Biosensors and Bioelectronics 74 (2015) 199–206

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Real-time monitoring of geosmin and 2-methylisoborneol, representative odor compounds in water pollution using bioelectronic nose with human-like performance

Manki Son [a], Dong-guk Cho [b], Jong Hyun Lim [c], Juhun Park [b], Seunghun Hong [b,d], Hwi Jin Ko [e,*], Tai Hyun Park [a,c,f,**]

[a] Interdisciplinary Program for Bioengineering, Seoul National University, Seoul 151-742, Republic of Korea
[b] Department of Physics and Astronomy, Seoul National University, Seoul 151-747, Republic of Korea
[c] School of Chemical and Biological Engineering, Seoul National University, Seoul 151-742, Republic of Korea
[d] Department of Biophysics and Chemical Biology, Seoul National University, Seoul 151-742, Republic of Korea
[e] N-Bio Institute, Seoul National University, Seoul 151-818, Republic of Korea
[f] Advanced Institutes of Convergence Technology, Suwon 433-270, Republic of Korea

ARTICLE INFO

Article history:
Received 24 April 2015
Received in revised form
11 June 2015
Accepted 23 June 2015
Available online 25 June 2015

Keywords:
Bioelectronic nose
Human olfactory receptor
Carbon nanotube
Field-effect transistor
Water quality monitoring

ABSTRACT

A bioelectronic nose for the real-time assessment of water quality was constructed with human olfactory receptor (hOR) and single-walled carbon nanotube field-effect transistor (swCNT-FET). Geosmin (GSM) and 2-methylisoborneol (MIB), mainly produced by bacteria, are representative odor compounds and also indicators of contamination in the water supply system. For the screening of hORs which respond to these compounds, we performed CRE-luciferase assays of the two odorants in heterologous cell system. Human OR51S1 for GSM and OR3A4 for MIB were selected, and nanovesicles expressing the hORs on surface were produced from HEK-293 cell. Carbon nanotube field-effect transistor was functionalized with the nanovesicles. The bioelectronic nose was able to selectively detect GSM and MIB at concentrations as low as a 10 ng L^{-1}. Furthermore, detection of these compounds from the real samples such as tap water, bottled water and river water was available without any pretreatment processes.

1. Introduction

When water is contaminated by bacteria, various off-flavors are produced such as geosmin (GSM) and 2-methylisoborneol (MIB), which release an earthy and musty odor, respectively (Zaitlin and Watson, 2006; Parinet et al., 2010; Li et al., 2012). A human nose is extremely sensitive to these molecules (Polak and Provasi, 1992) and these compounds are regulated by law for safe drinking water. In addition, harmful algal bloom, which occurs with high bacterial densities and increased water temperature, can be recognized by the smells of GSM and MIB (Freeman, 2010).

Typically, solid-phase microextraction gas chromatography–mass spectrometry (SPME-GC/MS) is used for the detection of GSM and MIB in water supply system (Lloyd et al., 1998; Watson et al., 2000). Although conventional methods have the advantage of a quantitative component analysis, they have limitations in rapid on-site assessments of water quality due to their long and complex pretreatment procedures, and large-sized instrumentations. Additionally the equipment is complicated and requires expert operators.

Recently, the bioelectronic nose, which integrates olfactory receptors (ORs) found in natural system and nanomaterials such as carbon nanotubes (Goldsmith et al., 2012; Jin et al., 2012; Kim et al., 2009; Lim et al., 2013, 2014; Park et al., 2012a), conducting polymer (Lee et al., 2012; Yoon et al., 2009) and graphene (Park et al., 2012b), has been reported as an effective analytical tool for detecting various volatile organic compounds with high selectivity and sensitivity (Lee and Park, 2010; Oh et al., 2011) (Table S1).

Herein, we report a human olfactory receptor (hOR)-based bioelectronic nose that mimics the human smell sensing system for the fast and easy assessment of water quality by detecting GSM and MIB. Human nose can quickly identify odors with high sensitivity and selectivity. To overcome the drawbacks of conventional methods, hORs that bind with the unpleasant smell were selected with screening procedures. The olfactory nanovesicles were

* Corresponding author.
** Corresponding author at: School of Chemical and Biological Engineering, Seoul National University, Seoul 151-742, Republic of Korea.
E-mail addresses: hwijinko@snu.ac.kr (H.J. Ko), thpark@snu.ac.kr (T.H. Park).

http://dx.doi.org/10.1016/j.bios.2015.06.053
0956-5663/

functionalized with single-walled carbon nanotube (swCNT) field-effect transistor (FET) for a conversion of biological signals to electric signals. The sensor was designed to detect two different odorants at the same time for more accurate assessment of water contamination. We demonstrate that this bioelectronic nose can serve as a sensor to monitor water quality in real time and even on the spot by directly detecting water contaminants without any pretreatment process.

2. Materials and methods

2.1. Expression human olfactory receptors and formation of nanovesicles

Human ORs were cloned with sequence information from the Olfactory Receptor DataBase. All hORs were subcloned in pcDNA3 mammalian expression vectors (Invitrogen, USA) containing the first 20 amino acids of human rhodopsin (Rho-tag) (Zhuang and Matsunami, 2007). The sequences of the cloned genes were verified by sequencing (GenoTech, Korea). Human embryonic kidney (HEK)-293 cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) (HyClone, USA) supplemented with 10% Fetal Bovine Serum (FBS) (Gibco, USA) and 100 U/mL penicillin, 100 μg mL^{-1} streptomycin (Gibco, USA) at 37 °C and 5% CO_2. Transfection was performed with the Neon Transfection System (Invitrogen, USA). The cells were harvested and resuspended in PBS and 2×10^6 cells were mixed with 5 μg of OR, 2 μg of pCRE-Luc, 1 μg of pSV40-RL, 1 μg of RTP1S, 0.5 μg of Ric8b, 0.5 μg of $G_{\alpha olf}$. Electric pulses were then applied three times at 1100 V with 10 ms per pulse. Transfected cells were cultured in DMEM supplemented with 10% FBS without antibiotics, with 5×10^4 cells per well in 96-well plates.

HEK-293 cells were resuspended in serum-free DMEM containing 10 μg mL^{-1} of cytochalasin B (Sigma, USA) and agitated at 300 rpm for 30 min. The nanovesicles were separated from the cells by centrifugation at 500 g for 10 min, and the supernatant was centrifugated to collect the nanovesicles at 15000 g for 30 min. The produced nanovesicles were resuspended in PBS with 1 μg mL^{-1} of total protein concentration, stored at −70 °C, and melted before being use. Morphology of the nanovesicles was observed by Scanning Electron Microscopy. HEK-293 cell-derived nanovesicles were applied on poly-D-lysine-coated slide glass and fixed with 4% paraformaldehyde in PBS at room temperature for 20 min. The fixed nanovesicles were dehydrated with increasing concentrations of ethanol and lyophilized by freeze-drying. The lyophilized nanovesicles were coated with Pt (10 nm) by a sputtering method and visualized by SEM. For the analysis of nanovesicle size, 1 mL of nanovesicles in PBS solution was analyzed by an ELS spectrophotometer (ELS-8000, Otsuka Electronics, Japan).

2.2. Immunocytochemistry and western blot analysis

HEK-293 cells were fixed with 4% paraformaldehyde in PBS at room temperature for 20 min. The cells were blocked with 5% BSA diluted in PBS and incubated with the Rho-tag antibody at a 1:500 dilution in PBS with 5% BSA at 37 °C for 1 h. The cells were then washed three times with PBS and incubated with the Alexa Fluor 594 (Invitrogen, USA) at 1:1000 dilution in PBS with 5% BSA at 37 °C for 1 h.

HEK-293 cells and nanovesicles were washed twice with PBS and lysed by sonication (2 s on/off, 5 min). The supernatant and membrane fractions were separated by centrifugation at 15000 g for 30 min. Protein samples were loaded onto 10% SDS-PAGE gels and transferred to PVDF membranes (Bio-Rad, USA) under 0.15 A of constant current for 60 min. The membranes were blocked with 5% BSA diluted in PBS and 0.1% Tween-20 at room temperature for 1 h and incubated overnight with the Rho-tag antibody at a 1:1000 dilution in PBS with 5% BSA and 0.1% Tween-20 at 4 °C. The membranes were then washed three times for 5 min with PBS with 5% BSA and 0.1% Tween-20 and incubated with anti-rabbit IgG-HRP (Invitrogen, USA) at 1:1000 dilution in PBS with 5% BSA and 0.1% Tween-20 at room temperature for 1 h. ECL solution (Thermo Scientific, USA) was used to detect proteins expressed on the membrane.

2.3. Biological activity assay

The Dual-Glo Luciferase Assay System (Promega, USA) was used to measure receptor response. Approximately 24 h after transfection, the medium was replaced with 50 μL of DMEM and incubated for 30 min at 37 °C. After stimulation with 25 μL of odorants for 4 h at 37 °C, luminescence was measured with Luminoskan Ascent Microplate Luminometer (Thermo Scientific, USA). The luciferase activity was normalized with the formula [CRE/Renilla(N) − CRE/Renilla(0)]/[CRE/Renilla(FSK) − CRE/Renilla (0)]. 1 μM forskolin (FSK), an adenylyl cyclase activator, was used as a positive control and a no odorant solution was used as a negative control for each hOR.

HEK-293 cells were incubated for 30 min with 10 μM of Fura-2 AM (Invitrogen, USA) in Ringer's solution (140 mM NaCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM KCl, 5 mM glucose, 10 mM HEPES (pH 7.4)) at 37 °C. The Fura-2 AM-loaded nanovesicles were produced as described for the formation of regular nanovesicles and placed on a 96-well plate. Calcium-dependent fluorescence was measured at 510 nm with dual excitations at 340 nm and 380 nm using a GENios Pro microplate reader (TECAN, Switzerland).

2.4. Fabrication of swCNT-FET

Purified swCNTs (Hanwha, Korea) were dispersed in 1,2-dichlorobenzene solution by applying ultrasonic vibration for 1 h. To create swCNTs patterns on a silicon oxide wafer, the octadecyltrichlorosilane (OTS) self-assembled monolayer (SAM) technique was used. First, we patterned AZ5214 photoresists on a silicon oxide wafer by using a conventional photolithographic technique. Then the wafer was placed in an OTS solution (1:500, v/v in hexane) for 5 min to create OTS SAM on the wafer. Subsequently, the OTS-coated wafer was dipped in acetone to remove the AZ5214 patterns. Next, the OTS-patterned wafer was placed in the swCNT solution for 30 s. These steps allowed swCNTs to be adsorbed selectively onto bare silicon oxide regions in the wafer, while OTS SAM regions blocked the adsorption of swCNTs. Source and drain electrodes (Au/Pd, 30 nm/10 nm) were fabricated via conventional photolithography, thermal evaporation, and lift-off process. Finally, the source and drain electrodes were passivated with a photoresist to prevent current leakage from the electrodes.

2.5. Construction of bioelectronic nose

For the immobilization of nanovesicles, 0.1 mg mL^{-1} of poly-D-lysine in PBS was added to swCNT channel regions and incubated at room temperature for 2 h. Then, 1 μL of nanovesicles in PBS was added to the swCNT channel and incubated at 4 °C for 2 h. All constructed bioelectronic noses were used immediately. For the electrical signal measurement, 49.5 μL of PBS solution was added to the nanovesicle-immobilized swCNT channel and 0.1 V DC was applied. Then, 0.5 μL of odorant solution was injected sequentially. Current changes between the drain and source electrodes were measured with a 2636A Dual-channel System Source Meter Instrument (Keithley, USA) and a MST 8000 probe station (MS TECH, Korea).