AIP | Applied Physics Letters

Bi nanowire-based thermal biosensor for the detection of salivary cortisol using the Thomson effect

Seunghyun Lee, Jung Hyun Lee, MinGin Kim, Jeongmin Kim, Min-Jung Song et al.

Citation: Appl. Phys. Lett. **103**, 143114 (2013); doi: 10.1063/1.4824015
View online: http://dx.doi.org/10.1063/1.4824015
View Table of Contents: http://apl.aip.org/resource/1/APPLAB/v103/i14
Published by the AIP Publishing LLC.

Bi nanowire-based thermal biosensor for the detection of salivary cortisol using the Thomson effect

Seunghyun Lee,[1] Jung Hyun Lee,[2] MinGin Kim,[1] Jeongmin Kim,[1] Min-Jung Song,[1] Hyo-Il Jung,[2,a)] and Wooyoung Lee[1,a)]
[1]*Department of Materials Science and Engineering, Yonsei University, 134 Shinchon, Seoul 120-749, South Korea*
[2]*Department of Mechanical Engineering, Yonsei University, 134 Shinchon, Seoul 120-749, South Korea*

(Received 1 July 2013; accepted 17 September 2013; published online 2 October 2013)

We present a study of a thermal biosensor based on bismuth nanowire that is fabricated for the detection of the human stress hormone cortisol using the Thomson effect. The Bi nanowire was grown using the On-Film Formation of Nanowires (OFF-ON) method. The thermal device was fabricated using photolithography, and the sensing area was modified with immobilized anti-cortisol antibodies conjugated with protein G for the detection of cortisol. The voltages were measured with two probe tips during surface modification to investigate the biochemical reactions in the fabricated thermal biosensor. The Bi nanowire-based thermal biosensor exhibited low detection limit and good selectivity for the detection of cortisol. © *2013 AIP Publishing LLC*.
[http://dx.doi.org/10.1063/1.4824015]

Thermal biosensors are based on the measurement of heat generated by enthalpy changes during biochemical reactions. Instead of the direct monitoring of heat, this variation is reflected as a temperature change because heat is proportional to variations in enthalpy or temperature.[1] Because the measured temperature difference (ΔT) is related to the potential difference (ΔV) by the Thomson effect, the substrate concentration reacted is proportional to the value of ΔV.[2–4] These biosensors have several advantages, such as high sensitivity, small sample volumes, low noise levels, and continuous monitoring of bioprocesses.[2,5] In addition, the proposed thermal biosensor based on bismuth nanowire has a high potential of overcoming the limitations of decay due to salt precipitation and pH alteration or variations among fabricated devices[6,7] in the well-known field effect transistor biosensors,[8,9] since the bioprocess does not specifically require a buffered environment to induce the voltage change, and the fabrication of both the nanowire and device can be well controlled. Maintaining adiabatic conditions during operation and thermally isolating the device with accurate control of the ambient temperature to prevent any loss of heat is critical because the results are dependent on temperature.[1] Therefore, many researchers have studied to discover or synthesize materials with low thermal conductivity to improve the thermoelectric efficiency.

Bismuth is a group-V semimetallic element that exhibits unique properties, such as small effective electron mass ($\sim 0.001\, m_e$), large thermoelectric power (50–100 μV/K), small thermal conductivity (8 W/mK), small carrier mean free path, highly anisotropic Fermi surface, and small energy overlap ($\sim$38 meV) between the L-point conduction band and the T-point valence band.[10–12] Especially Bi nanowire with low dimensionality enhances the thermoelectric figure-of-merit (ZT) due to the quantum confinement effect.[10–14] A high ZT value signifies low thermal conductivity because ZT is inversely proportional to thermal conductivity.[10,15,16] Thus, this nanowire shows potential as a promising material for thermoelectric applications.

Cortisol is a steroid hormone and a stress biomarker of various mental and physical diseases such as chronic fatigue syndrome, post-traumatic stress disorder, Cushing's syndrome, and Addison's disease.[17–19] Cortisol is also important for the regulation of blood pressure, glucose levels, and carbohydrate metabolism. Abnormal cortisol level can cause serious symptoms, including inflammation inhibition, depression of the immune system, weight loss, and fatigue.[20] Although the free cortisol level in saliva is lower than that in blood, saliva is usually used as a sample in the accurate measurement of cortisol because salivary cortisol levels are unaffected by physiological variations in saliva production, flow rates, salivary enzymes, and sample collections.[21]

Various biosensors that are based on electrochemical,[22–25] optical,[26] and surface plasmon resonance (SPR) techniques[27–29] have recently been developed to detect free cortisol in saliva. However, expensive laser instrumentation is required for these optical and SPR techniques, and electrochemical sensors need amplification of electrical signals in lower concentrations of cortisol owing to low signal-to-noise ratio (S/N).

In this study, a thermal biosensor based on a Bi nanowire was developed for the detection of free cortisol in saliva. Bismuth nanowires were synthesized using the on-film formation of nanowires (OFF-ON) method, and a thermal biosensor was fabricated through photolithography. The proposed thermal biosensor exhibited good selectivity and rapid response times for low concentrations of cortisol.

Cystein3-linked protein G (Cys3-protein G) was obtained from Bioprogen (Daejeon, Korea). Bovine serum albumin (BSA), cortisol antibody, and cortisol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Phosphate buffer (PB) solution, dibasic potassium phosphate (K_2HPO_4), and monobasic potassium phosphate (KH_2PO_4) were purchased from Yakuri Pure Chemicals (Kyoto, Japan). Megaposit™ SPR™

[a)]Authors to whom correspondence should be addressed. Electronic addresses: wooyoung@yonsei.ac.kr and uridle7@yonsei.ac.kr

3612 positive photoresist (PR) and microposit™ LOL™ 2000 liftoff layer (LOL) were provided by Rohm and Haas. All reagents were analytical grade.

The morphology of the Bi nanowire was analyzed using field-emission scanning electron microscopy (FESEM, JSM 7000 F, JEOL, Japan). The potential difference was measured by a Keithley 2182 nanovoltmeter (Keithley Instruments, USA). The electrical measurements were conducted at room temperature in an ambient air environment.

Bismuth nanowires were grown on Bi thin films via the on-film formation of nanowires (OFF–ON).[11] Thin Bi layer with a thickness of 50 nm was deposited onto a thermally oxidized silicon (Si) substrate by radio frequency (RF) sputtering and was subsequently heated at 250 °C for 5 h under ultrahigh vacuum (UHV). The synthesized Bi nanowires were dispersed on another Si substrate by direct contact through electrostatic attraction. Photolithography was used to develop a thermal device, and the schematic of its fabrication process is shown in Figure 1. The substrate with Bi nanowires was coated with a lift-off resist (LOR) using a spin coater, and the coated substrate was soft-baked at 115 °C for 2 min. The LOR coated substrate was again coated with a photoresist (PR; AZ1512) and soft-baked (at 95 °C for 2 min) using the same method. The baked substrate was exposed under UV light for 14 s and developed with a LOL developer. Prior to deposition of Cr/Au thin films, the surface of the Bi nanowires was etched for 5 min with Ar plasma to remove its oxide layer for ohmic contact. The Cr/Au thin films (5 nm/150 nm) were deposited by DC sputtering, and the deposited substrate was immersed in acetone for lift-off. To measure the biochemical reactions, a PR passivation layer (2 nm) was formed on the surface of all parts, with the exception of the sensing area and the bonding pads.

The principle of the thermal biosensor based on the Bi nanowire for the detection of cortisol is presented in Figure 2(a). Because all biological reactions are exothermic, a temperature difference occurs on the nanowire as a result of the binding of biomolecules, and this temperature difference

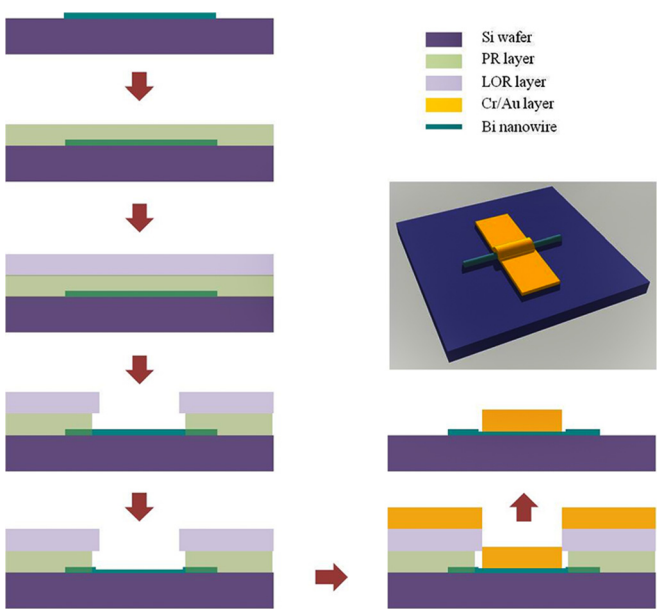

FIG. 1. Schematic of the process used to fabricate the thermal biosensor.

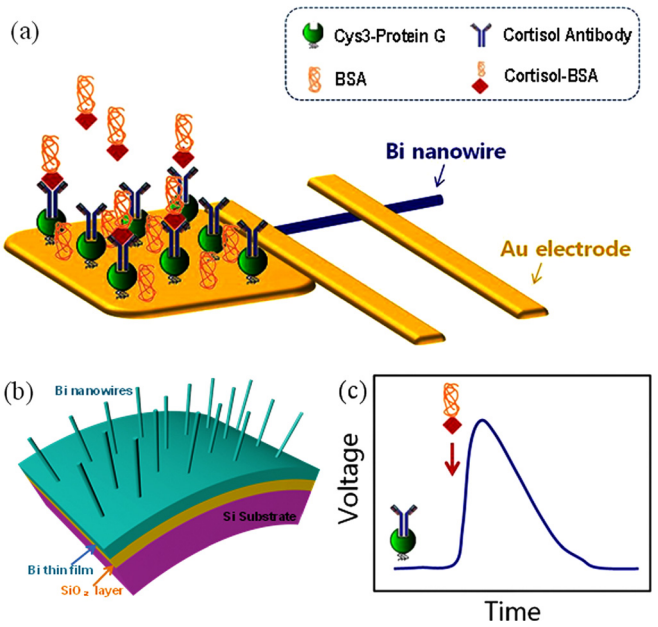

FIG. 2. (a) Schematic of the strategy and the principle of voltage detection for the thermal cortisol biosensor based on Bi nanowire. (b) Illustration of Bi nanowire growth using the OFF–ON method. (c) Schematic of the voltage difference response due to the antibody-antigen reaction.

leads to a potential difference due to the Thomson effect. Therefore, cortisol concentrations can be determined through the measurement of the voltage difference via the antibody–antigen reaction of cortisol. Figure 2(b) illustrates the OFF-ON method that was used to grow single-crystalline Bi nanowires.[11] The diameter of the nanowire used to fabricate the thermal biosensor was approximately 100 nm. The voltage difference response curve from the antibody–antigen reaction is shown in Figure 2(c).

Figure 3(a) shows the schematic of the fabricated device and the optical microscopic image of the electrodes with a Bi-nanowire bridge. The size of the electrode connected to the sensing area, with immobilize the biomolecules for the biochemical reaction, was $80 \times 120\ \mu m^2$. The practical scale of the overall device shown in Figure 3(b) was $2 \times 2\ cm^2$ and its performance was analyzed with 200 μL of a 100 ng/ml cortisol solution sample.

To evaluate the performance of this device, we continuously measured the voltage response for cortisol via step-by-step construction using the Keithley 2182 nanovoltmeter, as

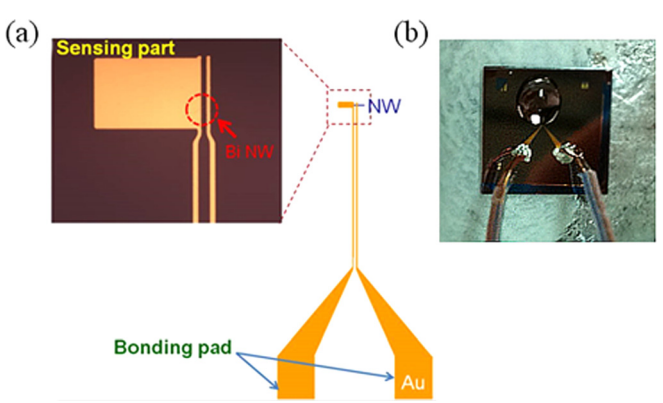

FIG. 3. (a) Schematic illustration and (b) optical microscope image of the thermal biosensor.

shown in Figure 4(a). In the case of phosphate buffered saline (PBS), no voltage change was observed as a result of any chemical reaction. Protein G with cysteine residue at the N-terminus has been widely used to enhance the orientation and stability of the antibody on a gold surface.[30] When 10 μg/ml of Cys3-protein G solution was added, a significant voltage change of 400 μV (ΔV_1) was observed after 100 s (τ_1). For these thermal biosensors, the response time (τ) represents the runtime required to transport the heat produced in biochemical reactions. Because Cys3-protein G with the thiol group was immobilized on the Au sensing area through an Au–S interaction, heat transfer dynamics occur between this biomolecule and Au. The indicated temperature difference (ΔT_1) was approximately 4.5 K (Fig. 4(b)). In the literature, the Au–S binding energy of ($\Delta H^\circ \approx -45$ kcal/mol) was investigated by the van't Hoff equation, which indicates an exothermic process.[31] The cortisol antibody was immobilized on the sensing area modified with Cys3-protein G with extreme orientation. Its construction was accomplished by specific binding between the heavy chains of the Fc region of the antibody and protein G.[32]

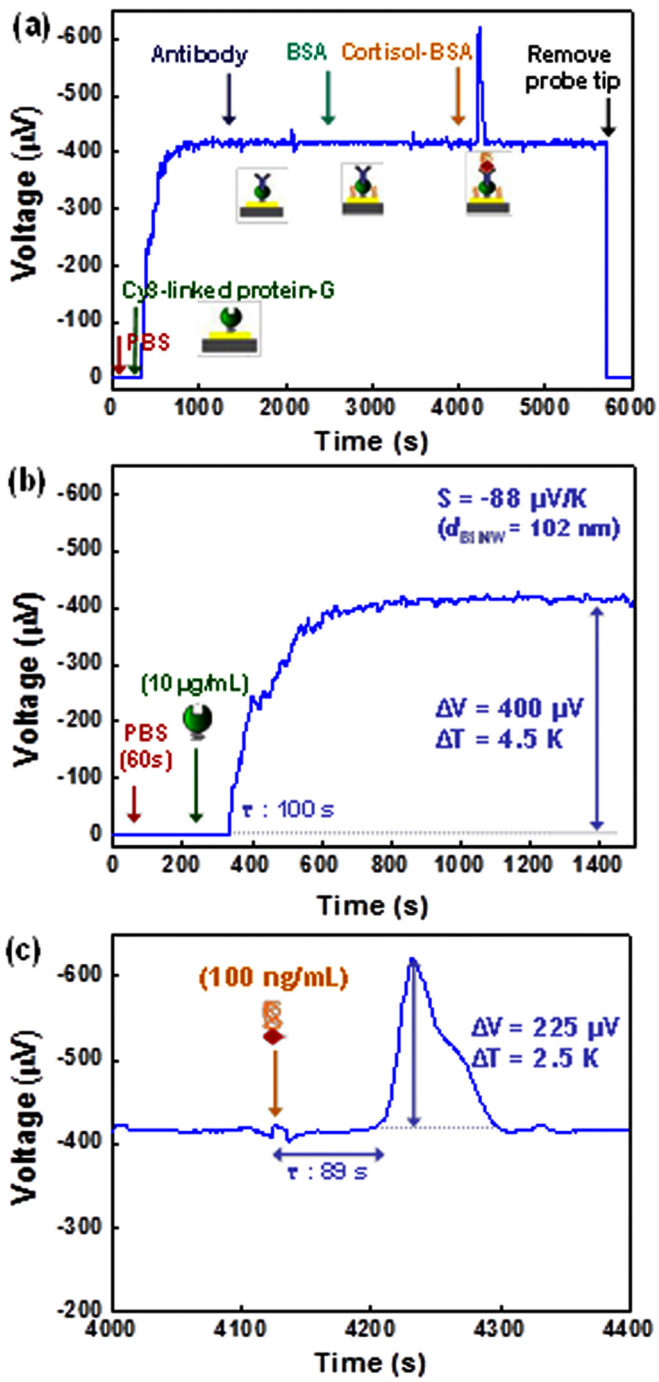

FIG. 4. Real-time thermal voltage response curves for the introduced biomolecules for each step-by-step construction: (a) complete reaction, (b) PBS, Cys3-protein G, and antibody, and (c) cortisol.

Although BSA (1 mg/ml) was added to deactivate and block excess reactive groups that remains on the Au sensing area, a voltage change was not observed because this step may be an isothermal adsorption process (Fig. 4(a)).[33] When 100 ng/ml cortisol solution was introduced onto the sensing area, its voltage changed significantly increasing 225 μV (ΔV_3) after 89 s (τ_3) with a temperature difference of 2.5 K (ΔT_3), as shown in Figure 4(c). The voltage difference is attributed to the specific binding of the cortisol to its antibody. The antigen–antibody reaction adheres to the lock-and-key model through an exothermic hydrogen bond, in which the exerted heat is less than the exerted heat of the Au–S bond.[34,35] The voltage for the antibody–antigen reaction exhibits an instantaneous increase and subsequently regains its original value (−410 μV),[36] which indicates that the heat produced from this reaction is exerted almost immediately. When the contacted tips were removed, the voltage drops to 0 V. Due to the Thomson effect, a Seebeck coefficient (S) of approximately 90 μV/K was measured for the Bi nanowire in this thermal biosensor.

A thermal biosensor based on a Bi nanowire was developed for the detection of cortisol. To investigate the biochemical reactions, we measured the voltage change in this thermal device during surface modification in small sample volume (200 μL) with two probe tips. The results showed that the thermal biosensor based on the Bi nanowire exhibited a low detection limit and adequate selectivity for the detection of cortisol. Therefore, the proposed thermal biosensor has strong potential for the detection of various biomolecules. Future studies of thermal biosensors based on Bi nanowires are suggested, with lower sample concentrations, different analytes, and smaller sensing areas.

The authors S. H. Lee and J. H. Lee contributed equally to this work. This work was supported by the Priority Research Centers Program (2009-0093823) and the Pioneer Research Center Program (2013008070) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology. H.I. Jung acknowledges the Development of Wellness Humancare Platform of the Ministry of Commerce, Industry and Energy the Ministry of Korea and the Future-Based Technology Development Program (Nano Fields, Grant No. 2010-0029297).

[1]P. Bataillard, E. Steffgen, S. Haemmerli, A. Manz, and H. M. Widmer, Biosens. Bioelectron. **8**, 89–98 (1993).
[2]B. Bjarnason, P. Johansson, and G. Johansson, Anal. Chim. Acta **372**, 341–348 (1998).
[3]K. Ramanathan and B. Danielsson, Biosens. Bioelectron. **16**, 417–423 (2001).

[4]H. J. Goldsmith, R. Hull, R. M. Osgood, Jr., J. Parisi, and H. Warlimont, *Springer Series in Materials Science 121: Introduction to Thermoelectricity* (Springer, New York, 2010), p. 1.
[5]U. Harborn, B. Xie, R. Venkatesh, and B. Danielsson, Clin. Chim. Acta **267**, 225–237 (1997).
[6]E. Stern, A. Vacic, and M. A. Reed, IEEE Trans. Electron Devices **55**, 3107–3118 (2008).
[7]J. M. Gamba, Ph.D. Dissertation (California Institute of Technology, 2012).
[8]G. Zheng, F. Patolsky, Y. Cui, W. U. Wang, and C. M. Lieber, Nat. Biotechnol. **23**, 1294–1301 (2005).
[9]Y. Cui, Q. Wei, H. Park, and C. M. Lieber, Science **293**, 1289–1292 (2001).
[10]A. Boukai, K. Xu, and J. R. Heath, Adv. Mater. **18**, 864–869 (2006).
[11]L. Li, Y. Zhang, G. Li, and L. Zhang, Chem. Phys. Lett. **378**, 244–249 (2003).
[12]W. Shim, J. Ham, K. Lee, W. Y. Jeung, M. Johnson, and W. Lee, Nano Lett. **9**, 18–22 (2009).
[13]X. Sun, Z. Zhang, and M. S. Dresselhaus, Appl. Phys. Lett. **74**, 4005–4007 (1999).
[14]Y. M. Lin, X. Sun, and M. S. Dresselhaus, Phys. Rev. B **62**, 4610–4623 (2000).
[15]M. S. Dresselhaus, G. Dresselhaus, X. Sun, Z. Zhang, S. B. Cronin, T. Koga, J. Y. Ying, and G. Chen, Microscale Thermophys. Eng. **3**, 89–100 (1999).
[16]J. W. Roh, K. Hippalgaonkar, J. H. Ham, R. Chen, M. Z. Li, P. Ercius, A. Majumdar, W. Kim, and W. Lee, ACS Nano **5**, 3954–3960 (2011).
[17]D. Hauer, F. Weis, T. Krauseneck, M. Vogeser, G. Schelling, and B. Roozendaal, Brain Res. **1293**, 114–120 (2009).
[18]R. C. Stevens, S. D. Soelberg, S. Near, and C. E. Furlong, Anal. Chem. **80**, 6747–6751 (2008).
[19]J. C. Zhou, M. H. Chuang, E. H. Lan, B. Dunn, P. L. Gillman, and S. M. Smith, J. Mater. Chem. **14**, 2311–2316 (2004).
[20]S. S. C. Tai and M. J. Welch, Anal. Chem. **76**, 1008–1014 (2004).
[21]R. F. Vining, R. A. McGinley, J. J. Maksvytis, and K. Y. Ho, Ann. Clin. Biochem. **20**, 329–335 (1983).
[22]K. Sun, N. Ramgir, and S. Bhansali, Sens. Actuators B **133**, 533–537 (2008).
[23]S. K. Arya, G. Chornokur, M. Venugopal, and S. Bhansali, Biosens. Bioelectron. **25**, 2296–2301 (2010).
[24]M. Venugopal, S. K. Arya, G. Chornokur, and S. Bhansali, Sens. Actuators, A **172**, 154–160 (2011).
[25]S. K. Arya, A. Dey, and S. Bhansali, Biosens. Bioelectron. **28**, 166–173 (2011).
[26]T. T. Nikiforov and J. M. Beechem, Anal. Biohem. **357**, 68–76 (2006).
[27]W. D. Wilson, Science **295**, 2103–2105 (2002).
[28]J. S. Mitchell, T. E. Lowe, and J. R. Ingram, Analyst (Amsterdam) **134**, 380–386 (2009).
[29]M. Frasconi, M. Mazzarino, F. Bootrè, and F. Mazzei, Anal. Bioanal. Chem. **394**, 2151–2159 (2009).
[30]E. S. Kim, C. K. Shim, J. W. Lee, J. W. Park, and K. Y. Choi, Analyst (Amsterdam) **137**, 2421–2430 (2012).
[31]H. Gronbeck, A. Curioni, and W. Andreoni, J. Am. Chem. Soc. **122**, 3839–3842 (2000).
[32]L. Bjorck and G. Kronvall, J. Immunol. **133**, 969–974 (1984).
[33]C. E. Giacomelli and W. Norde, J. Colloid, Interface Sci. **233**, 234–240 (2001).
[34]J. Janin and C. J. Chothia, Biol. Chem. **265**, 16027–16030 (1990).
[35]D. R. Davies, E. A. Padlan, and S. Sheriff, Annu. Rev. Biochem. **59**, 439 (1990).
[36]J. M. Junge, C. O. Junge, and E. G. Krebs, Arch. Biochem. Biophys. **55**, 338–355 (1955).