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Bioelectronic nose with high sensitivity and selectivity using chemically functionalized carbon nanotube combined with human olfactory receptor

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ABSTRACT

Single-walled carbon nanotubes (swCNTs) hold great promise for use as molecular wires because they exhibit high electrical conductivity and chemical stability. However, constructing swCNT-based transducer devices requires controlled strategies for assembling biomolecules on swCNTs. In this study, we proposed a chemically modified swCNT. The swCNT was functionalized with 1,5-diaminonaphthalene via π-stacking, for reliable attachment of the human olfactory receptor 2AG1 (hOR2AG1). The human olfactory receptor was then anchored. We investigated the use of this functionalized CNT in the fabrication of a highly sensitive and selective bioelectronic nose. For the bioelectronic nose, the swCNT-field effect transistor (FET) platform was composed of polyethylene glycol (PEG)-coated regions to prevent nonspecific absorption and chemically modified swCNTs regions containing hOR2AG1, which can bind to the specific odorant. This approach allowed us to create well-defined micron-scale patterns of hOR2AG1 on the swCNTs. Our bioelectronic nose displayed ultrahigh sensitivity down to concentrations as low as 1 fM due to the enhanced hOR2AG1–odorant interaction through the tight binding of hOR2AG1 on the chemically modified swCNTs. In addition, the approach described here may provide an alternative route for multiplexed detection of diverse odorants and to improve the sensitivity of sensor devices.

1. Introduction

The integration of nanomaterials and biomolecules is one of the most significant and interesting challenges facing the interdisciplinary field of nanobiotechnology (Park et al., 2010). The vertebrate olfactory receptors (ORs) belong to the largest G protein-coupled receptor (GPCR) subfamily and plays a critical role in recognizing thousands of odorant molecules (Krautwurst et al., 1998). Individual ORs with distinct specificity and affinity can bind multiple odorants and serve as one component of the combinatorial code which could allow for the discrimination of unlimited number of odorants (Malnic et al., 1999). Thus, significant advances have been made to develop a bioelectronic nose that exhibits remarkable functional properties by utilizing ORs as the primary sensing elements (Lee and Park, 2010). A diverse range of sensing devices have been used as the transducers in bioelectronic noses including surface plasmon resonance (SPR), quartz crystal microbalance (QCM), microelectrode, nanotube-based field effect transistor (FET) sensor, and electrochemical impedance spectrometry (EIS) (Benilova et al., 2008b; Kim et al., 2009; Lee et al., 2009a,b; Sung et al., 2006; Vidic et al., 2006; Yoon et al., 2009). These bioelectronic nose platforms have great potential to be used as effective tools to detect and discriminate many different odorants with high sensitivity and selectivity. Despite the great potential of the bioelectronic nose, there are a few limitations such as the low expression level of OR, the lack of appropriate heterologous systems and assaying tools (Lee and Park, 2010). Currently, only a few cognate odorant-OR pairs are known, and this acts as a bottleneck. In consequence, most bioelectronic noses have been developed for only a few ORs such as rat I7, human OR (hOR) 17–40, and hOR2AG1 (Benilova et al., 2008a; Hou et al., 2007; Yoon et al., 2009).

Recently, carbon nanotube (CNT)-based devices as well as other nanomaterial devices have been fabricated and combined with biomolecules (Maehashi et al., 2007; Staii and Johnson, 2005). Single-walled CNTs (swCNTs) are molecular wires that exhibit interesting structural, mechanical, electrical, and electromechanical properties. Recent studies showed that swCNTs could be made into field effect transistors (FETs) (Kim et al., 2009; So et al., 2005). Thus, surface modification of swCNTs could alter their physical properties and increase their potential use in the development of novel electronic devices such as bioelectronic noses (Hirsch, 2002;

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Vosgueritchian et al., 2010). Selective patterning of biomolecules on the swCNTs surface has been pursued in the past with the goal of using nanotubes coupled with biomolecules that have specific recognition properties for the fabrication of an ideal hybrid sensor platform (Assali et al., 2009; Chen et al., 2001). For this purpose, the sidewalls of swCNT were functionalized by biofunctional molecules, 1-pyrenebutanoic acid, containing pyrenyl moiety via π-stacking, which preserved the primary structures. Then biological molecules were subsequently immobilized onto swCNT surface with a high degree of control and specificity (Chen et al., 2001).

In this study, we constructed a chemically modified swCNT-based biosensor with 1,5-diaminonaphthalene via π-stacking and covalently attached hORs to the surface, which were used as the recognition element. The potential of using the chemically functionalized swCNTs as a bioelectronic nose was also evaluated.

2. Materials and methods

2.1. Preparation of hOR2AG1 protein

hOR2AG1 was used in this study as a model hOR. It was heterologously expressed with a GST-tag fused at its N-terminus in *Escherichia coli* (*E. coli*). The expression of OR in *E. coli* has various advantages such as low cost, high-level expression, short generation time, and homogeneity of the recombinant protein (Sarramegna et al., 2003). The *E. coli* BL21 strain was transformed with pDEST15/GST-hOR2AG1, and then cultured in LB medium containing 50 μg/ml of ampicillin (culture volume: 1 L). The expression of hOR2AG1 was induced for 4 h with 0.5 mM of isopropyl β-D-thiogalactoside (IPTG) at OD_{600} of 0.5. Cultured cells were harvested and resuspended in PBS. The resuspended cells were then lysed by sonication for 5 min and the insoluble fraction including hOR2AG1 was collected by centrifugation at 15,000 × *g* for 30 min. As previously reported, the insoluble fraction was treated with 5% Triton X-100 and centrifuged at 15,000 × *g* to remove impurity proteins (Song et al., 2009). The remaining insoluble fraction containing hOR2AG1 was used as a sensing element. The expression of hOR2AG1 in *E. coli* was confirmed by western blot analysis (Fig. 1a). The hOR2AG1 concentration was determined using the BCA reagent (Pierce) with bovine serum albumin as a standard.

2.2. Fabrication of biosensor platform

The starting substrate was a p-type silicon wafer that was covered with a 1000 Å thick oxide layer. A photolithography process was used to pattern a photoresist (PR, AZ5214) layer to mask the SiO_2 surface. The SiO_2 substrates containing the patterned PR were then incubated with PEG-toluene for 1 h under 2% humidity to prevent oxidation of PEG molecules (methoxy-terminated polyethylene glycol, MW 5000, Lsysan Bio). The solution was prepared by dissolving 2 g of PEG in 40 ml anhydrous toluene and 2.75 ml of tributylamine (Sigma–Aldrich), which was used as a catalyst (Lee et al., 2006). Since one end of this PEG contained a silanol group, the PEG molecules self-assembled onto the bare SiO_2 substrate (Fig. 1b). PR was then removed with acetone using a lift-off method. The PEG patterned substrate was immersed in a solution of swCNTs (0.1 mg/ml in 1,2-dichlorobenzene) for swCNT assembly. As a result, swCNTs were deposited onto the SiO_2 substrate. Afterwards, 1,5-diaminonaphthalene (100 μM in methanol, Sigma–Aldrich) was adsorbed onto the swCNTs. Thus, as shown in Fig. 1b, a self-assembled swCNTs pattern was created on the SiO_2 substrate. Afterwards, the functionalized swCNT pattern with 1,5-diaminonaphthalene was activated using 2% (v/v) glutaraldehyde (Sigma–Aldrich) for 3 h, and subsequently the hOR2AG1 protein immobilized to the swCNTs surface for 3 h at room temperature.

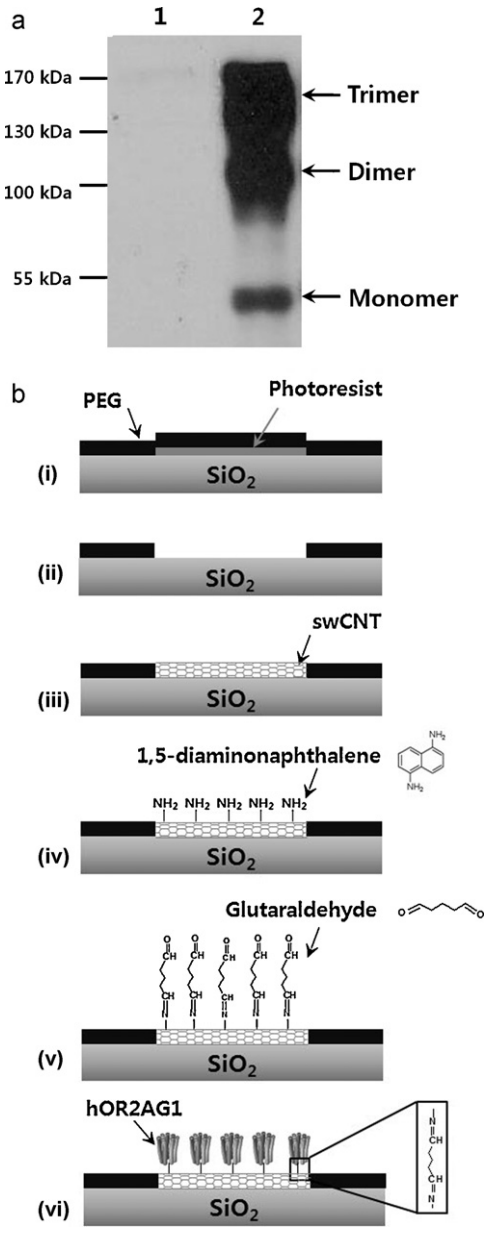

Fig. 1. (a) Western blot analysis of the hOR2AG1 expression. Lane 1: soluble fraction, lane 2: insoluble fraction. (b) Schematic diagram for patterning of a biofunctional surface. (i) Photoresist patterning on SiO_2 surface and self-assembly of PEG molecules on photoresist-patterned SiO_2 substrate. (ii) Lift-off of the photoresist. (iii) Self-assembly of single-walled carbon nanotube (swCNT) on the bare SiO_2 substrate. (iv) 1,5-diaminonaphthalene functionalization of the exposed swCNT substrate. (v) Activation of swCNTs surface functionalized with 1,5-diaminonaphthalene using glutaraldehyde. (vi) Deposition of hOR2AG1 on the functionalized swCNT substrate.

2.3. Characterization of hOR2AG1 on chemically functionalized swCNT surface

To determine the most reliable method of depositing the hOR2AG1 protein on the functionalized swCNTs surface, various condensing agents, including glutaraldehyde, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, Sigma–Aldrich), dicyclohexyl-carbodiimide (DCC, Sigma–Aldrich), and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM, Sigma–Aldrich), were tested. The functionalized swCNT pattern with 1,5-diaminonaphthalene was activated using 2% (v/v) glutaraldehyde in PBS for 3 h at room temperature. And then, the