiol; maleimide; ester (preferably N-hydroxysuccinimide ester group); and activated functional groups including carboxyl group (activated by the formation of hydroxy-succinimide ester) and hydroxyl group (activated by cyanogen bromide. Most preferable examples of a functional group in a third monomer include carboxyl group.

<51> Carboxyl group as a functional group in a third monomer is activated by preferably succinimide, succinimidyl ester, sulfosuccinimidyl ester, 2,3,5,6-tetrafluorophenol ester, 4-sulfo-2,3,5,6-tetrafluorophenol ester, aldehyde, acid anhydride, azide, azolide, carboimide, epoxide, ester, glycidyl ether, halide, imidazole or imidate. Most preferably, carboxyl group as a functional group is activated by succinimide or succinimidyl ester.

<52> In a preferred embodiment of the present invention, a polymer-coated solid substrate is represented by the following Formula 1.

<53>

<54> [Formula 1]



<55>

<56> wherein R_1 is selected from the group consisting of a silylalkyl group, an (alkoxysilyl)alkyl group, a (hydroxysilyl)alkyl group, a substituted or unsubstituted alkyl group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl group and a substituted or unsubstituted alkaryl group; R_2 is selected from the group consisting of polyethylene glycol (PEG), polyalkylene oxide, polyphenylene oxide, a copolymer of PEG and polyalkylene oxide, poly(methoxyethyl methacrylate), poly(methacryloyl phosphatidylcholine), perfluoropolyether, dextran and polyvinylpyrrolidone; R_3 is selected from the group consisting of aldehyde, epoxy, haloalkyl, a primary amine, thiol, maleimide, ester, carboxyl group and hydroxyl group; each of R_4, R_5 and R_6 is independently H or C_1-C_5 alkyl group; each of X, Y and Z is independently oxygen, sulfur or nitrogen atom; and each of l, m and n is independently an integer of 1-10,000.

<57> As used in the definition of R_1, the term "silylalkyl" group is referred to a silyl group substituted with alkyl group. Examples of silylalkyl group include, but are not limited to silylmethyl, silylethyl, silylpropyl and silylbutyl groups. As used herein, the term "(alkoxysilyl)alkyl" group means an alkoxy-substituted silylalkyl group. Examples of (alkoxysilyl)alkyl group include, but are not limited to, trimethoxysilylethyl, trimethoxysilylpropyl, trimethoxysilylbutyl, trimethoxysilylpentyl, triethoxysilylethyl, triethoxysilylpropyl, triethoxysilylbutyl, trimethoxysilylpentyl, methyldimethoxysilylethyl, methyldimethoxysilylpropyl, dimethylmethoxysilylethyl and

dimethylmethoxysilylpropyl groups. As used herein, the term "(hydroxysilyl)alkyl group is referred to hydroxyl-substituted silylalkyl group such as trihydroxysilylethyl, trihydroxysilylpropyl, trihydroxysilylbutyl and trihydroxysilylpentyl groups.

<58> As used herein, the term "aralkyl" group refers to one or more alkyl groups with aryl substituents. Examples of aralkyl group include, but are not limited to, benzyl and phenylpropyl groups. The term "alkaryl" group refers to aryl groups that has alkyl substituents.

<59> In Formula 1, preferable examples of R_1 include, but are not limited to, (alkoxysilyl)alkyl group, substituted or unsubstituted C_{6-30} alkyl group, substituted or unsubstituted aryl group, substituted or unsubstituted aralkyl group and substituted or unsubstituted alkaryl group; more preferably (C_{1-6} alkoxysilyl)C_{1-10} alkyl group, substituted or unsubstituted C_{6-30} alkyl group, substituted or unsubstituted phenyl, substituted or unsubstituted aralkyl group (e.g., C_{1-5} alkyl group with phenyl substituent) and substituted or unsubstituted alkaryl group (e.g., a phenyl substituted with C_{1-5} alkyl group); most preferably (C_{1-3} alkoxysilyl)C_{1-5} alkyl group, substituted or unsubstituted C_{8-20} alkyl group, substituted or unsubstituted phenyl, C_{1-5} alkyl group substituted with phenyl group or phenyl with C_{1-5} alkyl substituent.

<60> Polymers prepared by using the aforementioned three kinds of monomers can be coated onto solid substrate surface according to various processes known in saccharide field, for example by immersing a solid substrate in a polymer solution.

<61> In a preferred embodiment of the present invention, polymers exist on solid substrate surface in the form of self-assembled monolayers. These self-assembled monolayers have a thickness of 0.8-2 nm, preferably 0.9-1.3 nm.

<62> In another preferred embodiment of the present invention, a process of the present invention can further comprise the step of (c) covalently binding the functional group derived from the third monomer with chemicals, proteins,

peptides, nucleotides (DNA or RNA) or saccharides.

<63> Functional groups in a third monomer of polymeric layer coated on solid substrate are covalently bound to proteins, peptides, nucleotides (DNA or RNA), saccharides or chemicals.

<64> For example, activated carboxyl group can be covalently bound to biologically active molecules (e.g., proteins, peptides, nucleotides, saccharides and chemicals through amine groups. When a functional group is an amine group, it can be covalently bound to biologically active molecules through carboxyl groups in the biologically active molecules.

<65> Further, biologically active molecules can also be bound to functional groups indirectly. For example, materials such as biotin with affinity toward the aforementioned biologically active molecules (e.g., proteins, peptides, nucleotides or saccharides) are first bound to functional groups, and biologically active molecules including proteins (e.g., streptavidin or avidin) are subsequently bound to the materials with affinity toward the biologically active molecules, preferably via non-covalent bond).

<66> Biologically active molecules can be immobilized onto solid substrate according to the following methods: contact pin printings, non-contact pin printings such as inkjet printing and aerosol printing, capillary printing, microcontact printing, pad printing, screen printing, silk printing, micropipeting and spraying methods.

<67> Various kinds of solid substrates can be used in the present invention regardless of the surface property. Examples of solid substrate herein include, but are not limited to, metals (e.g., gold, gold-copper alloy and aluminum), metal oxide, glass, ceramic, quartz, silicone, semi-conductors, Si/SiO_2 wafer, germanium, gallium arsenide, carbon, carbon nanotubes, polymer (e.g., polystyrene, polyethylene, polypropylene, polyacrylamide), sepharose, agarose and colloids.

<68> When a surface-anchoring part of a first monomer is substituted or unsubstituted silane group, glass, metal oxide, ceramic, quartz, silicone,

semi-conductors and Si/SiO_2 wafer substrate are preferred as solid substrate. When a surface-anchoring part of a first monomer is substituted or unsubstituted alkyl group or substituted or unsubstituted aryl group, carbon, carbon nanotube and polymer (e.g., polystyrene, polyethylene, polypropylene, polyacrylamide) are preferred as solid substrate.

<69> According to the present invention, polymeric self-assembled monolayers can be stably coated onto solid substrate, the functional groups of which enables to immobilize biomolecules onto solid substrate to give stable patterns, while preventing nonspecific adsorption due to the anti-biofouling property, thereby remarkably increasing the signal-to-noise ratio.

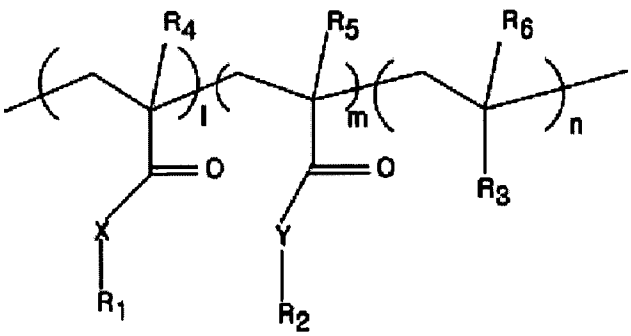
<70>

<71> In still another aspect, the present invention provides a biochip or biosensor comprising the aforementioned solid substrate herein.

<72> In a biochip or biosensor herein, biomolecules are immobilized onto solid substrate through functional groups of self-assembled monolayers.

<73> Preferable examples of a biochip herein include, but are not limited to, DNA microarray or protein chip. Description disclosed in U.S. patent Nos. 5,143,854, 5,242,974, 5,252,743, 5,324,633, 5,384,261, 5,405,783, 5,412,087, 5,424,186, 5,451,683, 5,482,867, 5,491,074, 5,527,681, 5,550,215, 5,571,639, 5,578,832, 5,593,839, 5,599,695, 5,624,

<74> [Formula 1]



<75>

<76> wherein R_1 is selected from the group consisting of a silylalkyl group,

an (alkoxysilyl)alkyl group, a (hydroxysilyl)alkyl group, a substituted or unsubstituted alkyl group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl group and a substituted or unsubstituted alkaryl group; R_2 is selected from the group consisting of polyethylene glycol (PEG), polyalkylene oxide, polyphenylene oxide, a copolymer of PEG and polyalkylene oxide, poly(methoxyethyl methacrylate), poly(methacryloyl phosphatidylcholine), perfluoropolyether, dextran and polyvinylpyrrolidone; R_3 is selected from the group consisting of aldehyde, epoxy, haloalkyl, a primary amine, thiol, maleimide, ester, carboxyl group and hydroxyl group; each of R_4, R_5 and R_6 is independently H or C_1-C_5 alkyl group; each of X, Y and Z is independently oxygen, sulfur or nitrogen atom; and each of l, m and n is independently an integer of 1-10,000.

<77>

<78> Representative features and advantages of the present invention can be summarized as follows:

<79> (a) The present invention relates to the use of triply functional polymer comprising a surface-anchoring, an anti-biofouling and a reactive parts.

<80> (b) In the present invention, polymeric self-assembled monolayers can be stably coated onto solid substrate.

<81> (c) In the present invention, signal-to-noise ratio is remarkably improved by means of superior anti-biofouling property.

<82> (d) In the present invention, desired biomolecules are efficiently immobilized onto solid substrate to give stable micropatterns through functional groups of a polymer herein.

<83> (e) In the present invention, bioreactivivity and anti-biofouling property can be tailored by simply changing initial feed ratios of each monomer in the synthesis of a polymer herein.

<84> (f) In the present invention, pSAMs are efficiently formed on solid substrate surface regardless of the kind of solid substrate.

<85> (g) The present invention discloses biosensors or biochips with a remarkably increased signal-to-noise ratio.

<86>

<87> The above features and advantages of the present invention will be apparent from or are set forth in more detail in the accompanying drawings, which are incorporated in and form a part of this specification, and the following Detailed Description, which together serve to explain by way of example the principles of the present invention.

【Mode for Invention】

<88> Reference will now be made in detail to the preferred embodiments of the present invention, examples of which are illustrated in the drawings attached hereinafter, wherein like reference numerals refer to like elements throughout. The embodiments are described below so as to explain the present invention by referring to the figures.

<89> The following examples illustrate the invention and are not intended to limit the same.

<90>

<91> EXAMPLES

<92> Experimental Section

<93> *Materials*

<94> N-Acryloxysuccinimide (NAS; 99%) was purchased from ACROS Organics (Noisy-le-Grand, France). 3-(Trimethoxysilyl)propyl methacrylate, poly(ethylene glycol) methyl ether methacrylate (average Mn = ca. 475), and 2,2′ azobisisobutyronitrile (AIBN) were purchased from Aldrich Chemical Co. (Milwaukee, WI). (+)-Biotinyl-3,6,9-trioxaundecanediamine (biotin-NH_2) and tetramethylrhodamine isothiocyanate (TRITC)-conjugated streptavidin were purchased from Pierce (Rockford, IL). All organic solvents were used as received without further purification. All substrates used herein such as Si/SiO_2 wafer, poly(dimethylsiloxane) (PDMS), and glass slide were first cleaned using detergent, followed by washing with deionized water and

methanol several times. Prior to forming the polymeric films, all of the substrates were treated with O_2 plasma (Expanded Plasma Cleaner; Harrick Plasma Corp., Ithaca, NY) for 1 min to generate -OH groups as well as to clean the surfaces.

<95>

<96> *Measurements*

<97> 1H NMR (300 MHz) spectra were recorded on a JEOL JNM-LA300WB FT-NMR (Tokyo, Japan). Organic phase gel permeation chromatography (GPC) was performed using a Waters 1515 series isocratic pump, a Rheodyne model 7725 injector with a 100 μL injection loop at a flow rate of 0.4 mL/min. The thicknesses of the monolayer films were measured with a Gaertner L116A ellipsometer (Gaertner Scientific Corporation, IL) at a 70° angle of incidence. A refractive index of 1.46 was used for all films, and a three-phase model was used to calculate thicknesses. Aphoenix 300, contact angle & surface tension analyzer (Surface electro optics, Kyonggi, Korea) equipped with viedo camera and monitor was used to measure contact angle. X-ray photoelectron spectroscopy (XPS) spectra were obtained using a Kratos AXIS Ultra Imaging X-ray Photoelectron Spectrometer with a monochromatized Al K X-ray source.

<98>

<99> *Synthesis of poly(TMSMA-r-PEGMA-r-NAS)*

<100> Prior to polymerization, neat PEGMA was flowed through the inhibitor removal column (Aldrich Chemical Co.). PEGMA(1.425 g, 3 mmol, 1 equiv), TMSMA (0.744 g, 3 mmol, 1 equiv), NAS (0.507 g, 3 mmol, 1 equiv) and AIBN (16.5 mg, 0.1 mmol, 0.01 equiv) were placed in a vial and dissolved in tetrahydrofuran (anhydrous, inhibitor free, 99.9%, 10 mL). The mixture was degassed for 20 min using an Ar gas stream, after which the vial was sealed with a Teflon-lined screw-cap. The polymerization reaction was carried out at 70 ℃ for 24 h. After evaporation of solvent under vacuum, the polymer was obtained as a viscous liquid. Different molar ratios of the bio-reactive group (NAS: 33%, 20%, and 10%, respectively) bearing copolymers were prepared. Initial feed

ratios of three monomers were 1:1:1 for poly-1, 2:2:1 for poly-2, and 4:5:1 for poly-3, respectively. Actual ratios of three monomer units incorporated in the final copolymers were calculated from comparison of the integration value of the peak at δ) 4.13 (CO_2-CH_2 at PEGMA), that of the peak at δ) 3.92 (CO2-CH2 at TMSMA) with that of the peak at δ) 2.82 (CO-CH_2CH_2-CO at NAS) in the 1H NMR spectra. 1H NMR (300 MHz, $CDCl_3$) of poly-1: δ= 4.13 (br, 2H, CO_2-CH_2 of PEGMA), 3.92 (br, 2H, CO_2-CH_2 of TMSMA), 3.66 (s, 30H), 3.63-3.55 (s, 9H; m, 2H), 3.40 (s, 3H), 2.82 (br, 4H, CO-CH_2CH_2-CO of NAS), 2.0-1.71 (br, 6H), 1.04 (br, 2H), 0.87 (br, 4H), 0.66 (br, 2H). poly-1 (Mn = 15784 with Mw/Mn = 1.58), poly-2 (Mn = 17245 with Mw/Mn = 1.71), poly-3 (Mn = 16571 with Mw/Mn = 1.67).

<101>

<102> *Synthesis of amphiphilic polymers*

<103> Poly-4 was synthesized by using dodecyl methacrylate as anchoring site. Dodecyl methacrylate (4 mmol, 1.016 g, 2 equiv), PEGMA (4 mmol, 1.9 g, 2 equiv) and methacrylic acid (2 mmol, 0.172 g, 1 equiv) were placed in a vial and dissolved in THF (anhydrous, 99.9%, inhibitor-free). This mixture was degassed for 15 min by bubbling with stream of N_2 gas. After 0.1 mmol AIBN (16.4 mg, 0.05 equiv) was added as an initiator, the vial was sealed with a Teflon-lined screw cap. The polymerization reaction was carried out at 70 ℃ for 24 h. The final product solution was stored at 4℃.

<104> Poly-5 and poly-6 were prepared by substituting dodecyl methacrylate at anchoring sites with fluoromonomer and benzyl methacrylate, respectively. Poly-4 (Mn = 19581 with Mw/Mn = 1.88), poly-5 (Mn = 21831 with Mw/Mn = 1.97), poly-6 (Mn = 16255 with Mw/Mn = 1.91). ^{1}H NMR (300.40 MHz, $CDCl_3$): poly-4 δ = 4.05 (br, 2H, CO_2-CH_2 of PEGMA), 3.82 (br, 2H, CO_2-CH_2 of dodecylMA), 3.66 (s, 30H), 3.40 (s, 3H), 2.0-1.71 (br, 10H), 1.5-1.1 (br, 20H), 0.87 (br, 3H); poly-6 δ = 7.30 (s, 5H), 4.93 (br, 2H, CO_2-CH_2 of benzylMA), 4.05 (br, 2H,

CO_2-CH_2 of PEGMA), 3.66 (s, 30H), 3.40 (s, 3H), 2.0-1.71 (br, 10H), 0.87 (br, 3H), 0.72 (br, 4H).

<105>

<106> *Modification of plastic surface by using amphiphilic polymers*

<107> THF was evaporated by using vacuum pump. After evaporation, the polymer was obtained as a viscous liquid. Acquired polymer (poly-4, poly-5 and poly-5) was well soluble in water presumably due to the presence of multiple PEG groups. Amphiphilic polymer (pSAMs) was formed on the surface of polystyrene-based substrate by immersing the substrates in aqueous solution of the amphiphilic polymers (20 mg/mL) at ambient temperature, followed by washing with pure water.

<108>

<109> *Anti-biofouling effects of the polymer-coated plastic surface*

<110> The general phenomenon of protein nonspecific binding on plastic surface is mainly due to hydrophobic interactions. To evaluate protein-resistance of the polymer coating, the polymer-coated plastic surface was immersed in bovine serum albumin (BSA) solutions (0.25 mg/mL in PBS, pH 7.4) for 2 h. The degree of nonspecific proteins adsorption onto the plastic surface was obtained from the high resolution N (1s) XPS spectrum.

<111>

<112> *Activation of polymer-coated plastic surfaces by using EDC/NHS*

<113> Polymer-coated plastic surface was activated for specific immobilization of biomolecules. The -COOH groups as functional part of poly-4, poly-5 and poly-6 were converted into NHS ester groups by using EDC/NHS reagent. A freshly prepared 1:1 mixture of EDC (0.4 M) and NHS (0.1 M) in distilled water was applied for 20 min to activate the -COOH groups on the surface of the polymer-coated plastic.

<114>

<115> *Microcontact Printing (μCP) of Biological Ligands*

<116> After inking, the amine-terminated biotin ligand (biotin-NH_2, 10 mM in

ethanol) was printed by contacting the PDMS stamp onto pSAMs for 60 s. The sample was then immersed immediately in a borate buffer (pH 9.0) for 2 h. After the pattern generation of biotin, the samples were immersed in a solution of TRITC-conjugated streptavidin (0.1 mg/mL) in phosphate buffered saline (PBS, pH 7.4) at room temperature. After 60 min, the samples were removed and washed several times with PBS and distilled water. In case of poly-1, poly-2 and poly-3, the patterns of streptavidin (λex = 488 nm, λem = 520 nm) were visualized using a Leica DMRBE microscope (Leica Microsystems AG, Wetzlar, Germany) equipped with a 40, 100, 200 (1.4 NA) oil objective and TRITC-optimized filter sets (Omega Optical Inc, Brattleboro, VT, U.S.A.). Images were acquired using a CoolSNAPfx CCD camera driven by MetaMorph imaging software (Universal Imaging Co, Downingtown, PA, U.S.A.). In case of poly-4, poly-5 and poly-6, the patterns of streptavidin (λex = 547, λem = 572 nm) was visualized using a Leica DMRBE microscope (Leica Microsystems AG, Wetzlar, Germany) equipped with a 200x, 400x objective and TRITC-optimized filter sets (Omega® Optical Inc, Brattleboro, VT, USA).

<117>

<118> *Application to ELISA of modified plastic surface by amphiphilic polymers*

<119> The amphiphilic polymer was coated on the surface by using a 96 well flat-bottom assay plate. Aqueous solution of the amphiphilic polymers (100 μL, 20 mg/mL) was added to each well for 1 h at ambient temperature. They were then washed four times with 150 μL deionized water, and soaked for 30 s between each wash. To activate -COOH group of functional part, 100 μL of EDC (400 mM) and NHS (100 mM) was added to each well for 2 h at 27 ℃, followed by washing four times. Then 100 μL of the amine-terminated biotin ligands (biotin-NH_2, 10 mg/mL in ethanol) was added per well for 1 h at 27 ℃, followed by washing three times with 150 μL ethanol, soaking for 30 s between each wash. 150 μL of borate buffer (pH 9.0) was then added immediately to each well for 2 h at 27 ℃. After removing the borate buffer,

100 μL of streptavidin in PBS (10 μg/mL) was added to each well for 1 h at 27℃, followed by washing with 150 μL PBS for three times. Then 100 μL of biotin-conjugated mouse anti-rabbit IgG monoclonal antibody in PBS was subsequently added with different concentration per well for 1 h at 27 ℃, followed by washing three times with 150 μL PBST (0.1% Tween-20) by using Immuno™ Washers (Nunc A/S, Roskilde, Denmark).

<120> 100 μL of HRP-conjugated anti-mouse IgG antibody in PBS (1 μg/mL) was then added to each well for 1 h at 27 ℃, followed by three times of washing. Finally, 100 μL of substrate solution (TMB) was added to each well, followed by the addition of 100 μL of 1 M HCl 30 min later, thereby terminating the enzymatic reaction. The intensity was calculated from absorption values obtained at 450 nm using an automated enzyme-linked immunosorbent assay (ELISA) reader.

<121>

<122> *Antibody immobilization by protein A on polymer-coated plastic surface*

<123> Amphiphilic polymer-coated plastic surface was prepared by immersing the substrates in aqueous solution of the amphiphilic polymers (20 mg/mL) at ambient temperature, followed by washing with pure water. Polymer-coated plastic surface was activated by EDC/NHS reagent. A freshly prepared 1:1 mixture of EDC (400 mM) and NHS (100 mM) in distilled water was applied for 20 min to activate -COOH groups on the surface of the polymer-coated plastic. After the activation, μCP was carried out to immobilize protein A.

<124> Protein A (100 μg/ml in PBS) was printed by contacting the PDMS stamp onto polymer-coated plastic surface for 1 h. The sample was then immersed immediately in a borate buffer (pH 9.0) for 2 h. After the pattern generation of protein A, the samples were added in a solution of anti-BSA (10 μg/ml in PBS) for 1h at room temperature. After washing with PBS, FITC-labeled BSA (50 μg/ml in PBS) was finally added onto the sample-coated plastic surface. After 1 h, the samples were washed several times with PBS. The patterns of BSA (λex = 494 nm, λem = 521 nm) was visualized using a Leica DMRBE microscope (Leica Microsystems AG, Wetzlar, Germany) equipped with a 200x,

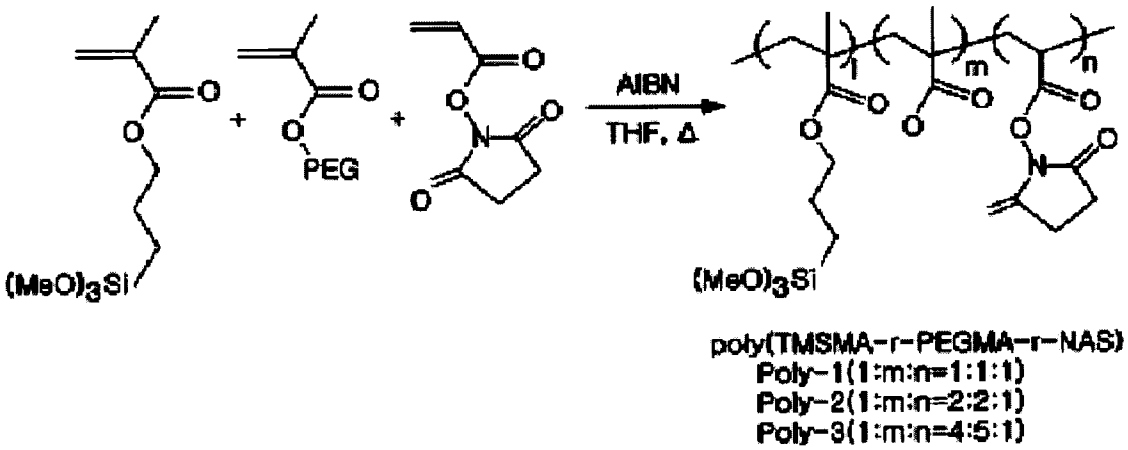
400x objective and TRITC-optimized filter sets (Omega® Optical Inc, Brattleboro, VT, USA).

<125>

<126> Results and Discussion

<127> The chemical structure of the triply functional copolymer is shown in Scheme 1.

<128> Scheme 1



<129>

<130> The copolymer, referred to as poly(TMSMAr-PEGMA-r-NAS), was synthesized from three kinds of monomers by radical polymerization: (i) (trimethoxy silyl)propyl methacrylate (TMSMA) as a surface-anchor part; (ii) polyethylene glycol methacrylate (PEGMA) as a protein-resistant part; and (iii) N-acryloylsuccinimide (NAS) as a bio-reactive part. Because the surface density of both the bio-reactive and anti-biofouling functionalities can be tailored by simply changing initial feed ratios of each monomer in the polymer synthesis, a series of random copolymers with three different NAS contents were synthesized as shown in Scheme 1: (i) poly-1 = 1:1:1, (ii) poly-2 = 2:2:1 and (iii) poly-3 = 4:5:1, respectively.

<131> The actual NAS content in each copolymer that was obtained on the basis of integration values in the 1H NMR spectrum (see Figure 2) turned out to be 35%, 21%, and 12% for poly-1, poly-2, and poly-3, respectively. It was ascertained that the compositions of the copolymers were well matched to the

initial feed ratios. The molecular weights of three copolymers synthesized herein ranged from 15,000 to 17,000 with a polydispersity index of 1.6 as measured by gel permeation chromatography (GPC) relative to monodisperse polystyrene standards.

<132> The polymeric self-assembled monolayers (pSAMs) were prepared by immersing a Si/SiO_2 wafer in a solution of the copolymer (10 mg/mL in methylene chloride) for 1 h at ambient temperature, followed by washing with the solvent. The pSAMs were then cured at 110 ℃ for 5 min to further ensure covalent bond formations via dehydration between the silane groups in the copolymer and the hydroxyl groups of the substrate that were generated after oxygen plasma treatment.[10]

<133> The self-assembled structure on a Si/SiO_2 surface and subsequent specific immobilization of biomolecules are shown in Figure 1. The pSAMs formed from poly-1 through poly-3 were characterized by ellipsometry and the contact angle measurement to measure the thickness and the hydrophilicity of the pSAMs, respectively (Table 1).

<134> 【Table 1】

<135> Thickness and Static Water Contact Angle of the pSAMs Constructed on a Si/SiO_2 Wafer Measured by Ellipsometry and Contact Angle and Surface Tension Analyzer

<136>

	Poly-1	Poly-2	Poly-3
Thickness(Å)	9.9 ± 0.1	10.4 ± 0.1	10.7 ±0.1
Static CA(°)	35.6 ± 0.4	35.3 ± 0.3	33.7 ± 0.5

Similar to the previously reported pSAMs of the copolymer lacking the NAS moiety,[10] uniform ultrathin films of the copolymers were generated overall the surface with an average thickness of 1 nm as measured by ellipsometry, suggesting that the films existed in the form of polymeric monolayers, not polymeric multilayers.

<137> The wettability of the pSAMs was attained by static water contact angle measurement at ambient temperature. The contact angle decreased with the

increasing ratio of hydrophilic PEG in the copolymer. The presence of the polymer layer was further confirmed by X-ray photoelectron spectroscopy (Figure 3 and Table 2).

<138>

<139> 【Table 2】

<140> Elemental composition of the pSAMs of poly-1 on a Si/SiO_2 wafer as measured by XPS

<141>

Substrate	Elemental composition(%)			
	C1s	N1s	O1s	Si2p
pSAM of poly-1	22.7	1.21	33.38	42.71

To examine the feasibility of the pSAMs as a dual functional surface (bio-reactive/bio-inert), a micropattern of biomolecules was prepared using a soft lithographic technique and a microcontact printing (μCP)[12] (Figure 4). In the first step, the amine-terminated biotin ink (biotin-NH_2, 10 mM in ethanol) was contact-printed for 1 min onto the pSAMs of poly-1 using a positive PDMS stamp with 50 ×50 μm circular patterns. Since amine groups of proteins can easily react with the activated NAS of the pSAMs, the resulting biotin-patterned pSAMs after removal of the stamp was immersed in borate buffer (pH 8.5) for 1 h to hydrolyze the unreacted NAS on the surface, and subsequently incubated with a solution of TRITC-labeled streptavidin (0.1 mg/mL of PBS buffer, pH 7.4). Figure 4 shows the fluorescence microscopic image of the pattern of TRITC-labeled streptavidin. As expected, streptavidin was deposited on the biotin-functionalized areas exclusively, indicative of specific interactions between them. It should be noted that no detectable nonspecific adsorption of streptavidin was observed on the areas where only a hydrolyzed polymeric layer existed without biotin, resulting in high contrast of signal-to-noise.

<142> When a smaller PDMS stamp was used (25 μm × 25 μm with a circle pattern), a similar result was obtained. Although either backprinting of PEG[13] or precoating step of bovine serum albumin (BSA)[14] has been reported as

necessary to minimize nonspecific adsorption of proteins onto any surfaces, a polymeric system of the present invention, however, does not need such additional steps due to preexisting PEG groups in the copolymer.

<143> To further confirm the protein resistance of the hydrolyzed form of the pSAMs,[15] the extent of protein adsorption was evaluated using BSA as a model plasma protein. The hydrolyzed form of pSAMs on a Si/SiO_2 wafer was incubated in BSA solution (0.1 mg/mL in PBS buffer, pH 7.4) for 1 h, washed, and air-dried. Table 3 shows the thickness of the hydrolyzed form of pSAMs of poly-1 through poly-3 as measured by ellipsometry before and after BSA adsorption.

<144> 【Table 3】

<145> Thickness of the Control (Unmodified) and the Hydrolyzed Form of pSAMs on the Si/SiO_2 Wafer before and after Bovine Serum Albumin Adsorption by Ellipsometry

<146>

	Control	Poly-1	Poly-2	Poly-3
Thickness(Å) (before)	Not available	10.6 ± 0.6	11.5 ± 0.6	11.9 ± 0.6
Thickness(Å) (after)	44.7 ± 0.1	11.5 ± 0.1	11.6 ± 0.1	11.6 ± 0.1

The thickness of the pSAMs after BSA adsorption maintained almost the same as the initial thickness. However, the thickness of unmodified silicon wafer as a control significantly increased. High-resolution nitrogen (1s) intensity on the pSAMs and control (unmodified Si/SiO_2 wafer) as measured by X-ray photoelectron spectroscopy revealed that the former showed 3% level of BSA adsorption (97% resistance) relative to that of the latter control. This result clearly demonstrates that the NAS-hydrolyzed pSAMs are highly resistant to nonspecific protein adsorption. All of the data presented above clearly suggests that biomolecules can be immobilized onto the pSAMs of poly(TMSMA-r-PEGMA-r-NAS) in a specific way with little nonspecific adsorption.

<147> Since spacing between biomolecules immobilized on the surface affects the interactions with target molecules, it is critical to control the density of the bio-reactive group on the surface for optimization of biochips.[16] In

this sense, the spacing effect was examined using a series of poly(TMSMA-r-PEGMA-r-NAS) bearing different molar ratios of the bio-reactive group (NAS): (i) poly-1 (35%), (ii) poly-2 (21%) and (iii) poly-3 (12%), respectively. Figure 5 shows the fluorescence microscopic images of the pattern of TRITC-labeled streptavidin onto each pSAM that was prepared by the same procedures of microcontact printing as described earlier. Streptavidin was efficiently immobilized onto each pSAM to give a micropattern with high signal-to-noise ratio but with different fluorescence intensity according to the density of the bio-reactive group on the surface. For better comparison, the relative signal intensity of the circle areas in the image of each pSAM was obtained and shown in Table 4.

<148> 【Table 4】

<149>

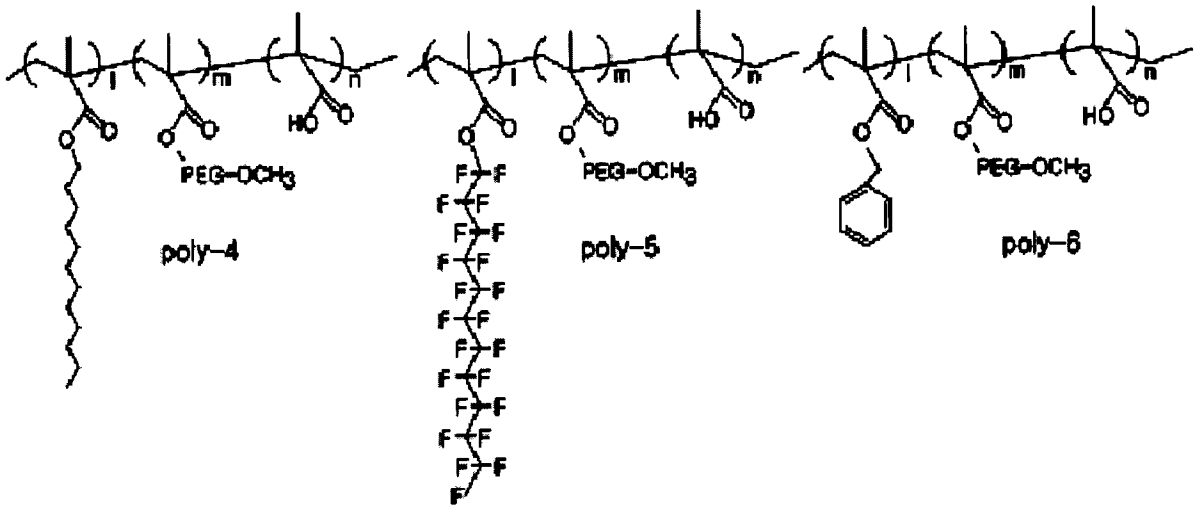
	Poly-1	Poly-2	Poly-3
Mean fluorescence intensity	231.77 ± 15.04	156.67 ± 45.82	99.01 ± 57

Poly-1 with a NAS content of 35% showed 232 ± 15 a.u. of the fluorescence intensity, whereas a much lower intensity was observed for poly-2 and poly-3 with 157 ± 46 and 99 ±57 a.u., respectively. As the NAS contents increase on the pSAMs of the polymers, a gradual increase was observed in the immobilization of streptavidin. This result indicates another favorable aspect of the present copolymer system: simple density control of the bioreactive portion by changing the initial feed ratio of each monomer in the synthesis, which may be useful in optimization of biochips.

<150>

<151> The chemical structure of an amphiphilic polymer has the following structure.

<152>



<153> Each polymer was synthesized from the corresponding monomers, with a molar feed ratio of 2:2:2, by radical polymerization. Dodecyl, fluoroalkyl and benzyl groups were selected as anchoring groups in poly-4, poly-5 and poly-6, respectively. Poly-4, poly-5 and poly-6 are anchored onto polystyrene plastic surface via hydrophobic interaction, via both hydrophobic and dipole interaction and via π-π stacking interaction, respectively. All the three polymers were well soluble in water presumably due to the presence of multiple PEG groups.

<154> The polymeric plastic surfaces coated with poly-4 and poly-6 were characterized by the static water contact angle analyzer to measure the wettability of the plastic surface at room temperature (Table 5).

<155> 【Table 5】

<156>

	Control	Poly-4	Poly-6
Static CA(°)	130 ±7	74 ± 1	79 ±2

The water contact angle of poly-4 and poly-6 decreased significantly from 130±7° to 74±1° and 79±2° after coating with amphiphilc polymers for 1 h. The significant decrease in contact angle indicates that plastic surface became more hydrophilic in nature with increasing hydrophilic PEG in the copolymer after polymer coating.

<157> To scrutinize the prospect of the polymer-coated plastic surface as a functional surface, a micropattern of biomolecules was prepared using a soft

lithographic technique. Figure 7 shows the fluorescence microscopic image of the pattern of TRITC-labeled streptavidin. As a result, streptavidin was efficiently immobilized onto each polymer-coated plastic surface to give a micropattern with high signal-to-noise ratio. However, a different level of fluorescence intensity was ascertained depending on the anchoring site of each polymer (dodecyl group in poly-4 and benzyl group in poly-6).

<158> To compare accurately between poly-4 and poly-6, the relative signal intensity of the circle areas in the image of each polymer-coated plastic surface was obtained and shown in Table 6.

<159> 【Table 6】

<160>

Polymer	Poly-4	Poly-6
Fluorescence intensity	149.9 ± 24.4	179.7 ± 34.3

Poly-4 with a dodecyl group showed 149.9 ± 24.4 a.u. of the fluorescence intensity, whereas a higher intensity was observed for poly-6 with 179.7 ± 34.4 a.u. Poly-4 has alkyl chain as anchoring site, thus enabling to attach on plastic surface via hydrophobic and van der waals interaction. In contrast, poly-6 can strongly attach on plastic surface via hydrophobic and van der waals and also via $\pi-\pi$ stacking interaction due to aromatic ring structure (benzyl group). Background signal was little observed on the areas where only a hydrolyzed polymeric surface existed without biotin, resulting in high contrast of signal-to-noise ratio. Although either backprinting of PEG[13] or precoating step of bovine serum albumin (BSA)[14] has been reported as necessary to minimize nonspecific adsorption of proteins onto any surfaces, a polymeric system of the present invention requires no such additional step due to preexisting PEG groups in the copolymer.

<161> To evaluate the anti-biofouling property of poly-4 through poly-6, polystyrene substrate coated with each polymer was immersed in BSA solution (0.25 mg/mL in PBS, pH 7.4) for 2 h. The degree of nonspecific protein adsorption onto the pSAMs was obtained from the high resolution N(1s) XPS spectrum (Figure 6). As compared to a control group, the polymer-coated polystyrene substrate of the present invention showed a significantly lower

protein adsorption (about 1% protein adsorption and 99% resistance).

<162> Surface of 96-well plates was treated with a polymer of the present invention. Biotin-amine was introduced onto the plates by EDC/NHS reagent in order to form streptavidin coated polymer surfaces for capturing and adsorbing biotin-conjugated mouse anti-rabbit IgG as shown in Figure 8. The biotin-conjugated mouse IgG was detected by ELISA method using HRP-conjugated anti-mouse IgG. A comparison has been made between thus prepared polymer-coated streptavidin plates and commercially available streptavidin plates. The commercially available streptavidin coated ELISA plates were treated with 100 μL of milk protein (2% w/v in distilled water) as blocking buffer for 2h.[15] However, such additional step was not required for the polymer-coated streptavidin plates herein because PEG groups block nonspecific binding of proteins. Figure 9 shows the dependence of absorbance on the concentration of biotin-IgG. The polymer-coated strepavidin plates shows a relatively higher intensity than the commercial streptavidin plates at the same concentrations, which indicates the superiority of the polymer-coated streptavidin plates herein in capturing biotin-IgG. Despite the non-treatment with blocking buffer, the polymer-coated plates herein showed a similar to the control in absorbance intensity at zero ng/ml concentration of biotin-IgG, which is due to PEG part.

<163> Antibody was immobilized by using protein A onto polymer-coated plastic surface. Protein A has been extensively used to immobilize different kinds of antibodies in the immunoassay. The protein A specifically captures the Fc part of an antibody and provides orientation of the bound antibody. In general, no antibody modification is required for the protein A-mediated antibody. Thus, the present invention provides a strikingly improved antigen detection method compared to the conventional antibody immobilization methods (e.g., the covalent conjugation or physical adsorption methods). Figures 10 and 11 schematically represent the anti-BSA immobilization by using protein A on polymer-coated plastic surface and the fluorescence microscopic image of the pattern of FITC-labeled BSA, respectively. As expected, FITC-labeled BSA

was deposited on the anti-BSA functionalized areas exclusively, indicative of specific interactions between them. It should be noted that no detectable nonspecific adsorption of BSA was observed on the areas where only a hydrolyzed polymeric layer existed without anti-BSA, resulting in high contrast signal-to-noise ratio. The relative signal intensity of the circle areas in the image of each polymer-coated plastic surface was obtained. Poly-4 with a dodecyl group showed 98.7 ± 15 a.u. of the fluorescence intensity, whereas a lower intensity of 42.6 ± 6.9 a.u was observed for poly-6. As mentioned above, a polymer system herein is advantageous for the orientations of antibody immobilization using protein A on plastic surface, which may be utilized in the development of protein chips.

<164> In conclusion, the present invention discloses that dual functionalities (i.e. anti-biofouling and bio-reactive properties) can be efficiently introduced onto SiO_2-based and plastic surface by forming polymeric self-assembled monolayers (pSAMs) with a novel PEG copolymer. According to the present invention, biomolecules are selectively immobilized onto plastic surface coated with amphiphilic polymers, while preventing nonspecific adsorption. Moreover, the surface density of both the bio-reactive and anti-biofouling functionalities can be tailored by simply changing initial feed ratios of each monomer in the polymer synthesis. Taken together, the present amphiphilic polymer-coated plastic surface platform may have warrant applications in the field of biosensors and biochips.

<165> The invention has been described in detail with reference to preferred embodiments thereof. However, it will be appreciated by those skilled in the art that changes may be made in these embodiments without departing from the principles and spirit of the invention, the scope of which is defined in the appended claims and their equivalents.

<166>

<167> REFERENCES

<168> All publications, patents and patent applications cited herein, whether supra or infra, are hereby 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.

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【CLAIMS】

【Claim 1】

<187> A process of preparing a polymer-coated anti-biofouling solid substrate comprising a covalently-bondable functional group, the process comprising the steps of:

<188> (a) preparing a polymer comprising a surface-anchoring part, a protein-repellent part and a reactive part by reacting (i) a first monomer comprising a surface-anchoring part that can be bound onto the surface of the solid substrate, (ii) a second monomer comprising an anti-biofouling polymer, and (iii) a third monomer comprising a covalently-bondable functional group; and

<189> (b) coating the polymer onto the surface of the solid substrate.

【Claim 2】

<190> The process of claim 1, wherein the first monomer is a methacrylate- or acrylate-based monomer, the surface-anchoring part of the first monomer is selected from the group consisting of a substituted or unsubstituted silane group, a substituted or unsubstituted alkyl group and a substituted or unsubstituted aryl group.

【Claim 3】

<191> The process of claim 2, wherein the substituted silane group is a silane group substituted with one or more alkoxy groups.

【Claim 4】

<192> The process of claim 2, wherein the alkyl group is a C_{6-30} alkyl group.

【Claim 5】

<193> The process of claim 2, wherein the substituted alkyl group is substituted with F, Cl or Br.

【Claim 6】

<194> The process of claim 1, wherein the second monomer is a methacrylate- or acrylate-based monomer, and the anti-biofouling polymer of the second monomer is selected from the group consisting of polyethylene glycol (PEG), polyalkylene oxide, dextran and polyvinylpyrrolidone.

【Claim 7】

<195> The process of claim 1, wherein the third monomer is a methacrylate- or acrylate-based monomer, and the functional group of the third monomer is a carboxyl group.

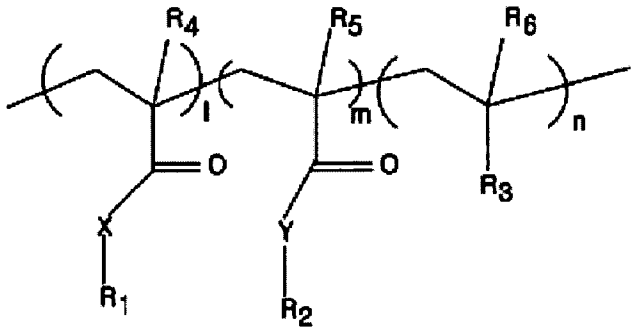
【Claim 8】

<196> The process of claim 7, wherein the carboxyl group is activated by at least one selected from the group consisting of succinimide, succinimidyl ester, sulfosuccinimidyl ester, 2,3,5,6-tetrafluorophenol ester, 4-sulfo-2,3,5,6-tetrafluorophenol ester, aldehyde, acid anhydride, azide, azolide, carboimide, epoxide, ester, glycidyl ether, halide, imidazole and imidate.

【Claim 9】

<197> The process of claim 1, wherein the polymer has is represented by Formula 1:

<198> Formula 1



<199>

<200> wherein R_1 is selected from the group consisting of a silylalkyl group, an (alkoxysilyl)alkyl group, a (hydroxysilyl)alkyl group, a substituted or unsubstituted alkyl group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl group and a substituted or unsubstituted alkaryl group; R_2 is selected from the group consisting of polyethylene glycol (PEG), polyalkylene oxide, polyphenylene oxide, a copolymer of PEG and polyalkylene oxide, poly(methoxyethyl methacrylate), poly(methacryloyl phosphatidylcholine), perfluoropolyether, dextran and polyvinylpyrrolidone; R_3 is selected from the group consisting of aldehyde, epoxy, haloalkyl, a primary amine, thiol, maleimide, ester, carboxyl group and hydroxyl group;

each of R_4, R_5 and R_6 is independently H or C_1-C_5 alkyl group; each of X, Y and Z is independently oxygen, sulfur or nitrogen atom; and each of l, m and n is independently an integer of 1-10,000.

【Claim 10】

<201> The process of claim 10, wherein the polymer is a monolayer self-assembled on the solid substrate.

【Claim 11】

<202> The process of claim 1 or 8, which further comprises the step of (c) covalently binding the functional group derived from the third monomer with one or more chemicals, proteins, peptides, nucleotides or saccharides.

【Claim 12】

<203> A dual functional solid substrate comprising a surface-anchoring part, an anti-biofouling part and a reactive part, the solid substrate being coated with a polymer prepared by the polymerization of the monomers of:

<204> (i) a first monomer comprising a surface-anchoring part that can be bound onto the surface of the solid substrate,

<205> (ii) a second monomer comprising an anti-biofouling polymer, and

<206> (iii) a third monomer comprising a covalently-bondable functional group.

【Claim 13】

<207> The solid substrate of claim 12, wherein the first monomer is a methacrylate- or acrylate-based monomer, and the surface-anchoring part of the first monomer is selected from the group consisting of a substituted or unsubstituted silane group, a substituted or unsubstituted alkyl group and a substituted or unsubstituted aryl group.

【Claim 14】

<208> The solid substrate of claim 13, wherein the alkyl group is a C_{6-30} alkyl group.

【Claim 15】

<209> The solid substrate of claim 13, wherein the substituted alkyl group is substituted with F, Cl or Br.

【Claim 16】

<210> The solid substrate of claim 12, wherein the second monomer is a methacrylate- or acrylate-based monomer, and the anti-biofouling polymer of the second monomer is selected from the group consisting of polyethylene glycol (PEG), polyalkylene oxide, dextran and polyvinylpyrrolidone.

【Claim 17】

<211> The solid substrate of claim 12, wherein the third monomer is a methacrylate- or acrylate-based monomer, and the functional group of the third monomer is carboxyl group.

【Claim 18】

<212> The solid substrate of claim 17, wherein the carboxyl group is activated by at least one selected from the group consisting of succinimide, succinimidyl ester, sulfosuccinimidyl ester, 2,3,5,6-tetrafluorophenol ester, 4-sulfo-2,3,5,6-tetrafluorophenol ester, aldehyde, acid anhydride, azide, azolide, carboimide, epoxide, ester, glycidyl ether, halide, imidazole and imidate.

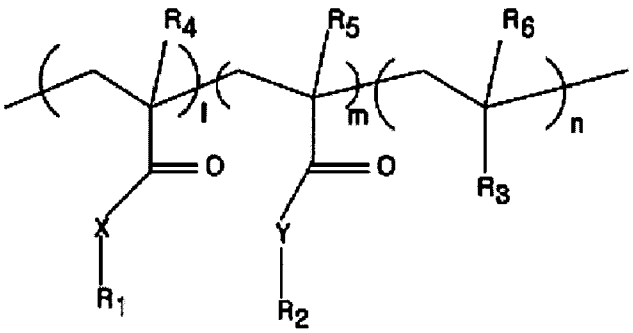
【Claim 19】

<213> The solid substrate of claim 12, wherein the reactive part is bound to one or more chemicals, proteins, peptides, nucleotides or saccharides.

【Claim 20】

<214> The solid substrate of claim 12, wherein the polymer is represented by Formula 1:

<215> Formula 1



<216>

<217> wherein R_1 is selected from the group consisting of a silylalkyl group,

an (alkoxysilyl)alkyl group, a (hydroxysilyl)alkyl group, a substituted or unsubstituted alkyl group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl group and a substituted or unsubstituted alkaryl group; R_2 is selected from the group consisting of polyethylene glycol (PEG), polyalkylene oxide, polyphenylene oxide, a copolymer of PEG and polyalkylene oxide, poly(methoxyethyl methacrylate), poly(methacryloyl phosphatidylcholine), perfluoropolyether, dextran and polyvinylpyrrolidone; R_3 is selected from the group consisting of aldehyde, epoxy, haloalkyl, primary amine, thiol, maleimide, ester, carboxyl group and hydroxyl group; each of R_4, R_5 and R_6 is independently H or C_1-C_5 alkyl group; each of X, Y and Z is independently oxygen, sulfur or nitrogen atom; each of l, m and n is independently an integer of 1-10,000.

【Claim 21】

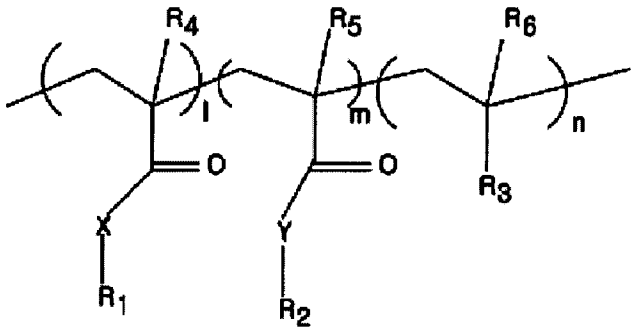
<218> A biochip or biosensors comprising a solid substrate of any of claims 12-20.

【Claim 22】

<219> A polymer represented by Formula 1:

<220> Formula 1

<221>

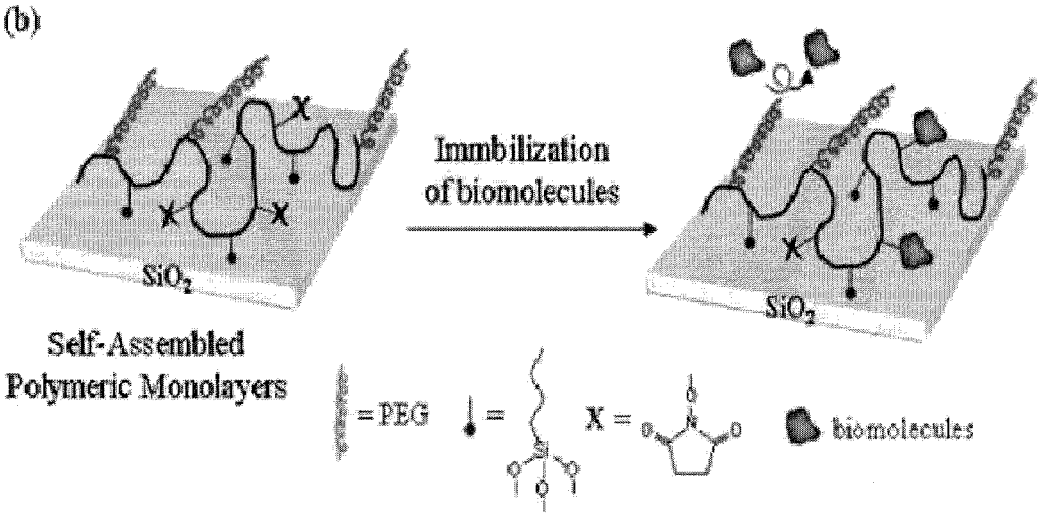


<222> wherein R_1 is selected from the group consisting of a silylalkyl group, an (alkoxysilyl)alkyl group, a (hydroxysilyl)alkyl group, a substituted or unsubstituted alkyl group, a substituted or unsubstituted aryl group, a substituted or unsubstituted aralkyl group and a substituted or unsubstituted alkaryl group; R_2 is selected from the group consisting of polyethylene glycol

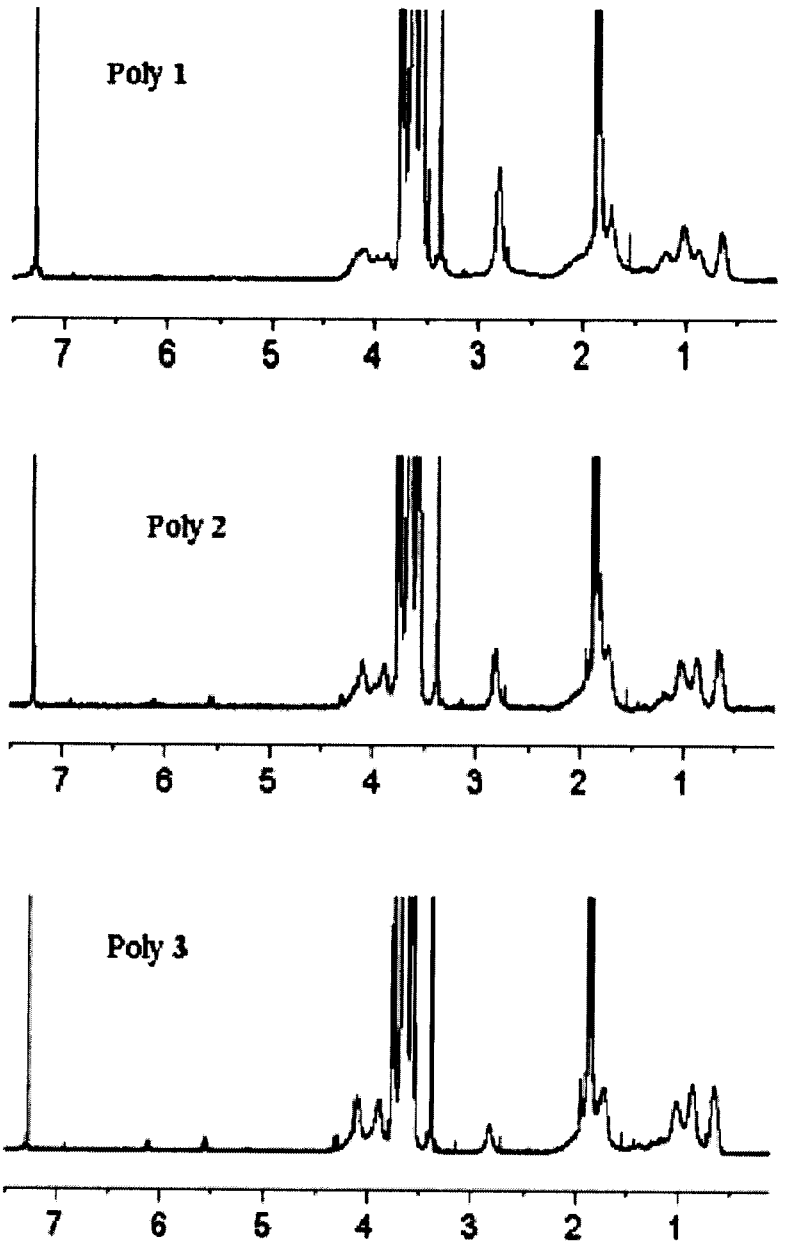
(PEG), polyalkylene oxide, polyphenylene oxide, a copolymer of PEG and polyalkylene oxide, poly(methoxyethyl methacrylate), poly(methacryloyl phosphatidylcholine), perfluoropolyether, dextran and polyvinylpyrrolidone; R_3 is selected from the group consisting of aldehyde, epoxy, haloalkyl, primary amine, thiol, maleimide, ester, carboxyl group and hydroxyl group; each of R_4, R_5 and R_6 is independently H or C_1-C_5 alkyl group; each of X, Y and Z is independently oxygen, sulfur or nitrogen atom; each of l, m and n is independently an integer of 1-10,000.

【DRAWINGS】

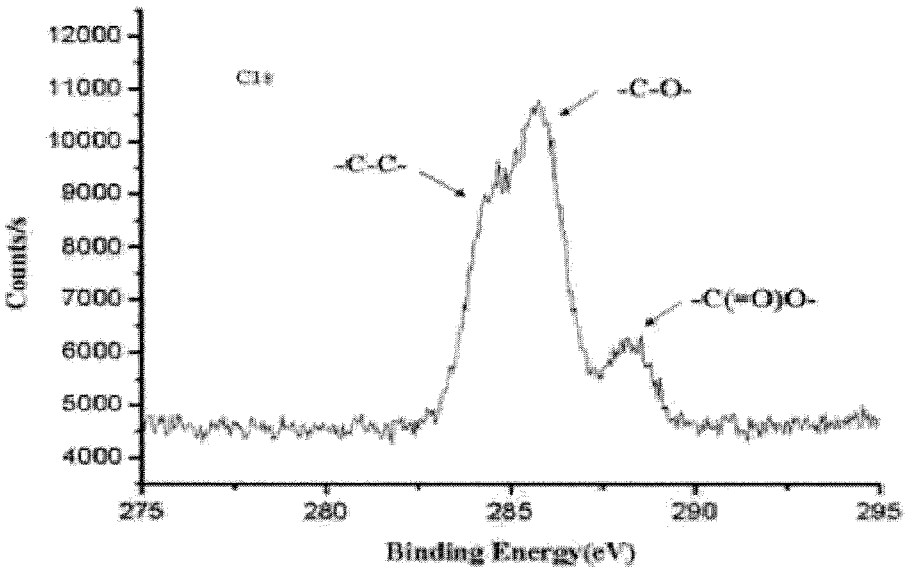
【Figure 1】



【Figure 2】



【Figure 3】



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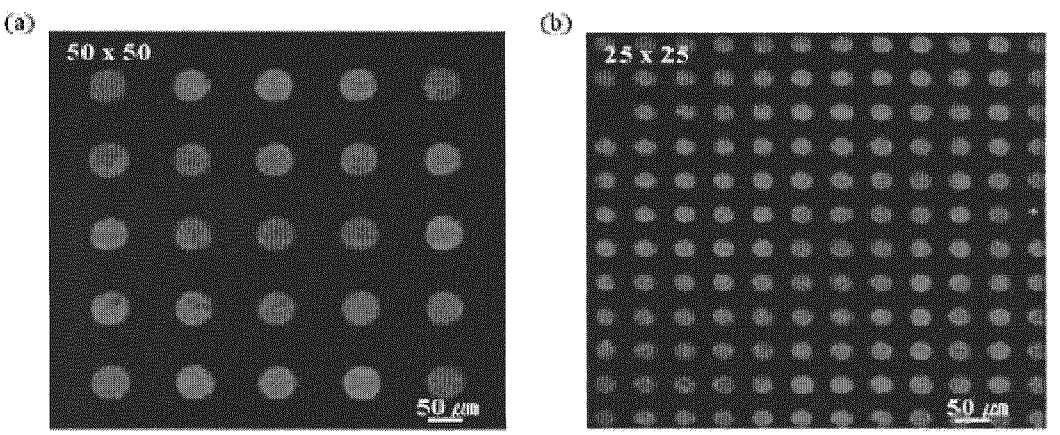
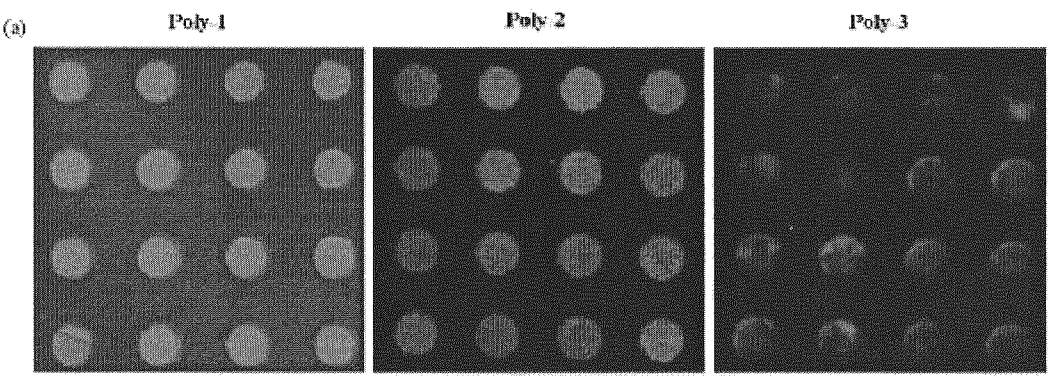
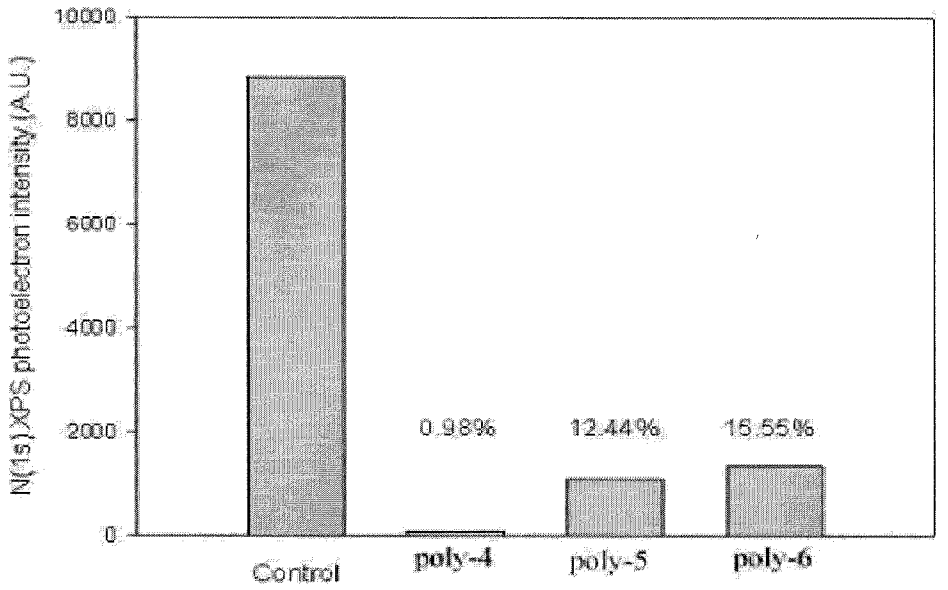


【Figure 5】



【Figure 6】



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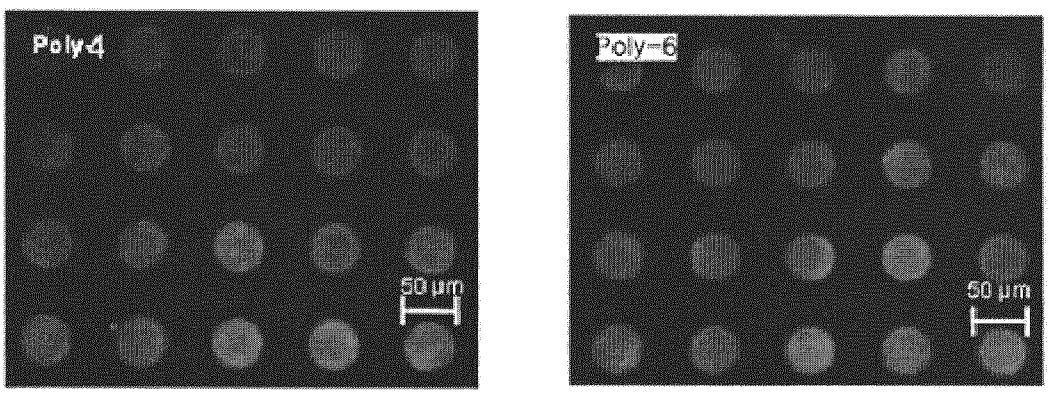

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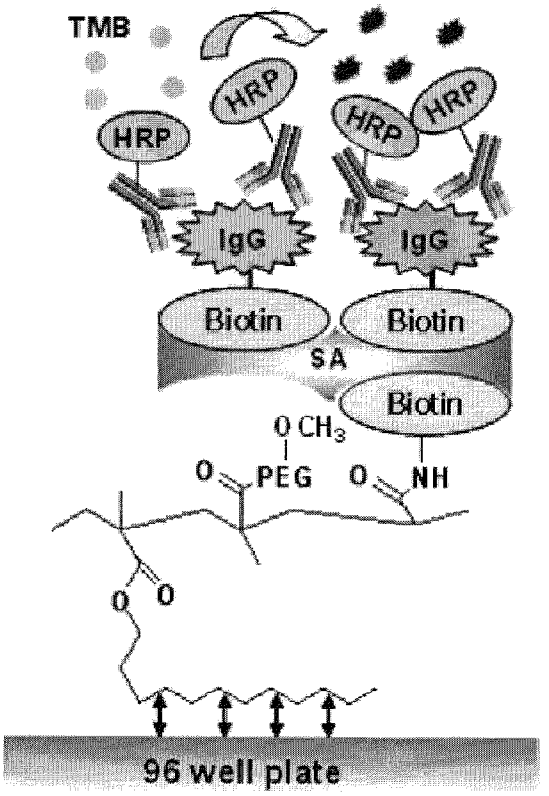
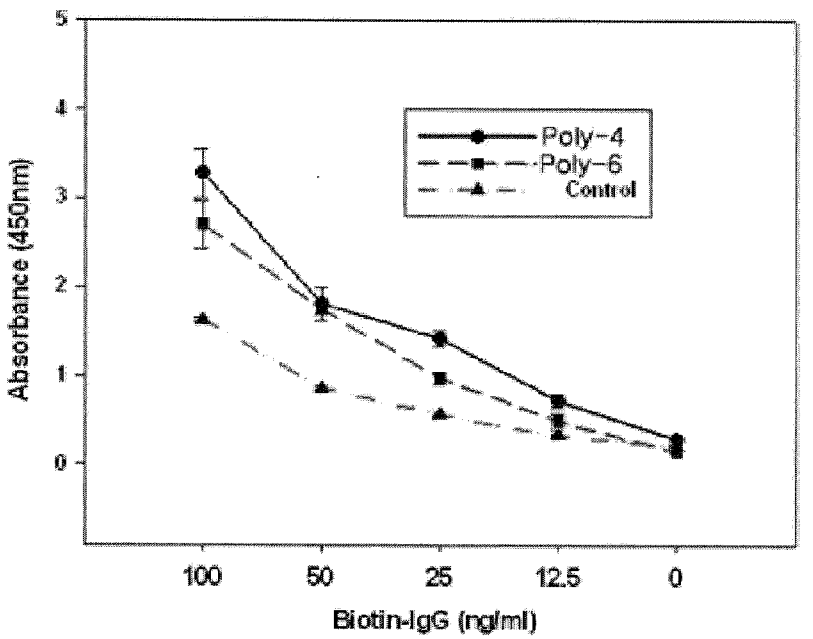
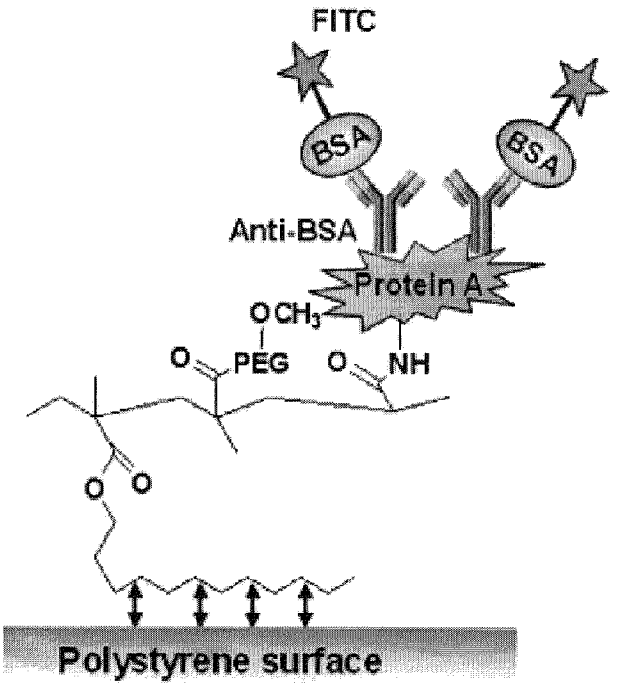


【Figure 9】



【Figure 10】


FITC
BSA
BSA
Anti-BSA
Protein A
O
CH3
O
PEG
O
NH
O
O
Polystyrene surface

【Figure 11】