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Graphene nano-ink biosensor arrays on a microfluidic paper for multiplexed detection of metabolites

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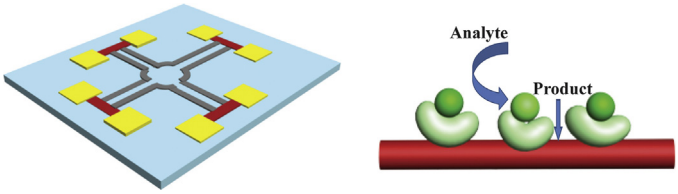
HIGHLIGHTS

- We report graphene-ink biosensor arrays on a microfluidic paper for metabolites.
- The device is able to detect multiple metabolites sensitively and rapidly.
- The device fabrication process is simple and inexpensive.

GRAPHICAL ABSTRACT



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ABSTRACT

The development of a miniaturized and low-cost platform for the highly sensitive, selective and rapid detection of multiplexed metabolites is of great interest for healthcare, pharmaceuticals, food science, and environmental monitoring. Graphene is a delicate single-layer, two-dimensional network of carbon atoms with extraordinary electrical sensing capability. Microfluidic paper with printing technique is a low cost matrix. Here, we demonstrated the development of graphene-ink based biosensor arrays on a microfluidic paper for the multiplexed detection of different metabolites, such as glucose, lactate, xanthine and cholesterol. Our results show that the graphene biosensor arrays can detect multiple metabolites on a microfluidic paper sensitively, rapidly and simultaneously. The device exhibits a fast measuring time of less than 2 min, a low detection limit of 0.3 μM, and a dynamic detection range of 0.3–15 μM. The process is simple and inexpensive to operate and requires a low consumption of sample volume. We anticipate that these results could open exciting opportunities for a variety of applications.

1. Introduction

Metabolites are the products of biological reactions inside cells, and are regulators which control the pace of metabolism. Metabolites have found wide applications in healthcare, pharmaceuticals, food science, and environmental monitoring. For example, glucose, lactate, xanthine and cholesterol, as metabolites, are important biomarkers for many diseases, such as diabetes, cardiovascular diseases, liver diseases, metabolic disorders, hyperuricemia, xanthinuria, and renal failure [1–4]. Despite these advances in the development of a variety of bioanalytical devices for metabolites, the ability to achieve rapid, sensitive, and low cost electrical detection of multiple metabolites remains unexplored. Paper is a low cost, easy-to-handle matrix which has recently attracted great recent interest as a platform for developing a variety of electronic [5,6], optical [7,8], sensing [9–11], and microfluidic devices [11–14]. A miniaturized biosensing platform on paper for multiplexed detection of metabolites is highly desired.

Graphene is a single-atom-thick, sp^2 carbon-based material that has attracted significant recent interest due to its remarkable electrical [15], optical [16], mechanical [17], sensing [18–21], and thermal [22] properties. Due to its single-atomic-layer structure, isolation of graphene has only recently been achieved, via epitaxial growth [23], chemical vapor deposition [24], sputtering [25], chemical exfoliation [26], and mechanical exfoliation [15,19]. Graphene-based ink is of particular interest due to its advantages of being processed in solution which facilitates the low-cost development of electronic and optical devices. Graphene sensors and biosensors have been developed for highly sensitive detection of a variety of analytes, including nitric oxide [20], ammonia [19],

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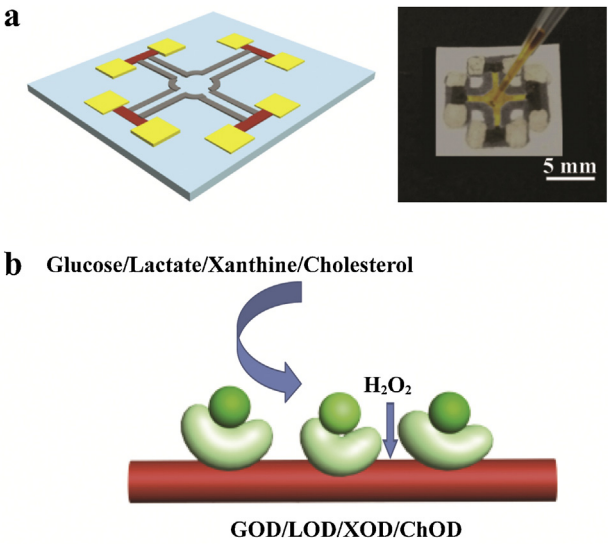

Fig. 1. (a) Schematic illustration (left) and camera image (right) of the multiplexed graphene-based electrical biosensor arrays on a microfluidic paper, and (b) biosensor detection principle. In (a) left and (b): red is graphene; yellow is silver paste; blue is paper; grey is microfluidic channel; light green is enzyme; dark green is analyte.

hydrogen [27], glucose [28], and glutamate [29]. Recently, we have also shown that graphene can be constructed as sensitive and selective biosensors for TNT, lactate, and 5-aminosalicylic acid [11,30–32].

Here, we present the development of graphene-based electrical biosensor arrays on a microfluidic paper for the multiplexed detection of metabolites. As shown in Fig. 1, graphene ink, silver paste and solid ink are printed on regular paper. Each individual channel is connected to its own enzyme-graphene electrode, which is connected to the two silver paste terminals, and all the channels share a common inlet. The solution containing the target metabolite is injected into the inlet, and flows into all the channels. When it is recognized by its own enzyme-graphene electrode, it changes the electrical signal on graphene electrode to generate a signal response. As an example, glucose, lactate, xanthine and cholesterol, which are typical important metabolites, are used as the sensor analytes. The device can also be constructed for the detection of a variety of other metabolites. The sensing principle is based on the oxidation of analyte by oxidase, e.g. glucose by glucose oxidase (GOD), lactate by lactate oxidase (LOD), xanthine by xanthine oxidase (XOD), and cholesterol by cholesterol oxidase (ChOD). The product of the enzymatic reaction, hydrogen peroxide, results in a detectable signal on graphene. Fig. 1 illustrates the basic design of the multiplexed channel structure. It shows the four graphene ink electrodes, each of them functionalized with different enzymes, namely GOD, LOD, XOD, and ChOD. The device can detect multiple metabolites simultaneously and requires a small sample volume due to the use of printed microfluidic channels, shows a high sensitivity and a fast measuring time due to the use of graphene-based electrical sensor, and has a low cost due to the use of paper-based substrate.

2. Material and methods

2.1. Apparatus and chemicals

The electrical measurement was done by an Autolab potentiostat with NOVA software (Metrohm USA, Riverview, FL) or a VSP-300 potentiostat with EC-lab software (Bio-logic USA, Knoxville, TN). A solid ink color printer, ColorQube 8570, was purchased from Xerox Corporation (Norwalk, CT) for printing the microfluidic channels. The optical microscope was purchased from Microscopes.com (Northbrook, IL). Graphene Nano Platelets (Grade 4 COOH rich, <4 layers) were from Cheap Tubes Inc. (Brattleboro, VT). PELCO Conductive Silver 187 used to form the terminals on the graphene electrode was purchased from Ted Pella. Inc. (Redding, CA). Lactate oxidase (LOD), xanthine oxidase (XOD) and cholesterol oxidase (ChOD) were purchased from Toyobo Co., Ltd. (Osaka, Japan). Glucose oxidase (GOD) was purchased from MP Biomedicals LLC (Solon, OH). Glucose and xanthine were purchased from Acros Organics (Pittsburgh, PA). Lactic acid, *N*-ethyl-*N*0-(3-dimethylaminopropyl) carbodiimide (EDC), and *N*-hydroxy succinimide (NHS) were purchased from Sigma–Aldrich (St. Louis, MO). Cholesterol was purchased from Alfa Aesar (Ward Hill, MA). Potassium phosphate monobasic and potassium phosphate dibasic were purchased from Fisher Scientific (Pittsburgh, PA). Human blood was purchased from Zen-Bio Inc. (Research triangle park, NC). All the solutions were prepared in deionized water obtained from Barnstead NANOpure® DIamond™ Ultrapure Water Systems (Thermo Scientific, Asheville, NC).

2.2. Biofunctionalization of graphene ink

The enzymes were immobilized covalently to graphene nano platelets using EDC–NHS chemistry. First, free and unbound—COOH groups of graphene nano platelets were activated by immersing them into phosphate buffer solution (PBS, 100 mM, pH 7.5) containing EDC (10 mM) and NHS (10 mM) for 6 h, then excesses of EDC and NHS were removed by washing with buffer. Finally, 6 mg of EDC–NHS treated graphene platelets was incubated with GOD (20 U), LOD (20 U), XOD (20 U), ChOD (20 U) individually at 4 °C overnight, then washed to remove the unbound enzymes. Enzyme-graphene ink concentrations from 0.05 to 0.6 mg μL^{-1} were investigated to obtain the optimal ink concentration.

2.3. Device fabrication and integration

Microfluidic channels were printed on paper with a solid color printer, and then baked at 110 °C for 5 min to melt the wax so that it penetrated the full thickness of the paper. One end of each channel (2.5 mm × 0.6 mm) was connected to a graphene sensor, and the other end was connected to the inlet of the device. A volume of 5 μL of the enzyme-graphene ink was printed on a paper sized 5 mm × 1 mm to form each graphene biosensor, positioned perpendicular to the direction of the sample flow. Highly conductive silver paste was printed as the positive and negative terminals to connect to the graphene electrode.

2.4. Sensing measurements

All measurements were carried out at room temperature by applying 0.5 V with the potentiostat to the silver ink terminals of the graphene sensor. 2.0 μL of PBS buffer (100 mM, pH 7.5) was first added at the inlet of the microfluidic channel to wet the graphene electrodes. After achieving a steady background current while wet, the measurement was started by adding 2.0 μL of analyte into the inlet to flow to the different enzyme-graphene electrodes at the end of the channels. The concentration of analyte (glucose, lactate, xanthine, and cholesterol) was determined by the increase in H_2O_2 oxidation current. Until a stationary current was obtained, current difference was recorded for plotting a calibration curve. After measuring, a washing step was performed by injecting 2.0 μL of buffer solution to flush the analyte from the enzyme-graphene electrodes before starting a new measurement. Blood samples were diluted 800, 500, 400 and 200 folds with PBS buffer, and dropped at the intersection of the device for the measurement of glucose, lactate, and cholesterol. Xanthine concentration was very low, and thus for