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(54) ELECTROCHEMICAL SENSOR FOR ANALYTE DETECTION

ELEKTROCHEMISCHER SENSOR ZUM NACHWEIS VON ANALYTEN

CAPTEUR ÉLECTROCHIMIQUE POUR LA DÉTECTION D'ANALYTE

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Description

RELATED APPLICATION

BACKGROUND

[0001] A typical disposable electrochemical sensor includes a substrate film upon which a layer of conductive material is deposited and patterned to form electrodes. Traditionally electrochemical cells, or biosensors, are comprised of three electrodes, a working electrode or sensing electrode, a reference electrode, and a counter electrode or auxiliary electrode. The working electrode is where the reaction of interest occurs at a fixed applied potential versus the reference electrode. The reference electrode functions to maintain a stable electrical potential on the working electrode. The counter electrode allows current to flow between the working electrode and the counter electrode so as not to disturb the reference electrode function. In cases when the system potential is inherently stable or small fluctuations in potential are not a concern, the reference and counter electrodes can be combined into a single reference/counter electrode paired with a working electrode. In some instances, electrochemical biosensors use amperometry to quantify specific analyte concentration(s). The working electrode provides a response proportional to its exposed surface area. During fabrication, the manufacture closely controls the process variation associated with the working electrode area.

[0002] Normally the working electrode is formed from two or more elements. One element is a conductive layer that forms the active element facilitating electron transfer to or from an electro-active species which are generated when the sample is applied to the sensor. A second element is a dielectric layer that defines, along with the first element, the actual dimensions of the working electrode that is in contact with the sample fluid. The second element forms a window over a portion of the conductive layer. Variation in either element may result in a variation in the sensor response. The second element or dielectric layer may therefore directly influence the accuracy of the reading.

[0003] In prior art electrodes, the surface areas may be defined by either conductive layer patterning or dielectric layer patterning and registration. EP 2 492 351 A2 discloses an electrochemical sensor for the detection of analytes in liquid media, which comprises four layers. US 2009/0017197 A1 discloses a fine resolution protein sensor fabricated with iridium oxide nanowire electrodes. WO 2004/061418 A2 discloses apparatuses, systems, kits and methods for conducting chemical, biochemical and/or biological assays on a sample, the apparatuses include assay cartridges and cartridge readers for conducting the assays. WO 2014/032044 A1 discloses a detection system for determining alpha-methylacyl-CoA (AMACR) levels in a bodily sample including at least one reaction solution for generating hydrogen peroxide upon combination with AMACR in the bodily sample and a biosensor for determining level of generated hydrogen peroxide. There is a need for a means of more accurately defining the sensor's working electrode to simplify the process of forming an accurate biosensor.

SUMMARY

[0004] Embodiments described herein relate to electrochemical biosensors that are capable of providing analysis of various analytes or biomolecules using biological recognition elements. The biosensor can produce a signal that is related to the presence or quantity of the analytes being detected in a sample, such as a biological sample. In some embodiments, the biosensor can be used to detect proteins, polypeptides, cytokines, micorganisms, polynucleotides (mRNA, DNA, cDNA, mRNA, etc.) that are present in a biological sample, such as a bodily fluid (*e.g.,* serum, blood, plasma, saliva, urine, mucous, breath, etc.).

[0005] In a first aspect, the present invention is directed to a sensor for the detection of an analyte in a biological sample comprising: a substrate, a working electrode formed on a surface of the substrate, a counter electrode formed on the surface of the substrate, a dielectric layer covering a portion of the working electrode and counter electrode and defining an aperture exposing other portions of the working electrode and counter electrode, a measuring device for applying voltage potentials to the working electrode and counter electrode and measuring the current flow between the working electrode and counter electrode, and a receptor functionalized or chemically functionalized onto a surface of the exposed portion of the working electrode, the receptor selectively binding to the analyte of interest and the analyte once bound being detectable by measuring the current flow between the working electrode and counter electrode, wherein the working electrode and the counter electrode are obtained from sputtering a metalized film on a plastic substrate and irradiating the metalized film using laser ablation to define the dimensions of the working electrode and the counter electrode, and wherein the receptor being functionalized onto the surface of the working electrode by cross-linking or biotinylating the surface and binding the receptor to the cross-linked or biotinylated surface.

[0006] In another aspect, the present invention is directed to a sensor for the detection of AMACR or an AMACR substrate thereof in a biological sample comprising: a substrate, a working electrode formed on a surface of the substrate, a counter electrode formed on the surface of the substrate, a dielectric layer covering a portion of the working electrode and counter electrode and defining an aperture exposing other portions of the working electrode and counter electrode, a measuring device for applying voltage potentials to the working electrode and counter electrode and measuring the current flow between the working electrode and counter electrode, and an antibody or antigen binding fragment to AMACR

or the AMACR substrate functionalized or chemically functionalized onto a surface of the exposed portion of the working electrode by cross-linking or biotinylating the surface and binding the antibody or antigen binding fragment to AMACR or the AMACR substrate to the cross-linked or biotinylated surface, the antibody or antigen binding fragment selectively binding to the AMACR or the AMACR substrate and the AMACR or the AMACR substrate once bound being detectable by measuring the current flow between the working electrode and counter electrode, wherein the working electrode and the counter electrode are obtained from sputtering a metalized film on a plastic substrate and irradiating the metalized film using laser ablation to define the dimensions of the working electrode and the counter electrode.

[0007] The interaction of the analyte and the receptor, *e.g.,* the bound analyte, can be detected using electrochemical analytical techniques, such as cyclic voltammetry (CV), differential pulse voltammetry (DPV), to determine the presence of the analyte in the biological sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008]

Fig. 1 is a schematic illustration of a biosensor in accordance with an aspect of the application.

Fig. 2 is a top plan view of an array of biosensors in a row manufactured by a screen-printing process.

DETAILED DESCRIPTION

[0009] Unless specifically addressed herein, all terms used have the same meaning as would be understood by those of skilled in the art of the subject matter of the application. The following definitions will provide clarity with respect to the terms used in the specification and claims.

[0010] As used herein, the term "monitoring" refers to the use of results generated from datasets to provide useful information about an individual or an individual's health or disease status. "Monitoring" can include, for example, determination of prognosis, risk-stratification, selection of drug therapy, assessment of ongoing drug therapy, determination of effectiveness of treatment, prediction of outcomes, determination of response to therapy, diagnosis of a disease or disease complication, following of progression of a disease or providing any information relating to a patient's health status over time, selecting patients most likely to benefit from experimental therapies with known molecular mechanisms of action, selecting patients most likely to benefit from approved drugs with known molecular mechanisms where that mechanism may be important in a small subset of a disease for which the medication may not have a label, screening a patient population to help decide on a more invasive/expensive test, for example, a cascade of tests from a non-invasive blood test to a more invasive option such as biopsy, or testing to assess side effects of drugs used to treat another indication.

[0011] As used herein, the term "quantitative data" or "quantitative level" or "quantitative amount" refers to data, levels, or amounts associated with any dataset components (*e.g.,* markers, clinical indicia,) that can be assigned a numerical value.

[0012] As used herein, the term "subject" refers to a human or another mammal. Typically, the terms "subject" and "patient" are used herein interchangeably in reference to a human individual.

[0013] As used herein, the term "bodily sample" refers to a sample that may be obtained from a subject (*e.g.,* a human) or from components (*e.g.,* tissues) of a subject. The sample may be of any biological tissue or fluid with, which analytes described herein may be assayed. Frequently, the sample will be a "clinical sample", *i.e.,* a sample derived from a patient. Such samples include bodily fluids, *e.g.,* saliva, breath, urine, blood, plasma, or sera; and archival samples with known diagnosis, treatment and/or outcome history. The term biological sample also encompasses any material derived by processing the bodily sample. Processing of the bodily sample may involve one or more of, filtration, distillation, extraction, concentration, inactivation of interfering components, and addition of reagents.

[0014] As used herein, the terms "normal" and "healthy" are used interchangeably. They refer to an individual or group of individuals who have not shown any symptoms of a disease, condition, or pathology to be detected, and have not been diagnosed with the disease, condition, or pathology. Preferably, the normal individual (or group of individuals) is not on medication. In certain embodiments, normal individuals have similar sex, age, body mass index-as compared-with the individual from, which the sample to be tested was obtained: The term "normal" is also used herein to qualify a sample isolated from a healthy individual.

[0015] As used herein, the terms "control" or "control sample" refer to one or more biological samples isolated from an individual or group of individuals that are normal (*i.e.,* healthy). The term "control", "control value" or "control sample" can also refer to the compilation of data derived from samples of one or more individuals classified as normal.

[0016] Embodiments described herein relate to electrochemical biosensors that are capable of providing analysis of various analytes or biomolecules using biological recognition elements. The biosensor can produce a signal that is related to the presence or quantity of the analytes being detected in a sample, such as a biological sample. In some embodiments, the biosensor can be used to detect proteins, polypeptides, cytokines, micorganisms, polynucleotides (mRNA, DNA, cDNA, mRNA, etc.) that are present in a biological sample, such as a bodily fluid (*e.g.,* serum, blood, plasma, saliva, urine, mucous, breath, etc.). The biosensors described herein can

provide a single use, disposable, and cost-effective means for simple point-of-care, real time assessment of analytes in biological samples, such as bodily fluids obtained by non-invasive or minimally invasive means.

[0017] Fig. 1 illustrates a biosensor 10 in accordance with an embodiment of the application. The sensor 10 is a three-electrode sensor including a counter electrode 12, a working electrode 14, and a reference electrode 16 that are formed on a surface of a substrate. A dielectric layer 40 covers a portion of the working electrode 12, counter electrode 14 and reference electrode 16. The dielectric layer 40 includes an aperture 20 that defines a detection region of the working electrode 12, counter electrode 14, and reference electrode 16, which is exposed to samples containing one or more analytes of interest to be detected. A receptor(s) for at least one analyte of interest can be functionalized or chemically functionalized to the working electrode. The receptor can bind selectively to one or more of the analytes of interest in the biological sample.

[0018] The biosensor can also include a voltage source 22 for applying a voltage potential to the working electrode, counter electrode, and/or reference electrode and a measuring device or current monitor 24 for measuring the current flow between the working electrode and counter electrode. The interaction of the analyte and the receptor can be detected using electrochemical analytical techniques, such as cyclic voltammetry (CV), -differential pulse voltammetry (DPV), to determine the presence of the analyte in the sample.

[0019] The working electrode 14 is poised at an appropriate electrochemical potential such that the current that flows through the electrode changes when the receptor binds to an analyte in the sample. The function of the counter electrode 12 is to complete the circuit, allowing charge to flow through the sensor 10. The working electrode 14 and the counter electrode 12 are preferably formed of the same material, although this is not a requirement. Examples of materials that can be used for the working electrode 14 and counter electrode 12 include gold, platinum, palladium, silver, carbon, alloys thereof, and composites thereof.

[0020] The receptor, which is functionalized or chemically functionalized to the working electrode, is a molecule that binds selectively to an analyte of interest. A molecule that binds selectively to an analyte is a molecule that binds preferentially to that analyte (*i.e.,* its binding affinity for that analyte is greater than its binding affinity for any other analyte). Its binding affinity for the analyte of interest may be 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 100-fold or more than its binding affinity for any other analyte. In addition to its relative binding affinity, the receptor must also have an absolute binding affinity that is sufficiently high to efficiently bind the analyte of interest (*i.e.,* it must have a sufficient sensitivity). Receptors having binding affinities in the picomolar to micromolar range are suitable. Such interaction can be reversible.

[0021] The receptor may be of any nature (*e.g.,* chemical, nucleic acid, peptide, lipid, combinations thereof). The analyte too may be of any nature provided there exists a receptor that binds to it selectively and in some instances specifically. In some embodiments, the analyte can be a charged species or molecule.

[0022] The term "functionalized" or "chemically functionalized," as used herein, means addition of functional groups onto the surface of a material by chemical reaction(s). As will be readily appreciated by a person skilled in the art, functionalization can be employed for surface modification of materials in order to achieve desired surface properties, such as biocompatibility, wettability, and so on. Similarly, the term "biofunctionalization," "biofunctionalized,", as used herein, means modification of the surface of a material so that it has desired biological function, which will he readily appreciated by a person of skill in the related art, such as bioengineering.

[0023] The receptors may be functionalized to the working electrode covalently or non-covalently. Covalent attachment of a receptor to working electrode may be direct or indirect (*e.g.,* through a linker). Receptors may be immobilized on the working electrode using a linker. The linker can be a linker that can be used to link a variety of entities.

[0024] In some embodiments, the linker may be a homo-bifunctional linker or a heterobifunctional linker, depending upon the nature of the molecules to be conjugated. Homo-bifunctional linkers have two identical reactive groups. Hetero-bifunctional linkers have two different reactive groups. Various types of commercially available linkers are reactive with one or more of the following groups: primary amines, secondary amines, sulphydryls, carboxyls, carbonyls and carbohydrates. Examples of amine-specific linkers are bis(sulfosuccinimidyl) suberate, bis[2-(succinimidooxycarbonyloxy)ethyl]sulfone, disuccinimidyl suberate, disuccinimidyl tartarate, dimethyl adipimate 2HCl, dimethyl pimelimidate 2HCl, dimethyl suberimidate HCl, ethylene glycol-bis-[succinimidyl-[succinate]], dithiolbis(succinimidyl propionate), and 3,3'-dithiobis(sulfosuccinimidylpropionate). Linkers reactive with sulfhydryl groups include bismaleimidohexane, 1,4-di-[3'-(2'-pyridyldithio)-propionamido)]butane, 1-[p-azidosalicylamido]-4-[iodoacetamido]butane, and N-[4-(p-azidosalicylamido)butyl]-3'-[2'-pyridyldithio]propionamide. Linkers preferentially reactive with carbohydrates include azidobenzoyl hydrazine. Linkers preferentially reactive with carboxyl groups include 4-[p-azidosalicylamido]butylamine.

[0025] Heterobifunctional linkers that react with amines and sulfhydryls include N-succinimidyl-3-[2-pyridyldithio]propionate, succinimidyl[4-iodoacetyl]aminobenzoate, succinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate, m-maleimidobenzoyl-N-hydroxysuccinimide ester, sulfosuccinimidyl 6-[3-[2-pyridyldithio]propionamido]hexanoate, and sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate.

Heterobifunctional linkers that react with carboxyl and amine groups include 1-ethyl-3-[3-dimethylaminopropyl]-carbodiimide hydrochloride. Heterobifunctional linkers that react with carbohydrates and sulfhydryls include 4-[N-maleimidomethyl]-cyclohexane-1-carboxylhydrazide HCl, 4-(4-N-maleimidophenyl)-butyric acid hydrazide 2HCl, and 3-[2-pyridyldithio]propionyl hydrazide.

[0026] Alternatively, receptors may be non-covalently coated onto the working electrode. Non-covalent deposition of the receptor to the working electrode may involve the use of a polymer matrix. The polymer may be naturally occurring or non-naturally occurring - and may be of any type including nucleic acid (*e.g.,* DNA, RNA, PNA, LNA, or mimics, derivatives, or combinations thereof), amino acids (*e.g.*, peptides, proteins (native or denatured), or mimics, derivatives, or combinations thereof, lipids, polysaccharides, and functionalized block copolymers. The receptor may be adsorbed onto and/or entrapped within the polymer matrix.

[0027] Alternatively, the receptor may be covalently conjugated or crosslinked to the polymer (*e.g.,* it may be "grafted" onto a functionalized polymer).

[0028] An example of a suitable peptide polymer is poly-lysine (*e.g.,* poly-L-lysine). Examples of other polymers include block copolymers that comprise polyethylene glycol (PEG), polyamides, polycarbonates, polyalkylenes, polyalkylene glycols, polyalkylene oxides, polyalkylene terepthalates, polyvinyl alcohols, polyvinyl ethers, polyvinyl esters, polyvinyl halides, polyvinylpyrrolidone, polyglycolides, polysiloxanes, polyurethanes, alkyl cellulose, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitrocelluloses, polymers of acrylic and methacrylic esters, methyl cellulose, ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, hydroxybutyl methyl cellulose, cellulose acetate, cellulose propionate, cellulose acetate butyrate, cellulose acetate phthalate, carboxylethyl cellulose, cellulose triacetate, cellulose sulphate sodium salt, poly(methyl methacrylate), poly(ethyl methacrylate), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate), polyethylene, polypropylene, poly(ethylene glycol), poly(ethylene oxide), poly(ethylene terephthalate), poly(vinyl alcohols), polyvinyl acetate, polyvinyl chloride, polystyrene, polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates), poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate), poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, polyanhydrides, poly(styrene-b-isobutylene-b-styrene) (SIBS) block copolymer, ethylene vinyl acetate, poly(meth)acrylic acid, polymers of lactic acid and glycolic acid, polyanhydrides, poly(ortho)esters, polyurethanes, poly(butic acid), poly(valeric acid), and poly(lactide-cocaprolactone), and natural polymers such as alginate and other polysaccharides including dextran and cellulose, collagen, albumin and other hydrophilic proteins, zein and other prolamines and hydrophobic proteins, copolymers and mixtures thereof, and chemical derivatives thereof including substitutions and/or additions of chemical groups, for example, alkyl, alkylene, hydroxylations, oxidations, and other modifications routinely made by those skilled in the art.

[0029] In one particular embodiment, the working electrode can comprise a gold working electrode that is crosslinked or biotinylated chemically in order to allow attachment of an antibody or biotin containing molecule. The gold working electrode can be cross-linked, for example, with dithiolbis(succinimidyl propionate) (DSP), which contains an amine reactive N-hydroxysuccinimide (NHS) ester that can react with amine groups of proteins and antibodies. Biotinylation can also be used for the attachment of biotin-containing molecules, including biotin containing aptamers, proteins, nucleic acid, or other molecule.

[0030] It will be appreciated, the flexibility of the chemical functionalization makes the biosensor useful for attaching essentially any receptor or ligand having an affinity for analytes. Examples of analytes for which receptors or ligands having affinity therefor that may be attached to the working electrode include DNA, RNA, oligonucleotides, proteins, biotin, and streptavidin. The receptors for these analytes functionalized or chemically functionalized to the working electrode can include ligands, such as antibodies or antigen binding fragments thereof. Antibodies, generally have several primary amines in the side chain of lysine (K) residues that can be available for cross-linking, such as NHS-ester cross-linking.

[0031] The chemical functionalization method also enables the bioconjugation of DNA aptamers having an amino group. These aptamers could potentially bind small molecules and proteins. Once bound, the change in the charge on the surface of the working electrode would enable the biosensor to detect the target biomolecule or small molecule.

[0032] In some embodiments, the receptor can be an antibody specific for an analyte of interest. Suitable antibodies for use in the biosensor described herein include monoclonal and polyclonal antibodies, immunologically active fragments (*e.g.,* Fab or (Fab)2 fragments), antibody heavy chains, humanized antibodies, antibody light chains, and chimeric antibodies. Antibodies, including monoclonal and polyclonal antibodies, fragments and chimeras, may be prepared using methods known in the art (see, for example, R. G. Mage and E. Lamoyi, in "Monoclonal Antibody Production Techniques and Applications ", 1987, Marcel Dekker, Inc.: New York, pp. 79-97; G. Kohler and C. Milstein, Nature, 1975, 256: 495-497; D. Kozbor et al., J. Immunol. Methods, 1985,81:

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[0033] Instead of being prepared, antibodies to be used in the methods described herein may be obtained from scientific or commercial sources.

[0034] In some embodiments, the receptor can be antibody or antigen binding fragment thereof that specifically binds to a cancer biomarker. Examples of cancer biomarkers include AMACR (prostate cancer), Carbohydrate antigen 125 (CA125) (ovarian cancer), human epididymis protein 4 (HE4) (ovarian cancer), BRAC1/BRAC2 (breast cancer, ovarian cancer), AFP (liver cancer), BCR-ABL (chronic myeloid leukemia), BRAF V600E (melanoma/colorectal cancer), KIT (gastrointestinal stromal tumor), PSA (prostate cancer), S100 (melanoma), KRAS (lung cancer), CIZL (lung cancer), and EGFR (colorectal/lung cancer).

[0035] It will be appreciated that the receptors include antibodies or antigen binding fragments to cancer biomarkers and that antibodies or antigen binding fragments to other biomarkers associated with other diseases, disorders, conditions, or pathologies, which can be detectable in a bodily sample can also be functionalized or chemically functionalized to the working electrode.

[0036] In-order to minimize any non-specific binding on the working electrode surface and blocking any open surface area of the working electrode at least one blocking agent can be applied to the surface of the working electrode once the receptor has been functionalized or chemically functionalized to the working electrode. The blocking agent can enhance the reproducibility and sensitivity of the biosensor by minimizing non-specific interactions on the working electrode. In some embodiments, the blocking agent can include dithiothreitol or casein. The blocking agent can be applied to the surface of the working at an amount effective to minimize non-specific binding of proteins or other molecules on the surface of the working electrode.

[0037] The voltage source 22 can apply a voltage potential to the working electrode 14 and reference and/or counter electrode 16, 12, depending on the design of the sensor 10. The current between the working electrode 14 and counter electrode 16 can be measured with the measuring device or meter 24. Such current is dependent on interaction of analyte with the receptor on the working electrode.

[0038] The amount or level of current measured is proportional to the level or amount of analyte in the biological sample. In some embodiments, where the sample is a bodily sample obtained from a subject, once the current level generated by the reaction solution tested with the sensor is determined, the level can be compared to a predetermined value or control value to provide information for diagnosing or monitoring of the condition, pathology, or disorder in a subject that is associated with presence or absence of the analyte.

[0039] The current level generated by sample obtained from the subject can be compared to a current level of a sample previously obtained from the subject, such as prior to administration of a therapeutic. Accordingly, the methods described herein can be used to measure the efficacy of a therapeutic regimen for the treatment of a condition, pathology, or disorder associated with the level of the analyte in a subject by comparing the current level obtained before and after a therapeutic regimen. Additionally, the methods described herein can be used to measure the progression of a condition, pathology, or disorder associated with the presence or absence of the analyte of interest in a subject by comparing the current level in a bodily sample obtained over a given time period, such as days, weeks, months, or years.

[0040] The current level generated by a sample obtained from a subject may also be compared to a predetermined value or control value to provide information for determining the severity or aggressiveness of a condition, pathology, or disorder associated with analyte levels in the subject. A predetermined value or control value can be based upon the current level in comparable samples obtained from a healthy or normal subject or the general population or from a select population of control subjects.

[0041] The predetermined value can take a variety of forms. The predetermined value can be a single cut-off value, such as a median or mean. The predetermined value can be established based upon comparative groups such as where the current level in one defined group is double the current level in another defined group. The predetermined value can be a range, for example, where the general subject population is divided equally (or unequally) into groups, or into quadrants, the lowest quadrant being subjects with the lowest current level, the highest quadrant being individuals with the highest current level. In an exemplary embodiment, two cutoff values are selected to minimize the rate of false positive and negative results.

[0042] The biosensor illustrated in Figs. 1 and 2 can be fabricated on a substrate 100 formed from polyester or other electrically non-conductive material, such as oth-

er polymeric materials, alumina (Al_2O_3), ceramic based materials, glass or a semi-conductive substrate, such as silicon, silicon oxide and other covered substrates. Multiple sensor devices 102 can thus be formed on a common substrate 100 (Fig. 2). As will be appreciated, variations in the geometry and size of the electrodes are contemplated.

[0043] The biosensor can be made using a thin film, thick film, and/or ink-jet printing technique, especially for the deposition of multiple electrodes on a substrate. The thin film process can include physical or chemical vapor deposition. Electrochemical sensors and thick film techniques for their fabrication are discussed in U.S. Pat. No. 4,571,292 to C. C. Liu et al., U.S. Pat. No. 4,655,880 to C. C. Liu, and co-pending application U.S. Ser. No. 20030155241.

[0044] In some embodiments, the working electrode, counter electrode, and reference electrode may be formed using laser ablation, a process which can produce elements with features that are less than one-thousandth of an inch. Laser ablation enables the precise definition of the working electrode, counter electrode, and reference electrode as well as electrical connecting leads and other features, which is required to reduce coefficient of variation and provide accurate measurements. Metalized films, such as Au, Pd, and Pt or any metal having similar electrochemical properties, that can be sputtered or coated on plastic substrates, such as-PET or polycarbonate, or other dielectric material; can be irradiated using laser ablation to provide these features.

[0045] In one example, a gold film with a thickness of about 300A to about 2000A can be deposited by a sputtering technique resulting in very uniform layer that can be laser ablated to form the working and counter electrodes. The counter electrode can use other materials. However, for the simplicity of fabrication, using identical material for both working and counter electrodes will simplify the fabrication process providing the feasibility of producing both electrodes in a single processing step. An Ag/AgCl reference electrode, the insulation layer, and the electrical connecting parts can then be printed using thick-film screen printing techniques.

[0046] The working electrode surface can then be cross-linked or biotinylated chemically in order to allow the attachment of an antibody or biotin-contained molecules. The crosslinking step can be accomplished by generating thiol bonds. This can be chemically accomplished using, for example, DSP (Dithiolbis[succinimidyl propionatel]) to produce the thiol bonds. DSP can be dissolved in DMSO, (Dimethyl sulfoxide). DSP contains amine-reactive *N*-hydroxysuccinimide (NHS) ester at each end of its carbon spacer arm. NHS can react with primary amine (At pH= 7-9) forming stable amide bonds. As an example, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-Hydroxysuccinimide (NHS) can be used forming semi-stable amine-reactive ester groups on the DSP modified gold electrode surface. Similar chemical methods can be used to produce semi-stable amine-ester groups to enhance the cross linking between the antibodies and the thiol groups. Other cross-linking agent, such as 3,3'-dithiobis[sulfosuccinimidylpropionate] (DTSSP), can also be used in this process.

[0047] Biotinylation is rapid, specific and is normally unperturb to the natural function of the molecule due to the relatively small size of biotin. Streptavidin and similar chemicals such as avidin can be immobilized on the working electrode surface for a biosensor for the detection of an interaction of antibody and antigen.

[0048] The working electrode of the biosensor can either be cross-linked or biotinylated with antibody receptor or biotin-contained protein (including antibody) using the immobilization approaches described above.

[0049] Following addition of an antibody or protein to the working electrode, the working electrode surface can be blocked using a blocking agent to minimize any non-specific molecule (*e.g.,* protein) bonding on the electrode surface. This step will enhance the reproducibility and sensitivity of the biosensor. In some embodiments, DTT (Dithiothreitol), casein, and/or other blocking agents can be used to cover the open surface area of the working electrode and minimize any non-specific protein coverage.

[0050] In other embodiments a plurality of biosensors can be provided on a surface of a substrate to provide a biosensor array. The biosensor array can be configured to detect analyte concentration changes in a host of chemical and/or biological processes (chemical reactions, cell cultures, neural activity, nucleic acid sequencing processes, etc.) occurring in proximity to the array. The biosensor array can include a plurality biosensors arranged in a plurality of rows and a plurality of columns. Each biosensor comprises a working electrode, a counter electrode, and a dielectric layer covering a portion of the working electrode and counter electrode and defining an aperture exposing other portions of the working electrode and counter electrode. A receptor(s) for at least one of the analytes of interest can be functionalized or chemically functionalized to the working electrode. The receptors can be the same or different for each biosensor of the array and can bind selectively to one or more of the analytes of interest. The biosensors of the array can be configured to provide at least one output signal representing the presence and/or concentration of an analyte proximate to a surface of the array. For each column of the plurality of columns or for each row of the plurality of rows, the array further comprises column or row circuitry configured to provide voltage potentials to respective biosensors in the column or row. Each biosensor in the row or column can potentially detect a different analyte and/or biased to detect different analytes.

[0051] In one example, the receptor can include an antibody and/or antigen binding fragment thereof that binds to alpha-methylacyl-CoA racemase (AMACR) or a substrate or metabolite of AMACR. AMACR is an enzyme involved in peroxisomal beta-oxidation of dietary

branched-chained fatty acids. AMACR has been consistently overexpressed in prostate cancer epithelium, hence it becomes an ideal specific biomarker for cancer cells within the prostate gland. Over-expression of AMACR may increase the risk of prostate cancer, because its expression is increased in premalignant lesions (prostatic intraepithelial neoplasia). Furthermore, epidemiologic, genetic and laboratory studies have pointed to the importance of AMACR in prostate cancer. Genome-wide scans of linkage in hereditary prostate cancer families have demonstrated that the chromosomal region for AMACR (5p 13) is the location of a prostate cancer susceptibility gene and AMACR gene sequence variants (polymorphisms) have been shown to co-segregate with cancer of the prostate in families with hereditary prostate cancer.

[0052] In some embodiments, the antibody can be a monoclonal antibody that specifically binds to AMACR. Examples of monoclonal antibodies that bind specifically to AMACR are AMACR Clone 13H4, commercially available from Dako, AMACR antibody, 2A10F3, commercially available from Thermoscientific, and AMACR antibody, 2A10F3, commercially available from Novus.

[0053] The antibody or antigen bind fragment thereof that binds to AMACR or a substrate or metabolite of AMACR can be attached to the working electrode covalently or non-covalently by, for example, cross-linking or biotinylation. The interaction of the antibody or antigen binding fragment thereof with AMACR or a substrate or metabolite of AMACR in a biological sample obtained from a subject having or suspected of having prostate cancer will produce a signal which can be detected electrochemically, electrically or optically, and the signal can then be used to quantify AMACR in a biological sample.

[0054] The amount or quantity of AMACR in the biological sample obtained from the subject suspected of having or at risk of prostate cancer can be measured using the biosensor to determine the level and quantity of AMACR in the bodily fluid and hence whether the subject has prostate cancer or an increased risk of prostate cancer.

[0055] The voltage source can apply a voltage potential to the working electrode and reference and/or counter electrode, depending on the design of the biosensor. The current between the working electrode and counter electrode can be measured with a measuring device or meter.

[0056] The amount or level of current measured can be proportional to the amount of AMACR or a substrate or metabolite of AMACR in the biological sample as well as the risk or presence of prostate cancer in the subject. Once the current level generated by the biological sample tested with the biosensor is determined, the level can be compared to a predetermined value or control value to provide information for diagnosing or monitoring of prostate cancer in a subject. For example, the current level can be compared to a predetermined value or control value to determine if a subject is afflicted with or has prostate cancer. An increased current level compared to a predetermined value or control value can be indicative of the subject having prostate cancer; whereas similar or decreased current level compared to a predetermined value or control value can be indicative of the absence of -prostate cancer in the subject

[0057] The current level generated by the biological sample obtained from the subject can be compared to a current level of a similar biological sample previously obtained from the subject, such as prior to administration of a therapeutic. Accordingly, the methods described herein can be used to measure the efficacy of a therapeutic regimen for the treatment of prostate cancer in a subject by comparing the current level obtained before and after a therapeutic regimen. Additionally, the methods described herein can be used to measure the progression of prostate cancer in a subject by comparing the current level in a biological sample obtained over a given time period, such as days, weeks, months, or years.

[0058] The current level generated by a biological sample of the subject may also be compared to a predetermined value or control value to provide information for determining the severity or aggressiveness of the prostate cancer in the subject. Thus, in some aspect, the current level may be compared to control values obtained from subjects with well known clinical categorizations, or stages, of histopathologies related to prostate cancer (*e.g.,* Gleason score of prostate cancer or indolent versus aggressive prostate cancer). In one particular embodiment, the current in a sample can provide information for determining a particular Gleason grade or score of prostate cancer in the subject.

[0059] A predetermined value or control value can be based upon the current level in comparable samples obtained from a healthy or normal subject or the general population or from a select population of control subjects. In some aspects, the select population of control subjects can include individuals diagnosed with prostate cancer. For example, a subject having a greater current level compared to a control value may be indicative of the subject having a more advanced stage of a prostate cancer.

[0060] The select population of control subjects may also include subjects afflicted with prostate cancer in order to distinguish subjects afflicted with prostate cancer from those with benign prostate disease. In some aspects, the select population of control subjects may include a group of individuals afflicted with prostate cancer.

[0061] The predetermined value can take a variety of forms. The predetermined value can be a single cut-off value, such as a median or mean. The predetermined value can be established based upon comparative groups such as where the current level in one defined group is double the current level in another defined group. The predetermined value can be a range, for example, where the general subject population is divided equally (or unequally) into groups, or into quadrants, the lowest quadrant being subjects with lowest current level, the highest quadrant being individuals with the highest current level. In an exemplary embodiment, two cutoff values

are selected to minimize the rate of false positive and negative results.

[0062] It will be appreciated that the detection of AMACR can be accomplished using any biological sample or physiological fluid obtained from the subject. Blood samples have been used, and the detection of AMACR can also be achieved using other physiological fluid, such as urine, saliva, and others. Thus, the detection of AMACR as a biomarker of prostate cancer in any type of physiological fluid can be provided by the biosensor described herein.

Claims

1. A sensor for the detection of an analyte in a biological sample comprising:

    - a substrate;
    - a working electrode formed on a surface of the substrate;
    - a counter electrode formed on the surface of the substrate;
    - a dielectric layer covering a portion of the working electrode and counter electrode and defining an aperture exposing other portions of the working electrode and counter electrode;
    - a measuring device for applying voltage potentials to the working electrode and counter electrode and measuring the current flow between the working electrode and counter electrode; and
    - a receptor functionalized or chemically functionalized onto a surface of the exposed portion of the working electrode, the receptor selectively binding to the analyte of interest and the analyte once bound being detectable by measuring the current flow between the working electrode and counter electrode,

    wherein the working electrode and the counter electrode are obtained from sputtering a metalized film on a plastic substrate and irradiating the metalized film using laser ablation to define the dimensions of the working electrode and the counter electrode, and wherein the receptor being functionalized onto the surface of the working electrode by cross-linking or biotinylating the surface and binding the receptor to the cross-linked or biotinylated surface.

2. The sensor of claim 1, wherein the working electrode and counter electrode independently comprise gold, platinum, palladium, silver, and alloys thereof.

3. The sensor of claim 1, further comprising a reference electrode on the surface of the substrate, the dielectric covering a portion of the reference electrode.

4. The sensor of claim 1, the receptor comprising an antibody or biotinylated antibody.

5. The sensor of claim 1, wherein the surface of the working electrode is cross-linked with dithiolbis(succinimidyl propionate) (DSP).

6. The sensor of claim 1, further comprising a blocking agent that covers open areas of the exposed portions of the working electrode.

7. The sensor of claim 1, wherein the blocking agent comprises dithiothreitol (DTT) or casein.

8. A sensor for the detection of AMACR or an AMACR substrate thereof in a biological sample comprising:

    - a substrate;
    - a working electrode formed on a surface of the substrate;
    - a counter electrode formed on the surface of the substrate;
    - a dielectric layer covering a portion of the working electrode and counter electrode and defining an aperture exposing other portions of the working electrode and counter electrode;
    - a measuring device for applying voltage potentials to the working electrode and counter electrode and measuring the current flow between the working electrode and counter electrode; and
    - an antibody or antigen binding fragment to AMACR or the AMACR substrate functionalized or chemically functionalized onto a surface of the exposed portion of the working electrode by cross-linking or biotinylating the surface and binding the antibody or antigen binding fragment to AMACR or the AMACR substrate to the cross-linked or biotinylated surface, the antibody or antigen binding fragment selectively binding to the AMACR or the AMACR substrate and the AMACR or the AMACR substrate once bound being detectable by measuring the current flow between the working electrode and counter electrode,

    wherein the working electrode and the counter electrode are obtained from sputtering a metalized film on a plastic substrate and irradiating the metalized film using laser ablation to define the dimensions of the working electrode and the counter electrode.

Patentansprüche

1. Ein Sensor für den Nachweis eines Analyten in einer biologischen Probe umfassend:

- ein Substrat;
- eine Arbeitselektrode, die auf einer Oberfläche des Substrats gebildet ist;
- eine Gegenelektrode, die auf der Oberfläche des Substrats gebildet ist;
- eine dielektrische Schicht bedeckend einen Teil der Arbeitselektrode und der Gegenelektrode und definierend eine Öffnung, die andere Teile der Arbeitselektrode und Gegenelektrode freilegt;
- eine Messeinrichtung zum Anlegen von Spannungspotentialen an die Arbeitselektrode und die Gegenelektrode und zum Messen des Stromflusses zwischen der Arbeitselektrode und der Gegenelektrode; und
- ein Rezeptor, welcher funktionalisiert oder chemisch funktionalisiert auf die Oberfläche eines exponierten Teils der Arbeitselektrode wird, wobei der Rezeptor selektiv an den zu untersuchenden Analyten bindet und der Analyt nach der Bindung durch Messen des Stromflusses zwischen der Arbeitselektrode und der Gegenelektrode nachweisbar ist,

wobei die Arbeitselektrode und die Gegenelektrode durch Sputtern eines metallisierten Films auf ein Plastiksubstrat und Bestrahlen des metallisierten Films mit Hilfe von Laserabtragung zur Definition der Maße der Arbeitselektrode und der Gegenelektrode erhalten werden, und wobei der Rezeptor durch Vernetzen oder Biotinylieren der Oberfläche und Binden des Rezeptors auf die Oberfläche eines exponierten Teils der Arbeitselektrode funktionalisiert oder chemisch funktionalisiert wird.

2. Der Sensor gemäß Anspruch 1, wobei die Arbeitselektrode und die Gegenelektrode unabhängig voneinander Gold, Platin, Palladium, Silber, und Legierungen davon enthalten.

3. Der Sensor gemäß Anspruch 1, zusätzlich umfassend eine Referenzelektrode auf der Oberfläche des Substrats, wobei das Dielektrikum einen Teil der Referenzelektrode bedeckt.

4. Der Sensor gemäß Anspruch 1, wobei der Rezeptor einen Antikörper oder einen biotinylierten Antikörper umfasst.

5. Der Sensor gemäß Anspruch 1, wobei die Oberfläche der Arbeitselektrode mit Hilfe von Dithiobis-Succinimidylpropionat (DSP) vernetzt ist.

6. Der Sensor gemäß Anspruch 1, zusätzlich umfassend ein Blockreagenz, welches offene Bereiche der exponierten Teile der Arbeitselektrode bedeckt.

7. Der Sensor gemäß Anspruch 1, wobei das Blockreagenz Dithiothreitol (DTT) oder Kasein umfasst.

8. Ein Sensor für den Nachweis von AMACR oder eines AMACR Substrats in einer biologischen Probe umfassend:

- ein Substrat;
- eine Arbeitselektrode, die auf einer Oberfläche des Substrats gebildet ist;
- eine Gegenelektrode, die auf der Oberfläche des Substrats gebildet ist;
- eine dielektrische Schicht, welche einen Teil der Arbeitselektrode und der Gegenelektrode bedeckt und eine Öffnung definiert, die andere Teile der Arbeitselektrode und der Gegenelektrode freilegt;
- eine Messeinrichtung zum Anlegen von Spannungspotentialen an die Arbeitselektrode und die Gegenelektrode und zum Messen des Stromflusses zwischen der Arbeitselektrode und der Gegenelektrode; und
- ein Antikörper oder ein antigen-bindendes Fragment eines Antikörpers für AMACR oder das AMACR Substrat, welcher/welches durch Vernetzen oder Biotinylieren der Oberfläche und Binden des Antikörpers oder des antigen-bindenden Fragments für AMACR oder das AMACR Substrat an die vernetzte oder biotinylierte Oberfläche funktionalisiert oder chemisch funktionalisiert auf die Oberfläche eines exponierten Teils der Arbeitselektrode wird, wobei der Antikörper oder das antikörperbindende Fragment selektiv an das AMACR oder das AMACR Substrat bindet und das AMACR oder das AMACR Substrat nach der Bindung durch Messen des Stromflusses zwischen der Arbeitselektrode und der Gegenelektrode nachweisbar ist,

wobei die Arbeitselektrode und die Gegenelektrode durch Sputtern eines metallisierten Films auf ein Plastiksubstrat und Bestrahlen des metallisierten Films mit Hilfe von Laserabtragung zur Definition der Maße der Arbeitselektrode und der Gegenelektrode erhalten werden.

Revendications

1. Capteur pour la détection d'un analyte dans un échantillon biologique comprenant :

- un substrat ;
- une électrode de travail formée sur une surface du substrat ;
- une contre-électrode formée sur la surface du substrat ;
- une couche diélectrique couvrant une partie

de l'électrode de travail et de la contre-électrode et définissant une ouverture exposant d'autres parties de l'électrode de travail et de la contre-électrode ;
- un dispositif de mesure pour appliquer des potentiels de tension à l'électrode de travail et à la contre-électrode et pour mesurer la circulation du courant entre l'électrode de travail et la contre-électrode ; et
- un récepteur fonctionnalisé ou chimiquement fonctionnalisé sur une surface de la partie exposée de l'électrode de travail, le récepteur se liant sélectivement à l'analyte présentant un intérêt et l'analyte, une fois lié, étant détectable par mesure de la circulation de courant entre l'électrode de travail et la contre-électrode,

dans lequel l'électrode de travail et la contre-électrode sont obtenues par pulvérisation cathodique d'un film métallisé sur un substrat plastique et irradiation du film métallisé au moyen d'une ablation au laser pour définir les dimensions de l'électrode de travail et de la contre-électrode, et dans lequel le récepteur est fonctionnalisé sur la surface de l'électrode de travail par réticulation ou biotinylation de la surface et liaison du récepteur à la surface réticulée ou biotinylée.

2. Capteur selon la revendication 1, dans lequel l'électrode de travail et la contre-électrode comprennent indépendamment de l'or, du platine, du palladium, de l'argent, et leurs alliages.

3. Capteur selon la revendication 1, comprenant en outre une électrode de référence sur la surface du substrat, le diélectrique couvrant une partie de l'électrode de référence.

4. Capteur selon la revendication 1, dans lequel le récepteur comprend un anticorps ou un anticorps biotinylé.

5. Capteur selon la revendication 1, dans lequel la surface de l'électrode de travail est réticulée avec du dithiol-bis(propionate de succinimidyle) (DSP).

6. Capteur selon la revendication 1, comprenant en outre un agent bloquant qui couvre des zones ouvertes des parties exposées de l'électrode de travail.

7. Capteur selon la revendication 1, dans lequel l'agent bloquant comprend du dithiothréitol (DTT) ou de la caséine.

8. Capteur pour la détection d'AMACR ou d'un substrat d'AMACR dans un échantillon biologique comprenant :
- un substrat ;
- une électrode de travail formée sur une surface du substrat ;
- une contre-électrode formée sur la surface du substrat ;
- une couche diélectrique couvrant une partie de l'électrode de travail et de la contre-électrode et définissant une ouverture exposant d'autres parties de l'électrode de travail et de la contre-électrode ;
- un dispositif de mesure pour appliquer des potentiels de tension à l'électrode de travail et à la contre-électrode et pour mesurer la circulation du courant entre l'électrode de travail et la contre-électrode ; et
- un anticorps ou un fragment se liant à un antigène dirigé contre l'AMACR ou le substrat d'AMACR fonctionnalisé ou chimiquement fonctionnalisé sur une surface de la partie exposée de l'électrode de travail par réticulation ou biotinylation de la surface et liaison de l'anticorps ou du fragment se liant à un antigène dirigé contre l'AMACR ou le substrat d'AMACR à la surface réticulée ou biotinylée, l'anticorps ou le fragment se liant à un antigène se liant sélectivement à l'AMACR ou au substrat d'AMACR et l'AMACR ou le substrat d'AMACR, une fois lié, étant détectable par mesure de la circulation de courant entre l'électrode de travail et la contre-électrode,

dans lequel l'électrode de travail et la contre-électrode sont obtenues par pulvérisation cathodique d'un film métallisé sur un substrat plastique et irradiation du film métallisé au moyen d'une ablation au laser pour définir les dimensions de l'électrode de travail et de la contre-électrode.

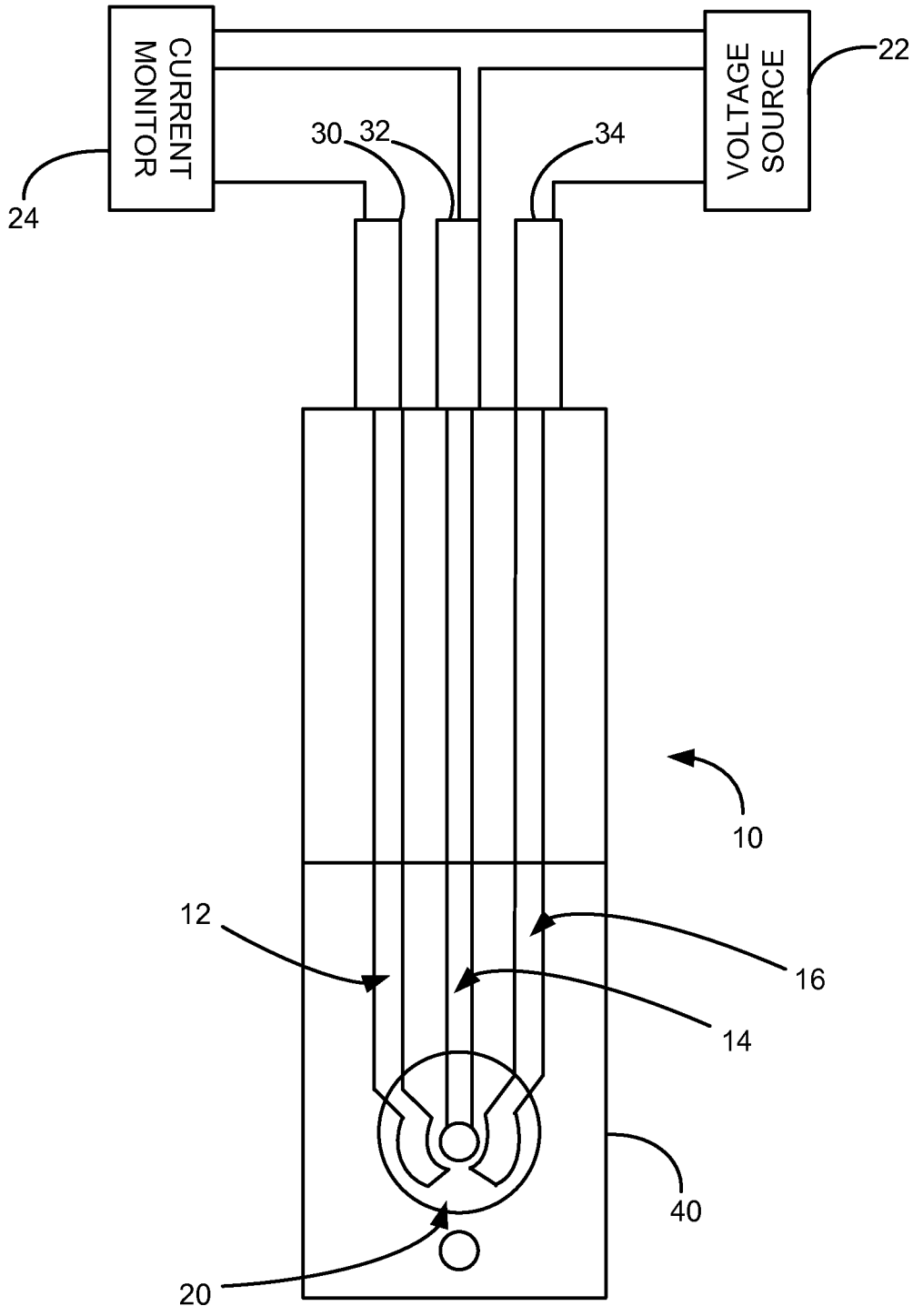

Fig. 1

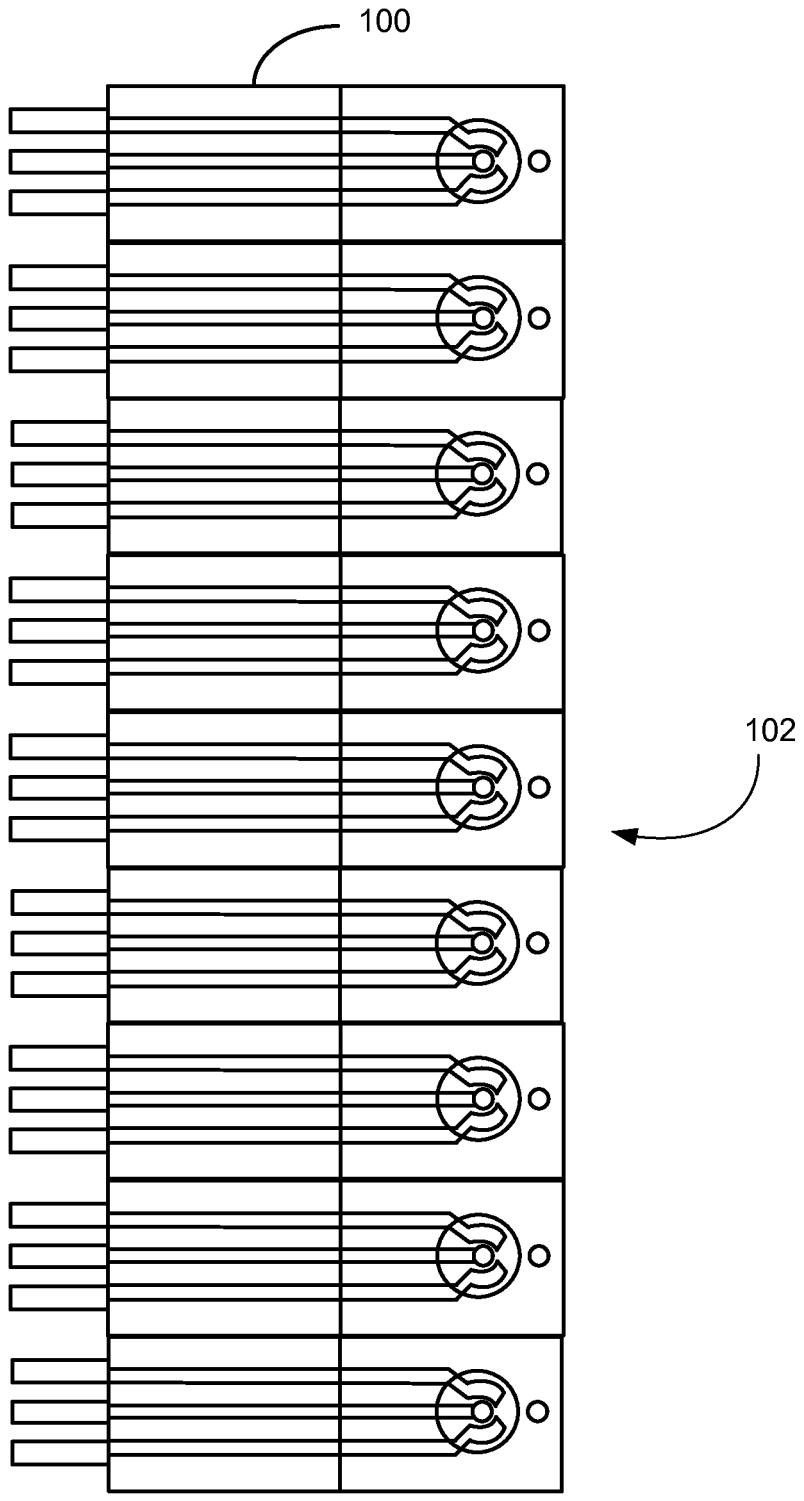

Fig. 2

REFERENCES CITED IN THE DESCRIPTION

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