TECHNOLOGICAL MONITORING OF LAB-ON-A-CHIP DEVICES FOR BIOTERRORISM PATHOGENS DETECTION

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Abstract: Biosensors, aptasensors and immunosensors are devices developed for detection or measurement of specific biological structures from microorganisms on many different matrices. A state-of-the-art search for the relevant technologies on biosensors conceived for lab-on-a-chip devices were performed in selected patent databases. The search results are then analyzed to answer specific questions about, for example, patterns of patenting activity or of innovation. The results are presented visually to assist understanding and conclusions based on the empirical evidence are provided. This paper provide a snapshot of the patent situation of the lab-on-a-chip biosensors, aptasensors and immunosensors technologies for detecting bioterrorism agents such as Bacillus anthracis and Yersinia pestis, either within a given country or region, or globally. This paper can serve as a tool to identify individual technological trends on biosensor development who are susceptible to came into the market and to qualify the differences between their technical features, which are important factors in the selection of R&D projects of potentially patentable unique lab-on-a-chip devices. Because of their sensitivity, substantial background suppression, and operational convenience, these lab-on-a-chip biosensors appear potentially well suited for pathogen detection.

Key-Words*: lab on a chip, biosensor, patent, bioterrorism*

Introduction: The Biological Defense Section of the Brazilian Army Institute for Chemical, Biological, Radiological and Nuclear Defense performed a technological monitorement of lab-on-a-chip devices for detection of bioterrorism pathogens using selected patent databases to improve the research and development of dual application products for both civilian and military personnel. The purpose of this article is to show a patent landscape study for review by prospective decision makers and researchers. All companies involved in lab-on-a-chip devices head research participate in a type of performance “arms race” in which the goal is to give every possible advantage to players. The patent landscape study helps to: monitor markets of interest, to identify gaps in and improve your research and development, to determine which of your prospective patents will have significant commercial value, to confirm which inventions are now in the public domain and to better understand current competitors and identify future ones.

Experimental: Data was collected in specialized databases such as Thomson Reuters Derwent Innovation Index and WIPO Patentscope using the classifications of the International Patent Classification that are connected to lab-on-a-chip device technologies. The results were used to report patenting trends, list of leading countries and companies, list of patent citations, list of top inventor teams and a list of patent abstracts associated with this study.

Results and discussion: Upon investigating lab-on-a-chip patents, inventions to do the following provided the most common classifications in International Patent Classification were B25J, B01L, H01L, G01N, B01J, A61B, H01F, C12Q, C12M, B03C, C12N and others (Table 1).

Table 1. Number of registers of patents of lab-on-a-chip devices in Patentscope database by International Patent Classification Code.

International Patent Classification Code	Number of registers
G01N	360
B01L	182
C12M	146
C40B	134
C12Q	102

B01J	47
B81B	45
C12N	32
B01F	29
H01L	27

The United States appears to dominate the total number of patent documents pertaining to lab-on-a-chip devices, followed by Republick of Korea, China, Germany, Japan, Australia, United Kingdom, Canada, Brazil, Mexico etc. Patents applied in PCT and in the European Patent Office were listed as well too (Table 2).

Table 2. Number of registers of patents of lab-on-a-chip devices in Patentscope database by country.

Country	Number of registers
United States	323
PCT	120
Republic of Korea	108
European Patent Office	79
China	47
Germany	27
Japan	19
Australia	18
United Kingdom	9
Canada	8
Brazil	2
Mexico	2
Denmark	1
EAPO	1
Egypt	1
Russian Federation	1

Further investigation shows that a substantial portion of the U.S. patent documents started by 2007 and the major part of them are from 2011, when almost half of the total patents of the country were publicized (Table 3).

Table 3. Number of U.S. registers of patents of lab-on-a-chip devices in Patentscope database by year.

Year	Number of U.S. Patents
2007	17
2008	13
2009	20
2010	20
2011	142
2012	20
2013	9
2014	13
2015	12
2016	15
2017	5

Table 4. Number of U.S. registers of inventors of lab-on-a-chip devices in Patentscope database.

Number of registers	Inventors list
116	Azimi Mehdi
114	Silverbrook Kia
61	Facer Geoffrey Richard
44	Moini Alireza
11	Worsman Matthew Taylor

9	AZIMI MEHDI
8	Ludwig Lester F.
8	SILVERBROOK KIA
7	FACER GEOFFREY RICHARD
7	MOINI ALIREZA

The US2017089899-A1 patent is a method of detecting a biological analyte in a subject, comprising obtaining a biological sample from a subject, passing the biological sample through the flow region of the microfluidic capture device, passing a redox solution through the flow region of the microfluidic capture device, applying a cyclic voltage to the first electrode of the microfluidic capture device, measuring the current flow and/or area of the voltage-current curve through the microfluidic capture device, and comparing the current flow and/or voltage-current curve to a corresponding control value to determine if the biological analyte is present in the biological sample, where the binding molecule of the microfluidic capture device specifically binds to the biological analyte being evaluated.

The biochemical analytical device of the WO2017001018-A1 patent has a sample port adapted to receive the test sample, and at least a sensor that analyzes the test sample and generates sensor data corresponding to the analyte in the analyzed test sample. A processor receives the sensor data from sensor, selects a non-linear function for the received sensor data, fits the selected non-linear function to sensor data, and compares the fitted non-linear function to a reference data to determine the analyte in test sample. The biochemical analytical device e.g. lab-on-a-chip device can be used for determining analyte in test sample such as blood and urine. Uses include but are not limited to drug, cell of host or foreign cell such as microbial cell e.g. bacteria, virus, toxin, byproducts of host cell or of foreign cell, allergens, products or byproducts of metabolic or enzymatic processes, chemical compounds.

The patent KR2015117110-A describes a lab-on-a-chip used for analyzing sample of target substance e.g. amino acid, peptides, polypeptide, protein, glycoprotein, lipoprotein, nucleoside, nucleotide, oligonucleotide, nucleic acid, carbohydrate, oligosaccharide, polysaccharide, fatty acid, lipid, hormone, metabolite, cytokines, chemokines, receptor, neurotransmitter, antigen, allergens, antibody, temperament, cofactor, inhibitors, drug, toxin, explosives, pesticides, biological risk agent, bacteria, virus, radioisotopes, vitamin, heterocyclic aromatic compound, amphetamines and hallucinogen (all claimed). This lab-on-a-chip consists of feeding portion for providing sample containing target material, substrate comprising micro-channel surface plasmon active layer on surface, and discharging portion for discharging sample.

The US2013157254-A1 patent method for detecting, identifying, analyzing and quantifying, by surface-enhanced Raman spectroscopy, involves providing support structure and a liquid reagent, functionalizing the surface-enhanced Raman (SER)-active metal with at least one binding agent, adding the obtained analyte sample to functionalized SER-active materials to effect attachment of designated target analyte, adding the liquid reagent to the support structure to attach to target analyte binding agent, irradiating, detecting and analyzing the detected SER spectrum to determine presence and quantity of target analyte.
The first SER-active material comprises at least one chemically synthesized porous material. The effects of the SER-active metals attached to at least one target analyte, in cooperatively generating the SER spectrum, are synergistic. The support structure comprises a substrate supporting the first SER-active material, where the substrate is fabricated from metal, glass, paper, or plastic, and is in the form of a substantially planar sheet, plate, or membrane. The substrate has a multiplicity of wells formed for receiving the analyte sample. The substrate is fabricated from glass or plastic and is in the form of a wall of a vial, capillary, or channel. The first and second SER-active materials is chosen from copper, gold, silver, nickel, platinum, rhodium, iron, ruthenium, cobalt, nickel, palladium, alloys and their mixtures. The SER-active metal of the first SER-active material is of particulate form or in the form of a surface having a morphology that is functionally equivalent to metal particles for generating a plasmon field when irradiated, where the SER-active metal of the second SER-active material is of particulate form. The SER-active metals of the first and second SER-active materials are different from one another. The binding agent, the liquid reagent comprised of the second SER-active material, the at least one designated target analyte, and any signature chemical that may be present, can readily pass through the at least one chemically synthesized porous material. The chemically synthesized porous material is effective to separate the at least one designated target analyte from other components of the analyte sample. The designated target analyte and binding agent is a chemical, biochemical, or biological substance. The binding agent and designated target analyte are paired with one another for effective interbonding, such pairs being chosen from (a)

antibodies and antigens, (b) peptides and biologicals, (c) drug receptors and drugs, (d) enzymes and their specific biochemical substrates, (e) carbohydrates and lectins, and (f) nucleic acid sequences and their complements. The designated target analyte is attached to the at least one binding agent by covalent, ionic, or hydrogen bonding, or by vanderwaals or electrostatic interactions between charged, polar, hydrophobic, or hydrophilic chemical groups on the surface of the at least one binding agent. , The target analyte sample comprises a biological agent. The biological agent is chosen from Bacillus anthracis, Clostridium botulinum A, Dengue fever, Ebola virus, Francisella tularensis, Leishmania, Marburg virus, Mycobacterium leprae, Plasmodium, Puumala hantavirus, ricin toxin, Variola virus, and Yersinia pestis. The agent is chosen from acetic acid, adipic acid, ascorbic acid, citric acid, formic acid, fumaric acid, lactic acid, malic acid, palmitic acid, peracetic acid, propionic acid, salicylic acid, sorbic acid, succinic acid, trihaloacetic acid, acetone, acetonitrile, benzene, chloroform, carbon tetrachloride, cyclohexane, dichloromethane, diethyl ether, dimethylsulfoxide, ethyl acetate, ethylene glycol, isopropyl ether, methyl ethyl ketone, n-hexane, phenolic derivatives, tetrahydrofuran, toluene, and their mixtures. Preferred Method: The binding agent is attached to the SER-active metal of the at least one functionalized SER-active material by covalent, ionic, or hydrogen bonding, or by vanderwaals or electostatic interactions between charged, polar, hydrophobic, or hydrophilic chemical groups on the surface of the at least one binding agent. The linker chemical or biochemical is interposed for attaching the at least one binding agent to the SER-active metal of the at least one functionalized SER-active material. The molecule of the linker chemical or biochemical contains sulfur. The spacer chemical or biochemical is interposed effectively between the at least one binding agent and the SER-active metal of at least one functionalized SER-active material to provide sufficient space for the at least one target analyte to attach to the binding agent. The blocking chemical or biochemical is added to at least one functionalized SER-active material to prevent extraneous SER-active chemicals, biochemicals, or biologicals contained in the analyte sample from interacting with the SER-active metal of the at lest one functionalized SER-active material so as to thus to minimize the production of SER spectra that would interfere substantially with a spectrum of the at least one designated target analyte. The method further involves adding to the analyte sample at least one reactive reagent that is effective to react, under the conditions existing or established, with a constituent of the analyte sample for releasing a signature chemical, a signature biochemical, or a signature biological, the signature chemical, biochemical, or biological constituting the at least one designated target analyte, where the target analyte is a released signature biochemical chosen from amino and nucleic acids, nucleotides, nucleosides, peptides, proteins, lipids, polysaccharides, haptans, antibodies, antigens, biomarkers, enzymes, steroids, hormones, lectins, aptamers, fragments and their polymers. The support structure is constructed to effectively enable irradiation of designated target analyte and the first and second SER-active materials, and detection of the cooperatively generated SER spectrum, where a chemometric technique is applied to augment at least one of detection, identification, analysis, and quantitation of the at least one target analyte, and augments analysis for the determination of pathogenicity, potency, toxicity or viability. Preferred Apparatus: The SER-active device component comprises a substrate supporting the first SER-active material, where the substrate is fabricated from metal, glass, paper, or plastic, and is in the form of a substantially planar sheet, plate, or membrane. The substantially planar sheet, plate, or membrane has a multiplicity of wells formed for receiving an analyte sample. The substrate is fabricated from glass or plastic and is in the form of a capillary, vial, disc, or lab-on-a-chip channel, the first SER-active material supported on a surface provided by the substrate. The support structure of the SER-active device component comprises a chemically synthesized porous structure through which the liquid reagent comprised of the second SER-active material, at least one designated target analyte, and any signature chemical that may be present, can readily pass. The apparatus comprises several collection components, contained by the packaging unit, chosen from eyedroppers, syringes, pipettes, micropipetters, swabs, and their various combinations. The apparatus additionally comprises (a) a water-supply component, contained by the packaging unit, comprised of a container containing distilled, deionized water, (b) at least one mixing vial component contained by the packaging unit, (c) a filtering component, contained by the packaging unit, for filtering an analyte sample, and (d) at least one second reagent supply component comprised of a container containing a second reagent, the second reagent chosen from buffered solutions, digesting agents, and agents that are reactive to degrade biological materials so as to effect release of signature analytes, where the second reagent is an agent that is reactive with constituents of analyte samples to degrade biological materials and release at least one signature chemical, signature biochemical, or signature biological so as to provide a signature analyte constituting the at least one designated target analyte.

The US2012051976-A1 patent describes devices utilizing zinc oxide nanotips, which enhances binding strength and immobilization of DNA, protein, and small biomolecules, and the wettability (from superhydrophobicity to superhydrophilicity or vice versa) of the zinc oxide nanotips can be controlled, where the superhydrophilic status of the zinc oxide nanotips can be obtained through UV shinning and which reduces the liquid sample

consumption and enhances the sensitivity greatly; exhibits high frequency operation thus leads to high sensitivity; integrates with silicon integrated circuits to built on the silicon substrates; is wireless and it can be operate in frequency domain; is compatible with thin film microelectronics processing technology, thus it is cost-effective and it can be mass-production; provides favorable binding sites to enhance immobilization, and increases effective sensing area, thus improves sensing and detection efficiency; has giant effective surface area and strong bonding sites; and has biological recognition elements, which serves to recognize analytes (comprising enzymes, microorganisms, tissues, antibodies, receptors, nucleic acids, organelles or whole cells); and provides zinc oxide nanotip arrays which is highly dense for diagnostic kits and flow-through systems, comprising zinc oxide UV bio testing bench (containing emitters, detectors, modulators, and filters), gene chip, lab-on-a chip and living-cell chip.

Conclusion: This paper can serve as a tool to identify individual technological trends on lab-on-a-chip development who are susceptible to came into the market and to qualify the differences between their technical features, which are important factors in the selection of R&D projects of potentially patentable unique lab-on-a-chip devices. Because of their sensitivity, substantial background suppression, and operational convenience, these biosensors appear potentially well suited for applications in pathogen detection on many matrices.

References and acknowledgements:

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