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Short communication

Paper-based chemiresistor for detection of ultralow concentrations of protein

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ABSTRACT

A new paper-based chemiresistor composed of a network of single-wall carbon nanotubes (SWCNTs) and anti-human immunoglobulin G (anti-HIgG) is reported herein. SWCNTs act as outstanding transducers because they provide high sensitivity in terms of resistance changes due to immunoreaction. As a result, the resistance-based biosensor reaches concentration detection as low as picomolar. The resulting paper-based biosensor is sensitive, selective and employs low-cost substrate and simple manufacturing stages. Since chemiresistors require low-power equipment and are able to detect low concentrations with inexpensive materials, the present approach may pave the way for the development of resistive biosensors at very low-cost with high performances.

1. Introduction

Affinity-based biosensors are one type of sensors with most promising potential applications in several fields: health care, food contamination, environmental safety and security, among others. For instance, detecting a human disease or infection in its early stage would represent a considerable advantage for the medical treatment to be effective. Ideally, part of this progress would be given by the development of simple, portable and low-cost biosensors. Importantly, these latter factors could extend the diagnosis to non-developed countries (Mabey et al., 2004; Peeling and Mabey, 2010).

Resistance-based biosensors emerged as an attractive complement to other electrochemical methods of detection. These label-free devices can measure small changes of the target analyte concentration with high precision and low power instrumentation (Esser et al., 2012; Das et al., 2011).

Initial chemiresistors based on nanostructured materials are composed of interdigitated microelectrodes containing one-dimensional nanostructures as transducer elements for gas sensing (Li et al., 2003). In addition to these nanostructured materials, the incorporation of molecular recognition elements, which confer selectivity to the system, considerably broaden the available sensing analytes from typical volatile organic compounds (VOC) to bacteria for instance (García-Aljaro et al., 2010). Such sensors are able to detect therefore chemicals, biomolecules and microorganisms of interest (Li et al., 2003; García-Aljaro et al., 2010; Cella et al., 2010; Wang et al., 2008). They provide high versatility since several nanostructured materials (e.g. carbon nanotubes or nanowires), and various receptors (e.g. aptamers, artificial receptors or antibodies), have been combined together with high reliability. Although the detection technique (i.e. measure of electrical resistance or intensity of electrical current) displays simplicity of operation, the conventional device construction still limits the real applications the sensors can address. These chemiresistors are commonly built on SiO_2/Si surfaces with manufacturing approaches that involve specialized equipment such as lithography techniques and trained personnel so that both the substrate and the construction strategy considerably raise the final cost of production. Other drawbacks of these devices are the lack of estimation of some performance parameters in most of the reported chemiresistors and the need to obtain better reproducibility, both in the measurement and the construction stages, which hinder the ultimate goal of these sensing devices: real sample measurement (Yáñez-Sedeño et al., 2010).

The possible integration of sensors in daily materials such as yarns, rubber or paper materials (Shim et al., 2008; Sekitani et al., 2008; Wang et al., 2009) emerged as an attempt to solve the cost-related issues. In this context, single-wall carbon nanotubes (SWCNTs) have proven to be suitable candidates to be used in sensing devices due to both excellent electronic transduction properties and effective deposition characteristics using carbon nanotubes-based ink. For instance, Shim et al. have taken

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advantage of cotton yarns to build a SWCNT based chemiresistor for protein detection (Shim et al., 2008). Recently, Ammu et al. reported on flexible chemiresistor vapor sensors based on cellulosic substrates and plastics (Ammu et al., 2012). Paper retains attention not only because it affords simplicity, low-cost and disposability but also because it allows developing very sensitive diagnostic systems. Although paper-based sensors operate for several detection techniques with relevant performances (Martinez et al., 2007, 2010; Jokerst et al., 2012; Miranda et al., 2011; Yu and White, 2010; Novell et al., 2012; Ge et al., 2012), to the best of our knowledge, such paper-based chemiresistors have not been developed so far for biomolecules of interest.

In this communication, we aim to demonstrate the development of a novel paper-based chemiresistor for rapid, direct and sensitive detection of proteins. We selected SWCNTs as efficient transducers and as a proof-of-concept for immunoreaction, we have used human immunoglobulin G (HIgG) as a target analyte. The conductive paper was generated by simply painting paper filter with SWCNT ink. Notably, this procedure is among the simplest, low-cost mass manufacturing methodologies (Gonzalez-Macia et al., 2010). The analytical performances of the resulting biosensors afford-picomolar detection of HIgG. Cost effective, facile use and easiness of construction are additional operational features that add on the performance parameters of the novel biosensor reported here.

2. Experimental part

The conductive paper (obtained painting filter papers with SWCNT ink) was cut into strips of 2 mm width and 10 cm long. Each strip was further trimmed so that a thinner part (1 mm width and 5 mm long) was left in the center of the strip. Then, each strip was coated with glue at 1.5 cm from the center in both sides keeping free the ending parts of the SWCNT-deposited paper where the clamps will be connected. The glue addition was necessary to avoid any water migration through the paper to the electrical connections. Finally an adhesive cover was incorporated on the upper side of the chemiresistor and sealed with glue to avoid any direct contact with the solution (Fig. 1a). Details about materials, methods and construction of the biosensor can be found in the Supporting information.

3. Results and discussion

The construction of the biosensor started by painting the filter paper with SWCNT-ink to convert it into conductive paper. The fast adhesion of the SWCNTs could be detected by naked eye due to a rapid color change of the paper from white to grey-black after a single painting. After each painting cycle, we removed most of the sodium dodecylbenzenesulfonate used in the ink by washing with water because the surfactant may interfere in the conduction path of the electrons (Hu et al., 2009) and in the further SWCNTs functionalization steps. The conductive paper obtained was folded and bended while the resistance monitored. However, these mechanical manipulations did not alter the electrical conductivity accounting for very robust conductive papers without specific requirements for particular maintenance or conditioning. We func;tionalized the SWCNTs by absorption of anti-HIgG followed by incubation with Tween 20 to avoid non-specific interactions, following an already reported procedure (Cid et al., 2008).

The chemiresistor was electrochemically characterized after each functionalization step. We recorded the current vs. voltage (I–V) curves for SWCNT immobilization onto the paper, anti-HIgG physisorption onto the SWCNT, Tween 20 blockage of the SWCNTs gaps and 62 pM HIgG addition (Fig. 1b). The current decreased after each functionalization step (from bare SWCNT to Tween 20) accounting for suitable functionalization of the paper-based biosensor. The immobilized molecules over the SWCNTs act as electron donators providing negative charges to the p-type SWCNT in presence of air, which lead to a reduction of the charge carriers so that the current value decreases (Wang et al., 2008; Star et al., 2003; Heller et al., 2008; Salehi-Khojin et al., 2011). The change in the addition of 62 pM concentration of HIgG corresponds to a change of about 18 nA in the response signal from the Tween 20 curve, which cannot be appreciated in Fig. 1b due to the scale. This change in intensity, however, can be clearly differentiated from other additions of HIgG (see Fig. 3a and further discussion below).

The instrumental response of chemiresistors depends on the change of the recorded resistance upon the addition of the target molecule. Therefore, among other factors, the sensitivity depends on the SWCNTs density (which is tuned by increasing or decreasing the number of painting cycles). Therefore, we selected this factor to optimize the biosensor performance. A batch of four biosensors with decreasing amounts of deposited SWCNTs, and therefore with increased resistances from 4 to 360 kΩ, was tested to select the optimal sensitivity. Fig. 2 shows the instrumental response (in terms of normalized resistance of the four biosensors $100 \times (R–R_0)/R_0$, where R and R_0 are the resistances of the biosensor after the exposure to 6.3 pM of HIgG and to buffer, respectively) as a function of the biosensor resistance (related to the density of the immobilized SWCNT). A resistance around

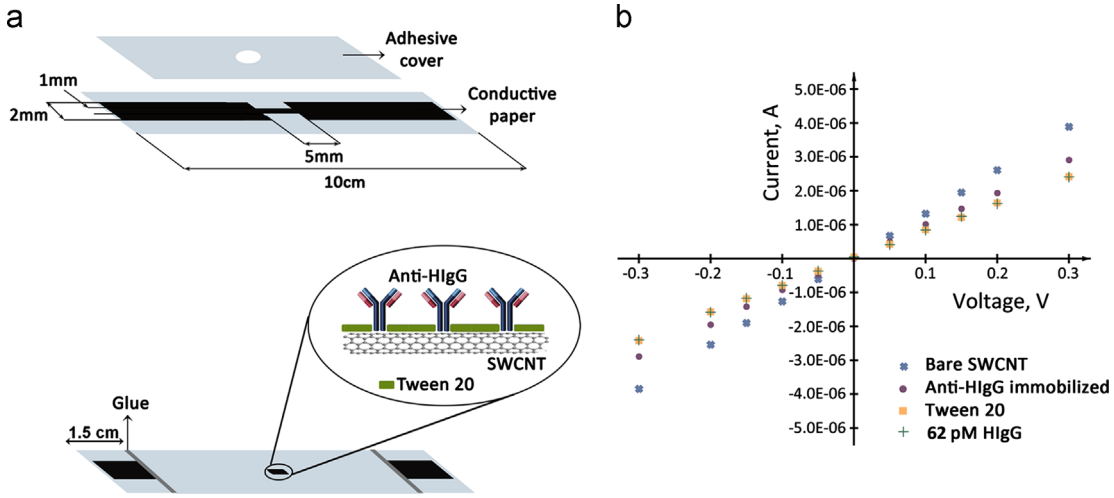

Fig. 1. (a) Schematic representation of the chemiresistor with the ideal attachment of the anti-HIgG onto the SWCNTs and (b) I–V characterization of the paper-based chemiresistor for the different construction steps and HIgG sensing.