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(54) DNA SENSOR AND MEASURING METHOD USING THE SAME

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(57) **ABSTRACT**

A DNA sensor is provided which is capable of identifying unknown DNA with enhanced detection sensitivity of the hybridization. A p-channel field-effect transistor having an electrolyte solution gate **8** and having as a p-channel **5** a diamond surface **2** which contains a mixture of at least a hydrogen-terminated surface and a surface terminated with an amino group or a molecule with an amino group is configured along with a probe DNA **11** constituted of a single-stranded DNA with known nucleotide sequence which is directly immobilized by a linker to the diamond surface **2** and with a target DNA constituted of an unknown single-stranded DNA which is dropped on the diamond surface **2.** When the target DNA is in complementary relationship to the probe DNA **11,** negative electric charge of the phosphate group of a double-stranded DNA produced by the hybridization of the probe DNA **11** with the target DNA both constituted of a single-stranded DNA is doubled, thereby resulting in increase of the hole density in the p-channel and shift of the threshold voltage of the p-channel field effect transistor toward positive direction. By detecting this shift of the threshold voltage toward positive direction, an identification can be made on whether or not the target DNA is in complementary relationship to the probe DNA **11.**

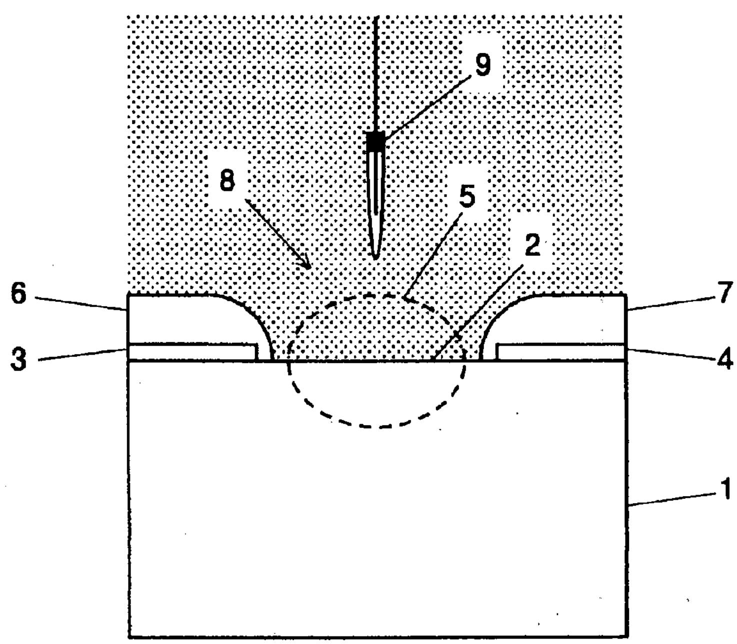


FIG. 1

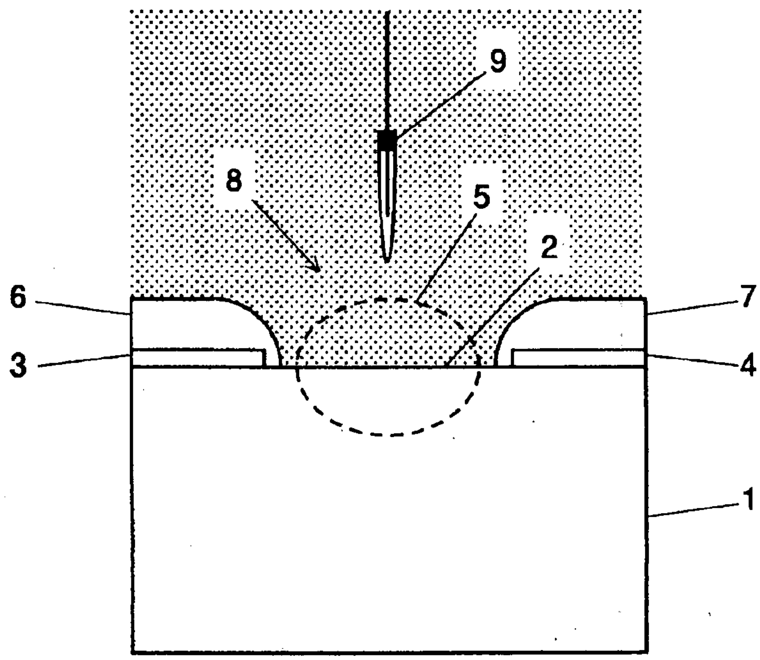


FIG. 2

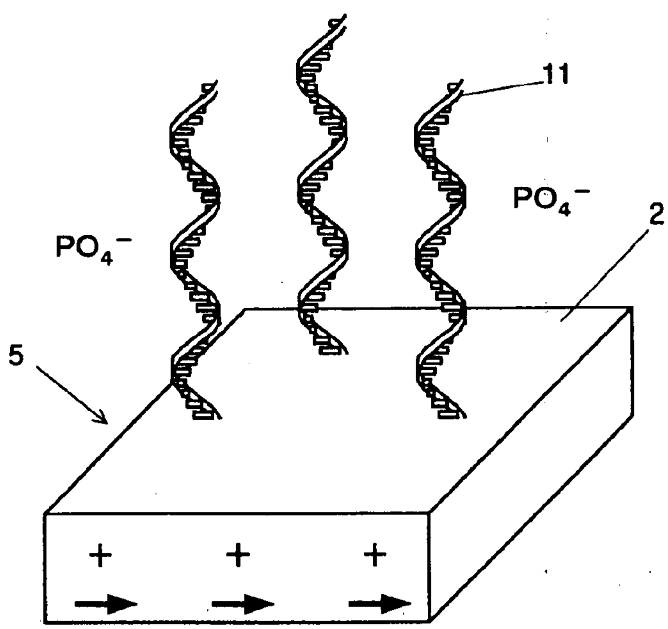


FIG. 3

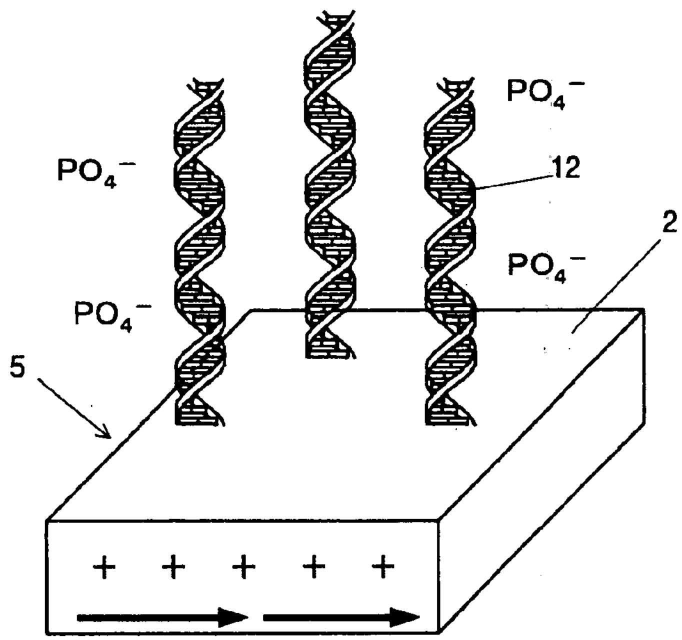

PO4−
PO4−
PO4−
PO4−
12
2
5
+ + + + +

FIG. 4

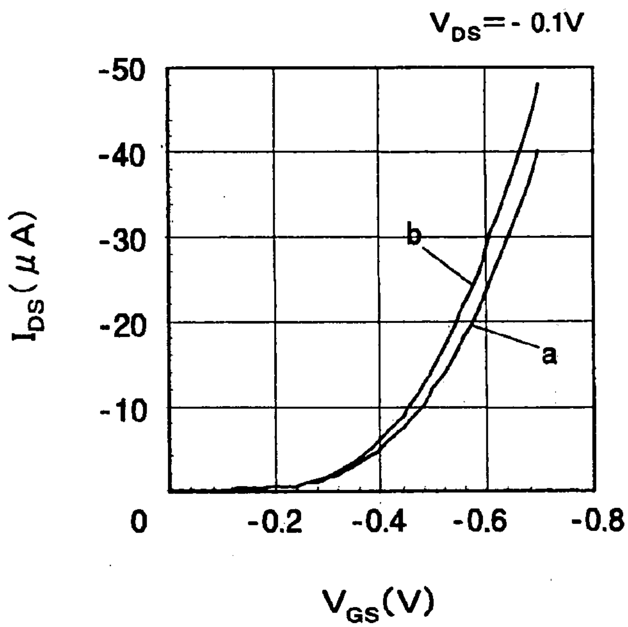
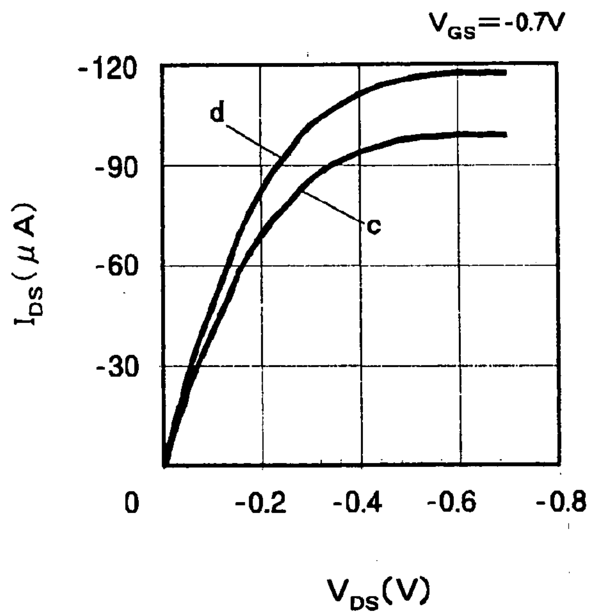
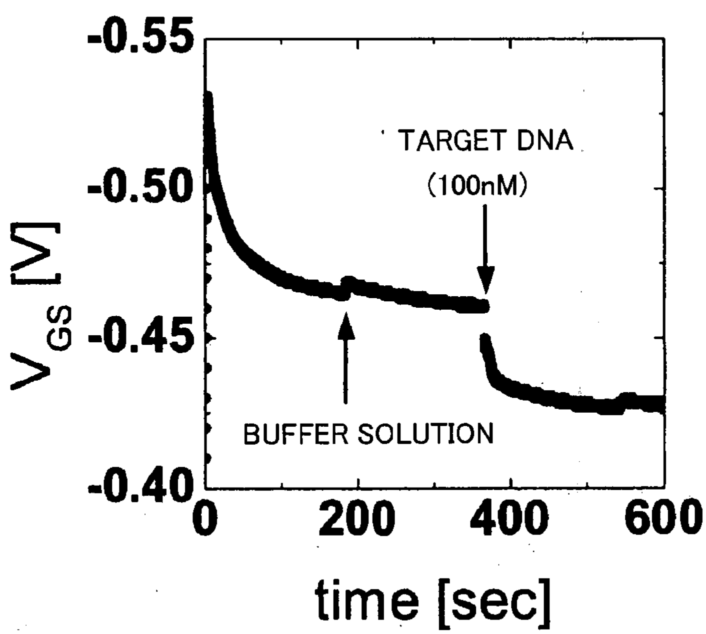

VDS = - 0.1V
-50
-40
-30
-20
-10
0
-0.2
-0.4
-0.6
-0.8
IDS (μA)
VGS (V)
b
a

FIG. 5



V_{GS}=-0.7V
-120
-90
-60
-30
d
c
I_{DS}(μA)
0
-0.2
-0.4
-0.6
-0.8
V_{DS}(V)

FIG. 6



-0.55
-0.50
-0.45
-0.40
V_{GS} [V]
TARGET DNA
(100nM)
BUFFER SOLUTION
0
200
400
600
time [sec]

FIG. 7

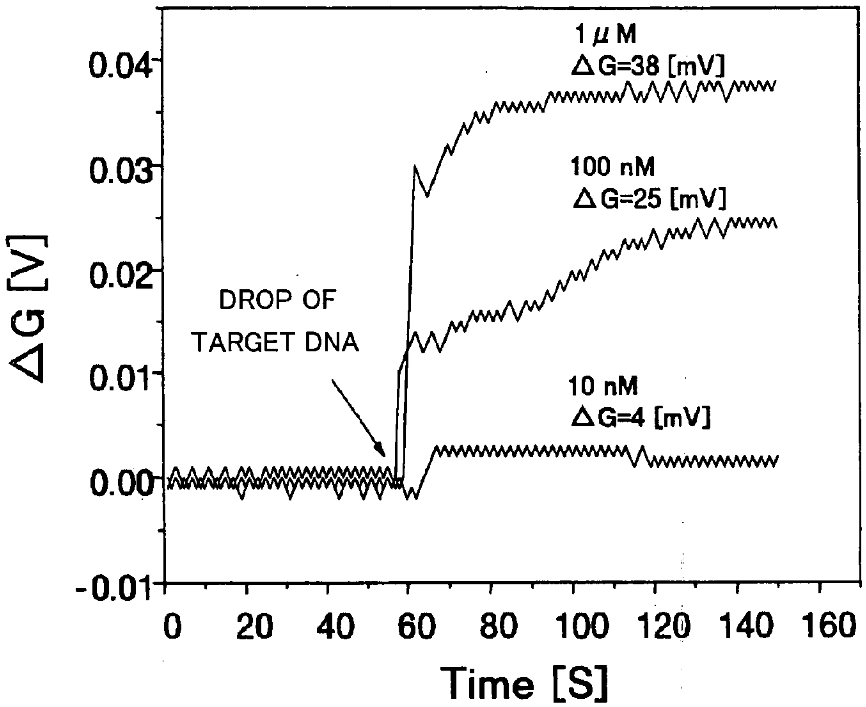

1 μM
ΔG=38 [mV]
100 nM
ΔG=25 [mV]
10 nM
ΔG=4 [mV]
DROP OF
TARGET DNA
ΔG [V]
0.04
0.03
0.02
0.01
0.00
-0.01
0
20
40
60
80
100
120
140
160
Time [S]

FIG. 8

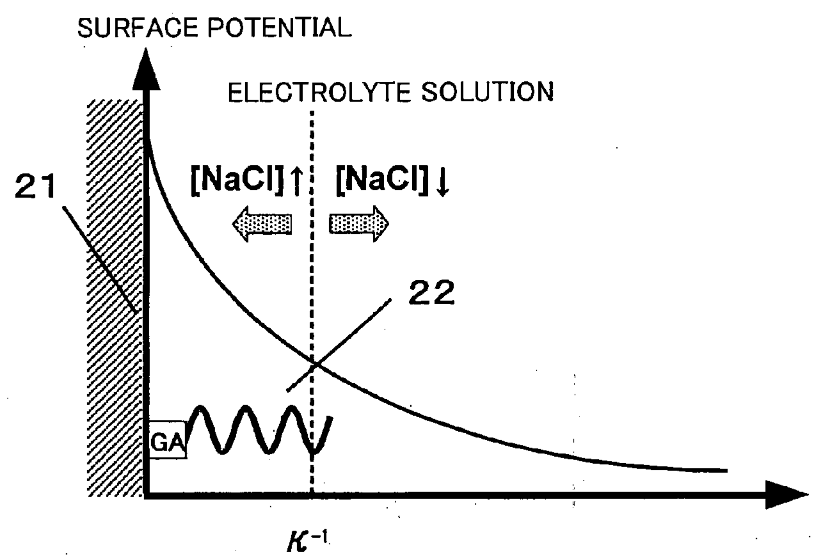
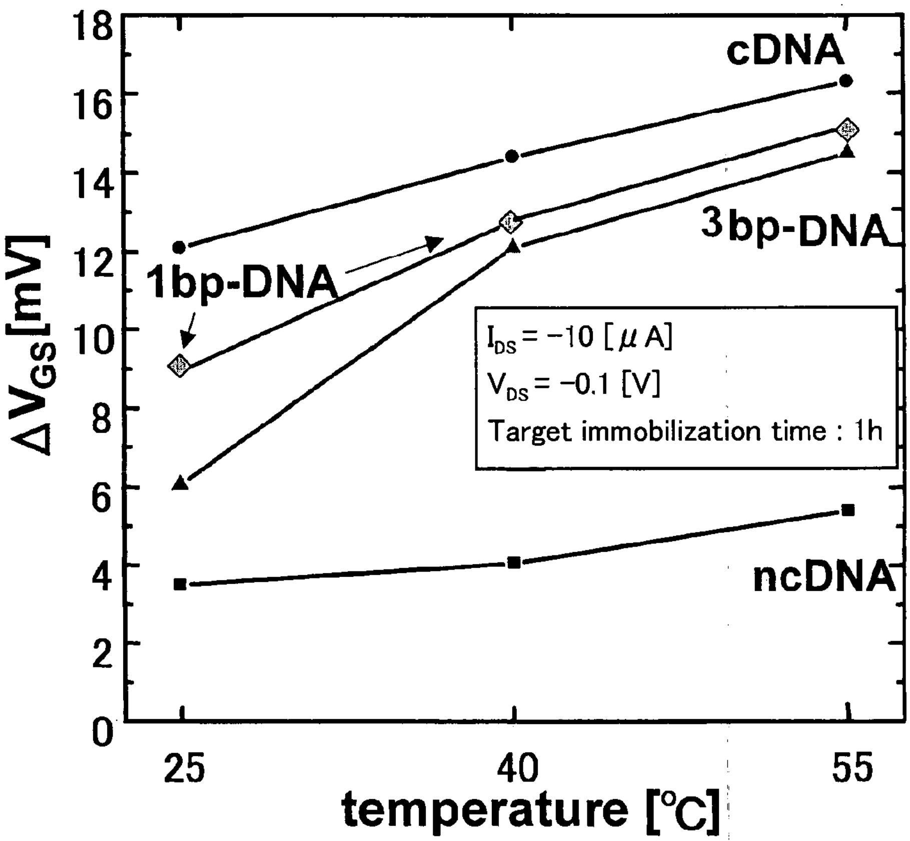

SURFACE POTENTIAL
ELECTROLYTE SOLUTION
21
[NaCl]↑
[NaCl]↓
22
GA
κ-1

FIG. 9


cDNA
1bp-DNA
3bp-DNA
ncDNA
I_{DS} = −10 [μA]
V_{DS} = −0.1 [V]
Target immobilization time : 1h
ΔV_GS[mV]
0
2
4
6
8
10
12
14
16
18
25
40
55
temperature [°C]

DNA SENSOR AND MEASURING METHOD USING THE SAME

TECHNICAL FIELD

[0001] The present invention relates to a biosensor, and more particularly relates to a DNA (Deoxyribonucleic Acid) sensor (DNA chip) having a p-channel field effect transistor and a measuring method using the DNA sensor.

BACKGROUND ART

[0002] Conventional technologies in this field are those described in the following.

[0003] (1) Fluorescence Detection Method

[0004] This fluorescence detection method is to immobilize a single-stranded DNA with known nucleotide sequence (probe DNA) on a glass substrate, silicon, diamond, or the like and to detect the hybridization (a phenomenon in which mutually complementary single-stranded DNAs are coupled to form a double-stranded DNA) with unknown single-stranded DNA (target DNA) by a fluorescent substance immobilized on the target DNA. A problem lies here, however, in large scale apparatus necessary to detect the hybridization by means of fluorescence in this method. Furthermore, a limitation is also present in realization in high density because the observation means is a fluorescence microscope.

[0005] (2) Electric Charge Detection Method

[0006] This electric charge detection method is based on a silicon ISFET(Ion Sensitive Field Effect Transistor). The sensitivity of the silicon ISFET is, however, too low to detect the doubling of the charge due to the hybridization of DNA.

[0007] (3) High Performance p-Channel Field Effect Transistor with Enhanced Threshold Voltage Resulting from Ozone Treatment, as Suggested by Present Inventors

[0008] This transistor is a p-channel field effect transistor having an electrolyte solution gate and having as a channel a diamond surface which is a mixture of a hydrogen terminated surface and an oxygen terminated surface produced by oxidization of the hydrogen terminated surface by an ozone treatment (see patent document 1 below).

[Patent Document 1]

[0009] Japanese Unexamined Patent Publication No. 2004-109020

DISCLOSURE OF INVENTION

[0010] The present invention is a further improvement of the charge detection method (2) (charge detection type DNA chip) described above, and aims at providing a DNA sensor and a measuring method using the DNA sensor capable of identifying an unknown DNA by increasing detection sensitivity of the hybridization by means of directly immobilizing a DNA on the diamond surface of a p-channel field effect transistor having an electrolyte solution gate and having as a channel a diamond surface which is a mixture of a hydrogen terminated surface and a surface terminated by an amino group or a molecule with an amino group as an amino termination.

[0011] To achieve the above objects, the present invention provides the following:

[0012] [1] A DNA sensor including a p-channel field-effect transistor having an electrolyte solution gate and having as a channel a diamond surface which contains a mixture of at least a hydrogen-terminated surface and a surface terminated by an amino group or a molecule with an amino group as an amino termination; a probe DNA constituted of a single-stranded DNA with known nucleotide sequence which is directly immobilized by a linker to the amino termination of the diamond surface; and a target DNA constituted of an unknown single-stranded DNA which is dropped on the diamond surface. When the target DNA is in complementary relationship to the probe DNA, due to a double-stranded DNA produced by the hybridization of the probe DNA with the target DNA both constituted of the single-stranded DNA, the threshold voltage of the p-channel field effect transistor shifts toward positive direction. The DNA sensor according to the present invention is characterized by that an identification on whether or not the target DNA is in complementary relationship to the probe DNA is performed by detecting the shift of this threshold voltage toward positive direction.

[0013] [2] In the DNA sensor according to the above item [1], the diamond surface contains an oxygen-terminated surface.

[0014] [3] In the DNA sensor according to one of the above items [1] and [2], the linker is divalent or trivalent carboxylic acid.

[0015] [4] In the DNA sensor according to one of the above items [1] and [2], the linker is divalent or trivalent aldehyde.

[0016] [5] In the DNA sensor according to one of the above items [1],[2],[3] and [4], the density of the probe DNA is equal to or greater than 10^{10} cm^{-2}, and the density of the target DNA is from 10^{-12}M to 10^{-6}M.

[0017] [6] In the DNA sensor according to one of the above items [1],[2],[3] and [4], the shifting difference of the threshold voltage toward positive direction is detected as a change in the gate voltage under a constant drain current.

[0018] [7] In the DNA sensor according to one of the above items [1],[2],[3] and [4], the shifting difference of the threshold voltage toward positive direction is detected as a change in the drain current under a constant gate voltage.

[0019] [8] In the DNA sensor according to one of the above items [1],[2],[3] and [4], the shifting difference of the threshold voltage toward positive direction is detected as a change in the drain current under a constant drain voltage.

[0020] [9] A measuring method using a DNA sensor including setting a p-channel field-effect transistor having as a gate an electrolyte solution and having as a channel a diamond surface which contains a mixture of at least a hydrogen-terminated surface and a surface terminated by an amino group or a molecule with an amino group as an amino termination; a probe DNA constituted of a single-stranded DNA with known nucleotide sequence which is directly immobilized by a linker to the amino termination of said diamond surface; and a target DNA constituted of an unknown single-stranded DNA which is dropped on said diamond surface, wherein an identification on whether or not said target DNA is in complementary relationship to said probe DNA is performed by detecting a shift of the threshold voltage of said p-channel field effect transistor toward positive direction, the shift resulting from a double-stranded DNA produced by hybridization of said probe DNA with said target DNA both constituted of the single-stranded DNA, the hybridization which occurs when said target DNA is in complementary relationship to said probe DNA.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. **1** illustrates a cross sectional view of an electrolyte solution gate field effect transistor (SGFET) according to the present invention.

[0022] FIG. **2** is a schematic diagram of a channel showing characteristics (No. 2).

[0023] (1) As shown in FIG. **1**, a p-channel **5** made of a diamond surface **2** is formed sandwiched between a source electrode **3** and a drain electrode **4** on the diamond surface **2** of an undoped polycrystalline diamond layer **1**. The diamond surface **2** contains a mixture of a hydrogen terminated and an amino terminated surfaces, and insulating layers **6** and **7** of polyimide resin are formed on the source electrode **3** and the drain electrode **4**, respectively. On the p-channel **5**, a gate **8** is formed of an electrolyte solution. Reference numeral **9** represents a reference electrode disposed in the electrolyte solution **8**. In addition, a substrate is not limited to the polycrystalline diamond layer, but a single crystal diamond layer or nano crystalline diamond may be used alternatively.

[0024] In this way, a p-channel field-effect transistor having an electrolyte solution as a gate **8** and having as a p-channel **5** a diamond surface **2** which contains a mixture of a hydrogen-terminated and an amino-terminated surfaces is prepared.

[0025] (2) Next, as shown in FIG. **2**, a probe DNA **11** constituted of a single stranded DNA with known nucleotide sequence is directly immobilized covalently onto the amino termination on the p-channel **5** of the diamond surface **2** by bridge reaction through a linker (for example, divalent or trivalent carboxylic acid (succinic acid, phthalic acid) or divalent or trivalent aldehyde (glutaraldehyde)). In this case the probe DNA **11** is immobilized at a density as high as equal to or greater than 10^{10} cm^{-2}.

[0026] In addition, an amino termination in the embodiment refers not only to the case where an amino group is stuck directly on the diamond surface **2** but also to the case where an amino group is stuck on an end of a molecule immobilized on the diamond surface. In this sense, description can be changed to a surface terminated by an amino group or a molecule containing an amino group.

[0027] Furthermore, as the linker, an acid or an acid compound is used for example, and divalent or trivalent carboxylic acid (COOH group) and divalent or trivalent aldehyde (COH group) are preferable. surface which contains a mixture of a hydrogen-terminated surface and an amino-terminated surface was prepared. A probe DNA with known nucleotide sequence and with a concentration of equal to or greater than 10^{10} cm^{-2} was directly immmobilized through glutaraldehyde (divalent aldehyde) to the diamond surface by bridge bonding. Target DNAs each complementary and not complementary to this probe DNA with a concentration between 10^{-12}M to 10^{-6}M were dropped on the above described SGFET to which the probe DNA was immobilized. Under a constant drain current condition, real time measurement of the gate voltage change due to the hybridization was performed. As a result, positive direction shift of the gate voltage by 4 mV, 25 mV and 38 mV could be observed at the target DNA concentration of 10 nM, 100 nM, and 1 μM, respectively. No shift was detected for the target DNA not in complementary relationship.

[0028] As described above, the DNA sensor in accordance with the present invention is suitable for real time detection of DNA, and along with biocompatibility of the diamond channel surface, it is expected as a device for practical usage in the clinical application.

[0029] Next, optimization in the concentration of buffer (NaCl) solution was examined experimentally. FIG. **8** is a diagram showing Debye length (Debye screening length) used immobilizing technology which is being made possible using diamond and by the size reduction in SGFET, the present invention can be expected for higher sensitivity detection than the conventional semiconductor biosensor or optical detection biosensor by using fluorescent label. Since reduction of specimen quantity necessary for measurement becomes possible by using the present DNA sensor, it can be used in the daily inspection and urgent inspection in the clinical inspection room. A device for detecting electric charge and electric potential suitable for integrated nanoscale device including other functions is also possible to realize.

[0030] In the above description, the diamond surface is referred to as a surface containing a mixture of a hydrogen-terminated surface and an amino-terminated surface. Additionally, an oxygen-terminated surface may be contained in the diamond surface as long as the occupancy rate of the oxygen-terminated surface is kept not to damage the function of the DNA sensor.

INDUSTRIAL APPLICABILITY

[0031] The DNA sensor in accordance with the present invention is suitable for realtime detection of DNA, and by making use of the biocompatibility of the diamond channel surface, this device can be utilized as a device for clinical application.

[0032] In addition, the present invention is expected to have a wide range of application as a mass consumption sensor not only in the medical field but also in food inspection, environmental measurement and the like, by reducing the device cost realized by mean of mass production. This invention is thus of great significance with economical and social influences.

[0033] Furthermore, due to the biocompatibility of carbon, the

1. A DNA sensor comprising:

(a) a p-channel field-effect transistor having as a gate an electrolyte solution and having as a channel a diamond surface which contains a mixture of at least a hydrogen-terminated surface and a surface terminated by an amino group or a molecule with an amino group as an amino termination;

(b) a probe DNA constituted of a single-stranded DNA with known nucleotide sequence which is directly immobilized by a linker to the amino termination of said diamond surface; and

(c) a target DNA constituted of an unknown single-stranded DNA which is dropped on said diamond surface,

wherein an identification on whether or not said target DNA is in complementary relationship to said probe DNA is performed by detecting a shift of the threshold voltage of said p-channel field effect transistor toward positive direction the shift resulting from a double-stranded DNA produced by hybridization of said probe DNA with said target DNA both constituted of the single-stranded DNA, the hybridization which occurs when said target DNA is in complementary relationship to said probe DNA.

2. The DNA sensor according to claim 1 wherein said diamond surface contains an oxygen-terminated surface.

3. The DNA sensor according to claim 1 or 2 wherein said linker is divalent or trivalent carboxylic acid.

4. The DNA sensor according to claim 1 or 2 wherein said linker is divalent or trivalent aldehyde.

5. The DNA sensor according to one of claims **1**, **2**, **3**, and **4** wherein the density of said probe DNA is equal to or greater than 10^{10} cm^{-2}, and the density of said target DNA is from 10^{-12}M to 10^{-6}M.

6. The DNA sensor according to one of claims **1**, **2**, **3**, and **4** wherein said shifting difference of the threshold voltage toward positive direction is detected as a change in the gate voltage under a constant drain current.

7. The DNA sensor according to one of claims **1**, **2**, **3**, and **4** wherein said shifting difference of the threshold voltage toward positive direction is detected as a change in the drain current under a constant gate voltage.

8. The DNA sensor according to one of claims **1**, **2**, **3**, and **4** wherein said shifting difference of the threshold voltage toward positive direction is detected as a change in the drain current under a constant drain voltage.

9. A measuring method using a DNA sensor comprising: setting

(a) a p-channel field-effect transistor having as a gate an electrolyte solution and having as a channel a diamond surface which contains a mixture of at least a hydrogen-terminated surface and a surface terminated by an amino group or a molecule with an amino group as an amino termination;

(b) a probe DNA constituted of a single-stranded DNA with known nucleotide sequence which is directly immobilized by a linker to the amino termination of said diamond surface; and

(c) a target DNA constituted of an unknown single-stranded DNA which is dropped on said diamond surface,

wherein an identification on whether or not said target DNA is in complementary relationship to said probe DNA is performed by detecting a shift of the threshold voltage of said p-channel field effect transistor toward positive direction, the shift resulting from a double-stranded DNA produced by hybridization of said probe DNA with said target DNA both constituted of the single-stranded DNA, the hybridization which occurs when said target DNA is in complementary relationship to said probe DNA.

10. (canceled)

11. (canceled)

12. (canceled)

13. (canceled)

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