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(54) **NANOHAIR STRUCTURE AND AN APPLICATION THEREFOR**

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B82Y 99/00	(2011.01)
B82Y 40/00	(2011.01)
B82Y 30/00	(2011.01)

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(57) **ABSTRACT**

There is provided a nanohair structure with the nanowires exposed on a nanotemplate; the method thereof; and a three-dimensional nanostructure-based sensor with ultra-sensitivity and greatly increased three-dimensional surface-to-volume ratio which immobilizes bio-nanoparticles to the nanohair structure and arranges antibodies to the nano surface with the controlled orientation by physical interaction.

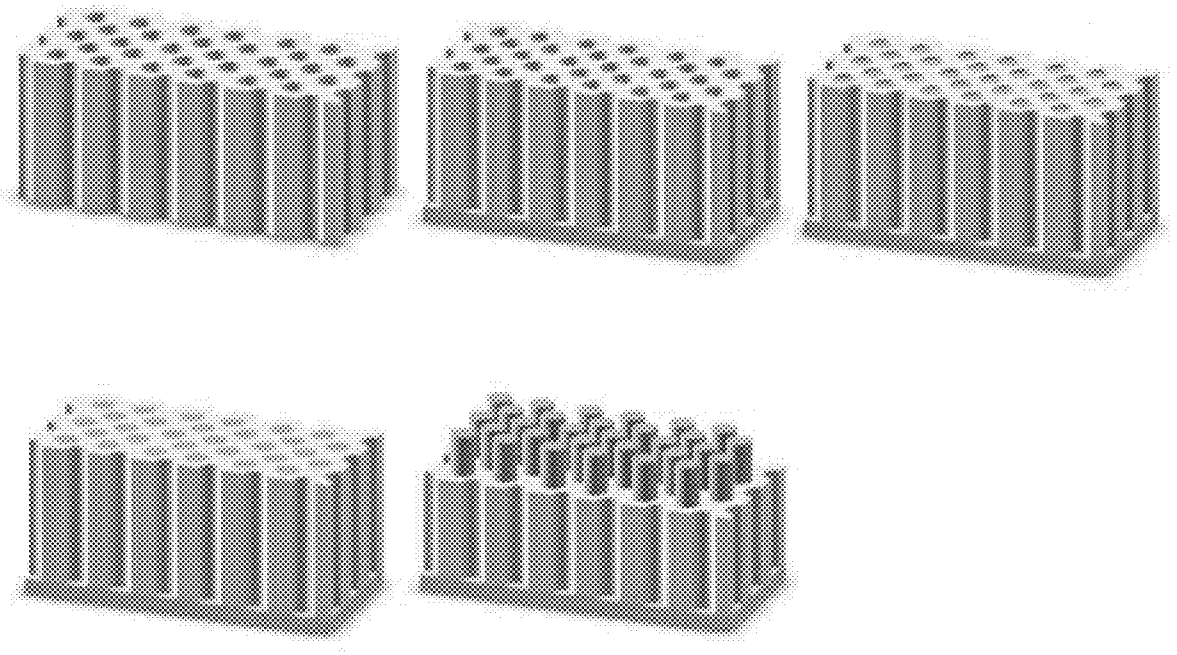


FIG. 1

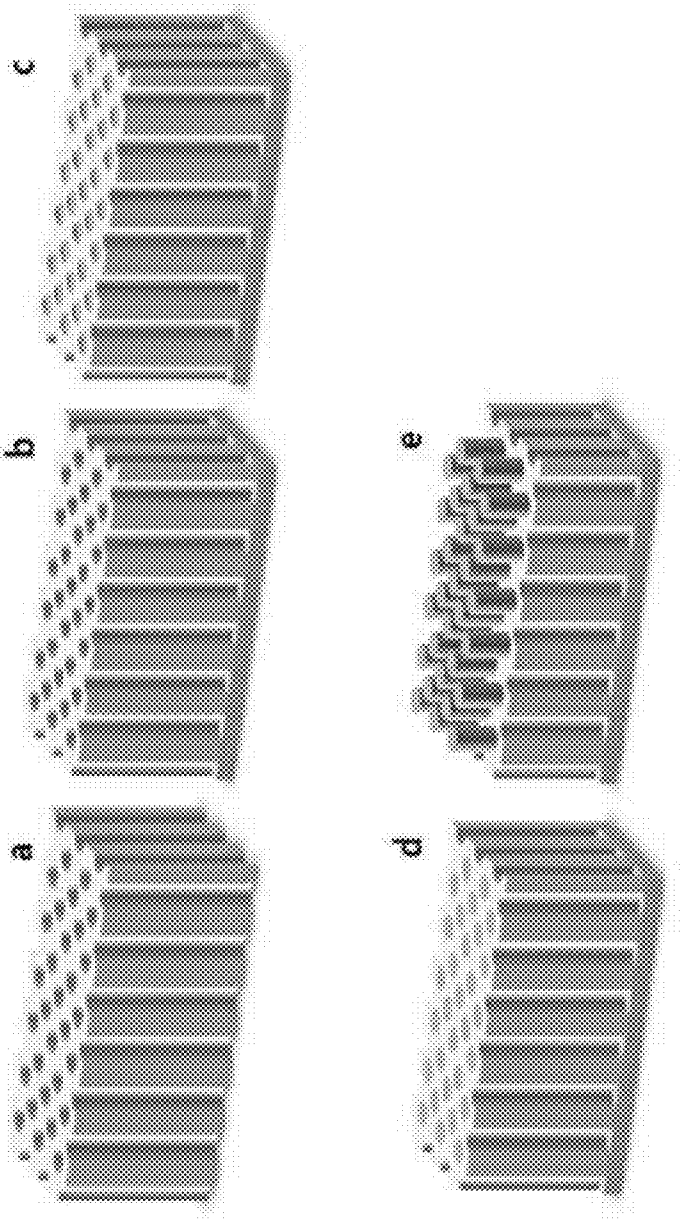


FIG. 2

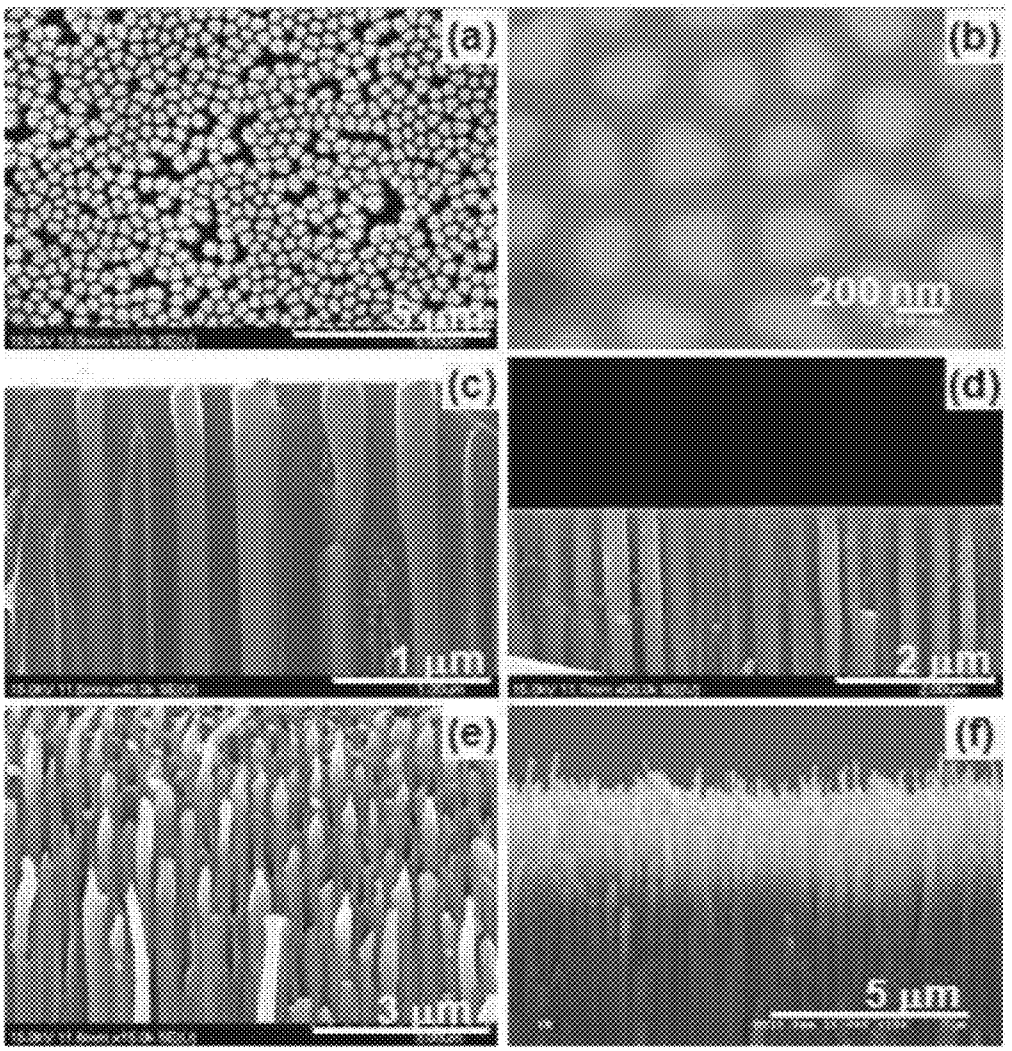


FIG. 3

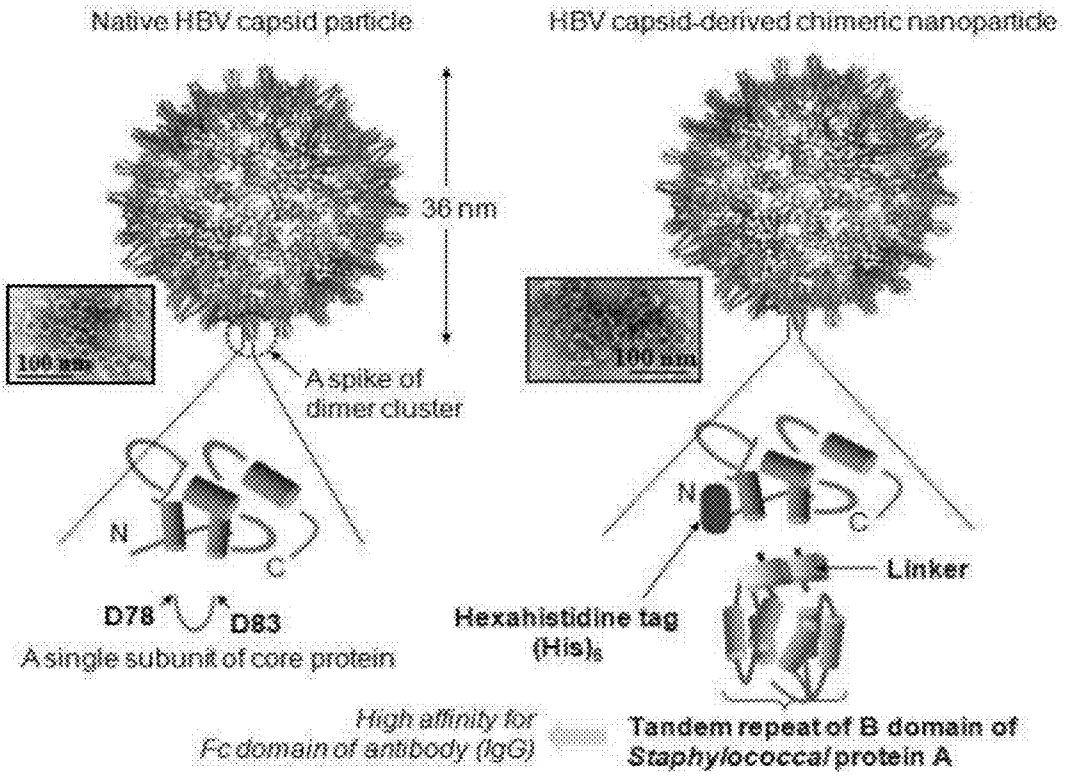

Native HBV capsid particle
HBV capsid-derived chimeric nanoparticle
36 nm
100 nm
100 nm
A spike of dimer cluster
N
C
N
C
Linker
D78
D83
A single subunit of core protein
Hexahistidine tag
(His)6
High affinity for Fc domain of antibody (IgG)
Tandem repeat of B domain of Staphylococcal protein A

FIG. 4

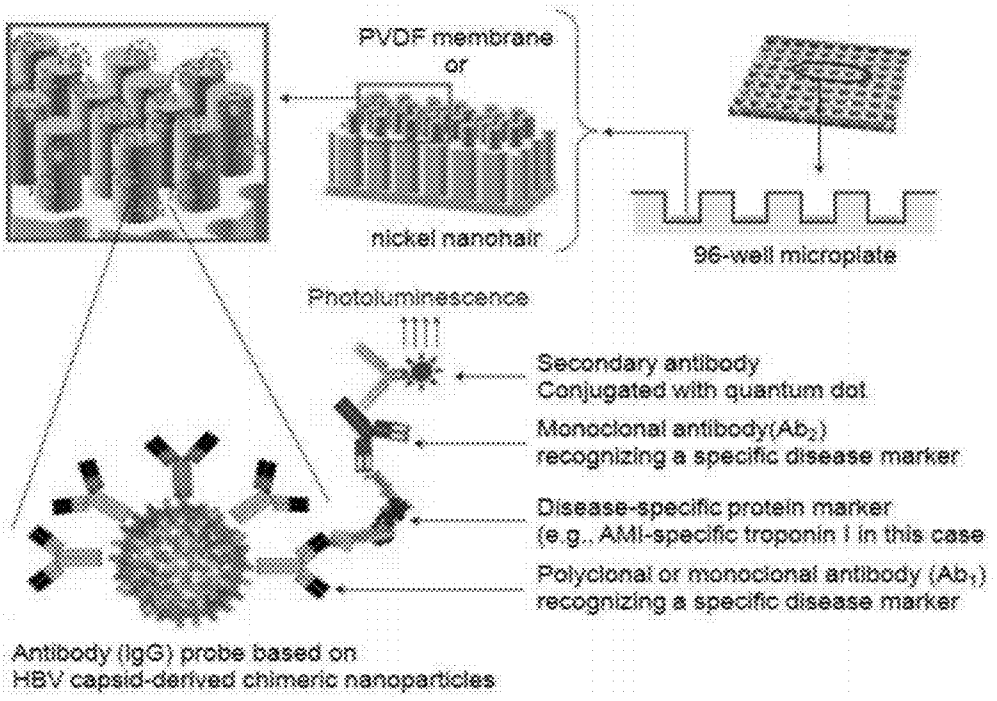

PVDF membrane
or
nickel nanohair
96-well microplate
Photoluminescence
Secondary antibody
Conjugated with quantum dot
Monoclonal antibody(Ab_2)
recognizing a specific disease marker
Disease-specific protein marker
(e.g., AMI-specific troponin I in this case
Polyclonal or monoclonal antibody (Ab_1)
recognizing a specific disease marker
Antibody (IgG) probe based on
HBV capsid-derived chimeric nanoparticles

FIG. 5

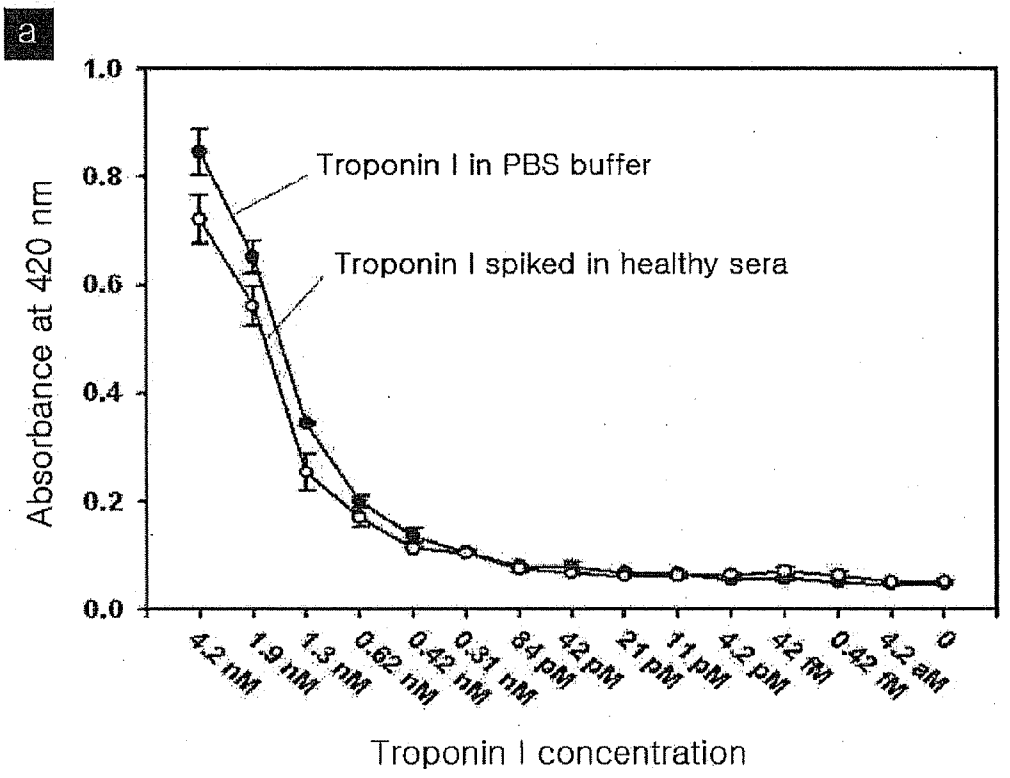

a
1.0
0.8
0.6
0.4
0.2
0.0
Absorbance at 420 nm
Troponin I in PBS buffer
Troponin I spiked in healthy sera
4.2 nM
1.9 nM
1.3 nM
0.62 nM
0.42 nM
0.31 nM
84 pM
42 pM
21 pM
11 pM
4.2 pM
42 fM
0.42 fM
4.2 aM
0
Troponin I concentration

FIG. 6

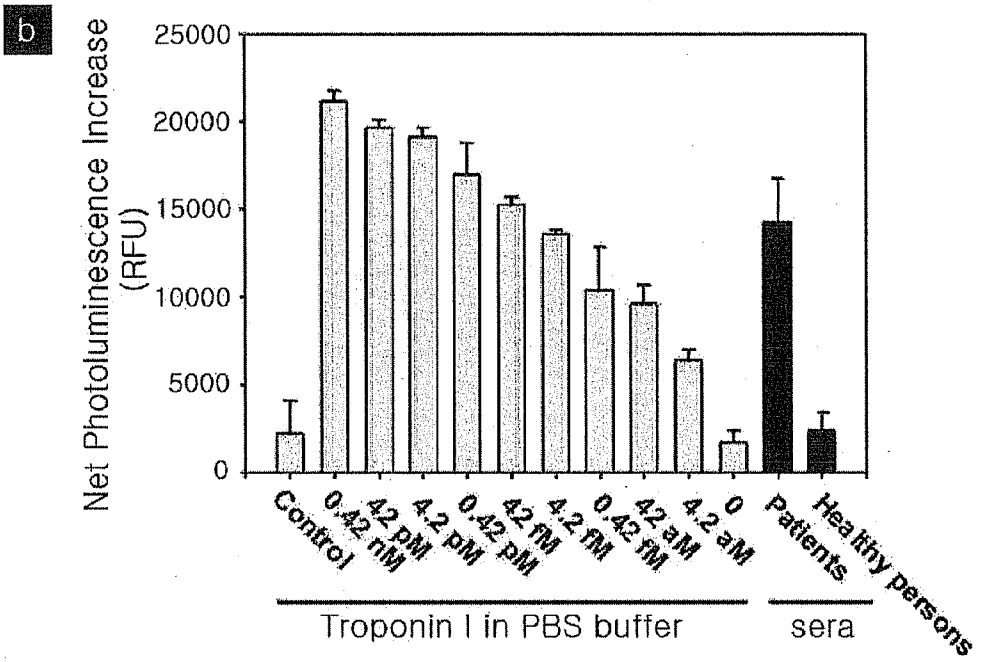

b
Net Photoluminescence Increase (RFU)
25000
20000
15000
10000
5000
0
Control
0.42 nM
42 pM
4.2 pM
0.42 pM
42 fM
4.2 fM
0.42 fM
42 aM
4.2 aM
0
Patients
Healthy persons
Troponin I in PBS buffer
sera

FIG. 7

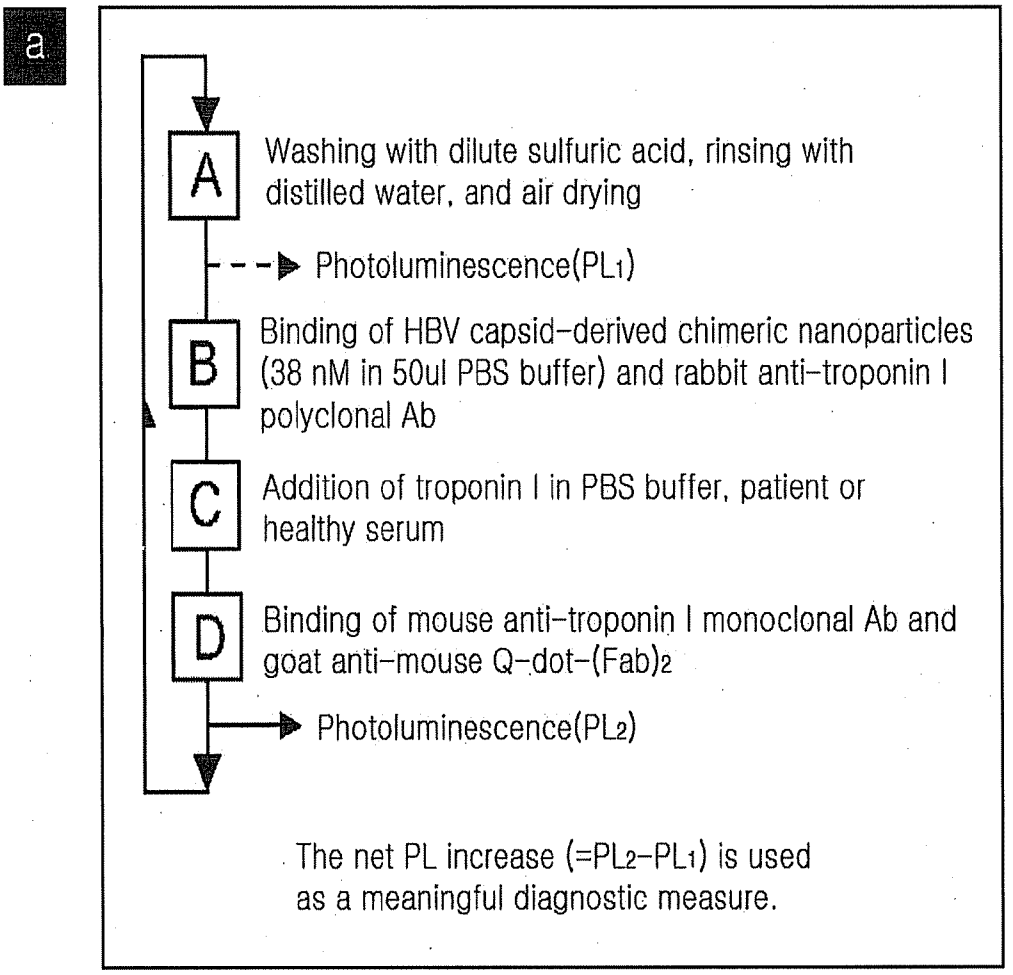

a
A
Washing with dilute sulfuric acid, rinsing with distilled water, and air drying
Photoluminescence(PL1)
B
Binding of HBV capsid-derived chimeric nanoparticles (38 nM in 50ul PBS buffer) and rabbit anti-troponin I polyclonal Ab
C
Addition of troponin I in PBS buffer, patient or healthy serum
D
Binding of mouse anti-troponin I monoclonal Ab and goat anti-mouse Q-dot-(Fab)2
Photoluminescence(PL2)
The net PL increase (=PL2-PL1) is used as a meaningful diagnostic measure.

FIG. 8

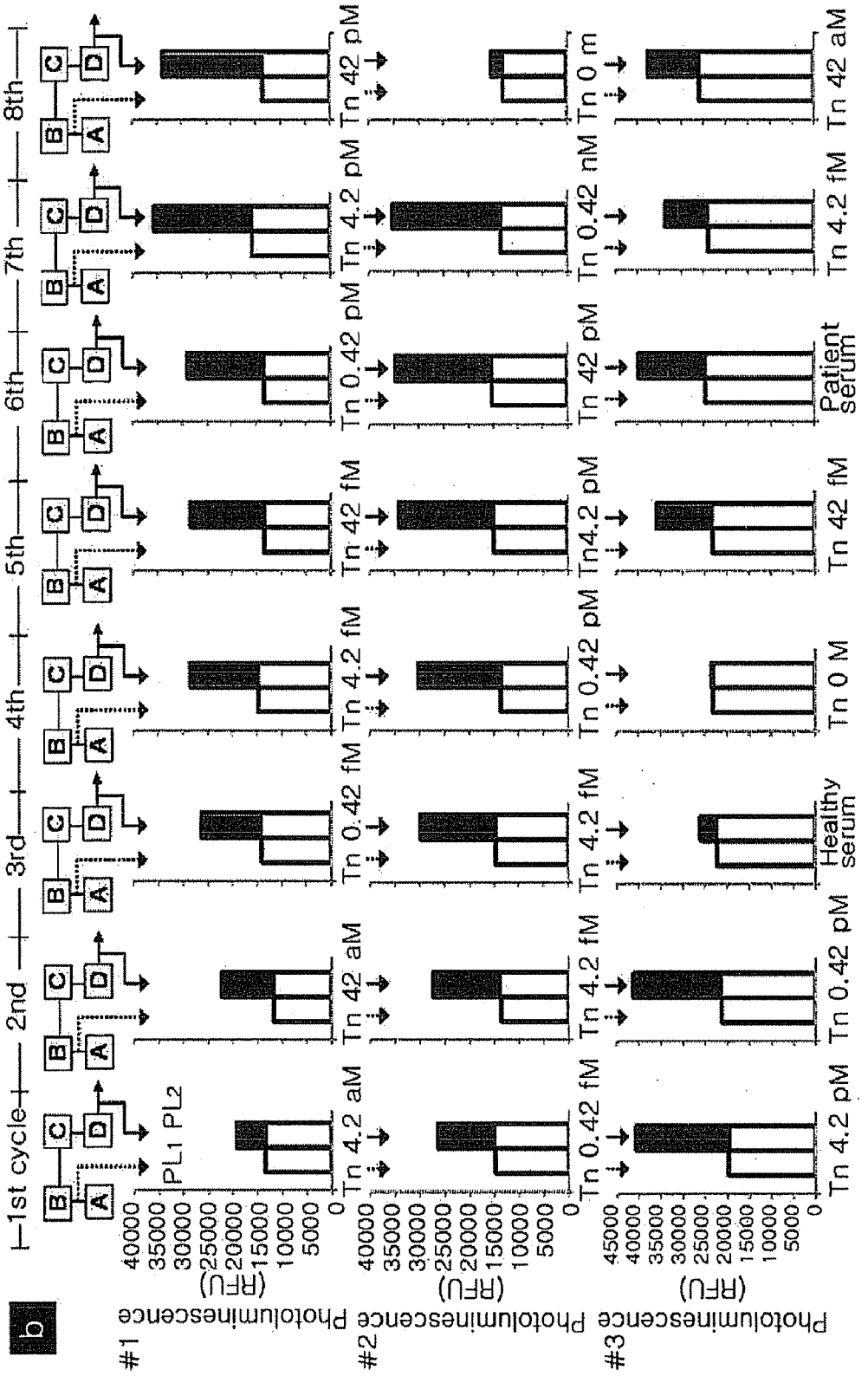

b
1st cycle
2nd
3rd
4th
5th
6th
7th
8th
A
B
C
D
#1
#2
#3
Photoluminescence (RFU)
PL1 PL2
Tn 4.2 aM
Tn 0.42 fM
Tn 42 aM
Tn 4.2 fM
Tn 0.42 fM
Tn 0.42 pM
Tn 4.2 fM
Tn 4.2 pM
Tn 0 M
Tn 0.42 pM
Tn 42 fM
Tn 4.2 pM
Tn 0.42 pM
Tn 42 pM
Tn 0.42 nM
Tn 0 m
Tn 4.2 pM
Tn 42 fM
Tn 4.2 fM
Tn 42 aM
Healthy serum
Patient serum

FIG. 9

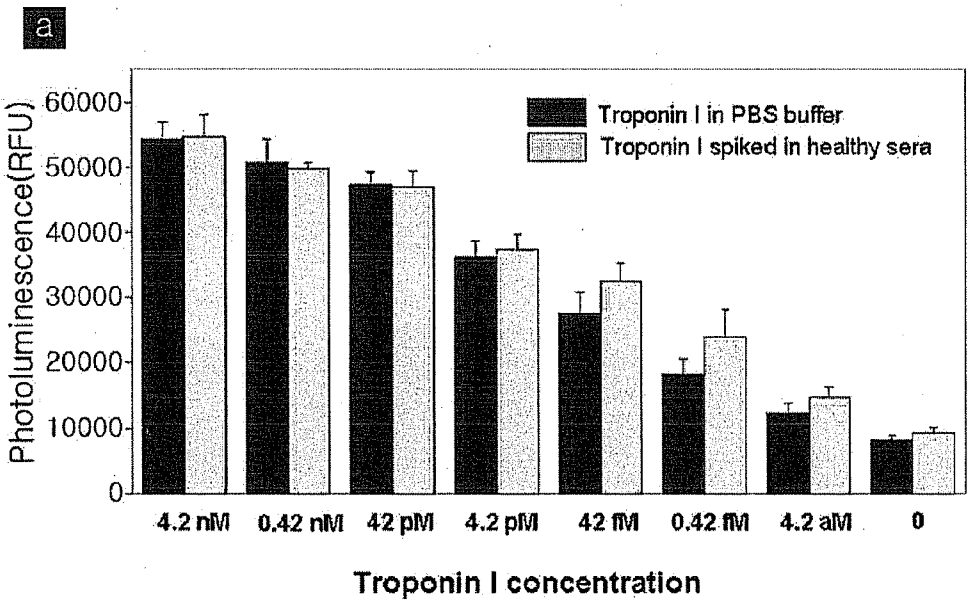

a
Photoluminescence(RFU)
60000
50000
40000
30000
20000
10000
0
Troponin I in PBS buffer
Troponin I spiked in healthy sera
4.2 nM
0.42 nM
42 pM
4.2 pM
42 fM
0.42 fM
4.2 aM
0
Troponin I concentration

FIG. 10

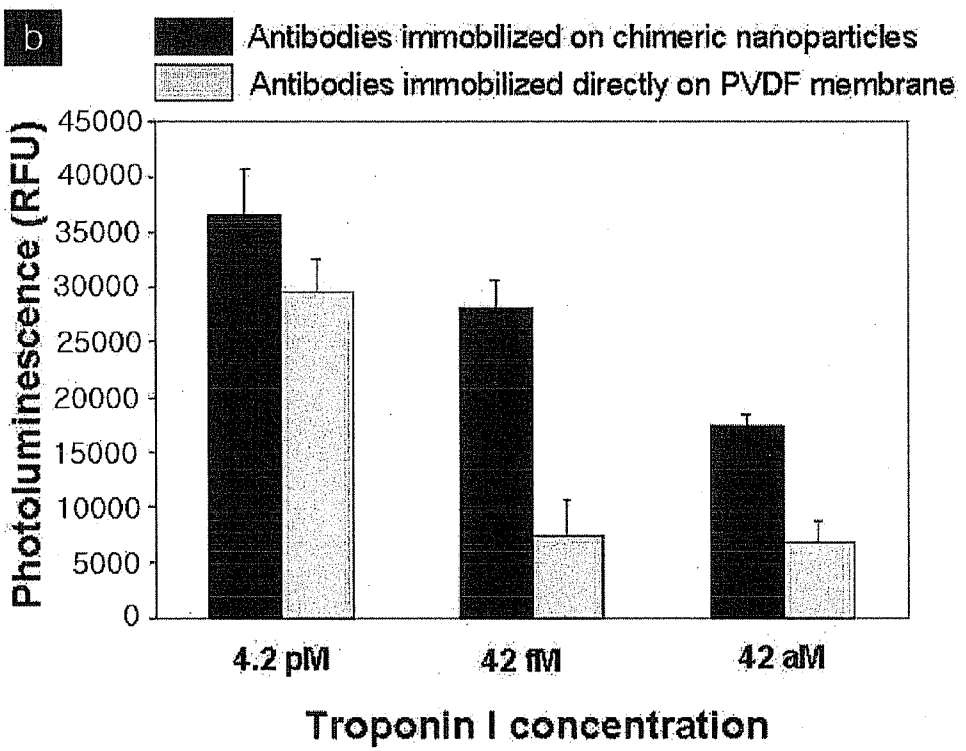

b
Antibodies immobilized on chimeric nanoparticles
Antibodies immobilized directly on PVDF membrane
Photoluminescence (RFU)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
4.2 pM
42 fM
42 aM
Troponin I concentration

FIG. 11

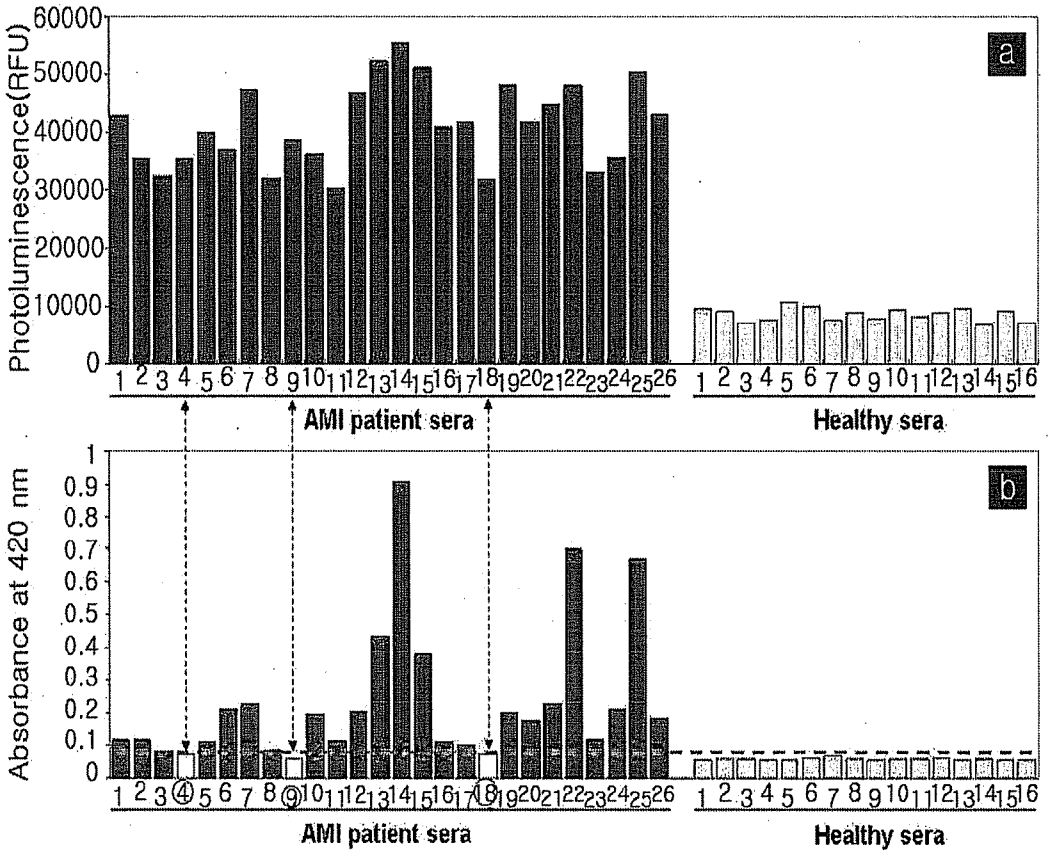

Photoluminescence(RFU)
60000
50000
40000
30000
20000
10000
0
a
AMI patient sera
Healthy sera
Absorbance at 420 nm
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
b
AMI patient sera
Healthy sera

FIG. 12

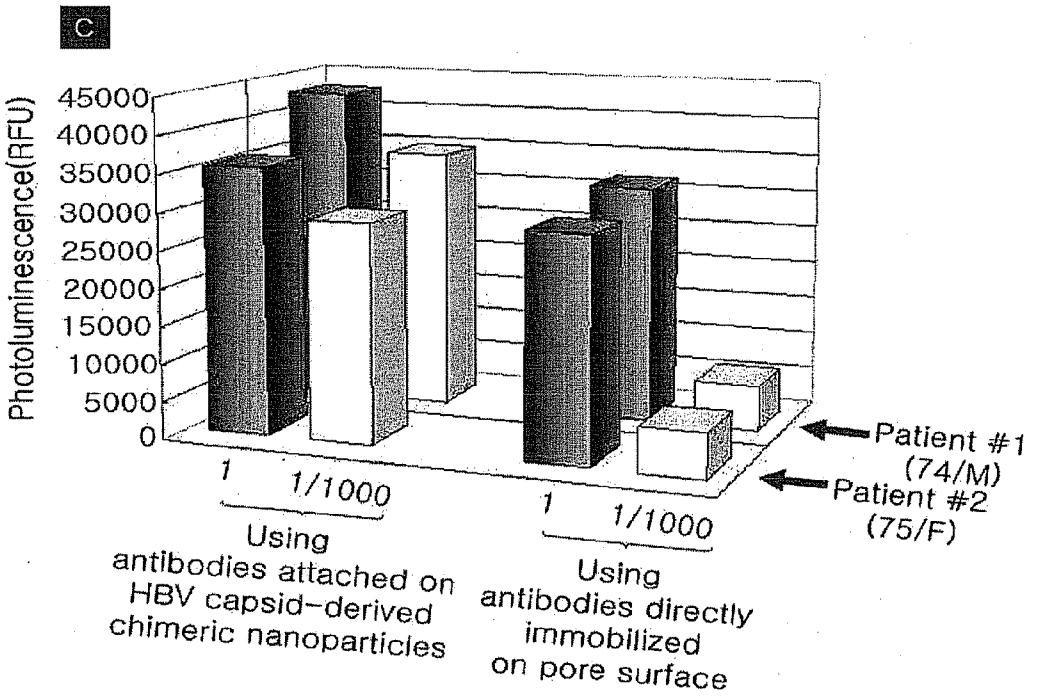
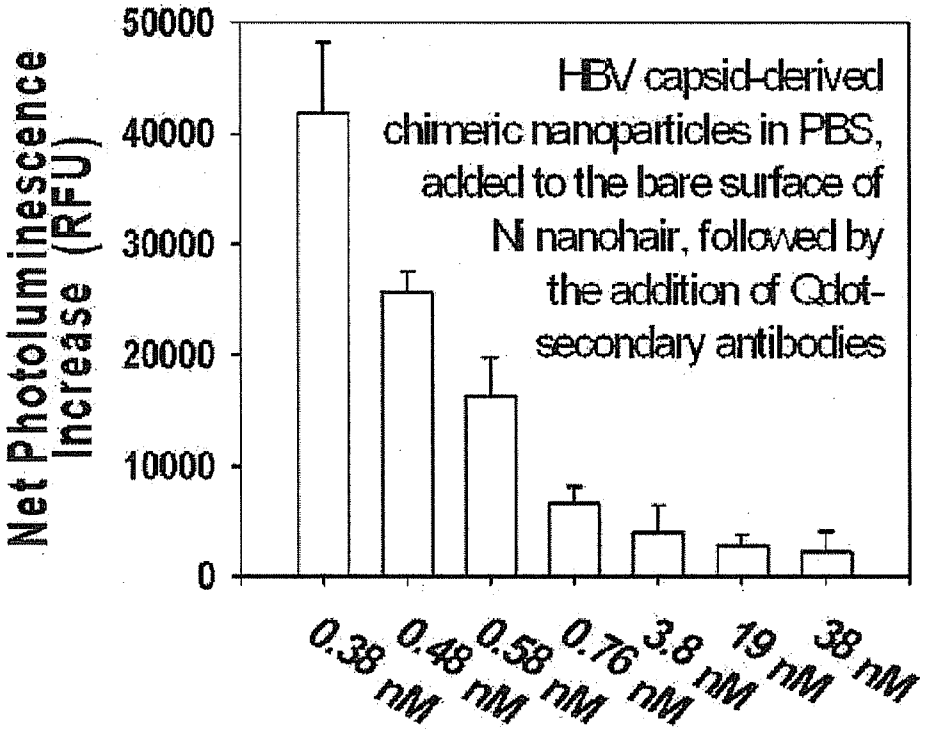

C
Photoluminescence(RFU)
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
1
1/1000
1
1/1000
Using antibodies attached on HBV capsid-derived chimeric nanoparticles
Using antibodies directly immobilized on pore surface
Patient #1 (74/M)
Patient #2 (75/F)

FIG. 13


Net Photoluminescence Increase (RFU)
50000
40000
30000
20000
10000
0
HBV capsid-derived chimeric nanoparticles in PBS, added to the bare surface of Ni nanohair, followed by the addition of Qdot-secondary antibodies
0.38 nM
0.48 nM
0.58 nM
0.76 nM
3.8 nM
19 nM
38 nM

NANOHAIR STRUCTURE AND AN APPLICATION THEREFOR

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of PCT/KR2010/001389 filed Mar. 5, 2010, which claims the benefit of Korean Application No. 10-2009-0019355 filed Mar. 6, 2009, the entire contents of which applications are incorporated herein by reference.

TECHNICAL FIELD

[0002] The present invention relates to a nanohair structure and a use thereof.

BACKGROUND ART

[0003] In general, there are many kinds of catalysts, such as a metal, an alloy, a metal oxide, and the like. In addition, a producing method can largely be classified into an infiltration method (after immersing a support into a solution dissolving an active material, the active material is supported at the support by evaporating or adding a precipitate), an ion exchange method (an active material is exchanged to a support by contacting the support with the solution dissolving the active material), a precipitation method (passing through a activating process by precipitating the active material in a solution state), and the like. Of these, the present invention, which is a specific method out of the infiltration method of metal catalyst, uses a metal nickel as a catalyst. In addition, since the nickel is easily made in desired shapes and desired sizes by using a nanotemplate as compared with a metal line, a metal thin film, a metal crystal, and the like by using the nickel are often used to study a catalytic action. For example, the surface of the nickel nanohair structure can be biofunctionalized to achieve applicability in the field of biotechnology (BT). Especially, the exposed part of nickel nanowire is possible to use in a biosensor using an affinity of antibody-antigen and biotin-avidin through a surface modification. Especially, the applicability of nickel is greatly improved because the nickel can be possible to selectively bind with a variety of ligands such as amine and histidine. In addition, it can also be possible to control the movement of nanostructure by using a magnetic property of nickel. However, it is extremely difficult to get the result of an individual nanowire property because an agglomeration phenomenon is generated due to a magnetic interaction and van der Waals forces for using the nickel nanowire that is completely separated from a nanotemplate with a plurality of nanopores such as an anodized aluminum oxide (AAO) membrane.

[0004] Here, the nickel nanohair structure according to the present invention is a very useful nanomaterial for chemically detecting because it is uniform in height; the agglomeration phenomenon is prevented by being inside the nanotemplate; and it has a high density. Accordingly, a method for synthesizing a nanostructure for studying the nickel nanohair structure is required.

[0005] Meanwhile, an early detection [Adams, J. E. et al. *Circulation* 88, 101-106 (1993); Adams, J. E., Schechtman, K. B., Landt, Y., Ladenson, J. H. & Jaffe, A. S. *Clin. Chem.* 40, 1291-1295 (1994); Thygesen, K., Alpert, J. S. & White, H. D. *J. Am. Coll. Cardiol.* 50, 2173-2195 (2007); Morrow, D. A. et al. *Clin. Chem.* 53, 552-574 (2007); Gibler, W. B. et al. *Ann. Emerg. Med.* 46, 185-197 (2005)] of Troponin I (Protein Marker) from a patient suffered with high risk acute myocardial infarction can reduce a risk rate of deaths from heart attack [Antman, E. M. et al. *N. Engl. J. Med.* 335, 1342-1349 (1996); Wu, A. H. B. & Jaffe, A. S. *Am. Heart J.* 155, 208-214 (2008); Benamer, H. et al. *Am. Heart J.* 137, 815-820 (1999); Heeschen, C., van den Brand, M. J., Hamm, C. W. & Simoons, M. L. *Circulation* 100, 1509-1514 (1999); Wong, G. C. et al. *Circulation* 106, 202-207 (2002)].

[0006] Most Troponin assays are currently based on the conventional Enzyme Linked Immunosorbent Assay (ELISA) and have detection limits in the nanomolar- and picomolar ranges [Rosi, N. L. & Mirkin, C. A. *Chem. Rev.* 105, 1547-1562 (2005)].

[0007] The above information disclosed in this Background section is only for enhancement of understanding of the background of the invention and therefore it may contain information that does not form the prior art that is already known in this country to a person of ordinary skill in the art.

SUMMARY OF THE DISCLOSURE

[0008] An object of the present invention, which is created by a necessity as mentioned above to solve the above problems, provides a nanohair structure which is necessary to prepare nanosensor.

[0009] Another object of the present invention provides a three-dimensional nanostructure-based ultra-sensitive biosensor based on the nanohair structure.

[0010] In order to achieve the above objects, the present invention provides a nanohair structure comprising:

(a) nanotemplate comprising a plurality of pores; and

(b) a plurality of nanowires grown through the pores of the nanotemplate, wherein one portion of the nanowires is embedded inside the nanotemplate, while the other portion of the nanowires is exposed vertically on top of the surface of the nanotemplate.

[0011] The 'nanohair structure' which is used as its widest mean, in the present invention means the structure of exposing the nanowire array equalizing the length of nanowire by using semiconductor process such as the chemical mechanical planarization (CMP) process and the reactive ion etching (RIE) process, after filling metal (for example, nickel) in nanotemplate (for example, AAO nanotemplate).

[0012] For an embodiment of the present invention, the exposed nanowire is derived from electrically conductive material such as transition metal species and alloy thereof comprising Ni, Fe, Co, Ni, Cu, Ag, Au, Pd and Pt or electrically conductive polymer. After preparing nanohair by using the metal or alloy thereof, the exposed part of the nanohair can be modified into oxide, nitride and carbide through oxidation, nitrification or carburization of the exposed part.

[0013] The 'electrically conductive polymer' in the present invention means a light electrically conductive polymer with easy processing. The polymer is a long chain molecule compared with prior low molecular material and become solid state (crystal) by aggregation of the polymer chains. For an embodiment of the present invention, the electrically conductive polymer is preferably polyacetylene, polyaniline, polypyrrole, polythiophene, poly sulfur nitride etc, but is not limited thereto.

[0014] For an embodiment of the present invention, the nanotemplate is preferably hard material like anodized aluminum oxide (AAO) or soft material like poly-carbonate, but is not limited thereto.

[0015] In addition, the present invention provides the method of the nanohair structure comprising, a) preparing a nanotemplate with a plurality of pores; b) generating a conductive electrode layer on one side of the nanotemplate; c) putting the nanotemplate into the solution containing the metal ion and growing the metal nanowire through pores of the nanotemplate by electrodeposition method employing it as a cathode; d) planarization of the metal nanowires through chemical mechanical planarization (CMP); and e) selective reactive ion etching (RIE) of the nanotemplate.

[0016] For the method for producing the nanohair structure, the thickness of deposition of step b) preferably is 250-350 nm, but is not limited thereto.

[0017] For the method for producing the nanohair structure, the metal ion is preferably selected from the group consisting of Ni, Fe, Co, Ni, Cu, Ag, Au, Pd and Pt, but is not limited thereto.

[0018] For the method for producing the nanohair structure, the solution containing the metal ion of step c) preferably is the mixture solution of metal sulfate, nickel chloride and boric acid, but is not limited thereto.

[0019] For the method for producing the nanohair structure, the process of reactive ion etching of step e) preferably is to etch the nanotemplate for 10 min in an etching rate of 0.25 μm/min using BCl_3 gas, but is not limited thereto.

[0020] For the method for producing the nanohair structure, precious metal such as Cu, Ag, Au, or Pt is mainly used in the electrode layer, but all kind of conductive thin film can be used in the electrode layer

[0021] For the method for producing the nanohair structure, Pt is mainly used in the anode layer, but Pd or Ir can be used in the anode layer, but is not limited thereto.

[0022] In addition, the present invention provides a three-dimensional nanostructure-based biosensor produced by adding chimeric nanoparticles on the surface of nanohair structure and immobilizing the chimeric nanoparticle to the structure.

[0023] For an embodiment of the present invention, the chimeric nanoparticle is preferably HBV derived-chimeric protein, but is not limited thereto.

[0024] For other embodiment of the present invention, the nanosensor preferably further comprises antibody which recognizes specific disease marker, but is not limited thereto.

[0025] For other embodiment of the present invention, the disease marker is preferably Troponin I, but is not limited thereto.

[0026] For an embodiment of the present invention, the producing method of the present invention preferably includes as follows: a) obtaining two gene clones that are derived from Hepatitis B virus core protein (HBVcAg) gene encoding synthesizes of N-NdeI-hexahistidine-HBVcAg(1-78)-G4SG4T-XhoI-C and N-BamH1-G4SG4-HBVcAg (81-149)-HindIII-C; b) producing other two clones, i.e., N-XhoI-SPA_B-EcoRI-C and N-EcoRI-SPA_B-BamHI-C in order to substituting P79A80 of HBVcAg with tandem repeat of 209-270 residues of B domain of Staphylococcal protein A (SPA_B); c) producing a plasmid expressing vector encoding the synthesis of N-His_6-HBVcAg (1-78)-SPA_B-SPA_B-HBVcAg (81-149)-C through a sequential ligation of the above four clones; and d) expressing the gene of chimeric protein by transforming the expression vector to a host, but it is not limited thereto.

[0027] The 'chimeric protein' or 'chimeric nanoparticle,' which is used as its widest mean, in the present invention means the protein or protein nanoparticles with various functionalities by combining a foreign bio material to the surface of the protein nanoparticles based on a genetic engineering and a protein engineering technique. Although HBV capsid of the present invention is used as a model virus scaffold for a surface display of SPA_B, other viruses or virus-like particles can be used for the production of chimeric protein or chimeric nanoparticles displaying a surface SPA_B.

[0028] For the present invention, the 'HBV-derived chimeric protein, means the protein or protein nanoparticles with various functionailities by combining a foreign protein to the HBV-derived protein.

[0029] The nanosensor in the present invention means device which detects specific compound, molecule or biomaterial like DNA or protein in the gas or liquid, or measures partial pressure or concentration of specific molecule or measures degree of vacuum of vacuum device or vacuum chamber or searching a site of gas leak.

[0030] In addition, the present invention provides nanohair-based electrodes which offer increased surface area compared to the conventional flat surface electrodes, and thereby increase the electrochemical or biological reactions of materials (for example, ions or enzymes).

[0031] The above and other features and advantages of the present invention will be apparent from or are set forth in more detail in the accompanying drawings, which are incorporated in and form a part of this specification, and the following Detailed Description, which together serve to explain by way of example the principles of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0032] The above and other aspects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

[0033] FIG. **1** shows a process mimetic diagram of a fabrication method of nanohair wire structure: FIG. **1**(*a*) shows the synthesize of anodized aluminum oxide nanotemplate; FIG. **1**(*b*) shows the deposition of conductive layer (Ag, 300~400 nm) by E-beam evaporation; FIG. **1**(*c*) shows the synthesize of nanowires inside the nanotemplate; FIG. **1**(*d*) shows the planarization process of nanowires and nanotemplate; and FIG. **1**(*e*) shows a selective etching process of nanotemplate for nanohair wire structure.

[0034] FIG. **2** shows Field Emission-Scanning Electron Microscope (FE-SEM) images of nickel nanohair wire structure according to Example of the present invention:

[0035] FIGS. **2**(*a*) and (*b*) show floor plans that are planarized by chemical mechanical planarization (CMP) process; FIGS. **2**(*c*) and (*d*) show cross-sectional diagrams that are planarized by the CMP process; and FIGS. **2**(*e*) and (*f*) show cross-sectional diagrams of nickel nanohair wire structure by selective reactive ion etching (RIE) process of nanotemplate.

[0036] FIGS. **3** and **4** show three-dimensional diagnostic assay based on virus nanoparticles. FIG. **3** shows schematic and TEM images of native hepatitis B virus (HBV) capsid particles and chimeric nanoparticles synthesized in *E. coli* and FIG. **4** shows schematic, of the diagnostic system performed in a 96-well microplate and the assay principle in detail. Briefly, antibodies that recognize the disease marker (Troponin I in this case) bind to the chimeric nanoparticle and are oriented in a specific way. Troponin I binds to the anti-

bodies and detection is achieved with secondary antibodies conjugated with quantum dots.

[0037] FIGS. **5** and **6** show the detection of Troponin I. FIG. **5** shows that the conventional ELISA assay could not detect Troponin I at concentrations lower than 0.1 nM, and FIG. **6** shows that the assay using chimeric nanoparticles and nickel nanohairs shows 10^{-18} (atto-molar, aM) sensitivities in both PBS and human sera. "Control" refers to the experiment in which only quantum-dot-secondary antibodies were added to the nickel nanohair surface, which was covered with a sufficient amount of chimeric nanoparticles (30 nM in PBS buffer 50

[0038] FIGS. **7** and **8** show a washable and reuseable assay system.

[0039] FIG. **7** shows Four-step protocol for washing and reusing the nickel nanohairs for detection. FIG. **8** shows the consecutive assays of eight different Troponin I (Tn) samples using three separate systems, showing good reproducibility. A, B, C, D, dotted and solid arrows, and PL1/PL2 correspond to those in FIG. **7**. Black areas of the rectangle represent net PL increase.

[0040] FIGS. **9** and **10** show Troponin I assay on PVDF membranes. FIG. **9** shows that chimeric nanoparticles immobilized on PVDF membranes show similar detection sensitivities in both PBS and human sera. FIG. **10** shows that antibodies immobilized directly on the PVDF membranes show significantly lower sensitivities than those immobilized on chimeric nanoparticles.

[0041] FIGS. **11** and **12** show clinical specificity and sensitivity of the viral chimeric nanoparticle-based assay. In assays of sera derived from 16 healthy individuals and 26 AMI patients, using the chimeric nanoparticles and PVDF membrane system [see FIG. **11**(*a*)] unambiguously detected Troponin I in all patients, but using an ELISA-based diagnosis [see FIG. **11**(*b*)] failed to detect three patients (Nos. 4, 9, and 18) and revealed nine ambiguous signals close to the clinical cutoff signal (horizontal dotted line). FIG. **12** shows assays with antibodies attached to chimeric nanoparticles, but not those immobilized directly on PVDF membranes, could detect Troponin I in a sample diluted 1,000 times. 74/M and 75/F represent the age and gender of the AMI patients.

[0042] FIG. **13** shows a graph for determining an amount of HVB capsid-derived chimeric nanoparticles added to bare nickel surface, preventing the binding of non-specific quantum-dot-secondary antibodies to the nickel surface.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0043] Hereinafter, the present invention will be described with embodiments of the present invention. Embodiments of the method for synthesizing a metallic nanohair structure will be described with Examples of the present invention.

[0044] The entire process of Example 1 is as depicted in FIG. 1. Example 1 of the present invention relates to a method for forming Nanohair structure having an exposed nanowire structure on the anodized aluminum oxide (AAO) nanotemplate after synthesizing the nickel nanowires in the AAO nanotemplate. An object of the present invention is to provide a development of bio-nano catalyst material that can be possible to apply in vitro by using the nanowire as a catalyst for the application in the biochemical field.

[0045] The technique according to Example 1 of the present invention is based on an electrochemistry, and relates to a producing method that would allow the mass production of a catalyst at low cost, in which the catalyst can increase the reaction rate, that is, can make the reaction of low activation energy by contacting with a reactant. The technique can be implemented by exposing the nickel nanowire through a selective isotropy reactive ion etching (RIE), after synthesizing the nickel nanowire in AAO nanotemplate produced by an electrodeposition method.

[0046] A producing process of the nickel nanostructure according to the present invention includes regularly planarizing the heights of nanowire and AAO through a chemical mechanical planarization (CMP), after synthesizing the nanowire in the AAO nanotemplate by using an electrochemical method. AAO nanotemplate of the sample resulted from the above steps is selectively etched through an etching process by using a reactive ion etching (RIE) apparatus. The nanohair structure (Ni nanohair structure) exposed on the final AAO nanotemplate like hairs can be synthesized through the processes as mentioned above. The nanohair structure implemented as mentioned above does not have an agglomeration phenomenon of nanowires, and has very high density (10^8 wires/cm^2) and a regular height (max. 60 μm) so that its applicability is largely increased as a catalyst in the biochemical and environmental fields.

[0047] The 'chemical mechanical planarization (CMP)' is one of the methods generally used in the process of planarizing, and includes pressing the action surface to the rotation polishing pad and then introducing a polishing and/or chemical reaction solution that is known as slurry on the polishing pad. The mechanical effect of pressure is applied through the polishing pad, and the chemical reaction caused by the input of slurry allows the materials to be selectively removed from the action surface thereby a little more equalizing the layer. Typically, deionized water having a high purity is applied to the polishing solution as base, and a particle and/or chemical additive having the effect of polishing is added therein. The more information about the chemical mechanical polishing, the slurry, and the like is disclosed in U.S. Pat. Nos. 6,914,001 and 6,887,137.

[0048] The 'Troponin I' disclosed in the specification of the present invention is a type of proteins found in the blood of patients suffered from a cardiac infarction, and when detecting in the existence of Troponin I, it can be judged to have a disorder of heart.

[0049] The 'nickel nanohair structure' disclosed in the specification of the present invention is the structure of exposing the nanowire by selectively etching AAO nanotemplate using the reactive ion etching (RIE) process, and equalizing the length of nanowire by using the chemical mechanical planarization (CMP) process, after synthesizing nickel nanowire in AAO nanotemplate.

[0050] The 'PVDF membrane (poly(vinyl difluoride) membrane)' disclosed in the specification of the present invention is the polymer membrane having small pores and hydrophobic property (no compatibility with water), and the present invention used the membrane having the pore of 450 nm size, in which the membrane has the property that allows the surface thereof to easily well take the nanoparticles.

[0051] The 'bio-nano-probe' disclosed in the specification of the present invention is used as the material of sensor that is accumulated with the probe for detecting a target to the protein nanoparticles.

[0052] The present inventors showed that combining the three-dimensional nanostructure including the nickel nanohair and virus nanoparticles that are designed to have a dual

affinity for antibodies and nickel can detect at low level of Troponin, i.e., 10^6~10^7 in human serum as compared with using the typical ELISA assay [Hirsch, L. R., Jackson, J. B., Lee, A., Halas, N. J. & West, *J. Anal. Chem.* 75, 2377-2381 (2003); Nam, J. M., Park, S. J. &Mirkin, C. A. *J. Am. Chem. Soc.* 124, 3820-3821 (2002); Niemeyer, C. M. & Ceyhan, B. Angew. *Chem., Int. Ed.* 40, 3685-3688 (2001); Chien, R. J. et al. *Proc. Natl. Acad. Sci.* 100, 4984-4989 (2003) Wang, J., Polsky, R., Merkoci, A. & Turner, K. L. Angew. *Chem., Int. Ed.* 43, 2158-2161 (2004)] in the present invention. The virus nanoparticles help the orientation of antibodies for the maximum capture of Troponin marker. When Troponin marker is largely bound to antibody in the high density combined to the nanostructure, the sensitivity of detecting is largely increased. The nickel nanohair is able to reproduce and also to regeneratively distinguish a healthy serum from unhealthy serum. The present inventors anticipate other virus nanoparticles forming a diagnosis assay with high similar sensitivity to other various protein markers.

[0053] Meanwhile, the HBV core protein, consisting of four long alpha-helix bundles (FIG. **3**), when expressed in bacteria, assembles into core-shell particles that closely resemble the native capsid structure of virus [Bottcher, B., Wynne, S. A. & Crowther, R. A. *Nature* 386, 88-91 (1997); Crowther, R. A. et al. *Cell* 77, 943-950 (1994)]. Through the electron cryomicroscopy analysis, Bottcher and colleagues demonstrated that a single HBV core protein truncated after residue 149 forms the core-shell particles that contain 240 subunits and have an overall diameter of 36 nm [Bottcher, B., Wynne, S. A. & Crowther, R. A. *Nature* 386, 88-91 (1997)]. The dimer clustering of the subunits produces spikes on the surface of the shell particle, and the immunogenic epitope is located at the tips of prominent surface spikes (FIG. **3**). The surface-exposed spike tip corresponds to the loop segment consisting of the residues from D78 to D83 of the single core protein. The present inventors made that the P79A80 in the loop segment was replaced with the tandem repeated SPA_B sequences (NCBI nucleotide accession No. M18264 nucleotide sequence 625-813, Sequence No. 11), which were subsequently exposed on the surface of the synthesized chimeric nanoparticles with high density (FIG. **3**). In the chimeric nanoparticle synthesis, the present inventors also added the hexahistidine sequence to the N-terminus of the truncated HBV core protein so that the chimeric nanoparticles would have a strong affinity for nickel (FIG. **3**). Transmission electron microscopy (TEM) image analysis (FIG. **3**) revealed that the HBV capsid-derived chimeric nanoparticles that were expressed in *E. coli* assembled into spherical nanoparticles with a nearly native diameter. Consequently, the chimeric nanoparticles have a dual affinity for the Fc domain of antibodies (IgG) and nickel. These viral particles can display on their surface various peptides and proteins that are used for detecting and/or quantifying bimolecular of interest.

[0054] As illustrated in FIG. **4**, a three-dimensional assay system was developed by combining the chimeric nanoparticles with a nickel nanohair structure or porous membrane, and then adding antibodies to specifically capture protein markers. For the present invention, the term 'nanohair' refers to an array of nanowires in which some wires are exposed to the air, the rest being embedded in the body of a supporting organic or inorganic template (FIG. **4**). In these structures, the air-exposed portion of the nanowires has a greatly increased surface-to-volume ratio.

[0055] When antibodies are added to the chimeric nanoparticles that are already attached to the nickel nanohair surface as a result of the affinity interaction between the hexahistidin and nickel (FIG. **3**), the Fc domain of antibody (IgG) is specifically bound to the surface SPA_S of the chimeric nanoparticles, and hence the antigen-specific variable domains of IgG are fully accessible to protein markers. Consequently, as illustrated in FIG. **4**, the efficient three-dimensional assay system was developed with the following significant advantages: (i) maximum accessibility of protein markers to antibodies, enabled both by the controlled orientation of the antibodies and the three-dimensional manner of protein capture, and (ii) a dramatically increased density and ratio of antibodies to protein markers on the three-dimensional nanohair surface. The captured markers were detected by sensing photoluminescence emitted by quantum dots conjugated to the secondary antibodies (FIG. **4**).

[0056] From FIG. **5**, it is clear that the ELISA-based assay (see Examples) did not detect Troponin I (in PBS buffer or healthy sera) at concentrations lower than about 0.1 nM, but gave highly reproducible signals at each Troponin I concentration. Using the chimeric nanoparticles and nickel nanohair system developed in the present invention, the sensitivity was surprisingly boosted to low 10^{-18} level (FIG. **6**), which represents about 100.000-fold higher sensitivity than the highest level (0.25 μM) reported to date [Apple, F. S., Smith, S. W., Pearce, L. A., Ler, R. & Murakami, M. M. *Clin. Chem.* 54, 723-728 (2008)] and also 10^8~10^7 greater sensitivity than current ELISA assays [Rosi, N. L. & Mirkin, C. A. *Chem. Rev.* 105, 1547-1562 (2005); Hirsch, L. R., Jackson, J. B., Lee, A., Halas, N. J. & West, *J. Anal. Chem.* 75, 2377-2381 (2003); Nam, J. M., Park, S. J. & Mirkin, C. A. J. *Am. Chem. Soc.* 124, 3820-3821 (2002); Niemeyer, C. M. & Ceyhan, B. DAngew. *Chem., Int. Ed.* 40, 3685-3688 (2001); Chien, R. J. et al. *Proc. Natl. Acad. Sci.* 100, 4984-4989 (2003); Wang, J., Polsky, R., Merkoci, A. & Turner, K. L. Angew. *Chem., Int. Ed.* 43, 2158-2161 (2004)]. As shown in the binding of the chimeric nanoparticles (i.e., in the step B of FIG. **7**), to prevent false signals of photoluminescence arising from non-specific binding of quantum dot secondary antibodies to the bare nickel surface, a sufficient amount of chimeric nanoparticles were added to the washed surface of the nickel nanohair as shown in the result of control of FIG. **6** (FIG. **13**). Furthermore, Troponin I in AMI patient sera was successfully detected using the same assay system and procedure, while the Troponin I-free PBS buffer and healthy sera gave only negligible signals (FIG. **6**).

[0057] One of the distinct advantages of this assay system according to the present invention is that one nickel nanohair structure can be repeatedly used for multiple samples. Through washing and rinsing (i.e., in the step A of FIG. **7**), the used nickel nanohair was refreshed and reused for another sample assay. All of the three separate nickel nanohair structures were successfully used for the consecutive assay of eight different samples. Each consecutive assay showed reproducible and consistent signals for all the samples tested.

[0058] A polyvinylidene fluoride (PVDF) membrane with an average pore size of 450 nm was selected as a suitable nanostructure with a hydrophobic pore surface on which the chimeric nanoparticles were easily immobilized, and was used to construct another type of three-dimensional assay system. As seen in FIG. **9**, antibodies attached on the chimeric nanoparticles could reproducibly detect Troponin I, at all concentrations, in both PBS buffer and healthy sera spiked

with Troponin I. The atto-molar detection limit of Troponin I was also comparable to that of the nickel nanohair-based assay. Furthermore, the assay with antibodies directly immobilized on the PVDF surface showed significantly lower sensitivities than assays using the chimeric nanoparticles (FIG. **10**). This is probably due to the orientation of the antibodies; those immobilized directly on the PVDF surface may be random and have lower accessibility to Troponin I. The orientation of antibodies immobilized on the chimeric nanoparticles seems crucial for the sensitivity of the assay.

[0059] The present inventors also tested the PVDF-based assay system in the clinical diagnosis of 26 AMI patients (Table 1) who were confirmed to have experienced an AMI, and the assay results were compared with the ELISA-based assay (a and b of FIG. **11**). In the ELISA-based assay (b of FIG. **11**), three (Nos. 4, 9, and 18) AMI patient sera were not positively detected; that is, the absorbance signals were below the clinical cutoff value (indicated by the horizontal dotted line), and the signals from nine (Nos. 1, 2, 3, 5, 8, 11, 16, 17, and 23) AMI patient sera were positive but very close to the clinical cutoff. Meanwhile, the chimeric nanoparticles and PVDF-based assay gave clear positive signals for all 26 AMI patient sera, therefore indicating 100% clinical specificity (FIG. **11**). (The ELISA assay results were not surprising because the clinical specificity of the ELISA kit is reported by the supplier to be 87.5%.) Furthermore, antibodies directly immobilized on the PVDF surface failed to diagnose the 1,000-times diluted AMI patient sera, whereas the chimeric nanoparticles and PVDF-based assay could detect Troponin I in the diluted patient sera (FIG. **12**), indicating that this three-dimensional assay can discriminate the onset of AMI even with an extremely small quantity of patient sera.

[0060] Using the HBV capsid-derived chimeric nanoparticles and three-dimensional nanostructures (nickel nanohair), we were able to develop a highly sensitive and specific assay system for the specific AMI marker, Troponin I. Although HBV capsid according to the present invention was used in this study as a model viral scaffold for the surface display of SPA_B, other viruses or virus-like particles could also be used for the production of chimeric nanoparticles, displaying the surface SPA_B. Owing to the controlled orientation of densely immobilized antibodies and the three-dimensional manner of protein capture, the assay sensitivity and clinical specificity were significantly enhanced as compared to the conventional ELISA assay.

[0061] The nanohair wire structure according to the present invention, which is a method for chemically detecting that has a yield of a high efficiency due to the exclusion of agglomeration phenomenon among the nanowires, has a high applicability in the flied of Biotechnology (BT) as well as Nanotechnology (NT) by synthesizing the nanowire material having a biological functionality inside the nanotemplate. The present invention showed that the assay system according to the present invention using chimeric nanoparticles and three-dimensional nanostructure (nickel nanohair and PVDF membrane) has a very higher sensitivity and specificity as the use of detecting the disease diagnosis marker and has very high sensitivity and specificity to the protein marker, such as Troponin I or specific AMI marker.

EXAMPLES

[0062] The present invention will now be described in more detail with reference to the following non-limited Examples. However, the following Examples are only for illustration to explain the present invention, but the range of the present invention will not be limited to the following Examples.

Example 1

Method for Synthesizing Nickel Nanohair Structure

[0063] —Synthesis of Nickel Nanowires—

[0064] The anodized aluminum oxide (AAO) nanotemplate with uniform pore diameter (tens to hundreds nm) as shown in FIG. **1**(*a*) was synthesized. And then, Ag as a working electrode was deposited one side of AAO in a thickness of 250-350 nm using E-beam evaporator as shown in FIG. **1**(*b*). Then, after adding AAO to the solution of nickel sulfate ($NiSO_4 6H_2O$, 0.5 M)+nickel chloride ($NiCl_2.6H_2O$, 0.1M)+boric acid (H_3BO_3, 0.1M) as shown in FIG. **1**(*c*), Pt as a counter electrode was deposited to the nickel nanowires. Here, nickel sulfate is a main component of plating; nickel chloride was used for increasing an electrical conductivity; and boric acid was used as a buffer solution for pH homeostasis.

[0065] —Planarization of Nickel Nanowires—

[0066] Then, as shown in FIG. **1**(*c*), the polishing was performed about 10 μm through a chemical mechanical planarization (CMP) process for identifying AAO nanotemplate with the height of nanowires that were overflow or underflow-gtown inside the AAO.

[0067] —Exposure of Nickel Nanowires on AAO—

[0068] As a final step, a selective reactive ion etching (RIE) process of AAO nanotemplate was performed in order to expose the nickel nanowires. The process was performed by etching AAO for 10 min at an etching rate of 0.25 μm/min using BCl_3 (100%) gas. And then, a cleaning process (DI water: ultrapure water, ethanol) was completed, finishing the process of the nickel nanohairs structure with a clean surface.

Example 2

Biosynthesis of HBV Capsid-Derived Chimeric Nanoparticles

[0069] Following assembly PCR using the primers as disclosed in the following Table 1, the present inventors prepared the two gene clones derived from the HBV core protein (HBVcAg) gene and code for the synthesis of N-NdeI-hexahistidine-HBVcAg(1-78)-G4SG4T-XhoI-C and N-BamH1-G4SG4-HBVcAg (81-149)-HindIII-C. To replace the P79A80 of the HBV cAg with the tandem repeat of SPA_B (residues 209-271), the two different clones, N-XhoI-SPA_a-EcoRI-C and N-EcoRI-SPA_a-BamHI-C were prepared. Through the sequential ligation of the four above gene clones into plasmid pT7-7, we constructed the plasmid expression vector pT7-Chimera-HBV encoding the synthesis of N-His_6-HBVcAg(1-78)-SPA_B-SPA_B-HBVcAg(81-149)-C. After the complete DNA sequencing of gel-purified plasmid expression vector, *E. coli* strain BL21 (DE3) [F^-ompThsdS_B(rB^- mB^-)] was transformed with pT7-Chimera-HBV, and ampicillin-resistant transformants were selected. The gene expression, purification and TEM image analysis of chimeric nanoparticles were performed by using the same method as disclosed in Ahn, J. Y. et al. *Nucl. Acids Res.* 33, 3751-3762 (2005).

[0070] The information and the disclosure in more detail about the primer sequences and templates related to the fabrication of HBV capsid-derived chimeric nanoparticles are as follows:

TABLE 1

Gene Name		Primer	Seq. No.	Sequence
Hepatitis B virus capsid	N-terminus of HBV capsid (1-234 Gene Sequence)	Sense	Seq. No. 1	**cat atg** *cat cac cat cac cat cac* gac att gac ccg tat aaa gaa
		Anti sense	Seq. No. 2	ccc act ccc tcc gcc acc gtc ttc caa att act tcc cac cca
		Anti sense	Seq. No. 3	**ctc gag** agt acc gcc tcc ccc act ccc tcc gcc acc
	C-terminus of HBV capsid (241-447 Gene Sequence)	Anti sense	Seq. No. 4	**gga tcc** gga tcc ggt ggc gga ggg tct ggg gga ggc ggt
		Sense	Seq. No. 5	ggc gga ggg tct ggg gga ggc ggt tcc agg gga tta gta gtc agc tat
		Anti sense	Seq. No. 6	**aag ctt** tta aac aac agt agt ttc cgg aag
B domain of *Staphylococcal* protein A		Sense	Seq. No. 7	**ctc gag** gca ccg aaa gct gat aac
		Anti sense	Seq. No. 8	**gaa ttc** gtc agc ttt tgg tgc ttg
		Sense	Seq. No. 9	**gaa ttc** gca ccg aaa gct gat aac
		Anti sense	Seq. No. 10	**gga tcc** gtc agc ttt tgg tgc ttg

[0071] Table 1 shows primer sequences, in which bold types represent restriction enzymes sequences; underlined parts represent linker sequences; and italic types represent 6 histidine sequences.

[0072] HBV capsid-derived chimeric nanoparticles can be largely divided into 1-78 amino acid sequence regions of capsid protein, the region including continuously two Staphylococcal protein A, and 81-149 amino acid sequences regions of capsid protein (1-78 sequences of capsid protein is NCBI Nucleotide accession number: AF286594 sequences: 1901-2134 (Sequence No. 12) and amino acid sequence (Sequence No. 13); 81-149 sequences of capsid protein is 2141-2347 sequences (Sequence No. 14) of AF286594; and the amino acid sequence is Sequence No. 15, and Protein A sequence is NCBI nucleotide accession No. M18264, nucleotide sequence 625-813 (Sequence No. 11); and the amino acid is Sequence No. 16).

[0073] The first region was subjected to extension PCR using the primer sequence region 1 including 6 histidines using the gene sequence of HBV capsid protein (1901-2452 sequences of NCBI Nucleotide accession number: AF286594 sequences) as a template, and the primer sequences 2 and 3 including a linker sequence (amino sequences GGGGSGGGGT). Firstly, after performing PCR using the primer sequences 1 and 2, PCR was performed by using the primer sequences 1 and 3 using the synthesized PCR products as the templates. As the results, PCR product consisting of 5'-NdeI-HBV capsid protein (1-78 amino acid sequences)-linker sequence (GGGGSGGGT)-XhoI-3' was obtained.

[0074] Using the second region, two protein A and B domains forming 5'-XhoI-SPA_B-EcoRI-3' and 5'-EcoRI-SPA_B-BamHI-3' using the primer sequences 7 and 8, and 9 and 10 through using B domain part of Protein A (SPA) sequence (NCBI Nucleotide accession number: M18264) of *Staphylococcus aureus* (SPA_B) were obtained as PCR products.

[0075] The third region was subjected to extension PCR using the primer sequence 6, and the primer sequences 4 and 5 including the linker sequence (Amino acid sequence GGGGSGGGG) through using HBV capsid protein gene sequence as a template. Firstly, after performing PCR using the primer sequences 5 and 6, PCR was performed by using the primer sequences 4 and 6 through using the synthesized PCR products as a template. As the results, PCR product consisting of 5'-BamHI-linker sequence (GGGGSGGGG)-HBV capsid protein (81-149 amino acid sequence)-HindIII-3' was obtained.

Example 3

Construction of the Three-Dimensional Diagnostic Systems Using Virus Nanoparticles

[0076] Nickel Nanohair-Based System:

[0077] The nickel nanohair structure prepared by the above Example 1 was placed in the Costar 96-well plate (Cat. No. 3599, Corning, N.Y., USA). Before immobilizing the chimeric nanoparticles, the nickel nanohair in each well was washed four times for 15 min using 0.3 M sulphuric acid and then six times for 10 min using distilled water, then completely dried. Next, the background photoluminescence from the nickel nanohair structure was measured using a microplate reader (GENios, Tecan, Austria) with excitation and emission at 420 and 650 nm, respectively. PBS buffer (50 μl) containing the 38-nM chimeric nanoparticles prepared from Example 2 was added to the nickel nanohair structure, followed by slow agitation for 30 min, after which it was washed with 50 mM Tris buffer (pH 7.4). Rabbit anti-troponin polyclonal antibody (5 μg/ml, Cat. No. ab470003, Abcam, Cambridge, UK) in 200 μl PBS buffer was added to the chimeric nanoparticles that were already immobilized on the nickel nanohair, by slowly stirring the nickel nanohair in the antibody-containing PBS buffer for 2 h.

[0078] PVDF-Based System:

[0079] PVDF membrane (Immobilion-FL, IPFL 10100, Millipore, Mass., U.S.A.) in a Costar 96-well plate was prewetted with methanol for 1 min and washed with a PBS buffer (137 mM, NaCl; 2.7 mM, KCl; 10 mM, Na_2HPO_4; 2 mM, KH_2PO_4; pH, 7.4) for 5-10 min. Before the PVDF membrane was completely dried, 10 a of PBS buffer containing the chimeric nanoparticles purified in Example 2 was dropped onto a designated spot on the membrane. The membrane was then slowly stirred for 1 h in the blocking solution (1% skimmed milk) and washed twice with the PBS buffer for 30 min. Goat anti-Troponin I polyclonal antibodies (20 μg/ml in PBS buffer; Cat. No. 70-XG82, Fitzgerald, Mass., USA) was added to the chimeric protein nanoparticles that were already

immobilized onto the PVDF membrane, by slowly stirring the membrane in the antibody-containing 200 μl PBS buffer for 2 h.

Experimental Example 1

Detection of Troponin I and Diagnosis of AMI Patients

[0080] To the three-dimensional diagnostic system consisting of anti-Troponin I antibodies, HBV capsid-derived chimeric nanoparticles prepared in Example 2, and nickel nanohair structure (or PVDF membrane) prepared in Example 1, 200 μl Troponin (human cardiac Troponin I-T-C complex, Cat. No. 8T62, HyTest, Finland) that had been properly diluted in PBS buffer or human serum (AMI patient or healthy serum) was added, then stirred for 20 s, and incubated at room temperature for 1 h. After washing for 5 min using PBS buffer, 200 μl mouse anti-Troponin I monoclonal antibodies (3.2 μg/ml, Cat. No. 4T21, HyTest, Finland) in PBS buffer was added, stirred for 20 s, incubated at room temperature for 1 h, and then washed for 5 min using PBS buffer. 200 μl Q-dot (CdSe)-secondary Ab conjugate [1 nM, Qdot 655-Goat F(ab')2 anti-mouse IgG conjugate, Cat. No. Q11021MP, Invitrogen, Carlsbad, Calif., USA] was added, stirred for 20 s, incubated at room temperature for 1 h, and finally washed for 10 min with PBS buffer. Photoluminescence was then measured using a microplate reader (GENios, Tecan, Austria) with excitation and emission at 420 and 650 nm, respectively.

[0081] All the ELISA assay experiments in the present invention were conducted using the commercial ELISA Troponin assay kit (Troponin I EIA, Cat. No 25-TR1HU-E01, 96 wells, ALPCO Diagnostics, NH, USA) that was developed for in vitro diagnostic use. In short, it is as follows: 1) 100 μl of human serum (AMI patient or healthy serum) or Troponin (human cardiac Troponin I-T-C complex, Cat. No. 8T62, HyTest, Finland) in PBS buffer was added to antibody-coated 96-wells microplate provided by a provider; 2) 100 μl of enzyme conjugated reagent (containing HRP enzyme-conjugated anti-Troponin I antibodies) was added to each well, stirred sufficiently for 30 s, incubated at room temperature for 90 min, and then washed five times with distilled water; 3) after the well was allowed to strike hard it on an absorption paper in order to remove the entire remained water drop, 100 μl of "TMB reagent (containing the substrate to HRP enzyme)" was added to each well, mixed for 5 s, and then incubated at room temperature for 20 min; 4) 100 μl of "Stop solution" was added to each well in order to stop the enzyme reaction, then mixed for 30 s, and then the absorbance was measured by using the microplate reader (GENios, Tecan, Austria) at 420 nm.

[0082] Troponin I EIA provides a reliable assay for the quantitative measurement of human cardiac-specific Troponin I with a clinical specificity of 87.5 The procedure disclosed in Troponin I EIA protocol was strictly followed for the Troponin I assay, and the assay procedure is as follows. The entire list of AMI patients and healthy sera are disclosed in Table 2.

TABLE 2

No.	Age	Sex	Hospital
AMI Patient Serum			
1	74	M	KangNam Sacred Heart Hospital
2	75	F	
3	58	M	
4	68	M	
5	63	M	
6	80	M	
7	59	M	
8	68	M	
9	74	M	
10	77	F	
11	86	F	
12	85	F	
13	52	F	
14	43	M	
15	68	M	
16	59	F	
17	84	F	
18	58	M	
19	37	M	
20	42	M	Korea University Medical Center
21*	3	F	
22	91	M	
23	72	F	
24	79	F	
25	89	F	
26	57	M	
Healthy serum			
1	34	M	Korea University Medical Center
2	24	M	
3	28	M	
4	25	F	
5	45	M	
6	24	M	
7	27	M	
8	24	F	
9	31	F	
10	25	M	
11	30	F	
12	24	M	
13	26	F	
14	48	M	
15	26	F	
16	29	M	

[*Person experienced with a myocardium damage due to a heart septal defect operation using an extracorporeal circulation]

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aaagctgac 189

<210> SEQ ID NO 12
<211> LENGTH: 234
<212> TYPE: DNA
<213> ORGANISM: Hepatitis B virus

<400> SEQUENCE: 12

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tctgacttct ttccttctat tcgagatctc ctcgacaccg cctctgctct gtatcgggag 120

gccttagagt ctccggaaca ttgttcacct caccatacag cactcaggca agctattctg 180

tgttggggtg agttgatgaa tctggccacc tgggtgggaa gtaatttgga agac 234

<210> SEQ ID NO 13
<211> LENGTH: 78
<212> TYPE: PRT
<213> ORGANISM: Hepatitis B virus

<400> SEQUENCE: 13

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Met Asp Ile Asp Pro Tyr Lys Glu Phe Gly Ala Ser Val Glu Leu Leu
1               5                   10                  15

Ser Phe Leu Pro Ser Asp Phe Phe Pro Ser Ile Arg Asp Leu Leu Asp
            20                  25                  30

Thr Ala Ser Ala Leu Tyr Arg Glu Ala Leu Glu Ser Pro Glu His Cys
        35                  40                  45

Ser Pro His His Thr Ala Leu Arg Gln Ala Ile Leu Cys Trp Gly Glu
    50                  55                  60

Leu Met Asn Leu Ala Thr Trp Val Gly Ser Asn Leu Glu Asp
65                  70                  75


<210> SEQ ID NO 14
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<212> TYPE: DNA
<213> ORGANISM: Hepatitis B virus

<400> SEQUENCE: 14

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ttgtggtttc acatttcctg tcttactttt ggaagagaaa ctgttcttga gtatttggtg    120

tcttttggag tgtggattcg cactcctccc gcttacagac caccaaatgc ccctatctta    180

tcaacacttc cggaaactac tgttgtt                                        207


<210> SEQ ID NO 15
<211> LENGTH: 69
<212> TYPE: PRT
<213> ORGANISM: Hepatitis B virus

<400> SEQUENCE: 15

Ser Arg Glu Leu Val Val Ser Tyr Val Asn Val Asn Met Gly Leu Lys
1               5                   10                  15

Ile Arg Gln Leu Leu Trp Phe His Ile Ser Cys Leu Thr Phe Gly Arg
            20                  25                  30

Glu Thr Val Leu Glu Tyr Leu Val Ser Phe Gly Val Trp Ile Arg Thr
        35                  40                  45

Pro Pro Ala Tyr Arg Pro Pro Asn Ala Pro Ile Leu Ser Thr Leu Pro
    50                  55                  60

Glu Thr Thr Val Val
65


<210> SEQ ID NO 16
<211> LENGTH: 63
<212> TYPE: PRT
<213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 16

Ala Pro Lys Ala Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe
1               5                   10                  15

Tyr Glu Ile Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Gly
            20                  25                  30

Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu
        35                  40                  45

Ala Glu Ala Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys Ala Asp
    50                  55                  60
```

What is claimed is:

1. A nanohair structure comprising:

(a) nanotemplate comprising a plurality of pores; and

(b) a plurality of nanowires grown through the pores of the nanotemplate, wherein one portion of the nanowires is embedded inside the nanotemplate, while the other portion of the nanowires is exposed vertically on top of the surface of the nanotemplate.

2. The nanohair structure according to claim **1**, wherein the nanowire is derived from one of transition metal species which is selected from the group consisting of Ni, Fe, Co, Ni, Cu, Ag, Au, Pd and Pt, alloy thereof or oxide, nitride or carbide thereof.

3. The nanohair structure according to claim **1**, wherein the nanotemplate is anodized aluminum oxide (AAO) or poly-carbonate membrane.

4. The nanohair structure according to claim **1**, wherein the nanowire is derived from electrically conductive polymer.

5. A method of the nanohair structure comprising, a) preparing a nanotemplate with a plurality of pores; b) generating electrode layer on one side of the nanotemplate; c) putting the nanotemplate into the solution containing the metal ion and growing the metal nanowire through pores of the nanotemplate by electrodeposition employing it as a cathode; d) planarizing the metal nanowire through a process of chemical mechanical planarization (CMP); and e) selectively reactive ion etching (RIE) of the nanotemplate.

6. The method of the nanohair structure according to claim **5**, wherein the thickness of the layer of step b) is 250-350 nm.

7. The method of the nanohair structure according to claim **5**, wherein the metal ion is selected from the group consisting of Ni, Fe, Co, Ni, Cu, Ag, Au, Pd and Pt ion.

8. The method of the nanohair structure according to claim **5**, wherein the method further comprises the step of modifying the exposed part of the nanohair into oxide, nitride or carbide of metal or alloy thereof through post-treatment process of oxidation, nitrification or carburization of the exposed part after preparing the nanohair structure.

9. The method of the nanohair structure according to claim **5**, wherein the nanotemplate is anodized aluminum oxide or poly-carbonate membrane.

10. A three-dimensional nanostructure-based nanosensor fabricated by adding chimeric nanoparticles to the nanohair structure according to claim **1** and immobilizing the chimeric nanoparticles to the nanohair structure.

11. The three-dimensional nanostructure-based nanosensor according to claim **10**, wherein the chimeric nanoparticle is a HBV-derived chimeric protein.

12. The three-dimensional nanostructure-based nanosensor of claim **10**, wherein the nanosensor further comprises an antibody which recognizes a specific disease marker.

13. The three-dimensional nanostructure-based nanosensor according to claim **12**, wherein the specific disease marker is Troponin I.

14. A nanowire-based electrode comprising nanohair structure according to claim **1**.

* * * * *