

Analyte sensor and fabrication thereof

FIELD OF INVENTION

5 The invention relates generally to an analyte sensor.

BACKGROUND

10 Microelectrode technology is predominantly used in sensor work and is advantageous over conventional electrode sensors for several key reasons:

1. Capable of functioning in highly resistive environments and is more sensitive in detecting trace amounts of analyte;
2. Possess very little capacitance and solution resistance effects, leading to higher mass transport rates;
- 15 3. When fashioned into an array assembly, the effect is an amplification of the current which further enhances the limits of detection.

 It has been found that incorporating graphene into the design of microelectrodes can potentially enhance the performance of the microelectrode. This is due to several properties that graphene possess:

- 20 • A wide electrochemical window
- Strong affinity to single strand nucleic acids making it an ideal composite for biosensors
- Chemical stability
- Relatively inert electrochemistry
- 25 • Highly tunable to be selective to specific analytes
- Contains no metallic impurities which are common in conventional metallic based microelectrodes that can vary from batch to batch causing detection inconsistencies
- Flexible, giving the potential to be fabricated on plastic surfaces
- 30 • Low cost

35 Creating a single active graphene microelectrode site is relatively simple. For example, by isolating a single graphene sheet and attaching contact pads one can readily probe the characteristics of a single sheet and allow it to function as a microelectrode. However, repeating this process to isolate multiple sheets with the same size and shape, placing them on a substrate at precise distances and interconnecting these sheets to create an array configuration is a difficult task.

There is thus a need to address the above drawbacks to realise a sensor having a microelectrode array configuration.

SUMMARY

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According to a first aspect of the invention, there is provided an analyte sensor comprising a passivation layer comprising at least one nano-sized to micro-sized trench for receiving an analyte; an electrode disposed within the at least one nano-sized to micro-sized trench of the passivation layer, the electrode comprising
10 atomically thin electrode materials from any one or more of graphene, derivatives of graphene, silicene, molybdenum disulfide or boron nitride; and a conductive substrate layer having a portion in contact with at least the electrode.

According to a second aspect of the invention, there is provided an analyte sensor comprising an array of nano-sized to micro-sized electrodes to receive an
15 analyte, the array comprising an electrode indented in a passivation layer, the electrode comprising atomically thin electrode materials from any one or more of graphene, derivatives of graphene, silicene, molybdenum disulfide or boron nitride; and a conductive substrate layer having a portion in contact with at least the array of nano-sized to micro-sized electrodes.

According to a third aspect of the invention, there is provided a method of
20 fabricating an analyte sensor, the method comprising forming a conductive substrate layer; forming at least one electrode in contact with a portion of the conductive substrate layer, the electrode comprising any one or more of graphene, derivatives of graphene, silicene, molybdenum disulfide or boron nitride; and forming a passivation
25 layer having a nano-sized to micro-sized trench within which the at least one electrode is disposed.

BRIEF DESCRIPTION OF THE DRAWINGS

30 Example embodiments of the invention will be better understood and readily apparent to one of ordinary skill in the art from the following written description, by way of example only, and in conjunction with the drawings. The drawings are not necessarily to scale, emphasis instead generally being placed upon illustrating the principles of the invention, in which:

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Figure 1A shows a cross-section structure of an analyte sensor according to a preferred embodiment of the invention. Figure 1B shows a cross-section structure of an analyte sensor according to one embodiment of the invention.

Figure 2 shows cyclic voltammetry (CV) conducted on the analyte sensor of Figure 1A.

Figures 3A and 3B respectively show the response of a macroelectrode and a microelectrode array exposed to the same medium to which the analyte sensor of Figure 2 is exposed.

Figure 3C shows the CV response of the analyte sensor of Figure 2.

Figure 3D shows a CV comparison for the analyte sensor of Figure 1A and the microelectrode array used in Figure 3B. For Figures 3A to 3D, the E/V vs Ag/Cl axes represents a voltage being swept in a certain potential range determined by the reduction/oxidation potential of the redox analyte. In Figures 3A to 3D, ruthenium hexamine is used as the redox analyte, which has a CV potential range from -0.4V to 0V, as indicated by the reduction/oxidation peaks that occur in the graphs of Figures 3A to 3D.

Figures 4A and 4B show MCF-7 breast cancer cells that were grown on the surface of the analyte sensor of Figure 1A.

Figure 4C depicts the analyte sensor of Figure 1A under chronoamperometry.

Figure 5A shows a SEM (scanning electron microscope) image of a surface of an analyte sensor of Figure 1A.

Figure 5B shows an AFM (atomic force microscope) image of one electrode of the analyte sensor of Figure 1A.

Figure 6A shows a method to fabricate an analyte sensor, according to various embodiments of the invention.

Figures 6B and 6C show further steps of the method shown in Figure 6A.

Figures 7A to 7H each show a stage within an exemplary process, which implements the method described above with respect to Figures 6A to 6C, to fabricate the analyte sensor shown in Figure 1A.

DEFINITIONS

The following provides sample, but not exhaustive, definitions for expressions used throughout various embodiments disclosed herein.

The term "analyte" may refer to a substance, which may be a constituent of an electrolyte solution, so that embodiments of the present invention detect the presence of the substance in the electrolyte solution.

The term "nano-sized" to "micro-sized" may refer to the size of the trench, which is of nanometer to micrometer scale, leading to an electrode that is disposed inside the trench to have dimensions such as a diameter of around 10um and thickness of around 1 atom. The size of the analyte sensor depends on the number of such electrodes and can have a dimension that is in the micrometer scale.

The term "passivation layer" may refer to a passive, inert or insulating structure that prevents electron flow, so that such a structure provides for portions within the conductive substrate layer of the micro-sized sensor, according to various embodiments, that do not contribute to the exchange of electrons that occur when detecting the presence of the analyte.

The term "layer" may not necessarily refer to a flat planar structure and may take the shape of an earlier layer upon which the layer is formed. The layer may also not be continuous in that

The term "electrode" may mean an electrical conductor that acts as either an anode, when electrons leave the electrical conductor (i.e. the occurrence of oxidation); or a cathode, when electrons enter the electrical conductor (i.e. the occurrence of reduction).

The phrase "in contact with at least the electrode" may mean that - between the two structures of the passivation layer and the electrode - portions of the conductive substrate layer are in contact with at least the electrode, so that it is not essential that the passivation layer also contacts the conductive substrate layer, for the micro-sized sensor, according to various embodiments, to detect the presence of the analyte.

25 DETAILED DESCRIPTION

In the following description, various embodiments are described with reference to the drawings, where like reference characters generally refer to the same parts throughout the different views.

Figure 1A shows a cross-section structure of an analyte sensor 100 according to a preferred embodiment of the invention.

The analyte sensor 100 includes a passivation layer 102 comprising at least one nano-sized to micro-sized trench 104 for receiving an analyte (not shown). An electrode 106 is disposed within the at least one nano-sized to micro-sized trench 104 of the passivation layer 102. The electrode comprises atomically thin electrode materials from any one or more of graphene, derivatives of graphene, silicene, molybdenum disulfide or boron nitride. Derivatives of graphene may include reduced

graphene oxide and graphene oxide. The analyte sensor 100 also includes a conductive substrate layer 108, having a portion 110 that is in contact with at least the electrode 106.

Figure 1A shows that the micro-sized sensor 100 has a plurality of nano-sized to micro-sized trenches 104, with each nano-sized to micro-sized trench 104 having an indented electrode 106. However, the micro-sized sensor 100 would still work for an embodiment (not shown) having a passivation layer with a single nano-sized to micro-sized trench having an electrode disposed therein. The conductive substrate layer 108 is also provided on an optional layer of bulk substrate 112, which may be fabricated from, for example, glass.

In the preferred embodiment, the conductive substrate layer 108 is also in contact with the passivation layer 102. However, in another embodiment, such as that shown in Figure 1B, there is no contact between the conductive substrate layer and the passivation layer.

Figure 1B shows a cross-section structure of an analyte sensor 150 according to one embodiment of the invention.

In one such embodiment, the electrode 156 may be provided as an interface layer in full contact with the conductive substrate layer 108. The electrode layer interface 156 may have depressions/cavities 154 from which the passivation layer 102 extends, the passivation layer 102 also containing nano-sized to micro-sized trenches 104 that extend the entire thickness of the passivation layer 102 to expose portions of the electrode layer interface 156. Alternatively, in another embodiment (not shown) the electrode layer interface may have no such depressions/cavities, whereby the passivation layer is simply aligned onto the electrode layer interface.

Returning to Figure 1A, the electrode 106 indented in the passivation layer 102 provides an array of nano-sized to micro-sized electrodes to receive an analyte, with the conductive substrate layer 108 having a portion in contact with at least the array of nano-sized to micro-sized electrodes 106. Such an array configuration provides an enhancement property that increases the sensing capabilities of an electrode. The array configuration provides multiple active sites so that output from the analyte sensor 100 is enhanced, which allows for a lower limit of detection and enables the analyte sensor 100 to detect lower concentrations of analyte. This output enhancement is elaborated below with reference to Figures 2 and 3A to 3D below.

Figure 2 shows cyclic voltammetry (CV) conducted on the analyte sensor 100 of Figure 1A using 1M KCl (potassium chloride) electrolyte solution with a 2.5mM Ruthenium Hexamine redox agent (hereafter also referred to as the medium 218). In

the conducted CV, there are three electrodes required, of which only the third electrode which is being studied, i.e. the electrode 106, is shown. The other two electrodes which are not shown are a counter electrode (a platinum mesh) and a reference electrode (a Ag/Cl electrode). These two electrodes (the Platinum mesh
 5 counter electrode and the Ag/Cl reference electrode) allow the running of the CV experiment and are positioned in the medium 218 alongside the electrode 106. In the embodiment shown in Figure 2, graphene is used for the electrode 106, while conductive materials such as indium tin oxide (ITO), copper, platinum or gold, may be used for the conductive substrate layer 108 and polymethyl methacrylate (PMMA)
 10 used for the passivation layer 102. Other than PMMA, other suitable materials include those that are water insoluble, such as SU-8 (1-Methoxy-2-propanol acetate) and PDMS (polydimethylsiloxane).

Figure 2 shows a schematic of charge transfer and electron flow through the analyte sensor 100 when the redox agent is reduced. As the ruthenium hexamine
 15 comes into contact with the electrode 106 surface at potentials where oxidation or reduction occurs, a cathodic or anodic current is created, depending on whether the species is reduced or oxidised respectively. In the case of reduction, there is electron transfer from the redox species in the medium 218 to the surface of the active electrodes 106 of the analyte sensor 100. Increasing the number of active
 20 sites provides for a greater amount of electron movement. The electrons then flow from the electrode 106 into the conductive substrate layer 108 to be output from the analyte sensor 100. The electron flow direction depicted in Figure 2 is reversed when oxidation of the redox agent occurs. The response for the analyte sensor 100 of Figure 2 is shown in Figure 3C at different scan rates of 10, 25, 50, 100, 250 and
 25 500mV/s and is elaborated upon after the discussion below in respect of Figures 3A and 3B, which respectively show responses of a macroelectrode and a microelectrode array exposed to the same medium 218 shown in Figure 2.

In Figure 3A, the macroelectrode used (not shown) comprises an ITO conductive substrate layer provided on a glass bulk substrate (confer the analyte
 30 sensor 100 of Figure 1A, with the passivation layer 102 and the electrode 106 omitted). Similar to Figure 2, a counter electrode (a platinum mesh) and a reference electrode (a Ag/Cl electrode) are positioned in the 1M KCl electrolyte solution with a 2.5mM Ruthenium Hexamine redox agent alongside the macroelectrode. The CV
 302 exhibits standard macroelectrode CV behavior.

35 Figure 3B shows the response of a microelectrode array (not shown) with a structure similar to that of the analyte sensor 100, but omitting the electrode 106. Similar to Figure 2, a counter electrode (a platinum mesh) and a reference electrode

(a Ag/Cl electrode) are positioned in the 1M KCl electrolyte solution with a 2.5mM Ruthenium Hexamine redox agent alongside the microelectrode array. Figure 3B shows a CV 304 displaying sigmoidal like behavior for different scan rates of 10, 25, 50, 100, 250 and 500mV/s, where each has a relatively smaller redox peak than that of CV 302 of Figure 3A. This is because the hole-to-hole separation is not large enough to completely isolate the electrical hemisphere during the voltage scan. Some partial overlapping occurs and causes slight depletion of redox species, causing a drop in current.

Figure 3C shows the CV 306 of the analyte sensor 100 of Figure 2 using graphene for the electrode 106. At small scan rates there is sigmoidal behavior, and as scan rates increase the redox peaks become more prominent. This is due to the fact that as scan rates increase, a microelectrode will tend towards a mixing of planar and radial diffusion at the active sites, giving a peak. This is also further enhanced by the fact that the dominant active sites are covered by the basal plane form of graphene, further hindering charge transfer and causing a greater amount of depletion, which results in a larger drop in current. However the sigmoidal shapes that results from the microelectrode array patterning allowed for an increase in output enhancement of analyte detection. Figure 3D shows the response for H_2O_2 sensing capability for a scan rate of 50mV/s, as graphene is known to be a good catalyst of peroxidase. Figure 3D shows a CV comparison for the analyte sensor 100 of Figure 1A (the graphs 310) and the microelectrode array used in Figure 3B (the graph 308). The devices were tested in a 20mM of H_2O_2 in 1mM Phosphate buffer saline solution. The analyte sensor 100 displayed superior catalytic properties towards H_2O_2 as compared to the microelectrode array. Also, the current generated during the redox of H_2O_2 was significantly higher for the analyte sensor 100 when compared to the microelectrode array used in Figure 3B.

It was also found that the analyte sensor 100 can also used in the sensing of the presence of cancer cells. Figures 4A and 4B show MCF-7 breast cancer cells that were grown on the surface of the analyte sensor 100. Phorbol 12-myristate 13-acetate (PMA) was injected into the solution medium with the MCF-7 cancer cells on the analyte sensor 100 to trigger the release of H_2O_2 . Figure 4C depicts the analyte sensor 100 under chronoamperometry. The spikes in the graph show the times at which PMA was injected into the solution triggering the release of H_2O_2 , signifying an immediate increase in current. This H_2O_2 was then oxidised resulting in an increase in cathodic current. While only the detection of peroxidase excreted by MCF-7 Breast Cancer cells is shown, the analyte sensor 100 can be tailored to detect other analytes due to the highly tunable nature of graphene.

Figure 5A shows a SEM (scanning electron microscope) image of a surface of an analyte sensor 100 of Figure 1A, where each of the electrodes 106 is disc shaped and has a diameter of around 10um, to provide an array of 10um disc shaped graphene sites, when graphene is used for the electrode 106. However, it is not essential that the electrode 106 is restricted to graphene. Metallic electrodes may be used instead, fabricated from any one or more of the materials: boron nitride, molybdenum disulfide (grown one atom thick, so as to exhibit semiconductor characteristics) and silicene (a one atom thick silicon layer). As shown in Figure 5A, the electrodes 106 are visible through nano-sized to micro-sized trenches made in the passivation layer 102. Figure 5B shows an AFM (atomic force microscope) image of one electrode 106. The inset picture 522 of Figure 5B shows a cross-sectional view of the electrode 106 taken along the line A-A', which shows that the passivation layer 102 has a thickness of around 250nm, which is sufficient to passivate the underlying conductive substrate layer 108 from contributing to the output signal of the analyte sensor 100. The raised level of the passivation layer 102 also gives protection to the electrode 106 from physical damage such as scratching.

Figure 6A shows a method 600 to fabricate an analyte sensor, according to various embodiments of the invention. The method comprises the following steps:

In step 602, a conductive substrate layer is formed. In step 604, at least one electrode in contact with a portion of the conductive substrate layer is formed. In step 606, a passivation layer having a nano-sized to micro-sized trench within which the at least one electrode is disposed is formed. With reference to step 606, the conductive substrate layer is preferably also in contact with the passivation layer.

The formation of the at least one electrode and the passivation layer of steps 604 and 606 may further comprise the steps shown in Figure 6B.

In step 608, an electrode layer is formed over the conductive substrate layer. In step 610, portions of the electrode layer are removed to expose the conductive substrate layer. The remaining portions of the electrode layer form the at least one electrode of step 604. In step 612, the passivation layer of step 606 of Figure 6A is formed over the at least one electrode and the conductive substrate layer. In step 614, portions of the passivation layer are removed to form the nano-sized to micro-sized trenches that expose the electrode.

In addition to the steps shown in Figure 6B, the formation of the at least one electrode and the passivation layer of steps 604 and 606 may also further comprise the steps shown in Figure 6C.

In step 618, a mask layer is formed over the electrode layer after the formation of the electrode layer of step 608. In step 618, the mask layer is patterned to retain the remaining portions of the electrode layer when portions of the electrode layer are removed to expose the conductive substrate layer. The passivation layer from step 612 is formed over the patterned mask layer, from step 618, in addition to the at least one electrode and the conductive substrate layer. The patterned mask layer of step 618 is removed when exposing the electrode in step 614.

Figures 7A to 7H each show a stage within an exemplary process, which implements the method 600 described above with respect to Figures 6A to 6C, to fabricate the analyte sensor 100 shown in Figure 1A. One advantage that the method 600, described above with respect to Figures 6A to 6C, provides is that it allows the fabrication of atomically thin electrode materials from graphene, derivatives of graphene (such as reduced graphene oxide and graphene oxide), silicene, molybdenum disulfide or boron nitride. Conventional mature processes, which can fabricate electrodes using materials such as gold and platinum, cannot be used to fabricate such atomically thin electrode materials.

In Figure 7A, a conductive substrate layer 108 (comprising, for example, ITO) on a bulk substrate 112 (comprising, for example, glass substrate) is provided. The conductive substrate layer 108 and the bulk substrate 112 stack is cleaned.

An electrode layer (comprising, for example, graphene) is grown via a chemical vapour deposition (CVD) onto copper foil (not shown). In Figure 7B, the electrode layer 706 (which is later processed, see Figure 7E, to form the electrode 106) is then transferred onto the ITO and glass substrate structure of Figure 7A by forming over the conductive substrate layer 108.

In Figure 7C, a mask layer 716 (comprising, for example, 10% w/w Polyvinyl alcohol (PVA) solution) is spincoated onto the graphene, ITO and glass substrate stack of Figure 7B by forming the mask layer 716 over the electrode layer 706.

In Figure 7D, the mask layer 716 is imprinted, using for example a nanoimprint lithograph (NIL) technique, over a two-step process to pattern the mask layer 716. The first step is performed at 50 bars at 27°C for 3 minutes and the second step is performed at 50 bars at 110°C for 10 minutes. The imprinting results in a patterned mask layer 718.

Upon demolding, the patterned mask layer 718 is etched (using, for example, reactive ion etching), as shown in Figure 7E, to transfer the shape of the patterned mask layer 716 onto the electrode layer 706. The etch removes portions of the underlying electrode layer 706 and exposes the conductive substrate layer 108. The portions of the electrode layer 706 that remain are those covered by the residual

protective layer of the patterned mask layer 718. The remaining portions of the underlying electrode layer 706 form the electrodes 106.

In Figure 7F, a passivation layer 102 (comprising, for example, a 1µm thick layer of poly methyl methacrylate PMMA) is then formed (by, for example, spincoating) over the patterned mask layer 718, in addition to the electrodes 106 and the conductive substrate layer 108. A subsequent etch is carried out, as shown in Figure 7G, on the passivation layer 102, under controlled timing to remove a sufficient portion of the passivation layer 102 to expose the patterned mask layer 718

In Figure 7H, the sample 720 is placed in deionised (DI) water bath at 60°C overnight under gentle stirring to dissolve the patterned mask layer 718, thereby exposing the electrode 106. Accordingly, in Figures 7G and 7H, nano-sized to micro-sized trenches are formed in the passivation layer 102 to expose the electrodes 106, whereby in forming these nano-sized to micro-sized trenches, the patterned mask layer 718 is removed.

The methods shown in Figures 6A to 6C and 7A to 7H describe a method to fabricate a graphene based microelectrode array, according to various embodiments of the invention. Graphene based microelectrodes have more favourable characteristics than microelectrodes without graphene. Also, while an array configuration would further improve the performance of graphene based electrodes, a graphene microelectrode array has not yet been fully realised. Thus, the various embodiments of the invention described above provide a method to successfully fabricate a graphene based microelectrode array, being an improvement over single graphene electrodes that exist.

The various embodiments of the invention address the challenge of making graphene active sites with the same dimensions throughout an array and being able to place them on a conductive substrate in a manner that only the graphene is exposed to the environment, with the rest of the conductive substrate being protected by an insulating layer. An existing method deposits graphene on a conductive layer. However once the graphene has been shaped, passivating the underlying conductive layer becomes a problem.

Current techniques include randomly isolating a graphene layer and then attaching electrode contacts onto the graphene sheet. However this is a tedious process, having to first isolate a single graphene sheet and then manipulating the process to deposit the contacts in exact positions. Other limitations are that graphene sheets do not naturally occur in uniform size and shape unless they have undergone pre-fabrication. This poses a large hurdle in moving towards graphene sensor arrays.

Various embodiments of the present invention overcome the problem of fabricating a graphene microelectrode array by allowing:

- i) A graphene sheet to be tailored in size and shape to form the active area of the device;
- 5 ii) Tailoring of this active area can be scaled to multiple sheets per imprint, increasing the number of active areas on the device surface;
- iii) Utilization of two resists (PVA and PMMA) with differing soluble properties, allowing passivation of the conductive substrate and at the same time, allowing the graphene active area to be exposed to the environment;
- 10 and
- iv) Self alignment of the areas that need to be dissolved to expose the graphene and passivation of underlying conductive layer.

With reference to Figures 7A to 7H, nanoimprint technology is used due to the flexibility and relative ease of patterning a large array of active sites in one imprint.

15 This imprint pattern can also be easily controlled depending on the mold used. PVA is first used as the sacrificial layer that will allow patterning of the graphene sheet. PMMA is then employed to insulate the conductive ITO layer. The PMMA interfaces well with the Patterned PVA so that upon dissolution of the PVA, only graphene is exposed to the environment. Charge transfer then occurs between the graphene

20 interface and the underlying ITO layer that can be attached to a potentiostat to gather signal data.

Various embodiments of the present invention provide the following advantages:

- a) pattern and isolate graphene sheets on a conductive substrate. This allows for enhancement in sensor field due to the characteristics of graphene.
- 25 b) self alignment process through the use of PVA and PMMA at different fabrication stages, that allows multiple active sites to be fabricated on the sensor allowing an array characteristic without further manual alignment steps. This distinguishes graphene active areas that are required to be exposed and conductive substrate areas that need to be passivated so that
- 30 charge transfer occurs solely through graphene active sites.
- c) nanoimprint technology that allows a tunable resolution of the active sites by adjusting the pattern and shape of the graphene active areas. The size, shape and periodicity of the active sites can have an impact on analyte
- 35 selectivity.
- d) fabrication on a variety of substrates, e.g. glass, silicon, quartz, plastic, providing many possible applications for the sensor.

e) the possibility of using materials other than graphene for the active site material, i.e. a different electrode material, so as to tailor the sensor to detect different analytes.

5 f) provides a high yield fabrication process to create graphene (or any thin film) microelectrodes in an array configuration. This provides higher sensitivity and a lower detection limit, allowing detection of analytes where they occur in lower concentrations, e.g. glucose detection in tears/saliva instead of pinprick blood samples for diabetics. This facilitates detection of

10 It will be appreciated by a person skilled in the art that numerous variations and/or modifications may be made to the present invention as shown in the embodiments without departing from a spirit or scope of the invention as broadly described. The embodiments are, therefore, to be considered in all respects to be illustrative and not restrictive.

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CLAIMS

1. An analyte sensor comprising
 a passivation layer comprising at least one nano-sized to micro-sized trench
 5 for receiving an analyte;

an electrode disposed within the at least one nano-sized to micro-sized trench
 of the passivation layer, the electrode comprising atomically thin electrode materials
 from any one or more of graphene, derivatives of graphene, silicene, molybdenum
 disulfide or boron nitride; and

10 a conductive substrate layer having a portion in contact with at least the
 electrode.

2. The analyte sensor of claim 1 wherein there is no contact between the
 conductive substrate layer and the passivation layer.

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3. The analyte sensor of claim 1 wherein the conductive substrate layer is also
 in contact with the passivation layer.

4. The analyte sensor of any one of the preceding claims, wherein the
 20 conductive substrate layer comprises any one or more of the following materials
 indium tin oxide, copper, platinum or gold.

5. An analyte sensor comprising
 an array of nano-sized to micro-sized electrodes to receive an analyte, the
 25 array comprising an electrode indented in a passivation layer, the electrode
 comprising atomically thin electrode materials from any one or more of graphene,
 derivatives of graphene, silicene, molybdenum disulfide or boron nitride; and

a conductive substrate layer having a portion in contact with at least the array
 of nano-sized to micro-sized electrodes.

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6. A method of fabricating an analyte sensor, the method comprising
 forming a conductive substrate layer;
 forming at least one electrode in contact with a portion of the conductive
 substrate layer; and

forming a passivation layer having a nano-sized to micro-sized trench within which the at least one electrode is disposed.

5 7. The method of claim 6, wherein the conductive substrate layer is also in contact with the passivation layer.

8. The method of claim 7, wherein the formation of the at least one electrode and the passivation layer comprises the steps of:

forming an electrode layer over the conductive substrate layer;

10 removing portions of the electrode layer to expose the conductive substrate layer, wherein the remaining portions of the electrode layer form the at least one electrode;

forming the passivation layer over the at least one electrode and the conductive substrate layer; and

15 removing portions of the passivation layer to form the nano-sized to micro-sized trenches that expose the electrode.

9. The method of claim 8, wherein the formation of the at least one electrode and the passivation layer further comprises the steps of:

20 forming a mask layer over the electrode layer after the formation of the electrode layer;

patterning the mask layer to retain the remaining portions of the electrode layer when portions of the electrode layer are removed to expose the conductive substrate layer;

25 wherein the passivation layer is formed over the patterned mask layer in addition to the at least one electrode and the conductive substrate layer, and

wherein exposing the electrode further comprises removing the patterned mask layer.

30 10. The method of claims 6 to 9, wherein the electrode comprises any one or more of atomically thin electrode materials from graphene, derivatives of graphene, silicene, molybdenum disulfide or boron nitride.

Analyte sensor and fabrication thereof**ABSTRACT**

5 According to one aspect of the invention, there is provided an analyte sensor comprising a passivation layer comprising at least one nano-sized to micro-sized trench for receiving an analyte; an electrode disposed within the at least one nano-sized to micro-sized trench of the passivation layer, the electrode comprising atomically thin electrode materials from any one or more of graphene, derivatives of
10 graphene, silicene, molybdenum disulfide or boron nitride; and a conductive substrate layer having a portion in contact with at least the electrode.

Figure 1A