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<b>Title of Invention:</b>	FLEXIBLE ELECTRODE, BIOFUEL CELL USING SAME, AND METHOD FOR MANUFACTURING SAME
<b>First Named Inventor/Applicant Name:</b>	Jinhan CHO
<b>Customer Number:</b>	89980
<b>Filer:</b>	Stephen Thomas Boughner/Mark Gambriel
<b>Filer Authorized By:</b>	Stephen Thomas Boughner
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1	Applicant Response to Pre-Exam Formalities Notice	MISC20200831_0181590014_R esponsetoNTFMP.pdf	16115  6837b87a5d54d59906e29bcfc1090292bb7 3219f	no	2

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Information:					
2		MISC20200831_0181590014_A ppasFiled.pdf	150080  af3976564be42089db2f089f47308808db2 779b4	yes	22
	<b>Multipart Description/PDF files in .zip description</b>				
	<b>Document Description</b>		<b>Start</b>	<b>End</b>	
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	Specification		1	18	
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3	Drawings-only black and white line drawings	MISC20200831_0181590014_D rawingsasFiled.pdf	4654284  738ebb0b3f5c99021ac773d4107a5a1fa7f3 db39	no	8
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4	Oath or Declaration filed	MISC20200831_0181590014_E xecutedCDA.pdf	2501576  040fb97c11cb3f91ba7a9246d936f44f588d 8e7d	no	2
Warnings:					
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5	Power of Attorney	MISC20200831_0181590014_E xecutedPOA.pdf	154729  17a1f7871e3edcbe2d5b6920d113793de83 9a64c	no	1
Warnings:					
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6	Miscellaneous Incoming Letter	MISC20200831_0181590014_Tr anslatorsCertification.pdf	189041  7d6136ca2da9d7ed0fee94bf52829b1ba78 ed684	no	1
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**National Stage of an International Application under 35 U.S.C. 371**

**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

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**PATENT ASSIGNMENT COVER SHEET**

Electronic Version v1.1  
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<b>SUBMISSION TYPE:</b>	NEW ASSIGNMENT						
<b>NATURE OF CONVEYANCE:</b>	ASSIGNMENT						
<b>CONVEYING PARTY DATA</b>							
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Name	Execution Date						
JINHAN CHO	05/13/2020						
CHEONG HOON KWON	05/13/2020						
<b>RECEIVING PARTY DATA</b>							
<b>Name:</b>	KOREA UNIVERSITY RESEARCH AND BUSINESS FOUNDATION						
<b>Street Address:</b>	145, ANAM-RO, SEONGBUK-GU						
<b>City:</b>	SEOUL						
<b>State/Country:</b>	KOREA, REPUBLIC OF						
<b>Postal Code:</b>	02841						
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<table border="1"> <thead> <tr> <th>Property Type</th> <th>Number</th> </tr> </thead> <tbody> <tr> <td><b>Application Number:</b></td> <td>16763031</td> </tr> </tbody> </table>		Property Type	Number	<b>Application Number:</b>	16763031		
Property Type	Number						
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<b>Fax Number:</b>	(202)315-3758						
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<i>Correspondence will be sent to the e-mail address first; if that is unsuccessful, it will be sent using a fax number, if provided; if that is unsuccessful, it will be sent via US Mail.</i>	
<b>Correspondent Name:</b>	NSIP LAW
<b>Address Line 1:</b>	P.O. BOX 65745
<b>Address Line 4:</b>	WASHINGTON, D.C. 20035
<b>ATTORNEY DOCKET NUMBER:</b>	018159.0014
<b>NAME OF SUBMITTER:</b>	STEPHEN T. BOUGHNER
<b>Signature:</b>	/Stephen T. Boughner/
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**COMBINED DECLARATION AND ASSIGNMENT UNDER 37 CFR 1.63(e)****DECLARATION**

As a below-named inventor, I hereby declare that this declaration is directed to the application attached hereto, or to United States Application Number or PCT International Application Number PCT/KR2018/007846 filed on July 11, 2018 (if applicable), entitled:

**FLEXIBLE ELECTRODE, BIOFUEL CELL USING SAME, AND METHOD FOR MANUFACTURING SAME**

The above-identified application was made or authorized to be made by me.

I believe I am the original inventor or an original joint inventor of a claimed invention in the above-identified application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 USC 1001 by fine or imprisonment of not more than five (5) years, or both.

I have reviewed and understand the contents of the above-identified application, including the claims.

I am aware of the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information that became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

**ASSIGNMENT**

For valuable consideration, I, as a below-named assignor, hereby assign to:

Korea University Research and Business Foundation  
145, Anam-ro, Seongbuk-gu  
Seoul, 02841  
Republic of Korea


and its successors and assigns (collectively hereinafter called "the Assignee"), the entire right, title, and interest throughout the world in the inventions and improvements that are the subject of the application identified in the above declaration, which is United States Application Number or PCT International Application Number PCT/KR2018/007846 filed on July 11, 2018.

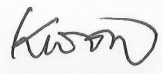
I authorize and request the attorneys appointed in the above-identified application to hereafter complete this Assignment by inserting above the application number and the filing date of the above-identified application when known, and to correct any typographical errors that may be discovered in this Assignment.

This Assignment includes the above-identified application, any and all United States and foreign patents, utility models, and design registrations granted for any of the inventions and improvements that are the subject of the above-identified application, and the right to claim priority based on the filing date of the above-identified application under the International Convention for the Protection of Industrial Property, the Patent Cooperation Treaty, the European Patent Convention, and all other treaties of like purposes; and I authorize the Assignee to apply in all countries in my name or in its own name for patents, utility models, design registrations, like rights of exclusion, and inventors' certificates for any of the inventions and improvements that are the subject of the above-identified application; and I agree for myself and my heirs, legal representatives, and assigns without further compensation to perform such lawful acts and to sign such further applications, Assignments, preliminary statements, and other lawful documents as the Assignee may reasonably request to effectuate fully this Assignment.

**COMBINED DECLARATION AND ASSIGNMENT UNDER 37 CFR 1.63(e)**

In witness whereof, I, as a below-named inventor and assignor, intending to be legally bound, have hereunto affixed my signature on the date indicated below next to my signature.

Inventor's Legal Name	<b>Jinhan CHO</b>		
Inventor's Signature		Date	May 13, 2020
Residence (City, Country)	Seoul, Republic of Korea		
Mailing Address	108-1001, 41, Sinbanpo-ro 23-gil, Seocho-gu, Seoul 06509 Republic of Korea		

Inventor's Legal Name	<b>Cheong Hoon KWON</b>		
Inventor's Signature		Date	May 13, 2020
Residence (City, Country)	Seoul, Republic of Korea		
Mailing Address	304-402, 8, Sangdo-ro 53-gil, Dongjak-gu, Seoul, 06977 Republic of Korea		

## POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in the accompanying transmittal letter (form PTO/AIA/15, PTO/SB/16, PTO/AIA/18, PTO/AIA/19, PTO/SB/29, PTO/AIA/50, PTO/AIA/82A, or equivalent).

I hereby appoint the practitioners of NSIP Law associated with the following Customer Number as my attorneys or agents to transact all business in the United States Patent and Trademark Office connected with the application identified in the accompanying transmittal letter:

**89980**

Please recognize or change the correspondence address for the application identified in the accompanying transmittal letter to the address of NSIP Law associated with the following Customer Number:

**89980**

I, the below-named Assignee, am the original Applicant (if there is only one Applicant) or an original Applicant (if there is more than one Applicant) of the application identified in the accompanying transmittal letter. The undersigned, whose title is supplied below, is authorized to act on behalf of the Assignee.

Name of Assignee	KOREA UNIVERSITY RESEARCH AND BUSINESS FOUNDATION		
Address of Assignee	145, Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea		
Signature	KIM BOMZEN	Date	September 31, 2020
Name	BOMZEN KIM		
Title	IP Manager		



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Inventors: Jinhan CHO et al.

Confirmation No. 3267

Application No.: 16/763,031

Art Unit: Unassigned

Filed: May 11, 2020

Examiner: Unassigned

For: FLEXIBLE ELECTRODE, BIOFUEL CELL USING SAME, AND METHOD FOR  
MANUFACTURING SAME

**RESPONSE TO NOTICE TO FILE MISSING PARTS**

Mail Stop Missing Parts  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

The application papers for the above-identified application were originally filed in the Korean language on May 11, 2020 and the application was assigned Application No. 16/763,031.

Under the provisions of 37 C.F.R. §§ 1.41(c) and 1.53(f), attached hereto is the executed Declaration of the inventor(s) (photocopy), necessary for completing the filing requirements in connection with the above-identified application.

The undersigned hereby declares that the inventors' Declaration, filed on September 3, 2020, corresponds to Appl. No. 16/763,031, filed May 11, 2020, entitled "FLEXIBLE ELECTRODE, BIOFUEL CELL USING SAME, AND METHOD FOR MANUFACTURING SAME."

Also attached hereto is an English Translation of the Korean Specification, eight (8) English drawing sheets, Power of Attorney for the Assignee Korea University Research and Business Foundation and a Statement of Accurate Translation.

No fee is believed due at this time as the Late Translation and late declaration fees were paid at the time of the New Application Filing. However, should the USPTO determine any fees are associated with the processing of this Request and any other documents filed concurrently with this Request, they may be charged to Counsel's Deposit Account 50-5113.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact the undersigned at the telephone number below.

Respectfully submitted,

Date: September 3, 2020

/Stephen T. Boughner/  
Stephen T. Boughner  
Registration No. 45,317

NSIP Law  
P.O. Box 65745  
Washington, DC 20035  
Telephone (202) 429-0020  
Facsimile (202) 315-3758  
CYP/STB/mag

Attachments: Executed Inventors' Combined Declaration and Assignment (CDA)  
English Translation of Specification  
English Drawings  
Statement of Accurate Translation  
Power of Attorney

## Translator's Certification

I, Hyochan, KIM of JUNG, KIM & PARTNERS, Seoul, Republic of Korea, do hereby affirm that I am well acquainted with the Korean and English languages. To the best of my knowledge, ability and belief, the English Language translation of the 371 National Stage Application filed on May 11, 2020, which entered as U.S. Application No. 16/763,031, entitled "FLEXIBLE ELECTRODE, BIOFUEL CELL USING SAME, AND METHOD FOR MANUFACTURING SAME", is true and accurate.

Signed



Date

September 3, 2020

## **DESCRIPTION**

### **TITLE OF THE INVENTION: FLEXIBLE ELECTRODE, BIOFUEL CELL USING SAME, AND METHOD FOR MANUFACTURING SAME**

#### **Technical Field**

The present invention relates to a flexible electrode, a biofuel cell using the flexible electrode, a method for manufacturing the flexible electrode, and a method for fabricating the biofuel cell. More specifically, the present invention relates to a technology in which an electrically non-conductive material is imparted with conductivity by coating with metal nanoparticles and is applied to a biofuel cell.

The present invention was made with the support of the Korean Ministry of Education, under Project No. 2017003192, which was conducted in the research project “Development of highly conductive nanocomposites based on metal nanoparticles and fibrous hybrid energy systems” within the project “Fostering Next-generation Researchers in Science and Engineering\_Presidential Post-doctoral Fellowship” and with the support of the Korean Ministry of Science, ICT & Future Planning, under Project No. 201805019452, which was conducted in the research project “Development of high performance nanocomposite electrode platforms based on polymer fabric materials and elastomers and their application to energy storage/generation systems” within the project “Promotion (Follow-up) of Mid-career Researchers in Science and Engineering”.

#### **Background Art**

A biofuel cell (BFC) is a type of fuel cell that uses a biomaterial as a fuel or utilizes a biocatalyst to oxidize a fuel. Biofuel cells have received attention mainly as power sources for implantable biomedical devices such as cardiac pacemakers, nerve stimulators, and drug delivery pumps. However, biofuel cells developed hitherto have the disadvantages of low power, short operation time, and poor mechanical properties. Since the performance of a biofuel cell is determined by the transfer of electrons from

an enzyme to an electrode, the surface structure of the electrode is of particular importance. The surface structure of the electrode should have high active surface area while possessing high conductivity such that an electric current can flow efficiently through the electrode.

Conventional biofuel cells use carbon nanotubes as electrode host materials. However, carbon nanotubes are very expensive and are not readily available, limiting their application to biofuel cells.

Thus, there is a strong need to develop host electrodes for biofuel cells that can replace carbon nanotubes.

### **Detailed Description of the Invention**

#### **Problems to be Solved by the Invention**

The present invention has been made in an effort to solve the problems of the prior art and one aspect of the present invention is to provide an electrode with controllable conductivity that is manufactured by coating metal nanoparticles on a cheap and readily available electrically non-conductive substrate based on layer-by-layer self-assembly, and a biofuel cell with improved power generation efficiency that is fabricated by applying the electrode to a cathode and an anode.

Particularly, the present invention provides a fibrous electrode in which a large amount of metal nanoparticles is coated on the surface of a cotton fiber as an electrically non-conductive substrate due to the hydrophilicity of the cotton fiber and the cotton fibrils are structurally twisted to form pores as passages for electron and mass transfer.

#### **Means for Solving the Problems**

An electrode of the present invention includes an electrically non-conductive substrate, a base layer disposed on the outer surface of the substrate, a nanoparticle layer including metal nanoparticles and disposed on the outer surface of the base layer, and a monomolecular layer including a monomolecular material having one or more amine groups and disposed on the outer surface of the nanoparticle layer.

The substrate is a cotton fiber.

The base layer is composed of polyethylenimine (PEI).

The metal nanoparticles are nanoparticles of at least one metal selected from the group consisting of gold, silver, aluminum, copper, and platinum.

The monomolecular material is tris(2-aminoethylamine) (TREN).

The electrode includes a plurality of nanocomposite thin films, each of which has a bilayer structure in which the monomolecular layer is formed on the nanoparticle layer.

A biofuel cell of the present invention includes: an anode including an electrode, an enzyme layer including an enzyme and disposed on the outer surface of a monomolecular layer of the electrode, and a linker layer including a monomolecular material having one or more amine groups and disposed on the outer surface of the enzyme layer; and a cathode including an electrode.

The enzyme is selected from the group consisting of glucose oxidase, D-fructose dehydrogenase, and a mixture thereof.

The monomolecular material is tris(2-aminoethylamine) (TREN).

The biofuel cell includes a plurality of oxidation layers, each of which has a bilayer structure in which the linker layer is formed on the enzyme layer.

A method for manufacturing an electrode according to the present invention includes (a) dipping an electrically non-conductive substrate in a solution of a polymer to form a base layer, (b) dipping the substrate, where the base layer is formed, in a solution of metal nanoparticles to form a nanoparticle layer, and (c) dipping the substrate, where the nanoparticle layer is formed, in a solution of a monomolecular material having one or more amine groups to form a monomolecular layer wherein a nanocomposite thin film having a bilayer structure consisting of the nanoparticle layer and the monomolecular layer is formed on the outer surface of the substrate.

Steps (b) and (c) are repeated sequentially such that a plurality of nanocomposite thin films are formed.

A method for fabricating a biofuel cell according to the present invention includes (a) dipping two electrically non-conductive substrates in a solution of a polymer to form base layers, (b) dipping the substrates, where the base layers are

formed, in a solution of metal nanoparticles to form nanoparticle layers, (c) dipping the substrates, where the nanoparticle layers are formed, in a solution of a monomolecular material having one or more amine groups to form monomolecular layers, (d) dipping one of the substrates, where the monomolecular layers are formed, in a solution of an enzyme to form an enzyme layer, and (e) dipping the substrate, where the enzyme layer is formed, in a solution of a monomolecular material having one or more amine groups to form a linker layer.

Steps (a) and (b) are repeated sequentially such that a plurality of nanocomposite thin films are formed on the base layer and each of the nanocomposite thin films has a bilayer structure in which the monomolecular layer is formed on the nanoparticle layer; and steps (c) and (d) are repeated sequentially such that a plurality of oxidation layers are formed and each of the oxidation layers has a bilayer structure in which the linker layer is formed on the enzyme layer.

The features and advantages of the present invention will become apparent from the following description with reference to the accompanying drawings.

Prior to the detailed description of the invention, it should be understood that the terms and words used in the specification and the claims are not to be construed as having common and dictionary meanings but are construed as having meanings and concepts corresponding to the technical spirit of the present invention in view of the principle that the inventor can define properly the concept of the terms and words in order to describe his/her invention with the best method.

### **Effects of the Invention**

The electrode of the present invention includes an electrically non-conductive material imparted with conductivity and can be manufactured in a relatively economical and easy manner compared to conventional electrodes using carbon nanotubes as electrode host materials.

In addition, the conductivity of the electrode according to the present invention can be easily controlled by varying the amount of metal nanoparticles coated on a cotton fiber. The electrode of the present invention can be applied to flexible devices due to its

sufficient electrochemical performance and high flexibility. The electrode of the present invention uses a cotton fiber and metal nanoparticles whose biocompatibility and biostability are demonstrated, thus being applicable to next-generation implantable systems.

Furthermore, the biofuel cell of the present invention based on the cotton fiber electrode contains a large amount of metal nanoparticles coated per unit area due to the hydrophilicity of the cotton fiber. Moreover, fuels and electrons are easily transferred to an anode and a cathode of the biofuel cell through micropores of the cotton fiber, resulting in an improvement in the power generation efficiency of the biofuel cell.

### **Brief Description of the Drawings**

FIG. 1 is a partial perspective view of an electrode according to one embodiment of the present invention.

FIG. 2 is a cross-sectional view of an electrode according to a further embodiment of the present invention.

FIG. 3 is an enlarged view of a cotton fiber.

FIG. 4 is a schematic view of a biofuel cell according to one embodiment of the present invention.

FIG. 5 is a process flow diagram illustrating a method for manufacturing an electrode according to one embodiment of the present invention.

FIG. 6 shows cross-sectional SEM and EDAX images of a cotton fiber electrode manufactured in Example 2.

FIG. 7 shows the electrical conductivities, SEM images, CV curves, and impedance spectra of cotton fiber electrodes manufactured in Example 2 as a function of the number of nanocomposite thin films.

FIG. 8 shows the polarization curves of electrodes for a biofuel cell produced in Example 3.

FIG. 9 shows the power densities of a biofuel cell fabricated in Example 3.

### **Best Mode for Carrying out the Invention**



Other objects, advantages, and novel features of the invention will become more apparent from the following detailed description and preferred embodiments with reference to the accompanying drawings. In the drawings, the same elements are denoted by the same reference numerals even though they are depicted in different drawings. Although such terms as “first” and “second,” etc. may be used to describe various elements, these elements should not be limited by above terms. These terms are used only to distinguish one element from another. In the description of the present invention, detailed explanations of related art are omitted when it is deemed that they may unnecessarily obscure the essence of the present invention.

Preferred embodiments of the present invention will now be described with reference to the accompanying drawings.

FIG. 1 is a partial perspective view of an electrode according to one embodiment of the present invention and FIG. 2 is a cross-sectional view of an electrode according to a further embodiment of the present invention.

As illustrated in FIGS. 1 and 2, the electrode of the present invention includes an electrically non-conductive substrate 10, a base layer 20 disposed on the outer surface of the substrate 10, a nanoparticle layer 31 including metal nanoparticles and disposed on the outer surface of the base layer 20, and a monomolecular layer 33 including a monomolecular material having one or more amine groups and disposed on the outer surface of the nanoparticle layer 31.

The present invention is directed to an electrode that includes an electrically non-conductive material imparted with conductivity by coating with metal nanoparticles and can be applied to biofuel cells and biosensors. Biofuel cells are devices that convert biological energy into electrical energy. Conventional biofuel cells use highly electrically conductive carbon nanotubes as electrode host materials. However, carbon nanotubes are not readily available and are expensive, limiting their application. The electrode of the present invention has the potential to replace conventional electrodes based on carbon nanotubes. However, the electrode of the present invention is not necessarily limited to specific applications such as biofuel cells and is applicable to any electronic device where electron transfer is needed.

As described above, the electrode of the present invention includes an electrically non-conductive substrate 10, a base layer 20, a nanoparticle layer 31, and a monomolecular layer 33.

The substrate 10 *per se* is not electrically conductive and its type and shape are not especially limited to those illustrated in FIGS. 1 and 2. However, the substrate 10 is composed of a cotton fiber that can improve the flexibility and electron transfer capability of the electrode, which will be described later. The base layer 20 is disposed on the outer surface of the substrate 10.

The base layer 20 is formed by coating and partially or entirely surrounds the outer surface of the substrate 10. The formation of the base layer 20 allows for effective coating of the nanoparticle layer 31 on the substrate 10. For example, the base layer 20 may be formed by coating polyethylenimine (PEI) on the substrate 10. However, the material for the base layer 20 is not necessarily limited to polyethylenimine (PEI). Any material that enables the formation of the nanoparticle layer 31 on the substrate 10 by coating may be used to form the base layer 20.

The nanoparticle layer 31 is introduced on the outer surface of the substrate 10 via the base layer 20. The nanoparticle layer 31 may partially or entirely surround the outer surface of the base layer 20. The nanoparticle layer 31 may be formed by layer-by-layer (LbL) self-assembly based on a solution process. The nanoparticle layer 31 includes metal nanoparticles to impart electrical conductivity to the electrically non-conductive substrate 10. The metal nanoparticles may be nanoparticles of at least one metal selected from the group consisting of gold, silver, aluminum, copper, and platinum but the metal of the metal nanoparticles is not necessarily limited to the above-mentioned type. Any metal that can impart electrical conductivity to the electrically non-conductive substrate may be used in the present invention. The monomolecular layer 33 is disposed on the outer surface of the nanoparticle layer 31.

The monomolecular layer 33 is composed of a monomolecular material having one or more amine groups and is formed by coating the monomolecular material so as to partially or entirely surround the outer surface of the nanoparticle layer 31. The monomolecular material may be coated on the nanoparticle layer 31 by layer-by-layer

(LbL) self-assembly based on a solution process. Metals have low resistance whereas films composed of metal particles exhibit insulating properties when the metal particles are surrounded by long organic ligands. Thus, the amine group-containing monomolecular material substituted with insulating ligands is used in the present invention to improve the bonding strength between the metal nanoparticles and to improve the electrical conductivity of the nanoparticle layer 31. The amine group-containing monomolecular material fixing the metal nanoparticles and improving the electrical conductivity of the nanoparticle layer 31 may be, for example, tris(2-aminoethyl)amine (TREN) or diethylenetriamine (DETA) but is not necessarily limited thereto. Any material that can impart electrical conductivity to the nanoparticle layer 31 may be used as the monomolecular material.

To sum up, the electrically non-conductive substrate 10 is imparted with electrical conductivity by a nanocomposite thin film 30 having a bilayer structure consisting of the nanoparticle layer 31 and the monomolecular layer 33 formed by layer-by-layer self-assembly.

According to a further embodiment of the present invention, the electrode includes a plurality of nanocomposite thin films stacked on one another (see FIG. 2). For example, the electrode may include a first nanocomposite thin film 30a in which a monomolecular layer 33a is formed on a nanoparticle layer 31a and a second nanocomposite thin film 30b in which a monomolecular layer 33b is formed on a nanoparticle layer 31b. The second nanocomposite thin film 30b is disposed on the first nanocomposite thin film 30a. As the number of the nanocomposite thin films increases, the electrical conductivity of the electrode increases. Accordingly, the number of the nanocomposite thin films 30a can be determined by the required electrical conductivity of a device using the electrode of the present invention.

A cotton fiber can be used as the electrically non-conductive substrate 10. The use of the cotton fiber enables the application of the electrode to flexible devices, the coating of a larger amount of the metal nanoparticles, and the application of the electrode to an anode and a cathode for a biofuel cell, resulting in improved power generation efficiency.

FIG. 3 is an enlarged view of a cotton fiber.

As illustrated in FIG. 3, the cotton fiber is a short single fiber having a structure in which a plurality of thin fibrils 11 are coiled and overlap with one another. The plurality of fibrils 11a and 11b are densely entangled to form micropores having a size of several tens of microns in the cotton fiber. Due to its structure, the cotton fiber is hydrophilic. According to a solution process, the cotton fiber dipped in a dispersion of metal nanoparticles is coated with a large amount of the nanoparticles. That is, the absolute amount of the metal nanoparticles per unit area of the electrode increases, resulting in effective electron transfer in the electrode. This makes the electrode suitable for use in a biofuel cell. The internal pores of the cotton fiber serve as passages for electron and mass transfer, facilitating the delivery of fuels and electrons to an anode and a cathode for a biofuel cell. Consequently, an anode and a cathode for a biofuel cell based on the cotton fiber electrode of the present invention have an outstanding ability to produce an electric current in response to changes in potential, resulting in a significant increase in current signal upon reaction with the anode fuel.

However, the non-conductive substrate 10 is not necessarily limited to a cotton fiber and can be selected from various types and shapes of non-conductive materials, including fiber-type non-conductive materials and disc-shaped non-conductive materials.

A detailed description will be given concerning the structure of a biofuel cell using the electrode of the present invention.

FIG. 4 is a schematic view of a biofuel cell according to one embodiment of the present invention.

As illustrated in FIG. 4, the biofuel cell includes: an anode 100 including an electrode 110, an enzyme layer 130 including an enzyme and disposed on the outer surface of a monomolecular layer of the electrode 110, and a linker layer 150 including a monomolecular material having one or more amine groups and disposed on the outer surface of the enzyme layer 130; and a cathode 200 including an electrode 110.

A biofuel cell includes an anode at which a hydrogen produced from the oxidation of glucose is used as a fuel and a cathode at which electron transfer is induced

by the reduction of oxygen to generate electricity. Representative examples of such biofuel cells include enzymatic biofuel cells in which glucose oxidase is directly immobilized on an electrode and microbial biofuel cells in which a microorganism having a good ability to oxidize glucose is used as a catalyst. The biofuel cell of the present invention may be an enzymatic biofuel cell. In this case, the performance of the enzymatic biofuel cell can be improved by increasing the amount of the enzyme attached to the electrode 110, increasing the active surface area of the electrode 110 using nanostructures or facilitating the transfer of electrons at the electrode 110.

Conventional enzymatic biofuel cells use redox electron carriers to efficiently deliver electrons generated by an enzymatic reaction to an electrode. The redox electron carriers contain non-biocompatible metal materials such as osmium, thus being unsuitable for *in vivo* applications. The unsafety of the electron carriers deteriorates the stability of the biofuel cells, leading to a short operation time of the biofuel cells. As a solution to the problems of conventional enzymatic biofuel cells, the biofuel cell of the present invention uses an electrode 110, in which metal nanoparticles are coated on an electrically non-conductive substrate, particularly a cotton fiber electrode.

As described above, the anode 100 of the biofuel cell according to the present invention includes an electrode 110, an enzyme layer 130, and a linker layer 150. The electrode 110 is the same as that described above.

The electrode 110 is particularly a cotton fiber electrode in which a plurality of nanocomposite thin films are stacked on one another. The other layers are the same as those described above and a repeated description thereof is omitted or only briefly presented herein.

The enzyme layer 130 includes an enzyme and is formed on the outer surface of the outermost monomolecular layer. The enzyme layer 130 may be formed by coating an enzyme so as to surround the monomolecular layer. The enzyme may be coated on the monomolecular layer by a solution process. For example, the enzyme may be selected from the group consisting of glucose oxidase (GOx), D-fructose dehydrogenase, and a mixture thereof but is not necessarily limited thereto. Any enzyme that can react with glucose as a fuel for the anode 100 to oxidize hydrogen may be used

in the present invention.

The linker layer 150 includes a monomolecular material having one or more amine groups and is disposed on the outer surface of the enzyme layer 130. The linker layer 150 may be formed partially or entirely surrounding the outer surface of the enzyme layer 130. The monomolecular material for the linker layer 150 may be, for example, tris(2-aminoethyl)amine (TREN) or diethylenetriamine (DETA). The linker layer 150 may be formed on the enzyme layer 130 by dipping the enzyme layer 130 in a solution of the monomolecular material.

The enzyme layer 130 and the linker layer 150 formed on the enzyme layer 130 form an oxidation layer having a bilayer structure. The oxidation layer may be provided in plurality. In this case, the biofuel cell may include a first oxidation layer in which a linker layer 150 is formed on an enzyme layer 130 and a second oxidation layer which another linker layer 150 is formed on another enzyme layer 130. The second oxidation layer is disposed on the first oxidation layer.

The cathode 200 of the biofuel cell may use an electrode 110 including a substrate coated with metal nanoparticles without enzyme introduction. The electrode 110 is particularly a cotton fiber electrode in which a plurality of nanocomposite thin films are stacked on one another.

The mode of operation of the biofuel cell based on the cotton fiber electrode 110 is discussed below. When assuming that glucose is used as a fuel for the anode 100 and GOx is used for the enzyme layer 130, the glucose is oxidized to gluconolactone at the anode 100 and electrons migrate from the GOx to the cotton fiber electrode 110. Electrons are transferred from the cotton fiber electrode 110 through an electrolyte by a reduction reaction at the cathode 200. The reduction reaction can be caused in the presence of oxygen by the introduction of gold nanoparticles, eliminating the need to use an expensive cathode catalyst.

There is a high possibility that the cotton fiber electrode 110 can be applied to subminiature platforms for energy production and devices for energy storage, ensuring its wide application to a variety of electrical/electronic devices and biomedical devices.

## **Mode for Carrying out the Invention**

A method for manufacturing an electrode and a method for fabricating a biofuel cell according to the present invention are described below.

FIG. 5 is a process flow diagram illustrating a method for manufacturing an electrode according to one embodiment of the present invention.

As illustrated in FIG. 5, the method includes (a) dipping an electrically non-conductive substrate in a solution of a polymer to form a base layer, (b) dipping the substrate, where the base layer is formed, in a solution of metal nanoparticles to form a nanoparticle layer, and (c) dipping the substrate, where the nanoparticle layer is formed, in a solution of a monomolecular material having one or more amine groups to form a monomolecular layer wherein a nanocomposite thin film having a bilayer structure consisting of the nanoparticle layer and the monomolecular layer is formed on the outer surface of the substrate.

An electrode manufactured by the method of the present invention is the same as that described above and a repeated description thereof is omitted or only briefly presented herein.

According to the method of the present invention, an electrode is manufactured by layer-by-layer self-assembly based on a solution.

First, an electrically non-conductive substrate is dipped in a solution of a polymer to form a base layer (step (a)). The polymer may be polyethylenimine (PEI). In this case, polyethylenimine (PEI) is dissolved in a solvent such as ethanol. However, there is no particular restriction on the kinds of the polymer and the solvent. The substrate may be a cotton fiber, as illustrated in FIG. 5, but is not necessarily limited thereto.

Next, the substrate, where the base layer is formed, is dipped in a solution of metal nanoparticles (step (b)). The metal nanoparticles are layer-by-layer self-assembled to form a nanoparticle layer in the form of a film on the outer surface of the base layer. The metal nanoparticles are gold nanoparticles protected with tetraoctylammonium bromide (TOABr) but are not necessarily limited thereto. For example, the metal nanoparticle may be nanoparticles of at least one metal selected from the group

consisting of silver, aluminum, copper, and platinum. The method may further include washing away uncoated metal nanoparticles with a suitable solvent such as pure toluene.

The substrate, where the nanoparticle layer is formed, is dipped in a solution of a monomolecular material having one or more amine groups (step (c)). The monomolecular material is layer-by-layer self-assembled on the nanoparticle layer to form a monomolecular layer. The nanoparticle layer and the monomolecular layer form a nanocomposite thin film having a bilayer structure. Weakly adsorbed molecules of the monomolecular material may be washed away with a suitable solvent such as ethanol. The substrate, where the nanocomposite thin film is formed, is dried, completing the manufacture of an electrode in which one nanocomposite thin film is formed. The monomolecular material is tris(2-aminoethyl)amine (TREN) that causes ligand exchange between TREN and TOABr. However, the monomolecular material is not necessarily limited to TREN and any monomolecular material having amine groups, such as diethylenetriamine (DETA), may be used without limitation.

The nanocomposite thin film may be provided in plurality. To this end, steps (b) and (c) are repeated sequentially. The method may further include washing and drying before each of the dipping steps.

A method for fabricating a biofuel cell according to the present invention includes (a) dipping two electrically non-conductive substrates in a solution of a polymer to form base layers, (b) dipping the substrates, where the base layers are formed, in a solution of metal nanoparticles to form nanoparticle layers, (c) dipping the substrates, where the nanoparticle layers are formed, in a solution of a monomolecular material having one or more amine groups to form monomolecular layers, (d) dipping one of the substrates, where the monomolecular layers are formed, in a solution of an enzyme to form an enzyme layer, and (e) dipping the substrate, where the enzyme layer is formed, in a solution of a monomolecular material having one or more amine groups to form a linker layer.

A biofuel cell fabricated by the method of the present invention includes an anode and a cathode. According to the method of the present invention, the anode and



the cathode are produced using two electrically non-conductive substrates. Since the electrode of the present invention *per se* is used as the cathode, an enzyme layer is not present in the cathode. The cathode is produced by the method for manufacturing the electrode (including steps (a)-(c)).

On the other hand, the anode is produced by the following procedure. First, a substrate, where a nanocomposite thin film is formed, is prepared through steps (a)-(c) and dipped in a solution of an enzyme to form an enzyme layer on the outermost monomolecular layer of the nanocomposite thin film. The solvent may be phosphate buffer (PB buffer) but is not necessarily limited thereto. The enzyme can be selected from the group consisting of glucose oxidase, D-fructose dehydrogenase, and a mixture thereof.

Next, the substrate, where the enzyme layer is formed, is dipped in a solution of a monomolecular material to form a linker layer on the enzyme layer. The enzyme layer and the linker layer formed on the enzyme layer form an oxidation layer having a bilayer structure. The monomolecular material may be, for example, tris(2-aminoethyl)amine (TREN) or diethylenetriamine (DETA) and the solvent may be phosphate buffer (PB buffer).

The anode of the biofuel cell according to the present invention may have a structure in which a plurality of oxidation layers are formed. To this end, steps (d) and (e) are repeated sequentially.

The present invention will be explained in more detail with reference to the following examples.

#### Example 1: Synthesis of TOA-gold nanoparticles

In this example, gold nanoparticles protected with tetraoctylammonium bromide (TOABr) were synthesized. First, a 30 mM solution of  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$  in deionized water (30 ml) was mixed with a 20 mM dispersion of TOABr in toluene (80 ml) with stirring and a 0.4 M aqueous solution of  $\text{NaBH}_4$  (25 ml) was added thereto to reduce the  $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ . The toluene was separated from the aqueous solution, followed by washing several times with  $\text{H}_2\text{SO}_4$  (0.1 M, purity 95%),  $\text{NaOH}$  (0.1 M,

97%), and deionized water to afford 8-nm-diameter TOA-gold nanoparticles.

#### Example 2: Manufacture of cotton fiber electrodes

In this example, cotton fiber electrodes were manufactured. First, a cotton fiber was dipped in a solution of polyethylenimine (PEI) in ethanol ( $1 \text{ mg ml}^{-1}$ ). Then, the PEI-coated cotton fiber was dipped in a solution ( $10 \text{ mg ml}^{-1}$ ) of the TOA-gold nanoparticles synthesized in Example 1 for 40 min, washed with pure ethanol, dipped in an ethanolic dispersion of TREN ( $1 \text{ mg ml}^{-1}$ ) for 40 min, and washed with pure toluene to remove weakly adsorbed TREN molecules to afford a fiber electrode in which one bilayer nanocomposite thin film was formed (hereinafter referred to as “(TOA-Au/TREN)<sub>1</sub> fiber electrode”). Here, ligand exchange occurred between TOABr and TREN.

The above procedure was repeated several times to manufacture cotton fiber electrodes in which different numbers of nanocomposite thin films were stacked on one another.

#### Example 3: Production of anodes and cathodes for biofuel cells

In this example, anodes and cathodes for biofuel cells were produced. First, the cotton fiber electrode manufactured in Example 2 was dipped in a solution (GOx,  $5 \text{ mg ml}^{-1}$ ) of glucose oxidase (GOx) in phosphate buffer (PB buffer). Next, the (TOA-Au/TREN)<sub>20</sub> cotton fiber electrode including twenty nanocomposite thin films stacked on one another, which was manufactured in Example 2, was dipped in a solution of TREN in PB to afford a fiber anode ((TOA-Au/TREN)<sub>20</sub>(GOx/TREN)<sub>1</sub>) in which one enzyme bilayer was formed. The above procedure was repeated to produce fiber anodes in which different numbers of enzyme bilayers were stacked on one another. The (TOA-Au/TREN)<sub>20</sub> cotton fiber electrode was used without further processing as a cathode.

#### Evaluation Example 1: SEM imaging and EDAX of the cotton fiber electrode

FIG. 6 shows cross-sectional SEM and EDAX images of the fiber/(TOA-Au/TREN)<sub>20</sub> cotton fiber electrode manufactured in Example 2. In FIG. 6, (a) and (d)

are scanning electron images of the cotton fiber electrode and (b), (c), (e), and (f) shows the results of EDAX for the cotton fiber electrode (yellow: gold nanoparticles, red: carbon). In FIG 6, (a), (b), and (c) are high magnification images of the cotton fiber electrode and (d), (e), and (f) are low magnification images of the cotton fiber electrode. The SEM images reveal an internal structure of the cotton fiber in which pores serving were formed between the fibrils in the form of thin threads densely coated with the gold nanoparticles to provide passages, demonstrating a difference in the overall structure of the electrode. The EDAX images reveal that mass transfer and diffusion of fuels (*e.g.*, glucose and oxygen) can be promoted through the pores serving as passages.

#### Evaluation Example 2: Electrical conductivities of the cotton fiber electrodes

FIG. 7 shows electrical conductivities, (b)-(e) SEM images, (f) CV curves, and (g) impedance spectra of the cotton fiber electrodes (fiber/(TOA-Au/TREN)<sub>n</sub>) as a function of the number of nanocomposite thin films (n).

As shown in (a) of FIG. 7, as the number of the TOA-Au and TREN bilayers (*i.e.* the nanocomposite thin films) increased, the electrical conductivity of the cotton fiber electrode increased and the resistivity of the cotton fiber electrode decreased. This is because the electrically non-conductive cotton fiber was imparted with conductivity by coating with the gold nanoparticles.

In FIG. 7, (b) and (c) are SEM images of the bare cotton fiber and (d) and (e) are SEM images of the coated cotton fiber. The SEM images reveal that the gold nanoparticles were densely and uniformly coated on the fibrils of the cotton fiber.

The current density of the cotton fiber electrode had a dependence on potential with increasing number of the TOA-Au/TREN bilayers in the cotton fiber electrode bound with the gold nanoparticles (see (f) of FIG. 7). ESR decreased with increasing number of the TOA-Au/TREN bilayers (see (g) of FIG. 7). ESR is caused by equivalent distribution resistance associated with ion diffusion in the nanostructured electrode. When the number of the TOA-Au/TREN bilayers was increased from 5 to 20, the resistance was changed to 1,346 Ω (n=5), 40 Ω (n=10), and 25 Ω (n=20). The sum of internal resistances was electrochemically obtained by impedance tests. The low

resistance value indicates effective electron transfer.

#### Evaluation Example 3: Performance of anode and cathode for biofuel cell

Five oxidation layers ((GOx/TREN)<sub>5</sub>) were formed on the cotton fiber electrode bound with the metal nanoparticles to produce an anode for a biofuel cell. The cotton fiber electrode was used without further processing as a cathode. The performance of each of the anode and the cathode was evaluated. In FIG. 8, (a) shows the polarization curves of the anode when the concentration of glucose was increased from 0 to 300 mmol l<sup>-1</sup> and (b) shows the polarization curves of the cathode under different atmospheres.

Referring to FIG. 8, the anode and the cathode based on the cotton fiber electrode had an outstanding ability to produce an electric current in response to changes in potential, resulting in a significant increase in current signal upon reaction with glucose.

#### Evaluation Example 4: Power generation efficiency of the biofuel cell

FIG. 9 shows the power densities of the biofuel cell. The power densities of the biofuel cell were measured at different glucose concentrations of 10 and 300 mmol l<sup>-1</sup>. The red curve shows the power generation efficiency obtained using the higher concentration of glucose electrolyte and the blue curve shows the power generation efficiency obtained at a concentration similar to the glucose concentration in the body fluid considering the fact that the biofuel cell is implanted. As a result, the inventive biofuel cell was found to have higher power generation efficiency than biofuel cells reported to date. This is because the affinity between the surface of the cotton fiber and the metal nanoparticles coated on the cotton fiber can be maintained at a high level due to the hydrophilicity of the cotton fiber, enabling effective coating of a large amount of the metal nanoparticles on the surface of the cotton fiber. That is, the absolute amount of the metal nanoparticles per unit area of the electrode increases, resulting in effective electron transfer in the electrode. In addition, the internal micropores of the cotton fiber serve as passages for electron and mass transfer, facilitating the transfer of glucose or

oxygen and electrons.

Although the present invention has been described herein with reference to the specific embodiments, these embodiments do not serve to limit the invention and are set forth for illustrative purposes. It will be apparent to those skilled in the art that modifications and improvements can be made without departing from the spirit and scope of the invention.

Such simple modifications and improvements of the present invention belong to the scope of the present invention, and the specific scope of the present invention will be clearly defined by the appended claims.

### **Industrial Applicability**

The electrode of the present invention is manufactured by coating metal nanoparticles on an electrically non-conductive substrate based on layer-by-layer self-assembly. The electrode of the present invention can be applied to a cathode and an anode to fabricate a biofuel cell with improved power generation efficiency. Therefore, the present invention is considered industrially applicable.

## CLAIMS

[Claim 1] An electrode comprising an electrically non-conductive substrate, a base layer disposed on the outer surface of the substrate, a nanoparticle layer comprising metal nanoparticles and disposed on the outer surface of the base layer, and a monomolecular layer comprising a monomolecular material having one or more amine groups and disposed on the outer surface of the nanoparticle layer.

[Claim 2] The electrode according to claim 1, wherein the substrate is a cotton fiber.

[Claim 3] The electrode according to claim 1, wherein the base layer is composed of polyethylenimine (PEI).

[Claim 4] The electrode according to claim 1, wherein the metal nanoparticles are nanoparticles of at least one metal selected from the group consisting of gold, silver, aluminum, copper, and platinum.

[Claim 5] The electrode according to claim 1, wherein the monomolecular material is tris(2-aminoethylamine) (TREN).

[Claim 6] The electrode according to claim 1, wherein the electrode comprises a plurality of nanocomposite thin films, each of which has a bilayer structure in which the monomolecular layer is formed on the nanoparticle layer.

[Claim 7] A biofuel cell comprising: an anode comprising the electrode according to claim 1, an enzyme layer comprising an enzyme and disposed on the outer surface of a monomolecular layer of the electrode, and a linker layer comprising a monomolecular material having one or more amine groups and disposed on the outer surface of the enzyme layer; and a cathode comprising the electrode according to claim 1.

[Claim 8] The biofuel cell according to claim 7, wherein the enzyme is selected from the group consisting of glucose oxidase, D-fructose dehydrogenase, and a mixture thereof.

[Claim 9] The biofuel cell according to claim 7, wherein the monomolecular material is tris(2-aminoethylamine) (TREN).

[Claim 10] The biofuel cell according to claim 7, wherein the biofuel cell comprises a plurality of oxidation layers, each of which has a bilayer structure in which the linker layer is formed on the enzyme layer.

[Claim 11] A method for manufacturing an electrode comprising (a) dipping an electrically non-conductive substrate in a solution of a polymer to form a base layer, (b) dipping the substrate, where the base layer is formed, in a solution of metal nanoparticles to form a nanoparticle layer, and (c) dipping the substrate, where the nanoparticle layer is formed, in a solution of a monomolecular material having one or more amine groups to form a monomolecular layer wherein a nanocomposite thin film having a bilayer structure consisting of the nanoparticle layer and the monomolecular layer is formed on the outer surface of the substrate.

[Claim 12] The method according to claim 11, wherein steps (b) and (c) are repeated sequentially such that a plurality of nanocomposite thin films are formed.

[Claim 13] A method for fabricating a biofuel cell comprising (a) dipping two electrically non-conductive substrates in a solution of a polymer to form base layers, (b) dipping the substrates, where the base layers are formed, in a solution of metal nanoparticles to form nanoparticle layers, (c) dipping the substrates, where the nanoparticle layers are formed, in a solution of a monomolecular material having one or more amine groups to form monomolecular layers, (d) dipping one of the substrates,

where the monomolecular layers are formed, in a solution of an enzyme to form an enzyme layer, and (e) dipping the substrate, where the enzyme layer is formed, in a solution of a monomolecular material having one or more amine groups to form a linker layer.

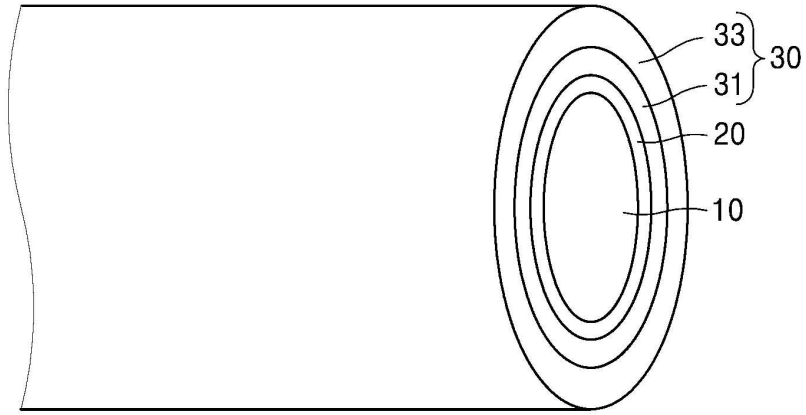
[Claim 14] The method according to claim 13, wherein steps (b) and (c) are repeated sequentially such that a plurality of nanocomposite thin films are formed on the base layer and each of the nanocomposite thin films has a bilayer structure in which the monomolecular layer is formed on the nanoparticle layer; and steps (d) and (e) are repeated sequentially such that a plurality of oxidation layers are formed and each of the oxidation layers has a bilayer structure in which the linker layer is formed on the enzyme layer.



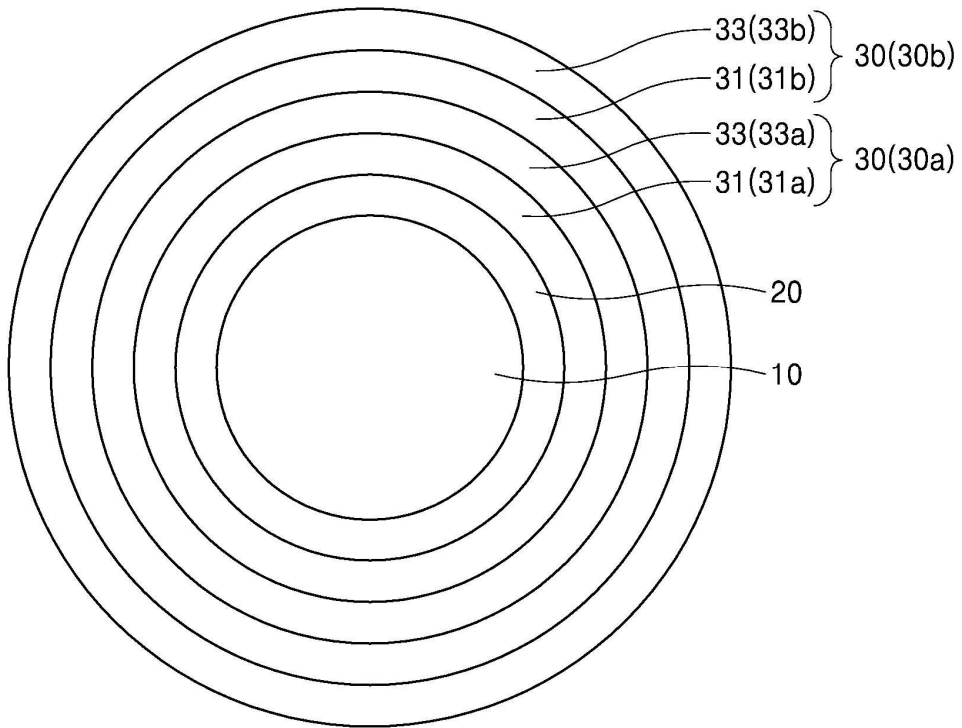
## **ABSTRACT**

The present invention relates to a flexible electrode, a biofuel cell using the same, and a method for manufacturing the same. The electrode according to the present invention comprises: a non-electrically conductive substrate (10); a base layer (20) disposed on the outer surface of the substrate (10); a nanoparticle layer (31) including metallic nanoparticles and disposed on the outer surface of the base layer (20); and a monomolecular layer (33) including a monomolecular material having an amine group and disposed on the outer surface of the nanoparticle layer (31).

[Fig. 1]



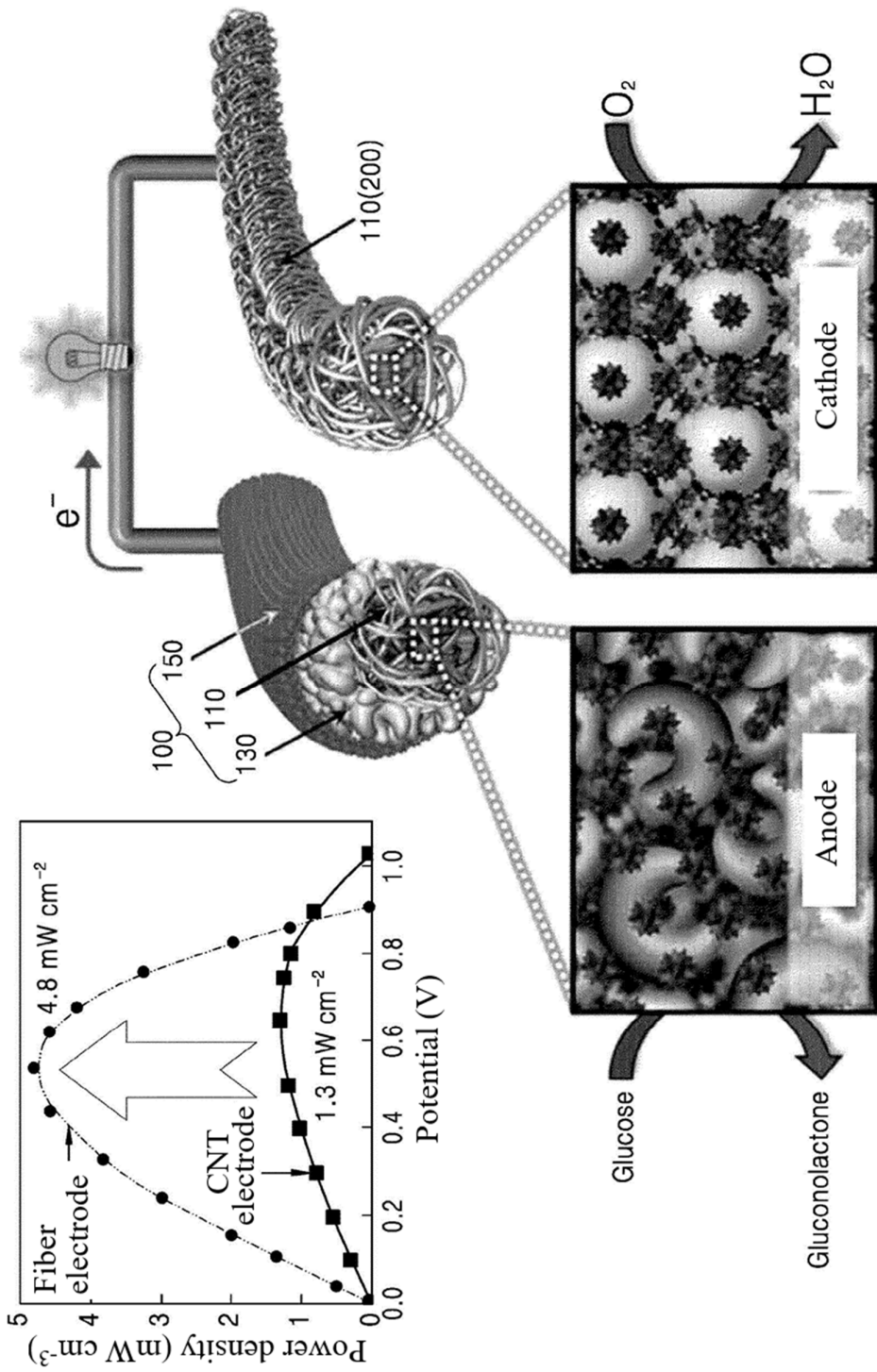
[Fig. 2]



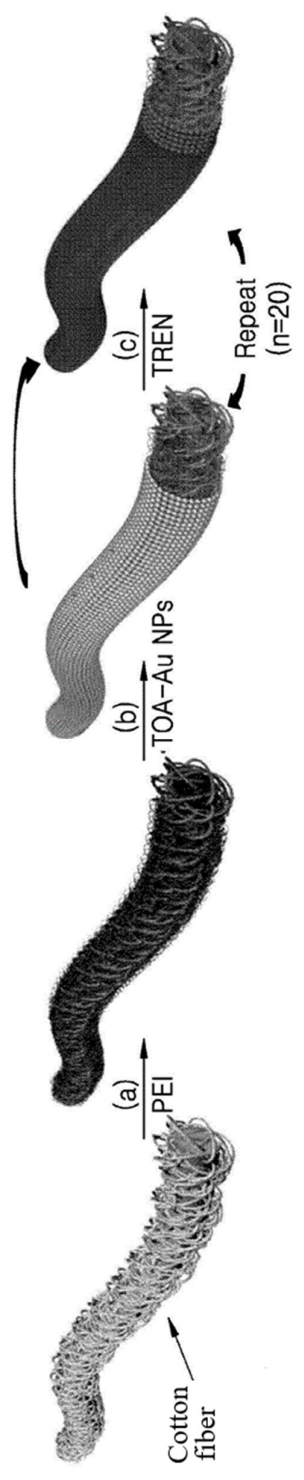
[Fig. 3]

**Cotton fiber**



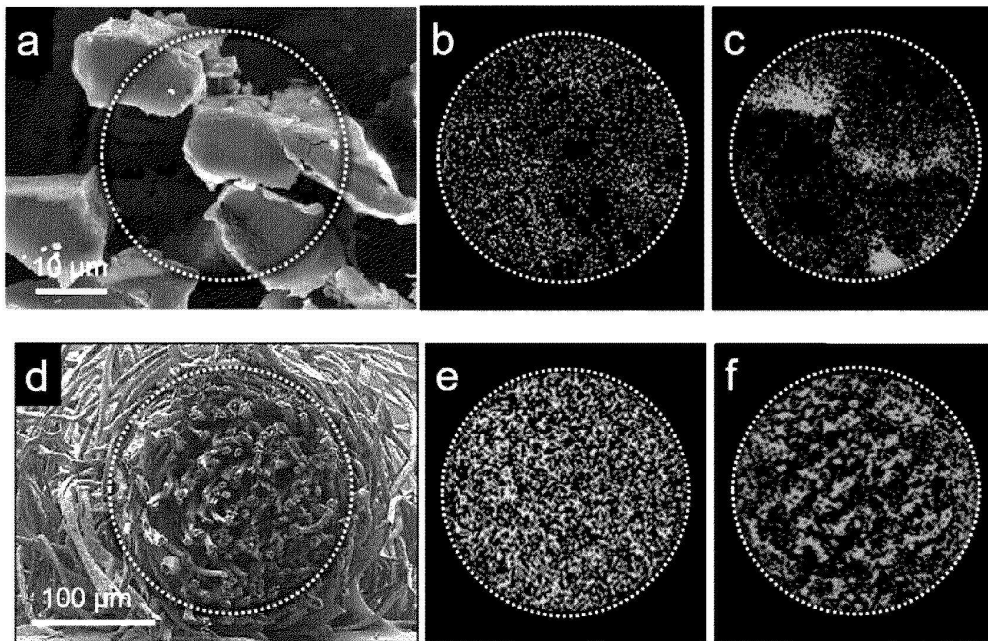


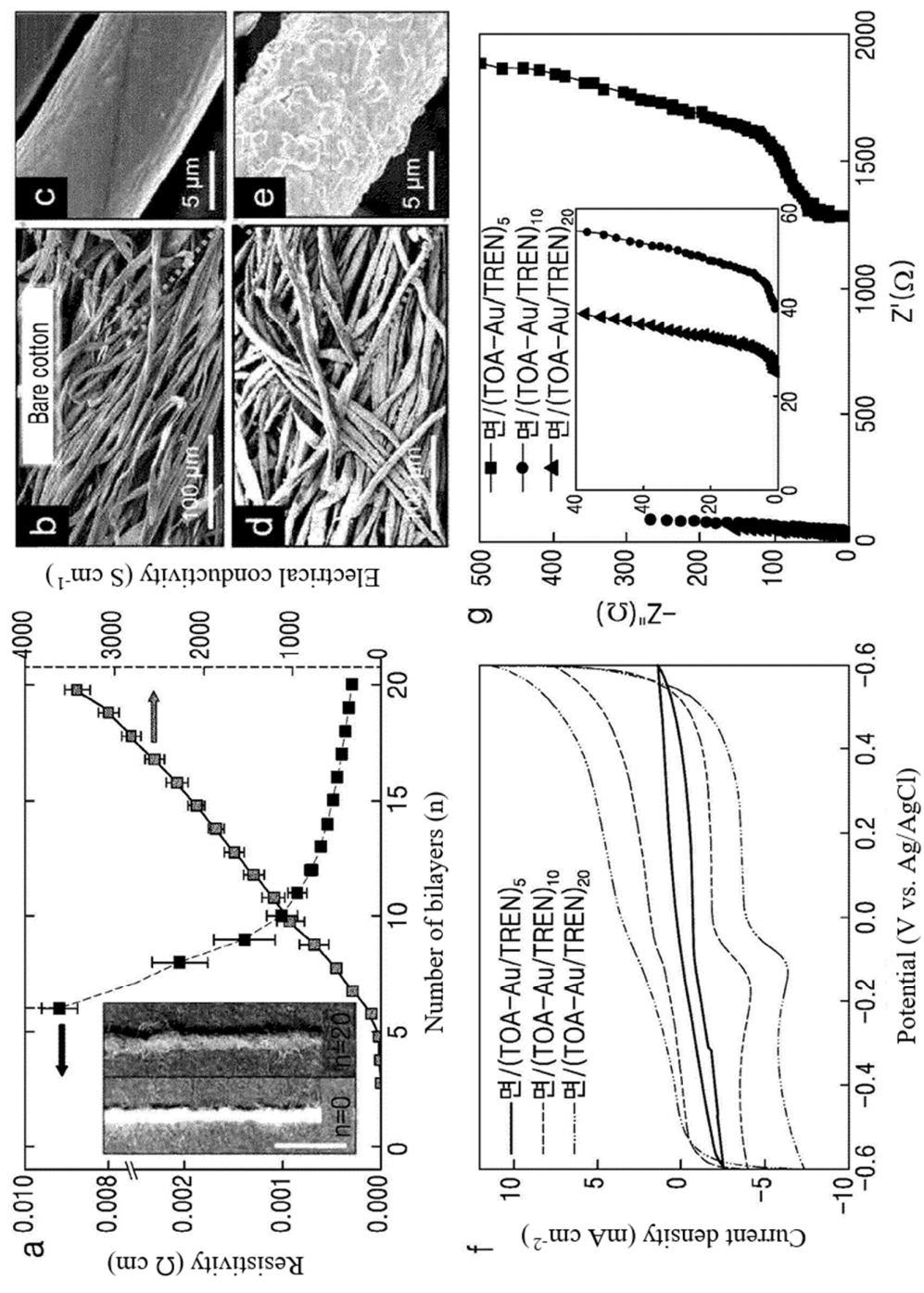
[Fig. 4]



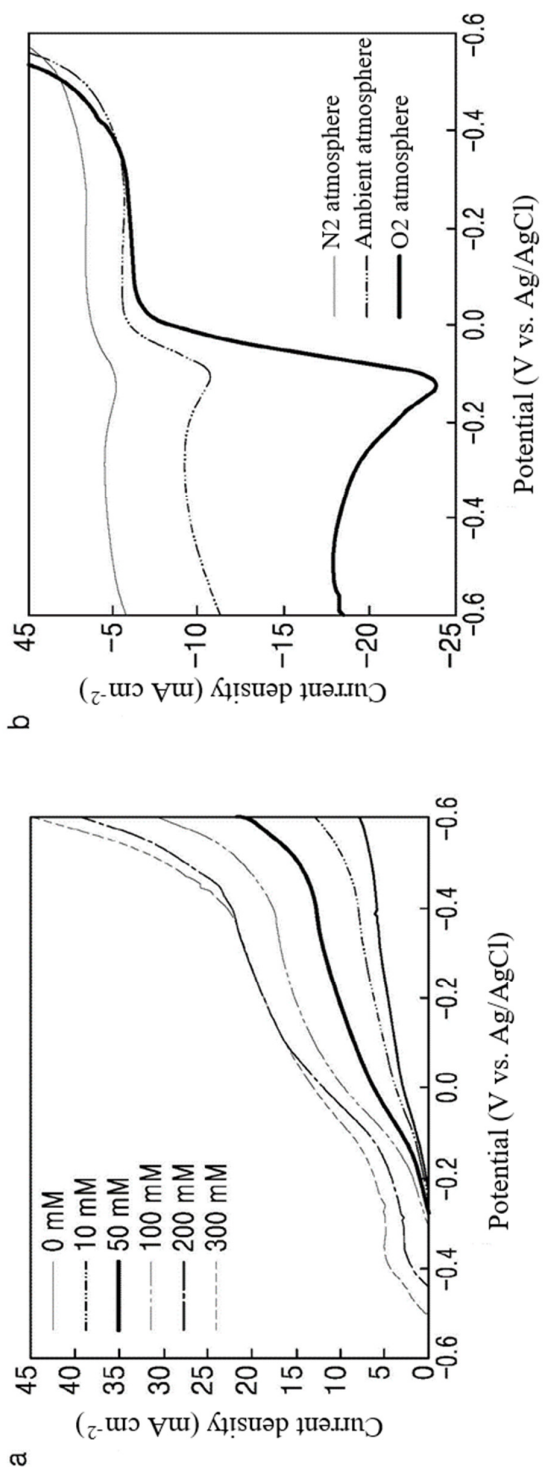
[Fig. 5]

[Fig. 6]





[Fig. 7]



[Fig. 8]



[Fig. 9]

