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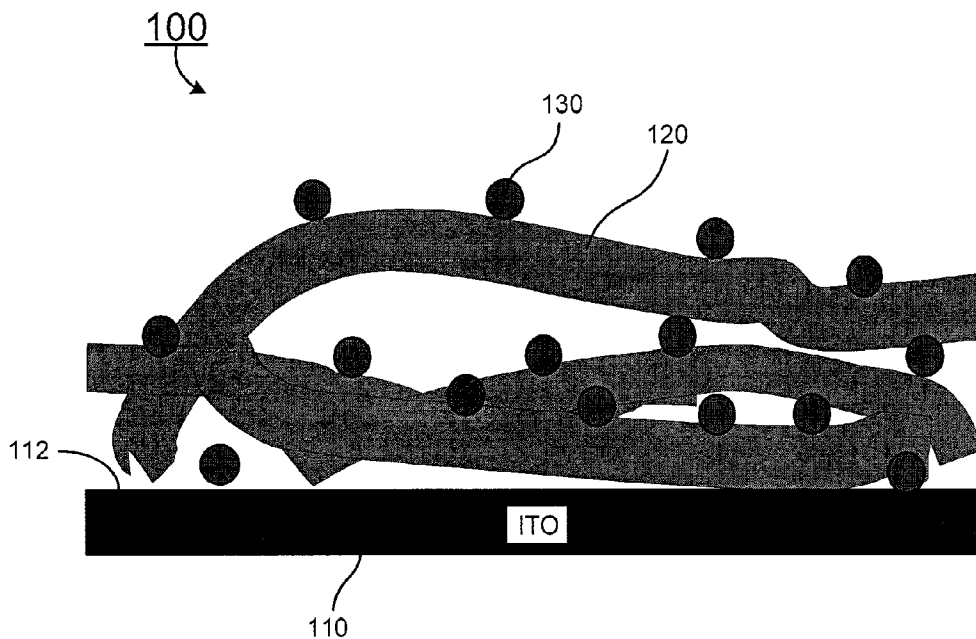
(43) International Publication Date  
24 July 2008 (24.07.2008)

PCT

(10) International Publication Number  
**WO 2008/088588 A2**

- (51) International Patent Classification:  
*C12M 3/00* (2006.01)
- (21) International Application Number:  
PCT/US2007/078634
- (22) International Filing Date:  
17 September 2007 (17.09.2007)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
11/653,189 12 January 2007 (12.01.2007) US  
11/653,192 12 January 2007 (12.01.2007) US
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- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:  
— without international search report and to be republished upon receipt of that report

(54) Title: **BIOCOMPATIBLE SCAFFOLD FOR SENSING PROTEINS**



(57) Abstract: This invention related to a biocompatible scaffold that can detect redox-active chemicals and biomolecules electrochemically. In one embodiment, the biocompatible scaffold includes a substrate, and a conductive layer of TiO<sub>2</sub>-containing nanowires or nano fibers formed on the substrate, wherein the conductive layer of TiO<sub>2</sub>-containing nanowires or nanofibers is formed with a pore structure, and wherein when the biocompatible scaffold is in contact with a biological analyte, one or more proteins of the biological analyte are immobilized on a surface of the conductive layer Of TiO<sub>2</sub>- containing nanowires or nanofibers so as to generate a measurable faradic current signal.

WO 2008/088588 A2

## BIOCOMPATIBLE SCAFFOLD FOR SENSING PROTEINS

This application is being filed as PCT International Patent application in the name of **University of Arkansas Technology Development Foundation**, a U.S. national corporation, Applicant for all countries except the U.S., and Z. Ryan Tian a U.S. resident, Applicant for the designation of the U.S. only, on 17 September 2007.

### CROSS-REFERENCE TO RELATED PATENT APPLICATION

This application is a continuation-in-part of U.S. patent application Serial No. 11/653,189 (“the ‘189 application”), filed January 12, 2007, entitled “TiO<sub>2</sub> NANOSTRUCTURES, MEMBRANES AND FILMS, AND METHODS OF MAKING SAME”, by Z. Ryan Tian, which itself claims the benefit, pursuant to 35 U.S.C. §119(e), of U.S. provisional patent application Serial Nos. 60/758,492, filed January 12, 2006, entitled “TiO<sub>2</sub> NANOFIBER MEMBRANES, METHODS OF MAKING SAME, AND APPLICATIONS OF SAME,” by Z. Ryan Tian and Wenjun Dong, and 60/785,649, filed March 23, 2006, entitled “TiO<sub>2</sub> NANOFIBERS, MEMBRANES, AND FILMS, METHODS OF MAKING SAME, AND APPLICATIONS OF SAME,” by Z. Ryan Tian and Wenjun Dong, which are incorporated herein by reference in their entirety.

This application is also a continuation-in-part of U.S. patent application Serial No. 11/653,192, filed January 12, 2007, entitled “TiO<sub>2</sub> NANOSTRUCTURES, MEMBRANES AND FILMS, AND APPLICATIONS OF SAME”, by Z. Ryan Tian, which itself claims the benefit, pursuant to 35 U.S.C. §119(e), of U.S. provisional patent application Serial Nos. 60/758,492, filed January 12, 2006, entitled “TiO<sub>2</sub> NANOFIBER MEMBRANES, METHODS OF MAKING SAME, AND APPLICATIONS OF SAME” by Z. Ryan Tian and Wenjun Dong, and 60/785,649, filed March 23, 2006, entitled “TiO<sub>2</sub> NANOFIBERS, MEMBRANES, AND FILMS, METHODS OF MAKING SAME, AND APPLICATIONS OF SAME” by Z. Ryan Tian and Wenjun Dong, and is also related to the co-pending ‘189 application, which are incorporated herein by reference in their entirety..

Some references, which may include patents, patent applications and various publications, are cited and discussed in the description of this invention. The citation

and/or discussion of such references is provided merely to clarify the description of the present invention and is not an admission that any such reference is “prior art” to the invention described herein. All references cited and discussed in this specification are incorporated herein by reference in their entireties and to the same extent as if  
5 each reference was individually incorporated by reference. In terms of notation, hereinafter, “[n]” represents the nth reference cited in the reference list. For example, [15] represents the 15th reference cited in the reference list, namely, Dong, W.; Cogbill, A.; Zhang, T.; Ghosh, S.; Tian, R. Z. *J. Phys. Chem.* 2006, 16819.

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### FIELD OF THE INVENTION

The present invention relates generally to a biosensor, and in particular to a biocompatible nanowire scaffold usable for detecting one or more proteins of a biological analyte and supporting the growth of cells.

15

### BACKGROUND OF THE INVENTION

Activities of certain proteins, like growth hormone (GH), in micrometer-scale pores of a bioscaffold are vital to the regenerations of tissues [1] including neuron [2] and bone [3, 4]. However, monitoring and detecting the protein activities inside such a bioscaffold have remained as a challenge. To develop a simple and economic  
20 electrochemical protein sensor to meet the challenge, a fast electron transfer (ET) between the proteins and the solid electrode surface is a must. However, polypeptide- and/or polymer-based bioscaffolds often have a poor electrical conductivity.

Redox proteins such as heme proteins have inherent electrochemical activity and are therefore capable of exchanging electrons directly with a working electrode to  
25 produce an electrochemical signal. This allows direct electrochemical detection and quantification without a requirement for a mediator means, such as via an enzyme mediated reaction.

Conventionally, the quantification of redox proteins has had to rely on laboratory based analytical techniques such as High Pressure Liquid Chromatography  
30 (HPLC) attached to a detector comprising a series of porous electrodes at different potentials. Although this system is suitably accurate, the size and expense of the equipment render it unsuitable for large scale sample testing or in-home consumer

usage. Furthermore, biological samples must commonly undergo extensive pre-treatment to provide them in a form that can be analyzed by HPLC.

Thus, the development of biosensing devices for quantifying redox proteins which are not only accurate, but are also conveniently portable, disposable and able to  
5 analyze a biological sample which has undergone minimal pre-treatment would be of great interest for advancing the existing technologies in multiplex real-time, label-free detection of proteins.

Therefore, a heretofore unaddressed need exists in the art to address the aforementioned deficiencies and inadequacies.

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### SUMMARY OF THE INVENTION

In one aspect, the present invention relates to a biocompatible scaffold.

In one embodiment, the biocompatible scaffold includes a substrate, and a  
15 conductive layer of TiO<sub>2</sub>-containing nanowires or nanofibers formed on the substrate, where the conductive layer of TiO<sub>2</sub>-containing nanowires or nanofibers is formed with a pore structure, and when the biocompatible scaffold is in contact with a biological analyte, one or more proteins of the biological analyte are immobilized on a surface of the conductive layer of TiO<sub>2</sub>-containing nanowires or nanofibers so as to generate a measurable faradic current signal.

20 The TiO<sub>2</sub>-containing nanofibers or nanowires are substantially in the TiO<sub>2</sub>-B phase or titanate phase, and have a typical diameter in the range of about 20-150 nm.

In one embodiment, the pore structure has a thickness in the range of about 1-  
25 50 μm. The nanowire or nanofiber structure may have a layered-titanate (Na<sub>2</sub>Ti<sub>3</sub>O<sub>7</sub>) structure with counter-cations (Na<sup>+</sup>) in the interlayer space. The substrate is formed of indium-tin-oxide (ITO) or a metal.

In one embodiment, the proteins comprise proteins with a redox-active center. The redox-active heme proteins comprise a cytochrome c.

The substrate can be conductive or semi-conductive. In one embodiment, the substrate is formed of indium-tin-oxide (ITO).

30

In another aspect, the present invention relates to a biocompatible scaffold. In one embodiment, the biocompatible scaffold has a substrate, and a layer of nanowires or nanofibers formed on the substrate.

The layer of nanowires or nanofibers is formed with a pore structure. The  
5 nanowires or nanofibers comprise oxide-containing nanowires or nanofibers. In one embodiment, the nanowires or nanofibers include TiO<sub>2</sub>-containing nanowires or nanofibers.

In yet another aspect, the present invention relates to a biosensor for detecting one or more proteins in a biological analyte. In one embodiment, the biosensor  
10 includes a substrate having a surface, a layer formed of nanowires or nanofibers on the surface of the substrate, where the layer of nanowires or nanofibers is formed with a pore structure, and a detector in communication with the substrate, where when the layer formed of nanowires or nanofibers is in contact with one or more proteins in the biological analyte, a measurable signal is generated and measured by the detector.

The biosensor further includes means for applying a potential to the biological  
15 analyte at a scan rate that is in the range of about 0.005-0.500 V/s. In one embodiment, the detector comprises a cyclic voltammetry (CV). When the layer formed of nanowires or nanofibers is in contact with one or more proteins in the biological analyte, one or more electrons transfer between the biological analyte and  
20 the substrate is to cause a measurable signal in the form of a faradic current.

In one embodiment, the one or more proteins in the biological analyte contain redox-active heme proteins. The redox-active heme proteins include cytochrome c. The biological analyte contains a buffer solution having a pH value in the range of about 6.2-9.0, such that the cytochrome c carries a net positive charge, and the  
25 nanowires or nanofibers carry a net negative charge, respectively.

The nanowires or nanofibers are oxide-containing nanowires or nanofibers. In one embodiment, the nanowires or nanofibers are TiO<sub>2</sub>-containing nanowires or nanofibers, where the TiO<sub>2</sub>-containing nanowires or nanofibers are substantially in the TiO<sub>2</sub>-B phase or titanate phase. In one embodiment, the nanowires or nanotubes have  
30 a typical diameter in the range of about 20-150 nm.

In one embodiment, the substrate is formed of indium-tin-oxide (ITO). The substrate can be conductive, or semi-conductive.

In a further aspect, the present invention relates to a method for detecting one or more proteins in a biological analyte. In one embodiment, the method includes the steps of providing a biosensor having at least one electrode and a scaffold formed of nanowires or nanofibers; introducing the biological analyte into the scaffold; and  
5 detecting electron transfers between the biological analyte and the surface of the scaffold so as to detect one or more proteins in the biological analyte. The detecting step, in one embodiment, is performed with a CV.

The method further includes the step of applying a potential to the biological analyte at a scan rate that is in the range of about 0.005-0.500 V/s.

10 In one embodiment, the biological analyte contains redox-active heme proteins, where the redox-active heme proteins comprise cytochrome c.

The biological analyte further contains a buffer solution having a pH value in the range of about 6.2-9.0, such that the cytochrome c carries a net positive charge, and the nanowires or nanofibers carry a net negative charge, respectively.

15 These and other aspects of the present invention will become apparent from the following description of the preferred embodiment taken in conjunction with the following drawings, although variations and modifications therein may be affected without departing from the spirit and scope of the novel concepts of the disclosure.

## 20 BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings illustrate one or more embodiments of the invention and, together with the written description, serve to explain the principles of the invention. Wherever possible, the same reference numbers are used throughout the drawings to refer to the same or like elements of an embodiment, and wherein:

25 Fig.1 shows schematically a biosensor according to one embodiment of the present invention;

Fig. 2 shows the characteristic of a titanate nanowire (NW) scaffold synthesized at temperature about 180 °C for about 3 days according to one embodiment of the present invention, (a) an SEM image of the titanate NW scaffold,  
30 (b) a TEM image of the titanate NW scaffold, (c) an XRD pattern of the titanate NW scaffold, where the titanate phase  $2\theta = 9.8^\circ$  (001),  $11.2^\circ$  (200),  $24.4^\circ$  (110) and  $29.7^\circ$  (003) (JCPDS card No.:47-0561), respectively, and (d) FT-IR spectra, where curve

210 is the FT-IR spectrum of cytochrome c immobilized onto the titanate-NW scaffold, which are mixed with KBr, and curve 220 is the FT-IR spectrum of cytochrome c in the pH 6.8 buffer solution, respectively;

Fig. 3 shows the characteristic of the titanate NW scaffold shown in Fig. 2, (a)

- 5 CV curves (anodic current,  $i_{pa}$ , and cathodic current,  $i_{pc}$ ) of the ITO glass electrode coated with titanate-NWs in a buffer solution of pH 6.8 (curve 330a for  $i_{pa}$  and curve 330c for  $i_{pc}$ ) and of 450 picomoles of cytochrome c immobilized onto the titanate NWs in the buffer solution of pH 6.8 (curve 340a for  $i_{pa}$  and curve 340c for  $i_{pc}$ ), where the scan rate is about 0.2V/s, (b) CV curves for various cytochrome c concentrations
- 10 in the pH 6.8 buffer, where curve 351a is  $i_{pa}$  for 45 picomoles, curve 352a is  $i_{pa}$  for 122 picomoles, curve 353a is  $i_{pa}$  for 243 picomoles, curve 354a is  $i_{pa}$  for 347 picomoles and curve 355a is  $i_{pa}$  for 450 picomoles, respectively, and the scan rate is about 0.2V/s, (c) the linear correlation between the *cyt c* concentrations and the anodic current  $i_{pa}$ , and (d) an SEM image of the PC-12 cell grown on the titanate NW-
- 15 scaffold after cultured on the titanate NW scaffold for about 72 hours;

Fig. 4 shows the characteristic of the titanate NW scaffold shown in Fig. 2, (a) an SEM image of the PC-12 cell grown on the titanate NW scaffold after cultured on the titanate NW scaffold for about 72 hours, and (b) CV curves of the cytochrome c immobilized onto the titanate NW scaffold at different potential scan rates and pH values;

Fig. 5 shows the characteristic of an NW scaffold according one embodiment of the present invention; and

Fig. 6 shows the characteristic of an NW scaffold according one embodiment of the present invention.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention is more particularly described in the following examples that are intended as illustrative only since numerous modifications and variations therein will be apparent to those skilled in the art. Various embodiments of the invention are now described in detail. Referring to the drawings of Figs. 1-6, like numbers indicate like components throughout the views. As used in the description herein and throughout the claims that follow, the meaning of “a”, “an”, and “the” includes plural reference unless the context clearly dictates otherwise. Also, as used in the description herein and throughout the claims that follow, the meaning of “in” includes “in” and “on” unless the context clearly dictates otherwise. Moreover, titles or subtitles may be used in the specification for the convenience of a reader, which shall have no influence on the scope of the present invention. Additionally, some terms used in this specification are more specifically defined below.

## DEFINITIONS

The terms used in this specification generally have their ordinary meanings in the art, within the context of the invention, and in the specific context where each term is used.

Certain terms that are used to describe the invention are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the apparatus and methods of the invention and how to make and use them. For convenience, certain terms may be highlighted, for example using italics and/or

quotation marks. The use of highlighting has no influence on the scope and meaning of a term; the scope and meaning of a term is the same, in the same context, whether or not it is highlighted. It will be appreciated that the same thing can be said in more than one way. Consequently, alternative language and synonyms may be used for any one or more of the terms discussed herein, nor is any special significance to be placed upon whether or not a term is elaborated or discussed herein. Synonyms for certain terms are provided. A recital of one or more synonyms does not exclude the use of other synonyms. The use of examples anywhere in this specification, including examples of any terms discussed herein, is illustrative only, and in no way limits the scope and meaning of the invention or of any exemplified term. Likewise, the invention is not limited to various embodiments given in this specification. Furthermore, subtitles may be used to help a reader of the specification to read through the specification, which the usage of subtitles, however, has no influence on the scope of the invention.

As used herein, “about” or “approximately” shall generally mean within 20 percent, preferably within 10 percent, and more preferably within 5 percent of a given value or range. Numerical quantities given herein are approximate, meaning that the term “about” or “approximately” can be inferred if not expressly stated.

As used herein, the term “isoelectric point” or its acronym “pI” refers to the pH at which a molecule or surface carries no net electrical charge. In order to have a sharp pI, a molecule (or surface) must be amphoteric, meaning it must have both acidic and basic functional groups. Proteins and amino acids are common molecules that meet this requirement.

Proteins can be separated according to their isoelectric point in a process known as isoelectric focusing. At a pH below the pI, proteins carry a net positive charge. Above the pI they carry a net negative charge. This has implications for running electrophoretic gels. The pH of an electrophoretic gel is determined by the buffer used for that gel. If the pH of the buffer is above the pI of the protein being run, the protein will migrate to the positive pole (negative charge is attracted to a positive pole). If the pH of the buffer is below the pI of the protein being run, the protein will migrate to the negative pole of the gel (positive charge is attracted to the

negative pole). If the protein is run with a buffer pH that is equal to the pI, it will not migrate at all.

As used herein, the term “cyclic voltammetry” or its acronym “CV” refers to a type of potentiodynamic electrochemical measurement. To obtain a cyclic  
5 voltammogram, the voltage is varied in a solution and the change in current is measured with respect to the change in voltage. It is a specific type of voltammetry used for studying the redox properties of chemicals and interfacial structures.

In a cyclic voltammetry experiment, a potential is applied to the system, and the faradic current response is measured (a faradic current is the current due to a  
10 redox reaction). The current response over a range of potentials (a potential window) is measured, starting at an initial value and varying the potential in a linear manner up to a pre-defined limiting value. At this potential (often referred to as a switching potential), the direction of the potential scan is reversed, and the same potential window is scanned in the opposite direction. This means that, for example, species  
15 formed by oxidation on the first (forward) scan can be reduced on the second (reverse) scan. This technique is commonly used, since it provides a fast and simple method for initial characterization of a redox-active system. In addition to providing an estimate of the redox potential, it can also provide information about the rate of electron transfer between the electrode and the analyte, and the stability of the analyte  
20 in the electrolyzed oxidation states (e.g., if they undergo any chemical reactions).

#### OVERVIEW OF THE INVENTION

Biocompatible scaffolds are found important in regenerations of tissue [1], such as bone [3, 4], under the guidance of proteins such as growth hormones. For monitoring proteins' activities inside the biocompatible scaffolds, there are needs to  
25 develop an electrochemical protein sensor having a scaffold that enables fast electron transfer between the proteins and sensor electrodes. However, scaffolds of peptides and/or polymers usually have a poor electrical conductivity.

Solid-state nanowires (NWs) are considered to be able to detect single viruses [5]. Among them, TiO<sub>2</sub>-based NWs are inexpensive to fabricate, highly  
30 biocompatible, chemically and photochemically stable, with negligible protein denaturation, thus being widely used in various applications [6-8]. For instance, heme proteins have been immobilized on TiO<sub>2</sub> nanoparticles and nanotubes (NTs) [9-14].

The present invention, among other things, discloses a biocompatible scaffold of titanate-NWs, which entangle each other on the surface of an indium-tin-oxide (ITO) substrate (electrode). The titanate-NW scaffold is utilized as a biosensor, or at least a part of it, for monitoring the electrochemical redox potentials of the cytochrome c (*cyt c*) that is pre-immobilized on the titanate-NW's surface. Additionally, the titanate-NW scaffold can also be utilized to support the growth of pheochromocytoma cells (PC-12).

The description will be made as to the embodiments of the present invention in conjunction with the accompanying drawings of Figs. 1-6. In accordance with the purposes of this invention, as embodied and broadly described herein, this invention, in one aspect, relates to a biocompatible scaffold usable for sensing proteins in a biological analyte and for supporting and directing the growth of stem cells.

The biocompatible scaffold includes a substrate, and a conductive layer of TiO<sub>2</sub>-containing nanowires or nanofibers formed on the substrate. The substrate can be conductive or semi-conductive. The substrate is formed of indium-tin-oxide (ITO) in one embodiment. The conductive layer of TiO<sub>2</sub>-containing nanowires or nanofibers is formed with a pore structure having a thickness in the range of about 1-50 μm. The pore structure may have a layered-titanate (Na<sub>2</sub>Ti<sub>3</sub>O<sub>7</sub>) structure with counter-cations (Na<sup>+</sup>) in the interlayer space. The TiO<sub>2</sub>-containing NWs are substantially in the TiO<sub>2</sub>-B phase or titanate phase, and have a typical diameter in the range of about 20-150 nm.

Other NWs containing the interlayer cations can also be utilized to practice the present invention.

When the biocompatible scaffold is in contact with a biological analyte, one or more proteins of the biological analyte are immobilized on a surface of the conductive layer of TiO<sub>2</sub>-containing nanowires or nanofibers, whereby electron transfers between the biological analyte and the surface of the conductive layer of TiO<sub>2</sub>-containing nanowires or nanofibers occur. The electron transfers generate a measurable faradic current signal.

The biocompatible scaffold can be utilized to detect redox-active heme proteins of a biological analyte. Cytochrome c (*cyt c*) is a redox-active heme protein, has 104 amino acids, and sits in the intermembrane space of mitochondria as an

electron carrier in the electron transport chain. It is a soluble protein, and capable of undergoing oxidation and reduction, but does not bind oxygen. In a buffer solution having a pH value in the range of about 6.2-9.0, the cytochrome *c* carries a net positive charge, while the nanowires or nanofibers carry a net negative charge. When  
5 the cytochrome *c* is immobilized on the surface of the TiO<sub>2</sub>-containing NW scaffold, one or more electrons transfer between the cytochrome *c* and the NW scaffold is to cause a measurable signal in the form of a faradic current, which is detected by a cyclic voltammetry (CV). Other types of proteins can also be utilized to practice the present invention.

10 Referring to Fig. 1, a biosensor 100 utilizing the above NW scaffold 120 for detecting one or more proteins in a biological analyte is shown according to one embodiment of the present invention.

The biosensor 100 has a substrate 110 having a surface 112, a scaffold 120 formed of NWs on the surface 112 of the substrate 110, and a detector (not shown) in  
15 communication with the substrate 110. In operation, the NW scaffold 120 is in contact with the biological analyte containing redox-active heme proteins 130 and a buffer solution. The buffer solution has a pH value in the range of about 6.2-9.0. The redox-active heme proteins such as *cyt c* 130 are immobilized on the surfaces of the NWs 120. The binding of *cyt c* on the NW scaffolds is attributed mainly to the  
20 electrostatic interaction between the negatively charged NW surface and the positively charged *cyt c* surface. At the pH 6.2-9.0, the NW surface is negatively charged due to its isoelectric point of 6.2 [21], while the *cyt c* surface is positively charged due to its isoelectric point of 10.0-10.5. Thus, the negative NWs surface shows a high affinity to the positive *cyt c*. Moreover, the shape of the *cyt c* in water is  
25 nearly spherical ( $a \times b \times c = 1.5 \text{ nm} \times 1.7 \text{ nm} \times 1.7 \text{ nm}$ ) [22], thus the *cyt c* can be easily immobilized in the voids of the NW scaffolds (diameter > 500 nm) for further enhancing the retention of the *cyt c* during the electrochemical redox processes.

Additionally, the biosensor 100 also includes means (not shown) for applying a potential to the biological analyte at a scan rate that is in the range of about 0.005-  
30 0.500 V/s. The detector in communication with the substrate 110 is used to detect electron transfers between the biological analyte and the surface 112 of the substrate 110. The detector includes a CV. The electron transfers are measured in terms of

faradic current,  $i_p$ . The presence and/or quantity of the proteins in the biological analyte can be determined by the faradic current  $i_p$ .

The NW scaffold can also be utilized to support the growth of cells, such as pheochromocytoma cells (PC-12). In one embodiment, the NW scaffold is at least partially coated with a plurality of biomolecules, including growth hormone.

Another aspect of the present invention relates to a method for detecting the one or more proteins of a biological analyte. The method includes the following steps: at first, a biosensor is provided, which has an electrode and a scaffold formed of NWs on the electrode. The biological analyte is then introduced into the scaffold. Electron transfers between the biological analyte and the surface of the substrate are detected by a CV, which is used determine the presence and/or quantity of the proteins in the biological analyte.

These and other aspects of the present invention are more specifically described below.

## IMPLEMENTATIONS AND EXAMPLES OF THE INVENTION

Without intent to limit the scope of the invention, exemplary methods and their related results according to the embodiments of the present invention are given below. Note that titles or subtitles may be used in the examples for convenience of a reader, which in no way should limit the scope of the invention. Moreover, certain theories are proposed and disclosed herein; however, in no way they, whether they are right or wrong, should limit the scope of the invention so long as the invention is practiced according to the invention without regard for any particular theory or scheme of action.

### EXAMPLE:

#### BIOCOMPATIBLE SCAFFOLD FOR DIRECT SENSING PROTEINS

In this exemplary embodiment, titanate-NWs are entangled on the surface of an ITO substrate into a scaffold, which is used to monitor the electrochemical redox potentials of the cytochrome c that is pre-immobilized onto the titanate-NWs' surface. The titanate-NWs are synthesized at temperature about 240 °C for about 3 days.

Figs. 2a and 2b show a scanning electron microscope (SEM) image and a

transmission electron microscopy (TEM) image of the titanate NW scaffold, respectively. A powder x-ray diffraction (XRD) pattern of the titanate NW scaffold is shown in Fig. 2c. The XRD pattern clearly demonstrates that the crystal structure of the NWs belongs to the titanate phase (i.e.  $\text{Na}_2\text{Ti}_3\text{O}_7$ ), which is characterized by the following lattice parameters:  $[2\theta = 9.8^\circ (001), 11.2^\circ (200), 24.4^\circ (110), \text{ and } 29.7^\circ (003)]$ , (JCPDS card No.: 47-0561). The edge-shared  $\text{TiO}_6$ -octahedron is the basic unit to form the negatively charged, layered titanate structure [15], with the sodium counter-cations ( $\text{Na}^+$ ) sitting in the interlayer space, thus resulting in variable interlayer distances depending on the size and the hydration degree of the cations. Accordingly, the titanate NW scaffold has a pore structure, as shown in Figs. 2a and 2b. After washing with the deionized distilled water (DDW to pH near 7), the sodium counter-cations are replaced with the protons.

Typically, the resistance of the air-dried NW-scaffold is above  $10^6$  Ohm, which may not be suitable for the electrochemical sensing. However, the hydroxyl (-OH) groups [4] on the NW surface at a buffer solution of pH 6.2–9 could act as “wet-electrons”, at the water-NW interface [16], to form a low-energy path for a fast ET on the NW surface, which ensures the signal transduction(s) across the entire biocompatible scaffold in biosensing. In other words, the surface of titanate NWs may be negatively charged and formed Ti-OH in the buffer solution [4]. The interface between water and metal-oxide can produce wet-electrons [16], thus, the conductivity of titanate-NWs is improved.

Cytochrome c is a redox-active heme protein, has 104 amino acids, and sits in the intermembrane space of mitochondria as an electron carrier in the electron transport chain. To verify whether the *cyt c* could be destabilized after binding to negatively charged surfaces [17-19], Fourier transform infrared spectroscopy (FT-IR) was used to characterize the protein's amide group that is sensitive to the polypeptide conformation. Usually, the amide I signal at  $1600\text{--}1700\text{ cm}^{-1}$  is primarily due to a stretching vibration of C=O group along the protein backbone. The FT-IR spectra are shown in Fig. 2d, where curve 210 is the FT-IR spectrum for cytochrome c-titanate-NWs mixed with KBr, while curve 220 is the FT-IR spectrum for cytochrome c in the pH 6.8 buffer solution. The FT-IR spectra show a peak at  $1648\text{ cm}^{-1}$  for amide I [20] of immobilized *cyt c* on the NWs, which is same as that obtained from native *cyt c* in

buffer of pH 6.8, showing the retained secondary structure for the *cyt c* immobilized on the titanate-NW scaffold.

The binding of *cyt c* on the titanate-NW scaffolds are attributed mainly to the electrostatic interaction between the negatively charged NW surface and the positively charged *cyt c* surface. At the pH 6.2–9.0, the NW surface is negatively charged due to its isoelectric point of 6.2 [21], while the *cyt c* surface is positively charged due to its isoelectric point of 10–10.5. Thus, the negative NWs surface shows a high affinity to the positive *cyt c*. Moreover, the shape of the *cyt c* in water is nearly spherical ( $a \times b \times c = 1.5 \text{ nm} \times 1.7 \text{ nm} \times 1.7 \text{ nm}$ ) [22], thus the *cyt c* can be easily immobilized in the voids of the scaffolds (diameter > 500 nm) for further enhancing the retention of the *cyt c* during the electrochemical redox processes.

The NW scaffold offers a desirable environment for the *cyt c* to undergo facile electron-transfer reactions. In the exemplary embodiment, the surface electrochemical properties of the NWs immobilized with cytochrome c are studied by means of a CV at various concentrations, which indicates a detection limit of 45 picomoles (or  $10^{-12}$  moles) of the *cyt c*.

Fig. 3a shows the CV signals acquired from an ITO glass electrode coated with the NWs (signal 330a for the anodic current  $i_{pa}$  and signal 330c for the cathodic current  $i_{pc}$ ) and the *cyt c*-NWs (signal 340a for the anodic current  $i_{pa}$  and signal 340c for the cathodic current  $i_{pc}$ ), respectively, in a potassium phosphate buffer solution of pH = 6.8. The scan rate is about 0.2V/s. A pair of reversible and well defined redox peaks from the *cyt c*-NW electrode with the formal peak potential ( $E^0$ ) of 0.03 V have been recorded. The  $E^0$  value (0.03 V), calculated from an average of anodic peak's and cathodic peak's potential values, is close to that reported by Stellwagen [23]. Based on its structure and morphology, the NWs behave like a conducting “nanocable” [16] to facilitate the electron transfers.

Measurement of the faradic current as a function of the scan rate can be used to diagnose whether a redox reaction on the electrode surface is controlled by diffusion. The cathodic and the anodic current peaks are both linearly proportional to the scan rate from 0.01 V/s to 0.2 V/s, implying that such an electrode has the typical characteristic of the thin-layer electrochemistry [24]. The ratio of  $i_{pc}$  to  $i_{pa}$  is about 2.0 and the separations between the redox peaks was about 58 mV (data not shown),

indicating that the electrochemical process on the *cyt c*-NW electrode surface may be quasi-reversible [24].

In the pH 6.8 buffer, the faradic current is increased linearly as the concentration of the *cyt c* in the scaffolds was increased, as shown in the Fig. 3b, where signal 351a is  $i_{pa}$  for 45 picomoles, signal 352a is  $i_{pa}$  for 122 picomoles, signal 353a is  $i_{pa}$  for 243 picomoles, signal 354a is  $i_{pa}$  for 347 picomoles and signal 355a is  $i_{pa}$  for 450 picomoles, respectively, and the scan rate is about 0.2V/s. The reversible, well-defined CV signals are observed in 15  $\mu$ L solutions between 3.0  $\mu$ M and 30.0  $\mu$ M, or 45 to 450 picomoles. The concentrations are all linearly correlated ( $R^2=$  0.9887) with the anodic currents, as shown in the Fig. 3c.

The results suggest that such a titanate-NW-modified ITO electrode could be used as a multiplexed sensing platform for developing robust, sensitive, economic nanobiosensors.

Additionally, the NW scaffold can also be utilized for supporting and directing the growth of pheochromocytoma cells (PC-12), thereby facilitating cellular activities. Figs. 3d and 4a are an SEM image of PC-12 cells cultured on the NW scaffold for about 72 hours, showing that PC-12 cells attached well and formed cell colonies on the NW scaffold. The cell appeared round in shape and maintained their morphologies, indicating a good compatibility between the cell and the NW scaffold. PC-12 cells attachment on the titanate-NW scaffold further suggests the adsorbed proteins do not lose any of their activities.

Fig. 4b shows CV signals of protein cytochrome c immobilized onto a thin-film of the NW scaffold at different potential scan rates and pH values. A reproducible detection limit of 45 picomoles of the *cytochrome c* has been achieved at the range of pH 6.8-9.0, revealing a greatly enhanced direct electron transfer for the cytochrome c on the titanate NW-modified ITO electrode. The formal potential of the *cytochrome c* on the NW is independent to the pH change. The results would shed new light on fabricating the NW-based affinity-specific bionanosensors via a functionalization on the ceramic nanowire surface could result in a multiplexed sensing platform for simple, economic, quick, sensitive, and selective biosensing.

Figs. 5 and 6 are CV signals of a NW scaffold according to another embodiment of the present invention.

Briefly, the present invention, among other things, discloses a biocompatible scaffold of the titanate NWs, and a titanate NW scaffold based biosensor. The titanate NW scaffold enhances the electron transfers between the ITO electrode and the *cyt c*. The titanate NW scaffold based biosensor can find applications in direct detection of  
5 immobilized redox-active proteins and monitoring biochemical processes during the growth of stem cells in real time within a confined environment of the titanate NW scaffold based scaffold.

The foregoing description of the exemplary embodiments of the invention has been presented only for the purposes of illustration and description and is not intended  
10 to be exhaustive or to limit the invention to the precise forms disclosed. Many modifications and variations are possible in light of the above teaching.

The embodiments are chosen and described in order to explain the principles of the invention and their practical application so as to enable others skilled in the art to utilize the invention and various embodiments and with various modifications as  
15 are suited to the particular use contemplated. Alternative embodiments will become apparent to those skilled in the art to which the present invention pertains without departing from its spirit and scope. Accordingly, the scope of the present invention is defined by the appended claims rather than the foregoing description and the exemplary embodiments described therein.

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## CLAIMS

What is claimed is:

1. A biocompatible scaffold, comprising:
  - a. a substrate; and
  - b. a conductive layer of TiO<sub>2</sub>-containing nanowires or nanofibers formed on the substrate, wherein the conductive layer of TiO<sub>2</sub>-containing nanowires or nanofibers is formed with a pore structure, and wherein when the biocompatible scaffold is in contact with a biological analyte, one or more proteins of the biological analyte are immobilized on a surface of the conductive layer of TiO<sub>2</sub>-containing nanowires or nanofibers so as to generate a measurable faradic current signal.
2. The biocompatible scaffold of claim 1, wherein the proteins comprise redox-active heme proteins.
3. The biocompatible scaffold of claim 2, wherein the redox-active heme proteins comprises a cytochrome c.
4. The biocompatible scaffold of claim 1, wherein the pore structure has a thickness in the range of about 1-50 μm.
5. The biocompatible scaffold of claim 1, wherein the TiO<sub>2</sub>-containing nanofibers or nanowires are substantially in the TiO<sub>2</sub>-B phase or titanate phase.
6. The biocompatible scaffold of claim 5, wherein the TiO<sub>2</sub>-containing nanofibers or nanowires have a typical diameter in the range of about 20-150 nm.

7. The biocompatible scaffold of claim 5, wherein the pore structure comprises a layered-titanate ( $\text{Na}_2\text{Ti}_3\text{O}_7$ ) structure with counter-cations ( $\text{Na}^+$ ) in the interlayer space.
8. The biocompatible scaffold of claim 1, wherein the substrate is formed of indium-tin-oxide (ITO).
9. The biocompatible scaffold of claim 1, wherein the substrate is conductive, semi-conductive or insulative.
10. A biocompatible scaffold, comprising:
  - a. a substrate; and
  - b. a layer of nanowires or nanofibers formed on the substrate, wherein the layer of nanowires or nanofibers is formed with a pore structure.
11. The biocompatible scaffold of claim 10, wherein the nanowires or nanofibers comprise oxide-containing nanowires or nanofibers.
12. The biocompatible scaffold of claim 11, wherein the nanowires or nanofibers comprise  $\text{TiO}_2$ -containing nanowires or nanofibers.
13. The biocompatible scaffold of claim 11, wherein the nanowires or nanofibers comprise ZnO-containing nanowires or nanofibers.
14. A biosensor 100 for detecting one or more proteins in a biological analyte, comprising:
  - a. a substrate 110 having a surface 112;

- b. a layer 120 formed of nanowires or nanofibers on the surface 112 of the substrate 110, wherein the layer of nanowires or nanofibers is formed with a pore structure; and
  - c. a detector 140 in communication with the substrate 110, wherein when the layer 120 formed of nanowires or nanofibers is in contact with one or more proteins in the biological analyte, a measurable signal is generated and measured by the detector 140.
15. The biosensor of claim 14, wherein the one or more proteins in the biological analyte comprise redox-active heme proteins.
16. The biosensor of claim 15, wherein the redox-active heme proteins comprise cytochrome c.
17. The biosensor of claim 16, wherein the biological analyte contains a buffer solution having a pH value in the range of about 6.2-9.0, such that the cytochrome c carries a net positive charge, and the nanowires or nanofibers carry a net negative charge, respectively.
18. The biosensor of claim 14, wherein the nanowires or nanofibers have a typical diameter in the range of about 20-150 nm.
19. The biosensor of claim 18, wherein the nanowires or nanofibers are oxide-containing nanowires or nanofibers.
20. The biosensor of claim 19, wherein the nanowires or nanofibers are TiO<sub>2</sub>-containing nanowires or nanofibers.

21. The biosensor of claim 20, wherein the TiO<sub>2</sub>-containing nanofibers or nanowires are substantially in the TiO<sub>2</sub>-B phase or titanate phase.
22. The biosensor of claim 19, wherein the nanowires or nanofibers are ZnO-containing nanowires or nanofibers.
23. The biosensor of claim 14, wherein the substrate is formed of indium-tin-oxide (ITO).
24. The biosensor of claim 14, wherein the substrate is conductive, semi-conductive or insulative.
25. The biosensor of claim 14, further comprising means for applying a potential to the biological analyte at a scan rate that is in the range of about 0.005-0.500 V/s.
26. The biosensor of claim 25, wherein the detector comprises a cyclic voltammetry (CV).
27. The biosensor of claim 26, wherein when the layer 120 formed of nanowires or nanofibers is in contact with one or more proteins in the biological analyte, one or more electrons transfer between the biological analyte and the substrate is to cause a measurable signal in the form of a faradic current.
28. A method for detecting one or more proteins in a biological analyte, comprising the steps of:
  - a. providing a biosensor having an electrode and a scaffold having a substrate and a layer of nanowires or nanofibers formed on a surface of the substrate;
  - b. introducing the biological analyte into the scaffold; and

- c. measuring electron transfers between the biological analyte and the surface of the substrate so as to detect one or more proteins in the biological analyte.
29. The method of claim 28, wherein the biological analyte contains redox-active heme proteins.
30. The method of claim 29, wherein the redox-active heme proteins comprise cytochrome c.
31. The method of claim 30, wherein the biological analyte contains a buffer solution having a pH value in the range of about 6.2-9.0, such that the cytochrome c carries a net positive charge, and the nanowires or nanofibers carry a net negative charge, respectively.
32. The method of claim 28, wherein the layer of nanowires or nanofibers is formed to have a pore structure.
33. The method of claim 28, wherein the nanowires or nanofibers are TiO<sub>2</sub>-containing nanowires or nanofibers.
34. The method of claim 28, wherein the substrate is formed of indium-tin-oxide (ITO).
35. The method of claim 28, wherein the substrate is conductive, semi-conductive or insulative.
36. The method of claim 28, further comprising the step of applying a potential to the biological analyte at a scan rate that is in the range of about 0.005-0.500 V/s.

37. The method of claim 36, wherein the measuring step is performed with a cyclic voltammetry (CV).

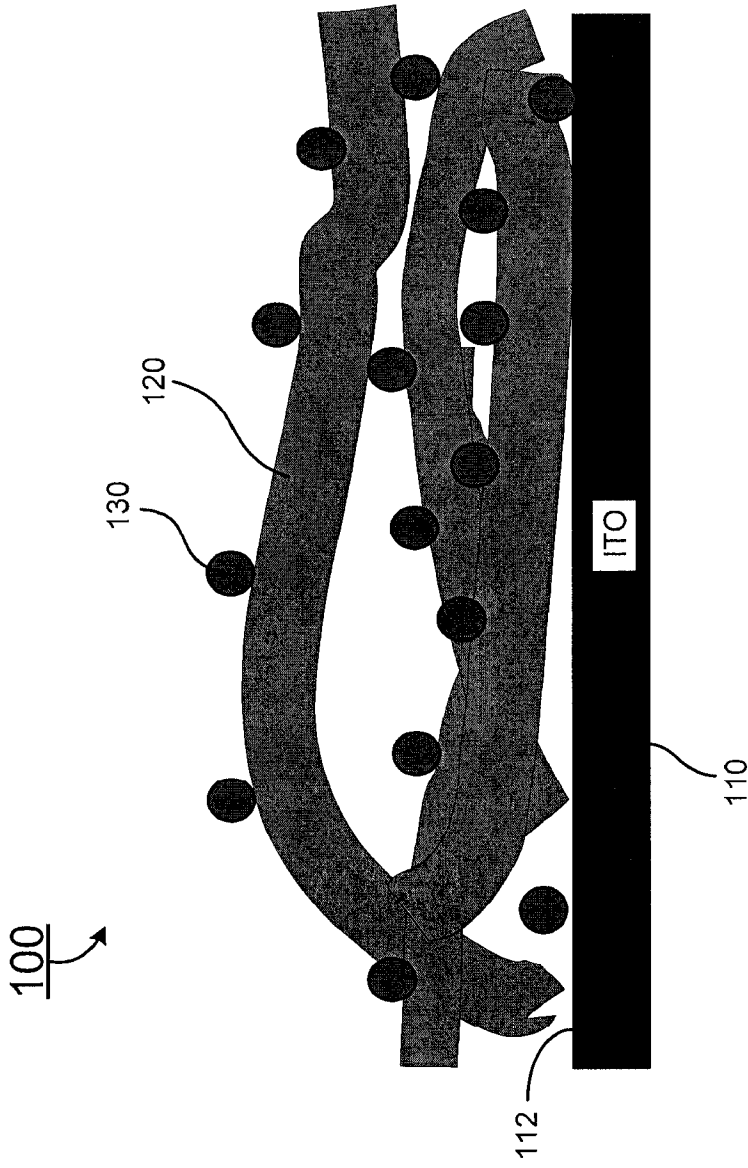


Fig. 1

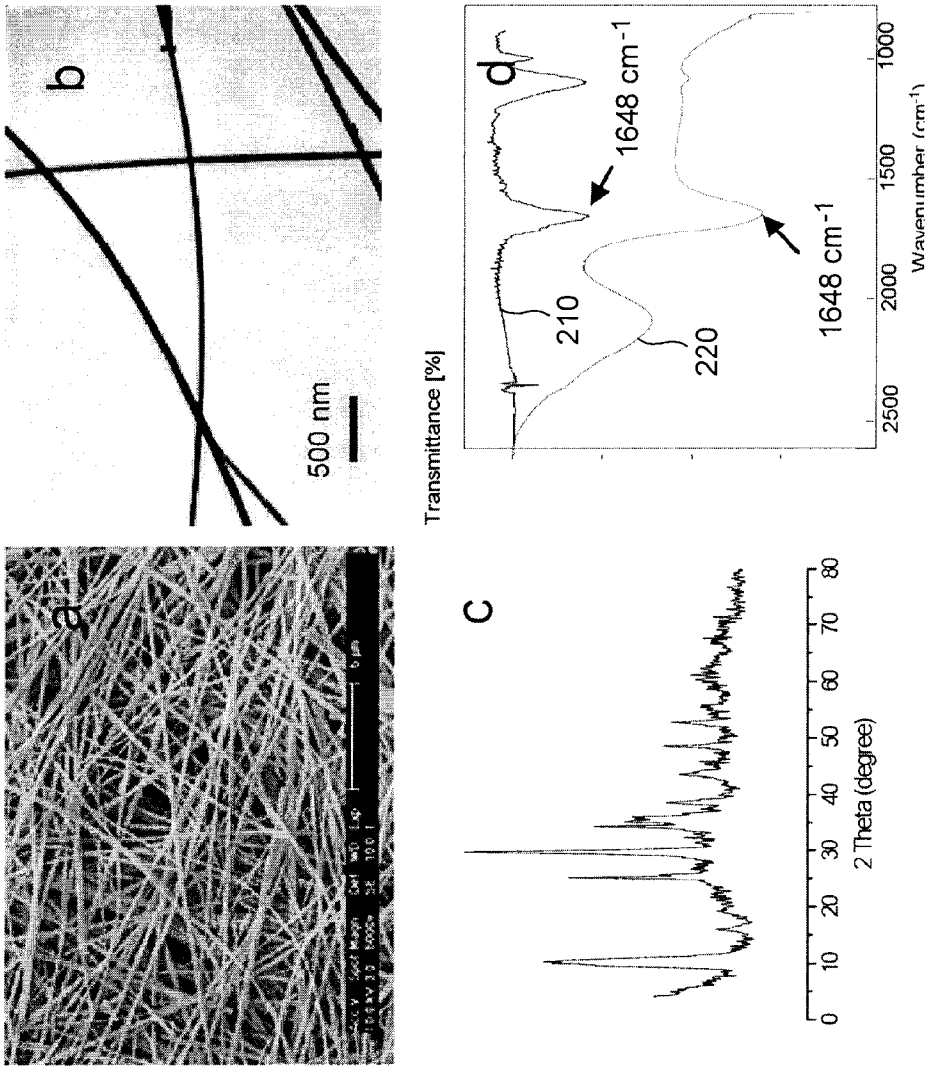


Fig. 2

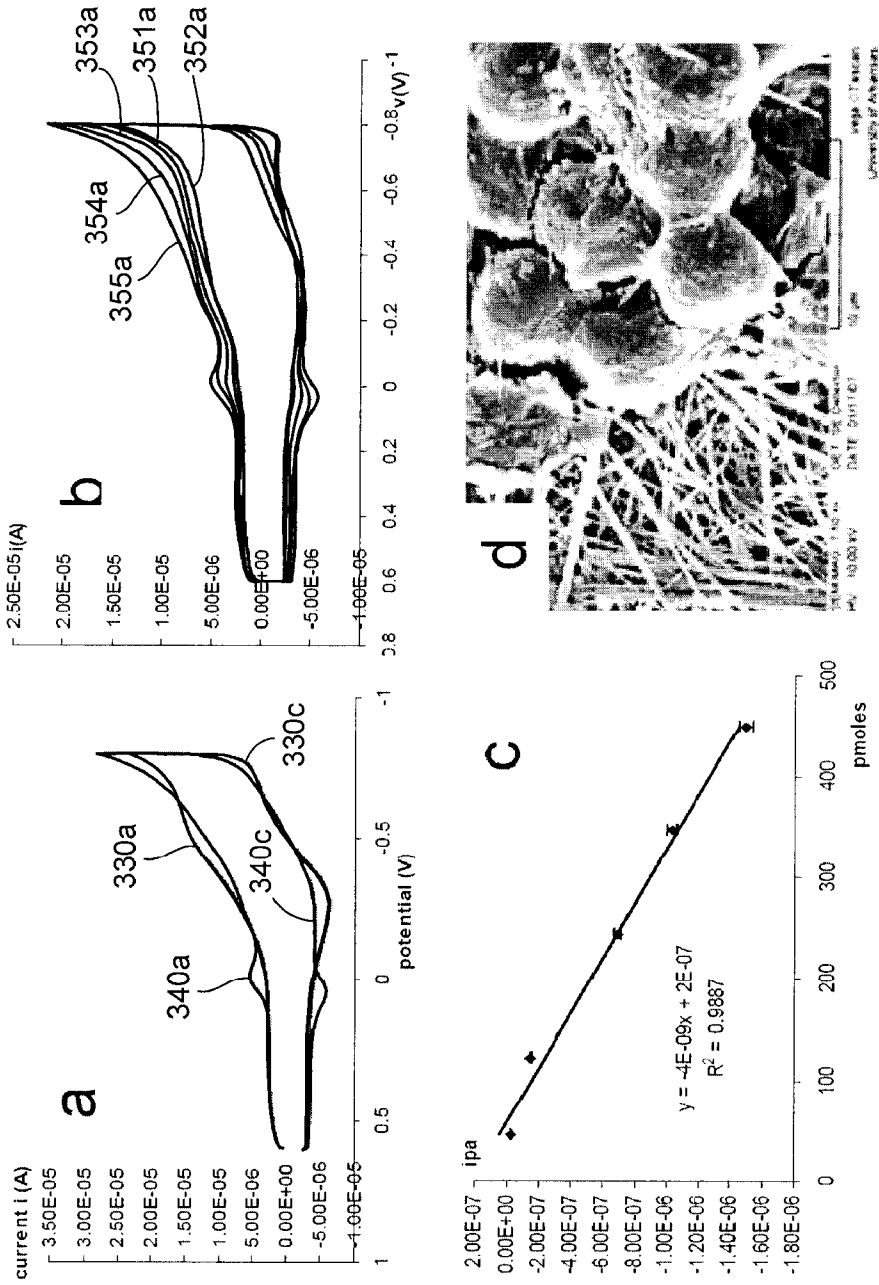


Fig. 3

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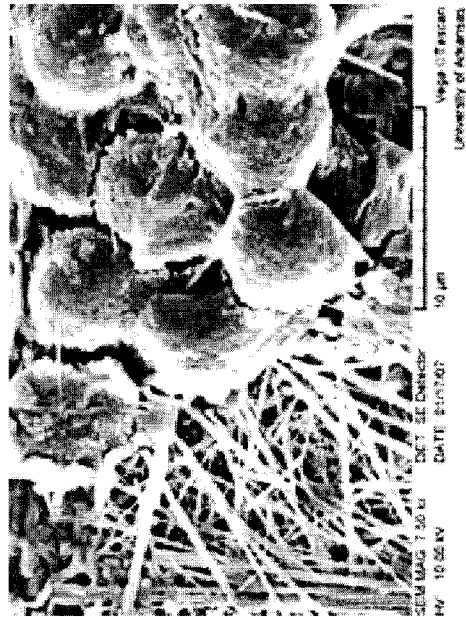
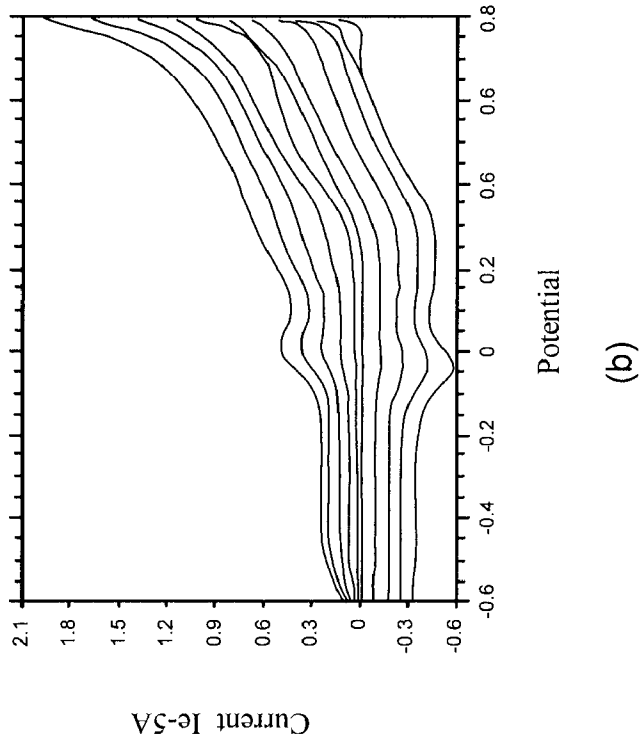


Fig. 4

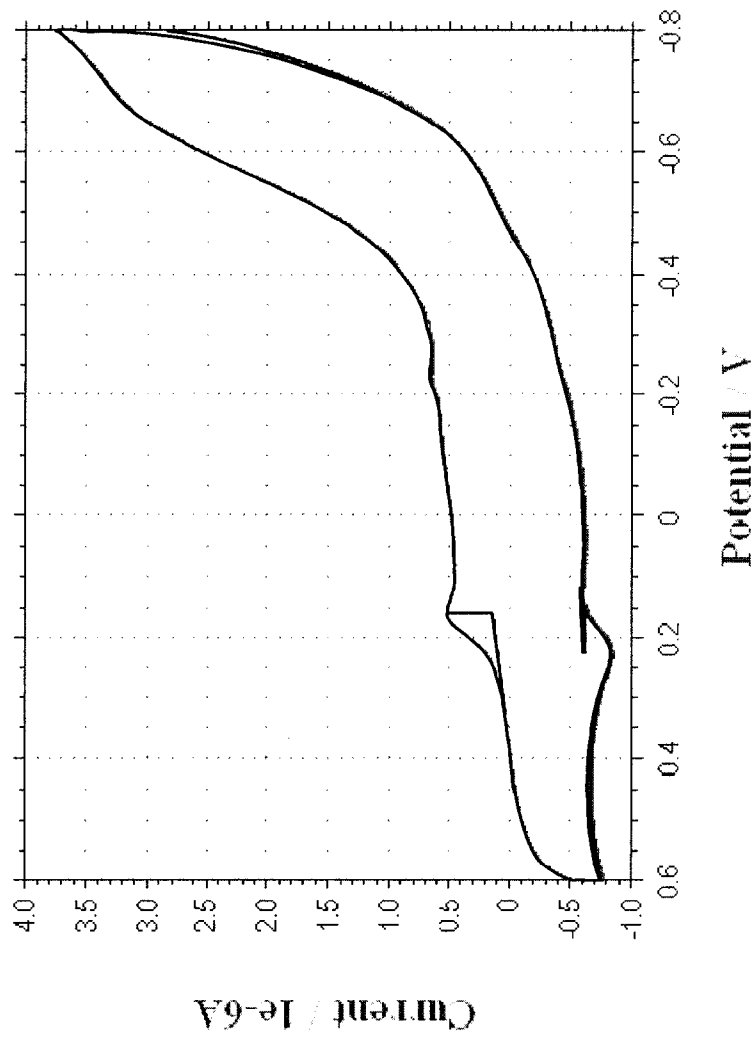


Fig. 5

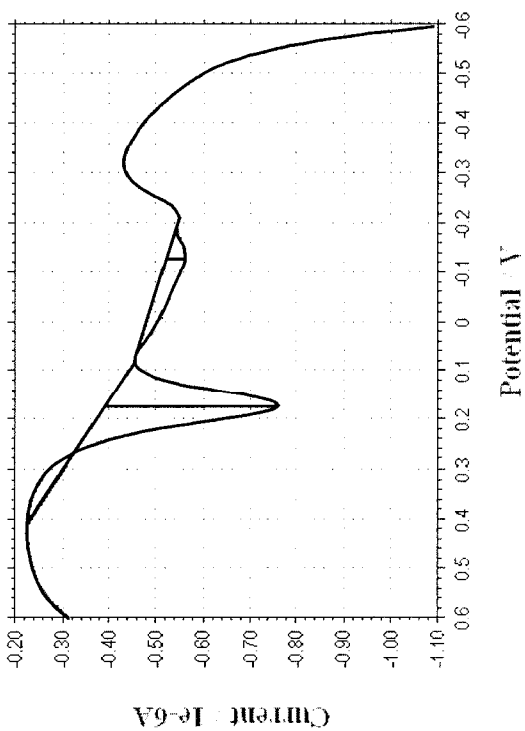
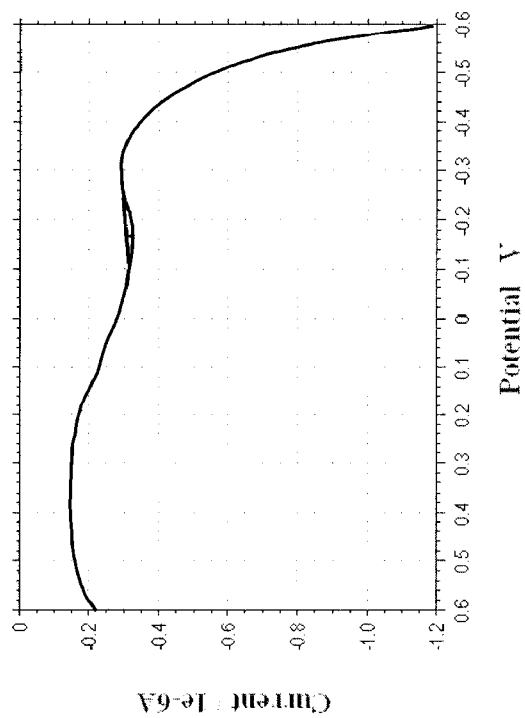


Fig. 6