Appl Biochem Biotechnol (2012) 167:1069–1075
DOI 10.1007/s12010-012-9693-9

Graphene-Based Waveguides: Novel Method for Detecting Biological Activity

Jangah Kim · Manasi Kasture · Taihyun Hwang · Atul Kulkarni · Rashid Amin · Sungha Park · Taesung Kim · Suresh Gosavi

Received: 31 October 2011 / Accepted: 10 April 2012 /
Published online: 9 May 2012

Abstract We demonstrate the fabrication of a biosensor based on graphene coupled with polydimethylsiloxane (PDMS) waveguide. Biosensors work on the principle of local evanescent graphene-coupled wave sensor. It is observed that the evanescent field shifts in the presence of chemical or biological species as evanescent waves are extremely sensitive to a change in refractive index. This method helps to monitor the target analyte by attaching the selective receptor molecules to the surface of the PDMS optical waveguide resulting in its optical intensity distribution shift. We monitor the electrical properties of graphene in the dark and under illumination of PDMS waveguide. The changes in photocurrent through the graphene film were monitored for blue, green, and red light. We observed that the fabricated graphene-coupled PDMS optical waveguide sensor is sensitive to visible light for the used bioanalytes.

Keywords Waveguide · Graphene · PDMS · Local evanescent wave · Biosensor

Introduction

With changing lifestyle and limitation in the traditional method of detection of diseases, drug discovery, proteomics, and environmental detection of biological agents are extremely

J. Kim · R. Amin · S. Park · T. Kim (✉)
Sungkyunkwan Advanced Institute of Technology (SAINT), Sungkyunkwan University,
Suwon 440746, South Korea
e-mail: tkim@skku.edu

M. Kasture · S. Gosavi (✉)
Department of Physics, University of Pune, Pune, India, 411007
e-mail: swg@physics.unipune.ac.in

T. Hwang · A. Kulkarni · T. Kim
School of Mechanical Engineering, Sungkyunkwan University, Suwon 440746, South Korea

T. Hwang
e-mail: taihyun@skku.edu

A. Kulkarni · S. Park
Department of Physics, Sungkyunkwan University, Suwon 440746, South Korea

significant problems [1]. Traditional methods used for detecting bioactivity like DNA hybridization are too slow and labor intensive. Traditional biosensors find restricted use due to limiting detecting range. They require preprocessing resulting in economic and efficiency loss. Bulk size and complicated electronic methods employed to transduce the biological functions occurring in the operations of these biosensors have led to a new invention for the detection of bioactivity. The few methods used for monitoring bioactivities include difference at the electronic supports for electrical transduction, such as current [2], potential changes [3, 4], piezoelectric transduction [5–8], field-effect transistor transduction [9, 10], photo electrochemical transduction [11], and others [12–17]. To overcome these difficulties occurring in these methods, new biosensors are developed to improve the detection of bioactivity. New methods make use of metallic and semiconducting nanomaterials for the fabrication of biosensors. Graphene is currently the subject of intense research for condensed matter physicists and electronic engineers due to its exceptional properties. Graphene also holds great promise for novel photonic devices such as a photo detector [18–20]. Such graphene photo detectors have been deployed to optical data link applications. Recently, Guw Shi-Rui and coworkers have shown graphene-based field-effect transistor biosensors [21].

In this paper, we report the fabrication of biosensors using graphene coupled with polydimethylsiloxane (PDMS) waveguide. Working of this biosensor is based on the local evanescent graphene-coupled (LEGC) sensor. In the LEGC sensor, graphene serves as a photo detector. This gives us the advantage of a different refractive index for the analysis of different bioactivities on a single chip. The objective of our work is to fabricate a biosensor which has fast response and is sensitive to change in bioactivity. The biosensors developed are transparent and flexible and possess a high surface to volume ratio.

Experimental

Synthesis of Graphene Film

The large-area single and few-layer graphene sheets are synthesized by the chemical vapor deposition (CVD) of methane gas on Cu foils at 1,000 °C, as described in [22]. After the synthesis of graphene on Cu by CVD, the PDMS, 5 wt% in chlorobenzene, is spin coated at 3,000 rpm and annealed at 180 °C.

The graphene films are mostly monolayered, based on Raman spectra analysis results of one- and bilayer graphene films, as depicted in Fig. 1. However, an atomic force microscope (AFM) image (Fig. 1, inset) shows multilayer islands with few defects. As the graphene layers are transferred two times (bilayer), the intensities of the G- and 2D band peaks increase together, but their ratios do not change significantly because the hexagonal lattices of the upper and the lower layer are randomly oriented. Hence, the original properties of each monolayer remain unchanged even after a few layers are stacked. The optical transmittance is usually reduced by approximately 2.2 to 2.3 % for each additional transfer, implying that the average thickness is approximately that of a monolayer.

Waveguide Fabrication

The synthesized graphene film is then transferred on the microgap electrode chip using the dip casting method. In this process, the underlying Cu foil is etched using 0.5 M aqueous

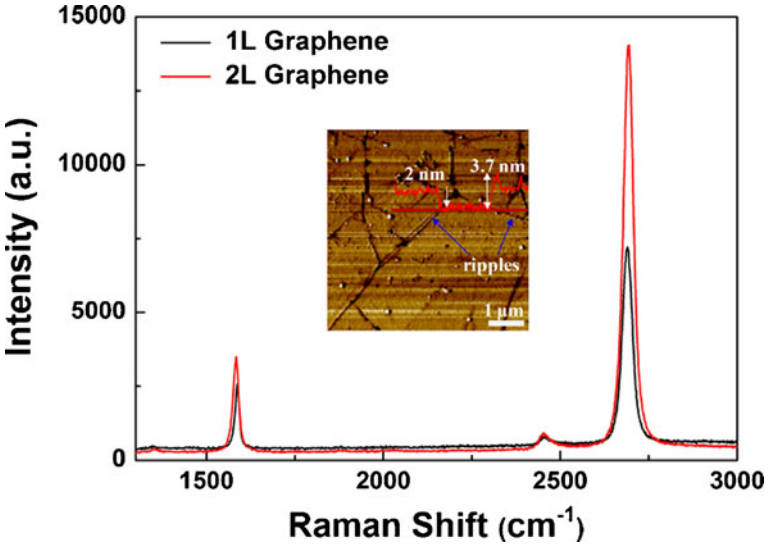

Fig. 1 Raman spectra of monolayer and bilayer graphene films synthesized by CVD with AFM image

ammonium persulfate ($(NH_4)_2\ S_2O_8$). Subsequently, the PDMS-supported graphene film is then transferred onto the DI water surface to facilitate the transfer of the graphene on to the micro gap electrode chip. The micro gap chip consists of electrodes that are fabricated on oxide-coated silicon substrate using standard electron-beam lithography and lift-off technique. The separation between the Au electrodes is 2 μm, and as such, seven Au electrodes were comprised in one single chip. For the fabrication of PDMS waveguide, a silicone elastomer kit (SYLGARD 184 SILICONE ELASTOMER KIT, Dow Corning) was used. The mixing ratio of the base to curing agent was 10:1, and the mixture was kept for 1 h curing at 60 °C. The fabricated waveguide has dimensions of $5\times1\times20$ mm ($W\times H\times L$). The fabricated PDMS waveguide is then placed on the graphene-deposited micro gap electrode chip to form a graphene-based PDMS waveguide device.

Bioanalytes Used

The biotinylated DX (DX biotin) lattice is designed based on the structure of immobile crossover branched junctions, and the DNA base sequence is designed to minimize the chances of error due to undesired complementary association and sequence symmetry, as shown in the Fig. 2 [23]. High performance liquid chromatography-purified synthetic biotinylated DNA oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA, USA). Complexes were produced by mixing a stoichiometric quantity of each DNA strand in physiological 1× TAE/Mg^{2+} buffer [Tris–acetate–EDTA (40 mM Tris, 1 mM EDTA) with 12.5 mM magnesium acetate]. For the annealing of the DX lattices, equimolar mixtures of strands were placed at 95 °C and gradually cooled to 25 °C by placing the test tube in 2 L of boiled water in a Styrofoam box for at least 24 h to facilitate hybridization. After this, the samples were stored overnight at 4 °C for structure stabilization. The final concentration of the DNA structure obtained was 400 nM. Streptavidin was purchased from Rockland Inc. (PA, USA). A 400-nM solution of streptavidin was prepared in deionized water.

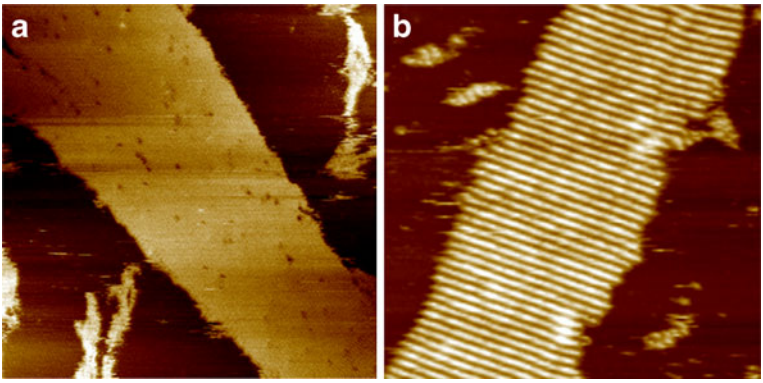

Fig. 2 **a** AFM image of DXB and **b** DX biotin with streptavidin (DXB+SA)

Experimental Setup

A schematic illustration of the experimental setup of the LEGC sensor is shown in Fig. 3. The different light sources viz. blue (λ=460 nm), green (λ=520 nm), and red (λ=660 nm) are used to guide the light through the PDMS waveguide. A 1-μl drop of each bioanalyte is placed on the PDMS waveguide, and dark and illuminated graphene photoconductance (I–V) is measured using an electrometer with and without the presence of bioanalytes. The variation in the photoconductance of graphene is then analyzed.

Result and Discussion

The sensing mechanism of the LEGC sensor relies on the specific binding of an analyte target to several localized regions of immobilized biological molecule probes to modify the waveguide cross section and thus the local evanescent field. A graphene below PDMS waveguide that serves as a photo detector can sense the modification in the evanescent field due to the local adlayers of bound analytes as depicted in the sensing scheme in Fig. 4. The sensing of targets such as proteins, DNA viruses, and bacteria is possible with this device.

It is well known that biomolecules are sensitive in the UV region. However, the fabrication of the miniature sensor device with UV light is cumbersome due to many reasons such as being hazardous to health and limitation to availability of high-efficiency UV LEDs. Hence, we evaluated the fabricated biosensor for the visible light sources viz. blue (400 nm), green (520 nm), and red (660 nm). I–V measurements carried out for these light sources are

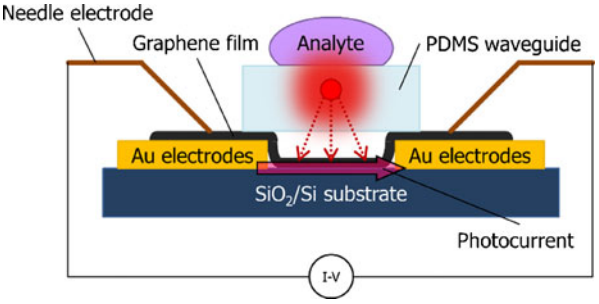

Fig. 3 Schematic representation of the experimental setup. The LEGC sensor is connected to an electrometer for the measurement of photocurrent (I–V)

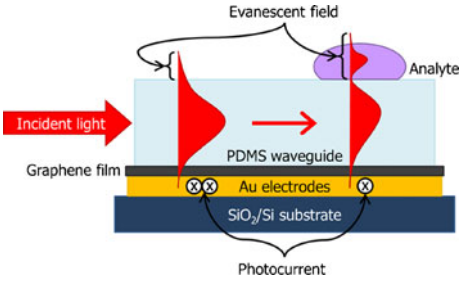

Fig. 4 Sensing scheme of the LEGC sensor

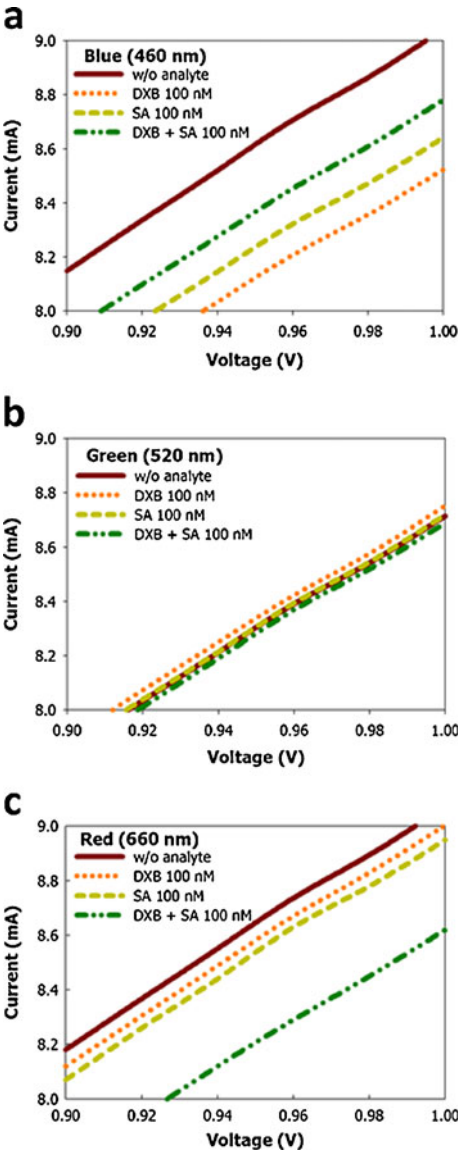

Fig. 5 Photocurrent (I–V) response of the LEGC sensor for **a** blue light, **b** green light, and **c** red light, for the DXB and streptavidin bioanalytes

shown in Fig. 5. Figure 5a represents the I–V curve for experiments carried out under the influence of blue light. From the I–V characteristics, we observe that the I–V response increases when biotin binds to streptavidin in the response for each individual analyte. Different trends are observed for experiments performed under the influence of other light sources. When illuminated with blue light, the resistance varies drastically for different analytes while a very minute chance in the resistance is observed for experiments carried out under the influence of green light as seen in Fig. 5b. For the red light condition, a combination of biotin and streptavidin decreases the resistance as compared to the individual analyte as seen in Fig. 5c. For the experiment carried out without higher resistance response as compared to experiments carried out in the presence of analytes. Here, we want to address that the photocurrent measurements were carried out after placing the analyte on the PDMS waveguide; hence, we cannot measure the response time of the fabricated sensor. However, as there is no direct interaction of the analyte and the graphene electrical response is in a few micro seconds, therefore, we can assume that the sensor response is in a few tens of microseconds.

Figure 6 shows the current response of different lights in the presence of analyte and in the absence of analyte. In the absence of analyte, an increase in current is observed which indicates reduced evanescent filed. A decrease in current is observed for the individual analyte, indicating enhancement in evanescent field. An increasing trend is observed when there is interaction of biotin and streptavidin decreasing the evanescent field. The variation in the sensor response for blue, green, and red light is ±3, ±5, and ±4 %, respectively, which is indicated by the error bars in Fig. 6. This variation may be due to the coupling losses of the waveguide and variation in the light intensities.

Conclusions

We developed a graphene-based waveguide biosensor based on a LEGC sensing mechanism. Herein, we also demonstrate that the graphene can be used as a photo detector. The response of the sensor to bioanalytes is encouraging for various light sources. Hence, we can satisfy the present need for a novel optical waveguide sensor with capabilities of detecting small volumes of multiple analytes while possessing comparable sensitivity to the conventional techniques for various medical, biological, and environmental situations. However, to improve the sensitivity or, in other words, to improve the detection limit, we are in the process of improving the waveguide dimensions and material.

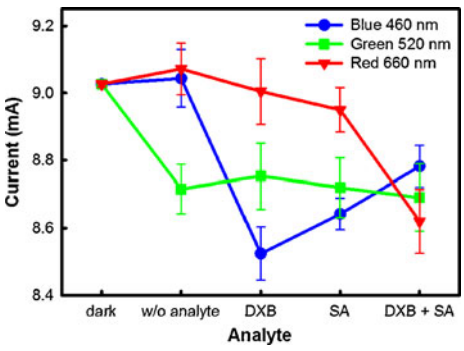

Fig. 6 Comparison of photocurrent for the used three light sources for the bioanalytes

Acknowledgements This work was partly supported by the GRRC program of Gyeonggi province [S-2011-1039-007-1, Development of integrated sensor Technology (2009-0083540)] and Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology (2009-0083540).

References

1. Turner, A. P. F. (2000). *Science, 290*, 1315–1317.
2. Bartlett, P. N., Tebbutt, P., & Whitaker, R. C. (1991). *Progress in Reaction Kinetics, 16*, 55.
3. Ianniello, R. M., Lindsay, T. J., & Yacynych, A. M. (1980). *Analytical Chemistry, 1982*, 54.
4. Ghindilis, A. L., & Kurochkin, I. N. (1994). *Biosensors and Bioelectronics, 9*, 353.
5. Lion-Dagan, M., Ben-Dov, I., & Willner, I. (1997). *Colloids and Surfaces B, 8*, 251.
6. Ben-Dov, I., Willner, I., & Zisman, E. (1997). *Analytical Chemistry, 69*, 3506.
7. Bardea, A., Dagan, A., Ben-Dov, I., Amit, B., & Willner,I. (1998). *Chemical Communications*, 839.
8. O'Sullivan, C. K., Vaughan, R., & Guilbault, G. G. (1999). *Analytical Letters, 32*, 2353.
9. Kazanskaya, N., Kukhtin, A., Manenkova, M., Reshetilov, A. N., Yarysheva, L., Arzhakova, O., et al. (1996). *Biosensors and Bioelectronics, 11*, 253.
10. Zayats, M., Kharitonov, A. B., Katz, E., Bückmann, A. F., & Willner, I. (2000). *Biosensors and Bioelectronics, 15*, 671.
11. Pardo-Yissar, V., Katz, E., Wasserman, J., & Willner, I. (2003). *Journal of the American Chemical Society, 125*, 622.
12. Amador, S. M., Pachence, J. M., Fischetti, R., McCauley, J. P., Jr., Smith, A. B., & Blasic, J. K. (1993). *Langmuir, 9*, 812.
13. Hobara, D., Niki, K., Zhou, C., Chumanov, G., & Cotton, T. M. (1994). *Colloids and Surfaces A, 93*, 241.
14. Liedberg, B., Nylander, C., & Lundström, I. (1995). *Biosensors and Bioelectronics*, 10, i.
15. Jordan, C. E., & Corn, R. M. (1997). *Analytical Chemistry, 69*, 1449.
16. Raitman, O. A., Katz, E., Bückmann, A. F., & Willner, I. (2002). *Journal of the American Chemical Society, 124*, 6487.
17. Raitman, O. A., Patolsky, F., Katz, E., & Willner, I. (1936). *Chemical Communications, 2002*.
18. Mak, K. F., et al. (2008). *Physical Review Letters, 101*, 196405.
19. Xia, F., Mueller, T., & Lin, Y. (2009). *Nature Nanotechnology, 4*, 839–843.
20. Rogalski, A., Antoszewski, J., & Faraone, L. (2009). *Journal of Applied Physics, 105*, 091101.
21. Guo, S.-R., Lin, J., Penchev, M., Yengel, E., Ghazinejad, M., Ozkan, C. S., et al. (2011). *Journal of Nanoscience and Nanotechnology, 11*(6), 5258–5263.
22. Lee, Y., Bae, S., Jang, H., Jang, S., Zhu, S. E., Sim, S. H., et al. (2010). *Nano Letters, 10*, 490.
23. Seeman, N. C. (1990). *Journal of Biomolecular Structure and Dynamics, 8*, 573.