Title: FUNCTIONALIZED POLY (3,4-ALKYLENEBRIDGEDTHIOPHENE) (PABT)

Abstract: A functionalized poly (3, 4-alkylenebridgedthiophene) (PABT) polymer of formula (I): where $A_i$ is a bridging alkyne chain; $Y_1$ and $Y_2$ are, independently, O, S or N-$R_2$, where $R_2$ is hydrogen, an alkyl group, alkenyl group, alkynyl group, a cycloalkyl, an aryl or heterocycle; and $R_1$ is a functional chain attached to the bridging alkyne chain, are described. In addition, a process for their preparation and a method for depositing functionalized PABT polymers on non-conductive support matrix or nanoparticle are described. These polymers are useful in the preparation of bionanointerfaces.
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FUNCTIONALIZED POLY(3,4-ALKYLENEBRIDGEDTHIOPHENE) (PABT)

This application claims the benefit of US Provisional Application No. 60/996,028, filed on October 25, 2007, which is incorporated herein by reference in its entirety.

FIELD

The invention generally relates to functionalized poly(3,4-alkylenebridgedthiophene) (PABT) polymers, a method for their preparation and uses thereof.

BACKGROUND


Poly(3,4-ethylenedioxythiophene) (PEDOT) is a known conducting polymer due to its electrical properties, long-term stability, and transparency to visible light at its doping state (Heywang, G.; F. Jonas, Adv. Mater. 1992,

Therefore, there is a need to investigate the utility of such polymers in bioengineering applications, and
a need to develop polymers that can interact with biological molecules, such as proteins and cells, for preparation of biointerfaces. Further, there is a need for functionalized polymers that would allow the development of biointerfaces having regions with functionalized PABT polymers, which form, for example, adhesive (i.e. fouling) or non-adhesive (i.e. non-fouling) surfaces for selective interaction with the biological molecules or targets of interest. This may permit development of, for example, biosensor devices for metabolite detection and membranes for cell patterning.

For polymerization of the 3,4-ethylenedioxythiophene (EDOT) monomers, most of the studies have utilized electropolymerization to deposit PEDOT directly on conductive and flat electrode surfaces. While electropolymerization offers a direct and convenient way to deposit PEDOT films, there is a need to develop a synthetic strategy to coat PABTs on non-conductive supporting matrix and nanoparticles. This would enable the construction of electroactive matrix or carriers for more versatile bioengineering applications.

**SUMMARY**

The present invention relates to functionalized poly(3,4-alkylenebridgedthiopene) (PABT) polymers and a method of depositing PABT polymer coatings on a non-conducting support matrix or a nanoparticle.

In a broad aspect the invention relates to a polymer comprising "n" subunits of formula (I):

3
wherein in each subunit:

$A_1$ is a bridging alkylene chain, optionally substituted, having 2, 3, 4, 5 or 6 carbon atoms;

$Y_1$ and $Y_2$ are, independently, $O$, $S$ or $N-R_2$, and wherein $R_2$ is hydrogen, an alkyl group, an alkenyl group, an alkynyl group, a cycloalkyl, an aryl or a heterocycle; and

$R_1$ is a functional chain attached to the bridging alkylene chain, and

wherein $n$ is an integer of 2 or more.

In a further aspect of the invention, it is contemplated that the sulphur ($S$) of the thiophene ring may be replaced by $O$, $N-R_2$ or another similarly functioning atom or group, as would be recognized by an artisan. Further, such a replacement could be made to all aspects of the invention.

In an embodiment, the polymer is, for example and without limitation, a co-polymer comprising "m" sub-units of formula (I) and "p" sub-units of Formula (II)
wherein $A_1$ and $A_2$ are independently a bridging alkylene chain, optionally substituted, having 2, 3, 4, 5 or 6 carbon atoms, and, "m" and "p" independently are integers of 2 or more, and $Y_1$, $Y_2$ and $R_1$ are as defined above.

In another embodiment, for example and without limitation, the co-polymer may have, primarily, the sub-unit of formula (I) and some quantity of sub-unit of formula (II). In a still further embodiment, the ratio of the sub-unit of formula (I) to formula (II) can range from 0.01 to 99.99%, 10 to 90%, 20 to 80%, 40 to 60%, 60% to 40%, 80% to 20% or 90% to 10%. In a further embodiment, for example and without limitation, about 5% of the units of the copolymer described above are sub-unit of formula (II). In a still another embodiment, for example and without limitation, the molecular weight of the polymer can range from 1000 - 1,000,000.

In a further embodiment, for example and without limitation, $A_1$ and $A_2$ are identical and are part of a glycol chain forming a five-membered ring with dioxythiophene.

In a still further embodiment, for example and without limitation, $R_1$ is a non-adhesive chain, wherein the non-adhesive chain is a phospholipid chain, an oligo-
ethylene glycol or a poly-ethylene glycol. In another embodiment, \( R_1 \) is an oligo-ethylene glycol having 2, 4, 6 or 8 carbon atoms.

In another broad aspect the invention relates to a compound of formula (III),

\[
\begin{array}{c}
\text{Formula (III)} \\
\end{array}
\]

wherein \( A_1, Y_1, Y_2 \) and \( R_1 \) are as described above.

In a further broad aspect the invention relates to a method of preparation of a compound of formula (III), as described above, the method comprising:

- reacting a compound of formula (IV), or a derivative thereof, with a compound of formula (V),

\[
\begin{array}{c}
\text{Formula (IV)} \\
\text{Formula (V)} \\
\end{array}
\]
wherein $A_2$ is a bridging alkylene chain, optionally substituted, having 2, 3, 4, 5 or 6 carbon atoms; and

wherein the compound of formula (IV), or derivative thereof, having a nucleophilic atom on the bridging alkylene chain for nucleophilic substitution on an electrophilic atom on $R_1$, or derivative thereof; and wherein LG is a suitable leaving group.

In a further broad aspect the invention relates to a bionanointerface for controlled adhesion of a biological molecule, the bionanointerface comprising:

- a surface having an adhesive region and a non-adhesive region;

- the adhesive region having a biologically adhesive agent for releasably binding the biological molecule; and

- the non-adhesive region having a polymer with "n" sub-units of formula (I) or a co-polymer having "m" sub-units of formula (I) and "p" sub-units of formula (II), wherein formula (I), formula (II), and n, m and p are as described above.

In an embodiment of the invention, the adhesive agent is, without limitation and for the purpose of illustration, a functionalized poly(EDOT-OH) or poly(EDOT-COOH) polymer. In an another embodiment of the invention, the polymer of formula (I), without limitation and for the purpose of illustration, is a functionalized poly(PEDOT-EG3-OH) polymer. In a further embodiment of the invention, the
polymer of formula (I), without limitation and for the purpose of illustration, is a co-polymer functionalized poly(PEDOT-EG3-OH) and poly(PEDOT-OH). In a still further embodiment of the invention, the nanobiointerface is formed by layer-by-layer electropolymerization.

In a further broad aspect the invention relates to a method of depositing functionalized poly(3,4-alkylenebridgedthiophene) (PABT) polymer coatings on a non-conductive support matrix or nanoparticle, the method comprising the steps of:

- dissolving a functionalized 3,4-alkylenebridgedthiophene (ABT) monomer in an aqueous medium to form a monomer solution;
- mixing the monomer solution with the non-conductive support matrix or nanoparticle; and
- polymerizing the monomers to form the functionalized PBDOT polymer.

In an embodiment, for example and without limitation, the method for depositing PABT polymer coatings, the polymerization is an oxidative chemical polymerization reaction. In a further embodiment, for example and without limitation, the monomer is a monomer having low solubility in water and which can be dissolved by sonicating. In a still further embodiment of the invention, without limitation and for the purpose of illustration, the non-conductive support matrix or nanoparticle is silica, a polystyrene nanoparticle, a metal oxide particle, nylon fibers, cellulose, a siliceous MCF or a chitosan alignate fiber. In an another embodiment of the invention, without limitation and for the purpose of illustration, the functionalized PABT polymer coating is poly(EDOT-OH) or poly
(EDOT-COOH). In another further embodiment of the invention, without limitation and for the purpose of illustration, the method is used for the preparation of functionalized PABT nanostructures. In another still further embodiment of the invention, without limitation and for the purpose of illustration, the nanostructure is a hollow poly(PEDOT-OH) sphere having a thickness of at least about 15 nm. In still another embodiment of the invention, without limitation and for the purpose of illustration, the functionalized poly PABT is a poly(PEDOT-OH) deposited on the non-conducting support matrix or nanoparticle having a layer thickness of 5-7 nm.

**BRIEF DESCRIPTION OF FIGURES**

Figure 1(A) displays, according to an invention embodiment, results of quartz crystal microbalance (QCM) studies of non-specific binding of glucose oxidase (GOX) to PEDOT nanobiointerfaces of various monomer compositions: (♦) original Au electrode surface, (●) EDOT-EG3-OH/EDOT-OH molar ratio = 9:1, (■) EDOT-EG3-OH/EDOT-OH molar ratio = 1:1, (▲) EDOT-OH, and (▼) EDOT-COOH.

Figure 1(B) displays, according to an invention embodiment, results of quartz crystal microbalance (QCM) studies of non-specific binding of bovine serum albumin (BSA) proteins to PEDOT nanobiointerfaces of various monomer compositions: (♦) original Au electrode surface, (●) EDOT-EG3-OH/EDOT-OH molar ratio = 9:1, (▲) EDOT-OH, and (▼) EDOT-COOH.

Figure 2 displays, according to an invention embodiment, the amperometric response of various functionalized PEDOT nanobiointerfaces in the presence of 40 mM of glucose upon non-specific adsorption of GOX and osmium-containing electroactive hydrogels.
**Figure 3(A)** displays, according to an invention embodiment, amperometric response of glucose at different concentrations on poly(EDOT-OH)-coated platinum electrode after adsorbing GOX on the surface of the electrode.

**Figure 3(B)** displays, according to an invention embodiment, amperometric response as a function of glucose concentration (■), and polynomial curve fit.

**Figure 4** displays in sections (a), (b), (c) and (d), and according to an invention embodiment, the adhesion of (top) NIH3T3 and (bottom) KB cells on PEDOT nanobiointerfaces of different monomer compositions: in section (a) EDOT, in section (b) EDOT-OH, in section (c) EDOT-COOH, and in section (d) EDOT-EG3-OH/EDOT-OH molar ratio = 9:1.

**Figure 5** displays, and according to an invention embodiment, the proliferation of NIH3T3 cells after (a) 2 h, (b) 15 h, and (c) 39 h of incubation on adhesive poly(EDOT-OH) biointerface, in sections (a), (b) and (c), respectively.

**Figure 6** shows a multi-layer PEDOT structure, according to an invention embodiment, where section (a) is legends of monomer composition of layered PEDOT nanobiointerfaces, and sections (b)-(g) are controlled cell adhesion from alternating layer-by-layer deposition of PEDOT nanobiointerfaces with adhesive and non-adhesive properties.

**Figure 7** shows the contact angles of PEDOT nanobiointerfaces from layer-by-layer deposition, according to an invention embodiment. The legends for the composition of PEDOT nanobiointerfaces are as shown in Figure 9.

**Figure 8** shows the controlled cell adhesion on poly(EDOT-OH) patterned on co-poly(EDOT-OH)-poly(EDOT-EG3-OH) surface, according to an invention embodiment. Section (a) is top and
side views of the device patterned by selective electropolymerization using PDMS mask, and magnified microscopic images of selective cell adhesion on the patterned surface are shown in sections (b) and (c).

Figure 9 illustrates a method for the coating of various substrates with functionalized PEDOT, according to an invention embodiment.

Figure 10(A) shows a transmission Electron Microscopy (TEM) micrograph of poly(EDOT-OH)-coated silica nanoparticles.

Figure 10(B) shows an energy Dispersive X-ray spectroscopy (EDX) results of poly(EDOT-OH)-coated silica nanoparticles.

Figure 11 displays in sections (a), (b), (c) and (d), a Scanning Electron Microscopy (SEM) micrographs of 200-nm polystyrene (PS) beads: in section (a) before coating with poly(EDOT-OH) layers, in section (b) after coating with thin (3-5 nm) poly(EDOT-OH) layers, and in section (c) after coating with thick (15-20 nm) poly(EDOT-OH) layers, and in section (d) is shown a SEM micrograph of 500-nm PS beads with intermediately thick (9-12 nm) poly(EDOT-OH) layers.

Figure 12 displays in sections (a), (b), (c) and (d), the structure and morphology of PEDOT-OH-coated PS beads after removal of the PS cores by toluene, where in section (a) TEM and in section (b) SEM micrographs of hollow beads obtained from 200-nm PS beads coated with thin poly(EDOT-OH) layers (< 5 nm) are shown, and in section (c) TEM and in section (d) SEM micrographs of hollow beads obtained from 500-nm PS beads coated with thick poly(EDOT-OH) layers (> 15 nm) are shown.

Figure 13 displays the N\textsubscript{2} adsorption-desorption isotherms of siliceous MCF (●), poly(EDOT-OH)-coated MCF after chemical
polymerization for (■) 4 h and (▲) 16 h, and a (inset) TEM micrograph of MCF after 4-h coating of poly(EDOT-OH).

**Figure 14** displays in sections (a), (b) and (c), the SEM and (inset) optical micrographs of chitosan-alginate fibers: in section (a) before chemical polymerization in EDOT-OH monomer solution and in section (b) and (c) after chemical polymerization in EDOT-OH monomer solution for 4 h and 16 h, respectively.

**DETAILED DESCRIPTION**

The invention relates to functionalized poly(3,4-alkylenebridgedthiophene) (PABT) polymers, methods for their preparation and their use in the preparation of, for example and without limitation, biointerfaces having regions with adhesive (fouling) and non-adhesive (non-fouling) surfaces, where the adhesive surface may bind to a biological target.

The term 'functionalized' in 'functionalized PABT polymer' is not limited and can be any atom or chain that imparts the desired characteristics of adhesiveness or non-adhesiveness due to the functional chain. In an embodiment, the adhesiveness and non-adhesiveness properties are towards adhesive and non-adhesive interaction with a target, such as, a biological target, which includes, without limitation and for the purposes of illustration, a cell or a protein. The functionalized PABT polymers of the invention may be obtained by polymerization of the appropriate functionalized ABT monomers, as would be understood by the skilled person.

In an embodiment, the invention relates to a polymer comprising "n" subunits of formula (I):
Formula (I)

wherein

$A_1$ is a bridging alkylene chain, optionally substituted, having 2, 3, 4, 5 or 6 carbon atoms;

$Y_1$ and $Y_2$ are, independently, O, S or N-$R_2$, and wherein $R_2$ is hydrogen, an alkyl group, an alkenyl group, an alkynyl group, a cycloalkyl, an aryl or a heterocycle; and

$R_1$ is a functional chain attached to the bridging alkylene chain, and

wherein $n$ is an integer of 2 or more.

In an embodiment, the polymer is, for example and without limitation, a co-polymer comprising "m" sub-units of formula (I) and "p" sub-units of Formula (II)
wherein $A_1$ and $A_2$ are independently, and optionally substituted, a bridging alkylene chain of 2, 3, 4, 5 or 6 carbon atoms, and, "m" and "p" independently are integers of 2 or more, and $Y_1$, $Y_2$ and $R_1$ are as defined above.

A co-polymer is generally considered to be a polymer derived from two (or more) monomeric species.

In the polymers described above, "n", "m" and "p" can be in the range of 2 or more. As the skilled person will understand, the length of the polymer may be dependent on the application, which can be assessed through routine experimentation. In one embodiment, the molecular weight of the polymer can range from 1000-1000000. In another embodiment, without limitation and for the purpose of illustration, the number of 'n', 'm' or 'p' subunits can range from 10 to 10000, 100 to 9000, 1000 to 8000, 2000 to 7000 or 3000 to 5000.

The following definitions of the various groups as described herein are the same for all occurrences of these groups throughout the entire specification unless otherwise indicated.

For the purpose of illustration and without limitation, a bridging alkylene chain that may be
substituted or unsubstituted, for example, and without limitation, include any straight or branched alkylene, for example, methylene, ethylene, n-propylene, i-propylene, sec-propylene, n-butylene, i-butylene, sec-butylene, t-butylene, n-pentylene, i-pentylene, sec-pentylene, t-pentylene, n-hexylene, i-hexylene, 1,2-dimethylpropylene, 2-ethylpropylene, 1-methyl-2-ethylpropylene, 1-ethyl-2-methylpropylene, 1,1,2-trimethylpropylene, 1,1-dimethylbutylene, 2,2-dimethylbutylene, 2-ethylbutylene, 1,3-dimethylbutylene, 2-methylpentylene, 3-methylpentylene cyclopropanylene, cyclobutanylene, cyclopentanylene, or cyclohexanylene.

Substitution on the bridging alkylene chain include, without limitation and for the purpose of illustration, one or more substituents which are the same or different, and may be, for example, and without limitation, a halogen atom, a hydroxyl group, an amine group, a thiol group, a nitro group, a nitrile group or a cyano group. One example of the substituted bridging alkylene chain, as disclosed herein, is a glycerol derivative.

For the purpose of illustration and without limitation, in context of the R₂ substituent on N-R₂, an alkyl group, which may be substituted, may be, for example, and without limitation, any straight or branched alkyl, for example, methyl, ethyl, n-propyl, i-propyl, sec-propyl, n-butyl, i butyl, sec-butyl, t-butyl, n-pentyl, i-pentyl, sec-pentyl, t-pentyl, n-hexyl, i-hexyl, 1,2-dimethylpropyl, 2-ethylpropyl, 1-methyl-2-ethylpropyl, 1-ethyl-2-methylpropyl, 1,1,2-trimethylpropyl, 1,1,2-triethylpropyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 2-ethylbutyl, 1,3-dimethylbutyl, 2-methylpentyl or 3-methylpentyl. The C₁₋₆
alkyl group may be, for example, and without limitation, interrupted by one or more heteroatoms which are independently nitrogen, sulfur or oxygen.

For the purpose of illustration and without limitation, in context of the \( R_2 \) substituent on \( N-R_2 \), an alkenyl group, which may be substituted, may be, for example, and without limitation, any straight or branched alkenyl, for example, vinyl, allyl, isopropenyl, 1-propene-2-yl, 1-butene-1-yl, 1-butene-2-yl, 1-butene-3-yl, 2-butene-1-yl or 2-butene-2-yl. The C2-6 alkenyl group may be, for example, and without limitation, interrupted by one or more heteroatoms which are independently nitrogen, sulfur or oxygen.

For the purpose of illustration and without limitation, in context of the \( R_2 \) substituent on \( N-R_2 \), an alkynyl group, which may be substituted, may be, for example, and without limitation, any straight or branched alkynyl, for example, ethynyl, propynyl, butynyl, pentynyl or hexynyl. The C2-6 alkynyl group may be, for example, and without limitation, interrupted by one or more heteroatoms which are independently nitrogen, sulfur or oxygen.

For the purpose of illustration and without limitation, in context of the \( R_2 \) substituent on \( N-R_2 \), a cycloalkyl group, which may be substituted, may be, for example, and without limitation, cyclopropanyl, cyclobutanyl, cyclopentanyl, cyclohexanyl or cycloheptanyl.

For the purpose of illustration and without limitation, in context of the \( R_2 \) substituent on \( N-R_2 \), an aryl group, which may be substituted, may be, for example, and without limitation, phenyl, pentalenyl, indenyl, naphthyl, azulenyl, heptalenyl, benzocyclooctenyl or phenanthrenyl.
For the purpose of illustration and without limitation, in context of the $R_2$ substituent on N-$R_2$, a non-aromatic heterocyclic group containing one or more heteroatoms which are independently nitrogen, sulfur or oxygen of the heterocyclic group containing one or more heteroatoms which are independently nitrogen, sulfur or oxygen and which group may be substituted, may contain, for example, and without limitation, from 1 to 4 heteroatoms which are independently nitrogen, sulfur or oxygen. The non-aromatic heterocyclic group containing one or more heteroatoms which are independently nitrogen, sulfur or oxygen may be, for example, and without limitation, pyrrolidinyl, pyrrolinyl, piperidinyl, piperazinyl, imidazolinyl, pyrazolidinyl, imidazolydinyl, morpholinyl, tetrahydropyranyl, azetidinyl, oxetanyl, oxathiolanly, phthalimide or succinimide.

For the purpose of illustration and without limitation, in context of the $R_2$ substituent on N-$R_2$, an aromatic heterocyclic group containing one or more heteroatoms which are independently nitrogen, sulfur or oxygen of the aromatic heterocyclic group containing one or more heteroatoms which are independently nitrogen, sulfur or oxygen and which group may be substituted, may contain, for example, and without limitation, from 1 to 4 heteroatoms which are independently nitrogen, sulfur or oxygen. The aromatic heterocyclic group containing one or more heteroatoms which are independently nitrogen, sulfur or oxygen may be, for example, and without limitation, pyrrolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, imidazoyl, thiazoyl or oxazoyl.

Each of the alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, non-aromatic heterocyclic and aromatic heterocyclic groups may be substituted with one or more
substituents which are the same or different. In an embodiment of the present invention, each of the above-mentioned groups may be substituted with, for example, and without limitation, one to three substituents.

The one or more substituents for each of the above-mentioned groups, which are the same or different, may be, for example, and without limitation, a halogen atom, a hydroxyl group, an amine group, a thiol group, a nitro group, a nitrile group or a cyano group.

The $R_1$ functional chain is not limited and can be, for example, substituted or unsubstituted, alkyl, alkenyl, alkynyl, cycloalkyl, cycoalkenyl, aryl, arylalkyl, arylalkenyl or a heterocycle, each of which may have one or more heteroatoms and/or organic functional groups within the functional chain, and additionally, may also be cationic, anionic or zwitterionic. The functional chain is selected based on the interaction desired with a target, such as, a biological cell or protein. It can be either adhesive (fouling) or non-adhesive (non-fouling). Whether a particular functional chain is adhesive or non-adhesive would depend upon the target. Examples of substituted alkyl chains having heteroatoms within the chain, include oligoethylene glycol, polyethylene glycol and an amine-substituted alkyl-phosphate. The molecular weight of the oligoethylene glycol can be, for example, up to about 1000 g/mol and the molecular weight of polyethylene glycol can, for example, range from about 1000 to about 40,000 g/mol. The functional chain can have and further be, different functional groups, such as, alcohols, ketones, aldehydes, carboxylic acid, esters, ethers, amide, amines, imine, phosphodiester, sulphide, thioether, sulfone, sulfoxide or thiols. The functional chain can be selected based on the design and properties desired for the synthesized polymer.
It can be selected, such that the functional group can adhesively (fouling) bind to a target cell or protein or has a non-adhesive (non-fouling) interaction with the target cell or protein.

In an embodiment of the invention, the bridging alkylene chain is a glycerol derivative linking oxygen atoms of a 3,4-dioxythiophene to form a five-membered ring, and resulting in 3,4-ethylenedioxythiophene (EDOT) monomers having a methylene-hydroxy group for attachment to the functional chain. In a further embodiment, the functional chain is a oligo or poly-ethylene glycol (OEG or PEG), a phospholipid or an acetic acid. These embodiments are further described below for the purpose of illustration.

In another aspect of this invention, preparation of 3,4-alkylenebridgedthiophene monomers of formula (III) are also disclosed.

![Formula (III)]

In the monomer of formula (III), $A_1$ and $R_1$ are the same as described above. In one embodiment, for the preparation of monomers of formula (III), a compound of formula (IV), or a derivative thereof, is reacted with a compound of formula (V).
Formula (IV)  Formula (V)

In formula (IV), $A_2$ is a derivative of $A_1$, such as, the protonated form of a hydroxyl group. The reaction may be performed by nucleophilic substitution reaction using the compound of formula (IV), or derivative thereof, having a nucleophilic atom on the bridging alkylene chain for nucleophilic substitution on an electrophilic atom on $R_1$, and where LG is a suitable leaving group.

A nucleophilic atom is generally considered, without limitation, as an atom that forms a bond with an electrophile by donating a pair of electrons for forming the bond. Suitable examples, without being limited to, include, oxygen, nitrogen and sulphur atoms.

Similarly, an electrophilic atom is generally considered, without limitation, as an atom that accepts a pair of electrons from the nucleophilic atom, and can include, without being limited to, carbon atoms bearing a strong electron withdrawing groups, phosphorous atoms and halides.

A leaving group is generally considered, without limitation, as an atom or group of atoms that detaches itself from the electrophile. Generally, the lower the $pK_a$ of the conjugate acid, the better the leaving group.
Suitable leaving groups include, without being limited to, halides, triflates or mesylate.

For the purpose for illustration, one example, as has been described below in further detail, the nucleophilic atom is the oxygen atom on a hydroxyl group and the electrophile is a derivatized oligoethylene glycol or a phospholane reagent having a leaving group, such as, chloride, iodide or mesylate.

In another broad aspect, a method of depositing functionalized poly(3,4-alkylenebridgedthiophene) (PABT) polymer coatings on non-conductive support matrix or nanoparticle has been described.

Suitable non-conductive support matrix include, without being limited to, colloidal silica, polystyrene beads, metal oxide particle, nylon fiber, cellulose, siliceous MCF or chitosan-alignate fibers.

A nanoparticle is generally understood as an object that behaves as a whole unit in terms of its transport and properties. It is normally classified based on size, ranging from about 1 to about 2500 nm, for example, and without limitation.

In a further embodiment, the coating is carried out by oxidative chemical polymerization. In a still further embodiment, the monomer is a monomer having low solubility in water, and which can be dissolved by sonicating in an aqueous medium.

An oxidative chemical polymerization is generally understood as, for example, a polymerization reaction that results in oxidation of the monomers used in the reaction.
Solubility is generally understood as, for example, the amount of a substance that can be dissolved in a given amount of solvent, and a compound having low solubility, would be generally understood as, for example, one that is sparingly soluble.

An aqueous medium is generally considered as a medium containing water and includes a medium that is predominantly water.

In a still further aspect, the invention relates to a bionanointerface for controlled adhesion of a biological molecule, the bionanointerface comprising:

- a surface having an adhesive region and a non-adhesive region;

- the adhesive region having a biologically adhesive agent for releasably binding the biological molecule; and

- the non-adhesive region having a polymer with "n" sub-units of formula (I) or a co-polymer having "m" sub-units of formula (I) and "p" sub-units of formula (II), wherein formula (I), formula (II), and n, m and p are as described above.

A biologically adhesive agent is understood to be an agent that would bind adhesively (fouling) to a biological target, such as, and without limitation, a cell or protein. The binding of the agent is generally, without limitation, reversible, such that under appropriate conditions, the biological target can be released from the biologically adhesive agent. In an embodiment of the
invention, the biologically adhesive agent would comprise a poly (EDOT-OH) or a poly (EDOT-COOH).

The invention and its different aspects are further illustrated by way of the following embodiments.

5 Synthesis of PABT

In one embodiment, the preparation of the non-adhesive functionalized PABT polymers was performed using oligoethylene glycol tethered EDOT monomers. It has been observed that the non-fouling properties of polyethylene glycol (PEG) and oligoethylene (OEG) grafted self-assembled monolayer (SAM) are dependent on the polymer molecular weight and graft density. Therefore, new monomer synthesis was performed for controlling the composition of PEDOT nanobiointerfaces. For example, triethylene glycol tethered EDOT monomer (EDOT-EG3-OH) was synthesized from 2-[2-(2-chloroethoxy)ethoxy]ethanol. The -OH group of the starting material was first protected by tetrahydrofuran, followed by transhalogenation reaction to yield THPO-EG3-I (Scheme 1). Nucleophilic substitution of THPO-EG3-I by deprotonated EDOT-OH in ambient, anhydrous conditions produced the protected product, EDOT-EG3-OTHP. The desired product EDOT-EG3-OH was obtained after the THP group was deprotected using acidic resin. The total yield over four steps was 30%, and the structure of EDOT-EG3-OH was confirmed by spectroscopic methods (NMR, infrared (IR) and mass spectrometry (MS)). In the schemes below, 'rt' stands for room temperature.
As an alternative embodiment, a different approach (Scheme 2) was applied for the synthesis of EDOT-EG4-OH and EDOT-EG6-OH. Starting from tetra- and hexaethylene glycol, one of the -OH groups was protected by trityl group. Subsequently, the other -OH group was mesylated to form a leaving group. Followed by nucleophilic substitution and deprotection, the desired products were derived, respectively. Electropolymerization of EDOT-EG3-OH was performed in 0.1 M of LiClO₄ aqueous solution containing 10 mM of the respective monomers and 1 mM of HCl/0.05 M of SDS. The dissolution of the deposited blue polymer was observed, which was most likely due to the increased water solubility of both the monomer and the polymer, due to the tethering hydrophilic ethylene glycol groups. The solubility of the polymer was reduced when EDOT-EG3-OH was copolymerized with another less soluble monomer. In the current example, about a minimum of 5% EDOT-OH in the monomer mixture enhanced the polymer deposition. Atomic force microscopy (AFM) images confirmed that the new PEDOT nanobiointerfaces containing EDOT-EG3-OH side chains displayed preferable smoothness ($R_{\text{rms}}$ < 5 nm). In general, the functionalized PEDOT films were thin (about < 100 nm in thickness), ultrasmooth ($R_{\text{rms}}$ about < 5 nm) and non-cytotoxic.
Scheme 2

In another embodiment, the non-adhesive functionalized PABT polymers were prepared using phospholipid tethered EDOT monomers. Post-polymerization functionalization of poly(EDOT-OH) surface was performed (Scheme 3) to tether phospholipid moieties. Poly(EDOT-OH) was first deposited on ITO-coated glass substrate. Afterwards, it was dipped into anhydrous THF solution containing 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP). After rinsing with THF, the substrate was dried and dipped into anhydrous CH$_3$CN in a pressure bottle. Anhydrous trimethylamine was introduced to the solution mixture, and the reaction was performed overnight. X-ray photoelectron spectroscopy (XPS) confirmed the successful grafting of phospholipid moiety.
Scheme 3

Enzyme Binding of PEDOT Nanobiointerfaces

The enzyme binding properties of functionalized PABT nanobiointerfaces were monitored by quartz crystal microbalance (QCM) and amperometric measurement of redox enzyme. In the QCM studies, various functionalized PEDOT thin films were deposited on Au crystals. Glucose oxidase (GOX) and bovine serum albumin (BSA) in PBS (0.1 wt %) solutions were prepared for enzyme binding tests. Figure 1 shows the binding processes of these two enzymes on various functionalized poly (3,4-ethyelendioxythiophene) (PEDOT) thin films and bare Au substrate. In QCM measurement, the decrease in frequency (ΔF) of a crystal is proportional to the mass increase on surface based on Sauerbrey relation. Poly(EDOT-OH) showed the strongest binding to GOX (Figure 1(A)). After copolymerization of EDOT-OH with EDOT-EG3-OH, the binding of GOX decreased as the ratio of EDOT-EG3-OH in copolymers increased. This indicated that the triethylene glycol functional groups on PABT thin films reduced non-specific binding, and that poly(EDOT-EG3-OH) formed a non-adhesive surface. The binding of BSA on poly(EDOT-OH) and co-poly(EDOT-OH)-poly(EDOT-EG3-OH) also indicates the same trend (Figure 1(B)). In contrast, poly(EDOT-COOH) demonstrated a very weak binding to GOX, but a strong binding to BSA. This illustrated that the carboxyl groups on PEDOT thin films played a role for enzyme binding. The carboxyl groups tend to dissociate into H⁺ cations and carboxylate in aqueous solution, which leads to a negative charge on PEDOT surface. Therefore, the binding property for poly(EDOT-COOH) thin films is determined by the Coulombic force between the functional groups on the enzyme surface and the negatively charged RCOO⁻ anions. The strong binding of BSA indicates the existence of positively charged
functional groups on BSA surface, and these functional groups have strong interactions with carboxyl groups. Analogous studies can be performed to understand the binding properties of enzyme on poly(EDOT-COOH) or other derivatives for developing a functional PBDOT polymer having the desired adhesive properties. In addition, the copolymerization of EDOT with various functional groups offers a controlled adhesion layer as well as a non-adhesive surface for proteins.

Another approach to evaluate the non-specific binding of enzymes is the current measurement of electrocatalytic oxidation by surface-bound GOX (Xie et al., 2004, 1611; Xie et al., 2004, 4023; Xie et al., 2004, e15). A greater amount of non-specifically bound GOX would display a larger amperometric output in the presence of glucose. As shown in Figure 2, adhesive poly(EDOT-OH) films yielded ~130 nA from physically absorbed GOX, whereas the PABT nanobiointerface composed of 10% EDOT-OH and 90% of EDOT-EG3-OH only recorded an amperometric measurement of < 25 nA under the same experimental condition. The trend of non-specific enzyme binding on PBDOT films of different compositions agreed with the QCM data. In addition, poly(EDOT-PC) from post-functionalization of poly(EDOT-OH) and poly(EDOT-EG3-OH) displayed no amperometric signal from this test, indicating the non-adhesive properties of these films.

The non-specific binding of redox enzymes on PABT surfaces also paths the way to electrocatalytic biosensing. As shown in Figure 3(A), with adhesive poly(EDOT-OH) film on Pt electrode, GOX was bound non-specifically to the surface. In traditional glucose sensor, a mediator is necessary to translate the electron from the redox activity of GOX active site to the surface of the electrode (Heller et al., 1990).
In the nanobiointerface of the invention, electrons from the glucose oxidation by GOX were observed to be transferred to the PABT-coated electrode without a mediator. When different concentrations of glucose were present in the solution, a polynomial relationship between the glucose concentration and the amperometric response was observed (Figure 3(B)). Such a curve could allow one to determine the glucose concentration in an unknown sample for biosensing applications.

Controlled Cell Adhesion on PEDOT Nanobiointerfaces.

Controlling the interfaces between cells and solid substrates can be of concern for cell-based biosensors and biochips. The interaction between cells and functionalized PABT substrates are illustrated by the cell adhesion on different functionalized PABT thin films. Cells were allowed to attach to PABT surfaces for 2 h under serum-free conditions. After 2 h of incubation, unattached and loosely attached cells were gently removed by washing three times with PBS. Substrates were then further incubated in full medium with 10% FBS overnight. As shown in Figure 4, both NIH3T3 and KB cells were selectively attached to polyEDOT and poly(EDOT-OH) surfaces. PABT surface electropolymerized from a mixture of 10% EDOT-OH and 90% EDOT-EG3-OH were cell-resistant, while the PEDOT-COOH surface characteristics were dependent on the cell type. Cell adhesion and proliferation were observed on poly(EDOT-OH) surface, as shown in Figure 5. These results support the possibility of engineering the surface "cell-adhesiveness" or "cell-resistance" through layer-by-layer deposition. To demonstrate this, multilayer PABT structures were constructed by electropolymerization of alternative layer of co-poly(EDOT-OH)-poly(EDOT-EG3-OH) and poly(EDOT-OH) from their respective monomer solutions (Figure 6). The formation of alternate layers was
characterized by the change in water contact angles. The advancing water contact angle decreased from 85° (ITO surface) to 48° with the deposition of the first layer of co-poly(EDOT-OH)-poly(EDOT-EG3-OH) due to the hydrophilic nature of the triethylene glycol side chains (Figure 7). It then increased to 62° when the second layer, poly(EDOT-OH), was deposited. The water contact angle decreased to 50° with the formation of the hydrophilic co-poly(EDOT-OH)-poly(EDOT-EG3-OH) third layer. This indicates that the surface property of PABT surfaces could be tuned through layer-by-layer electropolymerization. Similarly, cell adhesion experiments indicate that cells were selectively attached onto the poly(EDOT-OH) surface, and would resist attachment onto the co-poly(EDOT-OH)-poly(EDOT-EG3-OH) surface. In the case of multilayer structures, the cell-adhesion properties of the device was mediated by the top surface layer (Figure 6).

The non-adhesive PABT biointerfaces were also applied towards cell patterning by first covering the ITO-coated glass slides with non-adhesive co-poly(EDOT-OH)-poly(EDOT-EG3-OH) surface. Patterned, adhesive poly(EDOT-OH) was then electropolymerized using a PDMS mask to create the device configuration shown in Figure 8. After cell attachment and washing, cells were observed only on the poly(EDOT-OH)-covered regions, since the remaining regions were non-adhesive. This supports the engineering and patterning of the PABT nanobiointerfaces with cell-adhesive and cell-resistant properties through simple layer-by-layer electropolymerization and could be useful for cell patterning and chip-based biosensor applications where controlled adhesion can be an issue.

PABT nanobiointerface may be designed to have different properties, such as: (1) thin and smooth films are
deposited with < 100 nm in thickness and < 5 nm in roughness (R_{rms}); (2) interfaces grafted with a variety of functional groups that are capable of different bioconjugation pathways; (3) compositionally tunable biointerfaces prepared under uniform conditions; (4) films are conductive to provide electrical stimuli; (5) materials are deposited on selected electrode surfaces; (6) nanobiointerfaces synthesizable within seconds and amenable to large-scale manufacturing; and (7) low intrinsic cytotoxicity and low to no significant inflammatory response upon implantation.

**Process for polymerization of functionalized PBDOTs on non-conducting surface in an aqueous medium.**

The functionalized PABT monomers were initially polymerized by acid-catalyzed microemulsion electropolymerization. Although, electropolymerization offers a convenient way to deposit films, the variety of substrates on which such deposits can be made are limited, and there is a need to develop an alternate route, such as, chemical polymerization in an aqueous media, for depositing functionalized PEDOT coatings on non-conductive supporting matrix or nanoparticle. However, due to the low solubility of EDOT monomers, their chemical polymerization is generally performed in organic solutions or aqueous microemulsions for improved polymer formation. Although there has been some work on the surface coating procedure for PEDOT on nanomaterials (Han, 2004 et al., 2154; Han et al., 2004, 231), none of them describe coating functionalized EDOT monomers (EDOT-OH and EDOT-COOH) on nanomaterials. Switching from EDOT to functionalized EDOT is not trivial due to the additional side-chain interactions. The experimental parameters need to be determined and optimized, and different morphologies may result. Therefore, an alternative coating process was developed (Figure 9), that is also more
environmentally friendly, because the reaction was performed in sonicated aqueous solutions without surfactants. Due to the low solubility of functionalized EDOT monomers in water, sonication was performed to prepare an aqueous solution of EDOT monomers with a high concentration (generally > 10 mM). After adding the substrates to the monomer solutions, oxidants were added to initiate the reaction. HCl was used to lower the oxidation potential of EDOT, and Cl⁻ also served as dopants. After PEDOT was formed, it favored precipitation and adsorption onto the substrates due to its low solubility in water. Little side-products of homo-PEDOT clusters were observed in the reaction mixture. The degree of chemical polymerization was controlled by the monomer composition, concentration, and reaction time. Therefore, the thickness of PEDOT layers on the substrates could be varied by these three parameters.

Four different classes of materials were tested with this synthetic strategy. Core-shell nanoparticles attract great interest due to their unique and tailored properties for diverse applications. To date, there have been limited reports on PEDOT coatings with aqueous chemical polymerization. In addition, these studies focused on unfunctionalized PEDOT as coating layers, and silica or PS nanoparticles as templates. Besides applying PEDOT coatings on silica and PS nanoparticles, the deposition of functionalized PEDOT thin layers on other non-conductive materials with different shapes and porosities, including siliceous MCF and chitosan-alginate fibers were performed. Siliceous MCF with well-defined, ultralarge open pores of 30-50 nm is an attractive new material as catalyst support and separation medium, while chitosan-alginate fibers have been created as a new scaffold material for cell proliferation and tissue engineering. Deposition of
functionalized PABT on the surface of siliceous MCF and chitosan-alginate fibers provides for three-dimensional conductive systems for applications, such as advanced tissue engineering scaffolds and biofuel cells.

The various types of substrates all turned blue in color after polymerization, indicating the formation of functionalized PEDOT on their surface. The Transmission Electron Microscopy (TEM) micrograph of poly(EDOT-OH)-coated silica nanoparticles is shown in Figure 10(A). The poly(EDOT-OH) layer on silica nanoparticles was not so clear in the image; possibly due to the thin poly(EDOT-OH) layer and the poor contrast between the coating and the silica nanoparticle. Dynamic light scattering (DLS) measurements indicated the thickness of PEDOT-OH layer to be 5–7 nm. Energy dispersive X-ray analysis (EDX) confirmed the presence of sulfur peak after the poly(EDOT-OH) coating (Figure 10(B)). The chlorine peaks were presented since Cl⁻ ions served as dopants during the chemical polymerization. Elemental analysis gave the corresponding C/H ratio of poly(EDOT-OH), and provided additional evidence for the formation of poly(EDOT-OH) coatings on the surface.

The same coating method was also applicable to PS beads. Figure 11 shows the scanning electron microscopy (SEM) micrographs before and after the coating of poly(EDOT-OH) layers. The surface of poly(EDOT-OH)-coated beads was examined with various bead sizes and coating thicknesses. Formation of poly(EDOT-OH) layers on the surface of PS beads was clearly observed after the chemical polymerization process. Rougher surface was obtained from thicker poly(EDOT-OH) coatings (~ 20 nm) (Figure 11(section (c))). Similar surface roughness was noted when the polymerization was performed on larger beads (Figure 11(section (d))).
One advantage of applying PS beads as templates for PEDOT coatings is that PS can be easier dissolved by toluene under a mild condition. **Figure 12** shows the structure and morphology of poly(EDOT-OH) nanostructures after the PS core was removed. Hollow poly(EDOT-OH) spheres were clearly observed. The spherical structures collapsed after the removal of the PS cores in the case of thin poly(EDOT-OH) layers (< 5 nm) (see **Figure 12**(section (b))). For the thick poly(EDOT-OH) layers (> 15 nm), hollow and spherical particles were obtained. A single hole was observed on most of the hollow poly(EDOT-OH) particles (**Figure 12**(section (d))). This was most likely due to the single-point release of the toluene-dissolved polystyrene. This structure is of possible interest for potential applications in controlled release and delivery systems for drugs, genes and proteins.

Mesoporous silica materials have been recently applied as matrices for imaging tags and enzymes for biolabeling and biofuel cell applications due to their large surface-to-volume ratio. Characteristics of siliceous MCF before and after polymer coating were examined by N$_2$ adsorption-desorption isotherms (**Figure 13**). The pore size and pore volume of siliceous MCF were determined by the simplified Broekhoff-de Boer (BdB-FHH) method (Lukens, Jr., W. W.; P. Schmidt-Winkel, D. Zhao, J. Feng, G. D. Stucky, *Langmuir* 1999, 15, 5403) to be 48 nm and 1.98 cm$^3$/g, respectively. After coating with poly(EDOT-OH) for 4 h, the pore size and pore volume of MCF were reduced to 46 nm and 1.42 cm$^3$/g, respectively. TEM micrograph of the poly(EDOT-OH)-coated MCF illustrated homogeneous polymer distribution within the pores, without the formation of separate polymeric particles. When the chemical polymerization was extended to 16 h, the pore size and pore volume of MCF were
greatly reduced to 15 nm and 0.68 cm$^3$/g, respectively, indicating that more poly(EDOT-OH) was polymerized inside the pores. These results indicated that the thickness of poly(EDOT-OH) coating on MCF silica was controlled by the polymerization conditions.

Chitosan-alginate fibers derived from interfacial polyelectrolyte complexation have been applied as scaffolds for tissue engineering. Figure 14(section (a)) shows the chitosan-alginate fibers before poly(EDOT-OH) coating. They were white in color with a smooth surface. After the fibers was immersed in EDOT-OH solution in the presence of ammonium persulfate (APS) oxidants for 4 h, the fibers turned blue (Figure 14(section (b))) without changing in surface roughness, indicating the growth of poly(EDOT-OH) along the fiber surface. The surface of the fibers was noticeably rougher only after 16 h of chemical polymerization (Figure 14(section (c))). This suggested that the surface morphology was lost when a much thicker poly(EDOT-OH) layer was deposited through extended chemical polymerization.

Besides EDOT-OH, EDOT-COOH was also successfully coated onto the different substrates described with similar results. Compared to poly(EDOT-OH) coatings, the poly(EDOT-COOH) coatings of the same thickness generally required a longer polymerization period at the same monomer concentration. The poly(EDOT-COOH) coatings were also more uniform according to SEM and TEM observations.

In summary, a general synthetic approach for coating functionalized PABT in aqueous solutions onto non-conductive substrates has been developed. These functionalized coatings favor growth along substrate surfaces in aqueous solution. The PABT layers are smooth and homogeneous on the nanometer scale, and their thickness can
be controlled by the polymerization time and monomer/oxidant concentrations.

**EXAMPLES**

The following examples are intended as exemplary only and not in any way intended to limit the scope of the invention, which is defined in the claims.

**Materials and Reagents.** Ethylenedioxythiophene (EDOT, Sigma-Aldrich), lithium perchlorate (LiClO₄, Fluka), sodium dodecyl sulfate (SDS, Alfa Aesar) and D-(+)-glucose (Sigma) were used as received. Hydroxymethyl-functionalized EDOT (EDOT-OH) was synthesized according to the literature procedure (Lima et al., 1998). Carboxylic acid-functionalized EDOT (C₂-EDOT-COOH) was synthesized in the same way as described previously. A phosphate-buffered saline (PBS) consisting of 137 mM of NaCl (sodium chloride), 2.7 mM of KCl (potassium chloride), and 10 mM of phosphate buffer was used as the supporting electrolyte solution. Glucose oxidase-avidin D (GOD-A, Vector Laboratories) was diluted in PBS by 100, 1000 and 5000 times in volume to produce 50 µg/mL, 5 µg/mL and 1 µg/mL solutions. Indium tin oxide (ITO) coated glass (Delta-Technologies, Ltd.) was cleaned by standard procedure prior to use. Au and Pt disk working electrodes (CHI Instrument) were polished by Polishing Kits (PK-4, Bioanalytical Systems, Inc.) with 0.05-µm alumina (Gamma Micropolish, Buehler) before use. APS (Alfa Aesar) was used as received. Colloidal silica (Snowtex®, particle size = 40-50 nm, Nissan Chemical Industries, Ltd.) and polystyrene (PS) beads (Polybead®, particle size = 200 and 500 nm, Polysciences, Inc.) were purchased and used without further purification.

Siliceous mesocellular foam (MCF) and chitosan-alginate fibers were prepared as described elsewhere.(Schmidt-Winkel, P.; Lukens Jr., W. W.; Yang, P.; Margolese, D. I.; Lettow,

EXAMPLE 1

Preparation of THPO-EG3-I (see Scheme 1)

2-[2-(2-chloroethoxy)ethoxy]ethanol (7.25 mL, 8.43 g, 50 mmol) and 3,4-dihydro-[2H]-pyran (DHP) (9.1 mL, 8.41 g, 100 mmol) were added to a suspension of Amberlite IR-120 (H+ form, washed with acetone and dichloromethane (CH2Cl2) before use; 20.0 g) in CH2Cl2 (200 mL), and the mixture was refluxed overnight. The resin was filtered off and the solvent was evaporated. The oily residue was dried in high vacuum.

Sodium iodide (NaI) (18.73 g, 125 mmol) dissolved in acetone (150 mL) was added, and the solution was refluxed overnight. The solvent was removed under reduced pressure, and the mixture was partitioned between brine (100 mL) and CH2Cl2 (200 mL). The aqueous phase was further extracted with CH2Cl2 (4 x 50 mL). The combined organic phases were dried (MgSO4) and the solvent was removed. THPO-EG3-I (5.89 g, 68%) was obtained as a pale yellow oil after column chromatography with silica gel (80 g Combiflash cartridge; hexane/ethyl acetate = 0 to 100% over 20 min). 1H nuclear magnetic resonance (NMR) (400 MHz, CDCl3): δ 4.63 (t, J = 3.2 Hz, 1H), 3.86 (m, 2H), 3.74 (t, J = 6.8 Hz, 2H), 3.67 (m, 5H), 3.60 (m, 1H), 3.49 (m, 1H), 3.26 (m, 1H), 3.25 (t, J = 6.8 Hz, 2H), 1.79 (m, 1H), 1.70 (m, 1H), 1.62-1.48 (m, 4H). 13C-NMR (100 MHz, CDCl3): δ 98.9, 72.0, 70.6, 70.6, 70.3, 66.7, 62.3, 30.6, 25.4, 19.5, 3.0.

EXAMPLE 2
Preparation of EDOT-EG3-OTHP (see Scheme 1)

EDOT-OH (4.31 g, 25 mmol) was dissolved in dry dimethyl formamide (DMF) (50 mL) in a 250-mL round-bottomed flask under argon (Ar). Sodium hydride (NaH) (60% dispersion in mineral oil; 1.50 g, 37.5 mmol) was added slowly against a gentle stream of Ar, and the mixture was stirred under Ar for 15 min. THPO-EG3-I (8.60 g, 25 mmol) was then added, and the mixture was stirred overnight and partitioned between brine (250 mL) and ethyl acetate (100 mL). The organic layer was separated, washed with brine (3 x 250 mL), dried (MgSO₄) and evaporated. The crude product was purified by column chromatography on silica gel (80 g Combiflash cartridge; hexane/ethyl acetate = 0 to 100% over 20 min). Unreacted EDOT-OH (1.74 g, 40%) was recovered first, followed by elution of the THP-protected alcohol EDOT-EG3-OTHP (2.92 g, 50% yield based on recovered starting material). \(^1\)H-NMR (400 MHz, CDCl₃): \(\delta\) 6.34 (m, 2H), 4.64 (t, \(J = 3.2\) Hz, 1H), 4.33, (m, 1H), 4.26 (dd, \(J = 11.6, 2.0\) Hz, 1H), 4.07 (dd, \(J = 11.6, 7.6\) Hz, 1H), 3.88 (m, 2H), 3.80-3.59 (m, 14H), 3.51 (m, 1H), 1.72 (m, 1H), 1.61 (m, 2H), 1.54 (m, 3H). \(^13\)C-NMR (100 MHz, CDCl₃): \(\delta\) 141.6, 141.5, 99.7, 99.6, 99.0, 72.6, 71.2, 70.7, 70.6, 70.6, 70.6, 69.6, 66.7, 66.1, 62.3, 30.6, 25.4, 19.5.

Example 3

Preparation of EDOT-EG3-OH (see Scheme 1)

EDOT-EG3-OTHP (2.29 g, 5.9 mmol) was dissolved in methanol (20 mL), and Amberlite IR-120 (H⁺ form, washed with acetone and CH₂Cl₂ before use; 5.0 g) was added. The mixture was stirred overnight, the resin was filtered, and the solvent was evaporated. EDOT-EG3-OH was obtained as a colorless oil after column chromatography on silica gel (40
g Combiflash cartridge; hexane/ethyl acetate = 40 to 100% over 20 min). $^1$H-NMR (400 MHz, CDCl$_3$): δ 6.33 (m, 2H), 4.34 (m, 1H), 4.26 (dd, $J = 11.6$, 2.4 Hz, 1H), 4.07 (dd, $J = 11.6$, 7.6 Hz, 1H), 3.79–3.66 (m, 12H), 3.61 (m, 2H), 2.42 (broad s, 1H). $^{13}$C-NMR (100 MHz, CDCl$_3$): δ 141.5, 141.4, 99.8, 99.7, 72.6, 71.1, 70.7, 70.5, 70.3, 69.6, 66.1, 61.7, 60.5.

EXAMPLE 4

Preparation of C$_2$-EDOT-COOCH$_3$

10 A 100-mL round-bottom flask was charged with a stir bar, 2,3-dihydrothienol[3,4-b][1,4]dioxin-2-ylmethanol (EDOT-OH, 861 mg, 5.0 mmol), NaI (150 mg, 1.0 mmol) and NaH (60% suspension in mineral oil, 240 mg, 6.0 mmol), and the flask was backfilled with argon thrice. Dry tetrahydrofuran (THF, 20 mL) was introduced, and the suspension was stirred for 15 min and cooled in an ice bath. Methyl bromoacetate (0.57 mL, 0.92 g, 6.0 mmol) was added dropwise, and the reaction mixture was stirred for 18 h. The majority of THF was removed with a rotary evaporator; the crude product was partitioned between water and ethyl acetate, and further extracted twice with ethyl acetate. The combined organic layers were washed with brine, dried (MgSO$_4$) and evaporated. The crude product was purified with a silica gel column (hexane/ethyl acetate = 5:1).

EXAMPLE 5

Preparation of C$_2$-EDOT-COOH

C$_2$-EDOT-COOCH$_3$ (610 mg, 2.5 mmol) was dissolved in THF (10 mL) in a 100-mL round-bottom flask, and freshly prepared aqueous NaOH solution (2 M; 10 mL) was added. The mixture was stirred vigorously until the starting material
was completely consumed by thin layer chromatography (TLC) (3 h). The mixture was acidified to pH < 3, and then extracted with ethyl acetate (5×). The combined organic layers were washed with water until neutral, dried (MgSO₄), and evaporated. C₂-EDOT-COOH (480 mg; 83%) was obtained as a thick, colorless oil that solidified upon standing overnight.

**EXAMPLE 6**

**Electropolymerization and Film Syntheses.**

PBDOT films of different EDOT monomers obtained in Examples 1-3 were electropolymerized on Au, Pt and ITO electrodes using 10 mM of EDOT aqueous solution containing 0.1 M of lithium perchlorate (LiClO₄) as supporting electrolyte in the presence of 1 mM of hydrochloric acid (HCl) and 0.05 M of sodium dodecylsulphate (SDS) by applying cyclic potentials (-0.6 to 1.1 V vs. Ag/AgCl at a scan rate of 100 mV/s) or potentiostatic methods.

**EXAMPLE 7**

**Post-Polymerization Functionalization of Poly(EDOT-OH)**

Poly(EDOT-OH) was electropolymerized on ITO substrate. The phospholipid moieties were introduced similarly as in the synthesis of EDOT-PC. The poly(EDOT-OH)-coated glass substrate was dipped into 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP) solutions in anhydrous tetrahydrofuran (THF) at -20°C for 3 h. The film was then washed with anhydrous THF, dried and dipped into anhydrous acetonitrile (CH₃CN) in a pressure bottle. Anhydrous trimethylamine was bubbled quickly to the solution mixture. The pressure bottle was closed, and allowed to warm up to room temperature. After it was heated at 20°C for 16 h, the
bottle was cooled. The films were further washed with anhydrous CH₃CN, and ready for measurements.

**EXAMPLE 8**

**Cell Culture and Controlled Adhesion**

NIH3T3 cells and KB cells were cultured in DMEM and RPMI culture media, respectively, supplemented with fetal bovine serum (FBS) (10%), penicillin (200 units/mL) and streptomycin (200 μg/mL) (complete medium). The cultures were maintained at 37°C in a humidified atmosphere containing 5% of carbon dioxide (CO₂). At confluence, the cells were trypsinized, washed and resuspended in serum-free medium at a concentration of 50,000 cells/mL. PEDOTs were electropolymerized onto clean ITO substrates from a solution containing 10 mM of EDOT monomers, 0.05 M of SDS, 0.1 M of LiClO₄ and 0.01 M of HCl at 1.1 V (vs. Ag/AgCl) with a cut-off charge density of 5 mC/cm². Multilayered PEDOT structures were made in a similar manner using layer-by-layer growth. The substrates were then cut into 10 mm × 8 mm pieces, and loaded into a 24-well plate. PEDOT/ITO substrate was pre-sterilized for 1 h in 70% ethanol. 500 μL of cell suspension was added into each 24-well plate loaded with PEDOT/ITO substrate. After seeding, samples were kept for incubation at 37°C in a humidified atmosphere containing 5% of CO₂. Cell adhesion was evaluated at different time points (1 h, 2 h, 3 h and overnight). Non-adherent cells were removed by gently washing the wells three times with PBS. Samples were visualized under Olympus inverted optical microscope IX71 using phase contrast mode. Images were taken using a CCD camera.

**EXAMPLE 9**

Coating of PEDOT-OH and PEDOT-COOH films.
EDOT-OH and C2-EDOT-COOH monomers were dissolved in water by sonication in a water bath sonicator (Elma Transsonic 660/H, 35 kHz) for 30 min to prepare monomer solutions of 30 mM. For coating of PEDOT on nanoparticles, monomer solutions were added to aqueous solutions of colloidal silica and polystyrene (PS) beads. After the mixed solutions were stirred for 30 min, a solution containing 30 mM of APS and 10 mM of HCl was introduced. The chemical polymerization proceeded for 16 h at room temperature before it was quenched by methanol. For PEDOT coating on siliceous MCF, 200 mg of MCF was placed in a round flask and degassed by using a mechanical pump. Monomer solutions were then injected into this flask, and the entire system is stirred under vacuum for 30 min. After that, vacuum was released and a solution containing APS and HCl was added under the atmospheric pressure to initiate the chemical polymerization. For PEDOT coating on chitosan-alginate fibers, the fibers were first immersed in the monomer solutions for 30 min. Then the solution containing APS and HCl was added to start the coating of PEDOT.

**EXAMPLE 10**

**Polymer Film Analysis.**

The surface morphology of polymer films was observed with field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM). FESEM was carried out with JEOL JSM-7400 at a vacuum of \(10^{-8}\) torr and an accelerating voltage of 10 kV. Transmission electron microscopy (TEM) experiments were performed on a JEOL JEM-3010 electron microscope with an acceleration voltage of 300 kV. The nitrogen sorption isotherms were obtained with a Micromeritics ASAP 2020M system; the samples were degassed for 10 h at 150°C before the measurements. The carbon,
hydrogen and sulfur contents of PEDOT layers were examined with a CE440 CHN analyzer (Exeter Analytical).

Although the foregoing invention has been described in some detail by way of illustration and example, and with regard to one of more embodiments, for the purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes, variations and modifications may be made thereto without departing from the spirit or scope of the invention as defined in the appended claims.

It must be noted that as used in the specification and the appended claims, the singular forms of "a", "an" and "the" included plural reference unless the context clearly indicates otherwise.

Unless defined otherwise all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs.

All publications, patents and patent applications cited in this specification are incorporated herein by reference as if each individual publications, patents and patent applications were specifically and individually indicated to be incorporated by reference. The citation of any publication, patent or patent application in this specification is not an admission that the publication, patent and patent application is prior-art.
1. A polymer comprising "n" subunits of formula (I):

\[
\begin{array}{c}
\text{S} \\
\text{Y}_1 \text{A}_1 \text{Y}_2 \\
\text{R}_1
\end{array}
\]

Formula (I)

wherein

\(A_1\) is a bridging alkylene chain, optionally substituted, having 2, 3, 4, 5 or 6 carbon atoms;

\(Y_1\) and \(Y_2\) are, independently, O, S or N-R_2, and

wherein \(R_2\) is hydrogen, an alkyl group, an alkenyl group, an alkynyl group, a cycloalkyl, an aryl or a heterocycle; and

\(R_1\) is a functional chain attached to the bridging alkylene chain, and

wherein \(n\) is an integer of 2 or more.

2. The polymer of claim 1, wherein

\(R_1\) is a non-adhesive chain; and

\(A_1\) comprises a heteroatom for bonding to the non-adhesive functional chain, and wherein the heteroatom is O, N, S, P or B.
3. The polymer of claim 1 or 2, wherein the polymer is a co-polymer comprising "m" sub-units of formula (I) and "p" sub-units of formula (II):

![Chemical Structure](image)

Formula (I)                        Formula (II)

wherein $A_1$, $Y_1$, $Y_2$ and $R_1$ are as defined in claim 1, $A_2$ is independently a bridging alkylene chain, optionally substituted, having 2, 3, 4, 5 or 6 carbon atoms, and $m$ and $p$ independently are an integer of 2 or more.

4. The polymer of claim 3, wherein the co-polymer has at least about 5% of sub-units of formula (II).

5. The polymer of claim 3 or 4, wherein:

$A_1$ and $A_2$ are glycerol and forms a five-membered ring to form a 3,4-bridgeddioxythiophene; and the functional chain is an oligo- or a poly-ethylene glycol (OEG or PEG).

6. The polymer of any one of claims 3 to 5, wherein the functional chain is oligo-ethylene glycol having 2, 4, 6 or 8 carbon atoms.
7. The polymer of any one of claims 1 to 4, wherein
the functional chain is a phospholipid chain.

8. A compound of formula (III),

\[
\begin{array}{c}
\text{S} \\
\text{Y}_1 \\
\text{A}_1 \\
\text{Y}_2 \\
\text{R}_1
\end{array}
\]

wherein A₁, Y₁, Y₂ and R₁ are as defined in claim 1.

9. The derivative of claim 8, wherein
A₁ is glycerol and forms a five-membered ring to
form a 3,4-bridgeddioxythiophene; and

the functional chain is an oligo- or a poly-
ethylene glycol (OEG or PEG).

10. A process for preparation of the compound of
formula (III) as defined in claim 9, the method comprising:

- reacting a compound of formula (IV), or a
derivative thereof, with a compound of formula
(V), or a derivative thereof,
wherein $A_2$ is a bridging alkylene chain, optionally substituted, having 1-6 carbon atoms;

$Y_1$ and $Y_2$ are as defined in claim 1; and

wherein the compound of formula (IV), or derivative thereof, having a nucleophilic atom on the bridging alkylene chain for nucleophilic substitution on an electrophilic atom on $R_2$, or derivative thereof; and wherein

LG is a suitable leaving group.

11. A bionanointerface for controlled adhesion of a biological molecule, the bionanointerface comprising:

- a surface having an adhesive region and a non-adhesive region;

- the adhesive region having a biologically adhesive agent for releasably binding the biological molecule; and

- the non-adhesive region having a polymer as defined in any one of claims 1 to 7.

12. The biointerface of claim 11, wherein the adhesive agent is a functionalized poly(EDOT-OH) or poly(EDOT-COOH) polymer.
13. The biointerface of claims 11 or 12, wherein the nanobiointerface is formed by layer-by-layer electropolymerization.

14. A method of depositing a functionalized poly(3,4-alkylenebridgedthiophene) (PABT) polymer coatings on a non-conductive support matrix or nanoparticle, the method comprising the steps of:

- dissolving a functionalized 3,4-alkylenebridgedthiophene (ABT) monomer in an aqueous medium to form a monomer solution;

- mixing the monomer solution with the non-conductive support matrix or nanoparticle; and

- polymerizing the monomers to form the functionalized PABT polymer.

15. The method of claim 14, wherein the monomer is a monomer having low solubility in water dissolved by sonicating the monomer in an aqueous medium.

16. The method of claims 14 or 15, wherein the functionalized poly PABT is poly(EDOT-OH) or poly(EDOT-COOH).
Figure 1A

Figure 1B
Figure 2
Figure 3A

Figure 3B

\[ y = 26.883 + 5.2523x - 0.053513x^2 \]

\[ R^2 = 0.9963 \]
Figure 4

Figure 5
Figure 6
Figure 7

Figure 8
Figure 9

Figure 10(A)

Figure 10(B)
Figure 11 (A-D)

Figure 12
Figure 13

Figure 14
**INTERNATIONAL SEARCH REPORT**

A. **CLASSIFICATION OF SUBJECT MATTER**

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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)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Wpi, epodoc, caplas, medline: +dioxothiophene, ?EDOT, +ethylene glycol, +oxyalkylene, phospholipid?, functional+, modif», deriv«, aqueous, water+ , coat+ , polymer+ etc

C. **DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
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<tr>
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<td>X</td>
<td>US 4959430 A (JONAS et al.) 25 September 1990 See examples 15-17</td>
<td>1, 2, 8, 14</td>
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</table>

Further documents are listed in the continuation of Box C

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
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Date of the actual completion of the international search 12 December 2008

Date of mailing of the international search report 23 DEC 2008

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<tr>
<td>X</td>
<td>Han M. G. et al., &quot;Preparation of poly(3,4-ethylenedioxythiophene)(PEDOT) coated silica core-shell particles and PEDOT hollow particles&quot;, Chemical Communications, 2004, pages 2154-2155.</td>
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<td>US 2008224099</td>
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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX