Biosensors and Bioelectronics 65 (2015) 274–280

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Graphene oxide-based biosensor for detection of platelet-derived microparticles: A potential tool for thrombus risk identification

Jyotsna Kailashiya [a], Nitesh Singh [a], Sunil K. Singh [c], Vikas Agrawal [b], Debabrata Dash [a,*]

[a] Department of Biochemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India
[b] Department of Cardiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India
[c] Department of Biotechnology, Motilal Nehru National Institute of Technology, Allahabad 211004, India

ARTICLE INFO

Article history:
Received 8 July 2014
Received in revised form
15 October 2014
Accepted 16 October 2014
Available online 28 October 2014

Keywords:
Biosensor
Platelet-derived microparticles
Graphene oxide
Acute myocardial infarction
Electrochemical detection
Point-of-care testing

ABSTRACT

We report here design of a graphene oxide-based electrochemical biosensor for detection of platelet-derived microparticles (PMPs), a major risk factor for arterial pro-thrombotic pathologies like acute myocardial infarction and stroke. Electrodes were fabricated with immobilized layers of graphene oxide and a specific antibody targeted against active conformation of integrin $\alpha_{IIb}\beta_3$ on PMP surface. Results showed progressive rise in impedance in Nyquist plots with increasing number of PMPs in analyte. The sensor was highly specific for PMPs and did not identify microparticles originating from other cells. Blood obtained from patients diagnosed with acute myocardial infarction exhibited significantly higher values of impedance, consistent with larger number of circulating PMPs in these patients, as compared to samples from healthy individuals, thus validating biosensor as a specific, sensitive, label-free and cost-effective tool for rapid point-of-care detection of PMPs at bedside. Our biosensor is most ideal for mass population screening programs at periphery-level healthcare units with limited resources. It is aimed at early detection of individuals having higher imminent cardiovascular risk, as well as for routine analysis, which in turn would contribute to better management and survival of screened 'high-risk' subjects.

1. Introduction

Global status report of World Health Organization has categorized cardiovascular diseases, comprising of coronary artery disease, stroke, atherosclerosis and heart failure, as leading causes of death resulting in millions of casualties worldwide, followed by deaths due to diabetes (WHO, 2013). Although cardiovascular diseases affect people with advanced age, risk factors may appear relatively early (McGill et al., 2008). One such risk factor is circulating platelet-derived microparticles (PMPs) in blood of patients suffering from myocardial infarction or peripheral arterial diseases (van der Zee et al., 2006). Elevated plasma levels of PMPs have been associated with enhanced risk of coronary heart disease in healthy individuals (Ueba et al., 2010).

Platelet activation is central to the pathogenesis of arterial thrombosis. PMPs are membrane vesicles of less than 1 μm diameter released from stimulated platelets (Siljander, 2011). They play significant role in hemostatic response and their presence in circulation represents serious procoagulant risk (Nomura, 2001; Sinauridze et al., 2007; Skeppholm et al., 2012; Suades et al., 2012; VanWijk et al., 2003). Detection of PMPs at early stage of disease would aid in diagnosis, prevention and management of the pathology. Flow cytometry is the available technique for PMP detection (Lacroix et al., 2010) but is associated with major drawbacks that include underestimation of PMP count, lack of universal standardization, time consuming experiments, high cost of the equipment and need for skilled operator (Barteneva et al., 2013; Dey-Hazra et al., 2010; Leong et al., 2011; van der Pol et al., 2012). In contrast electrochemical biosensing stands far superior chance as an efficient and affordable tool for PMP detection.

'Biosensing' is an emerging concept based on amperometry, potentiometry and impedance analysis, which detects transduction of biological events to electrochemical signals with high sensitivity (Higgins and Lowe, 1987). Nanoscale particles of gold, iron and silicon, as well as graphene and carbon nanotubes have been employed as immobilization matrices during fabrication of electrodes (Chen and Chatterjee, 2013; Hakim et al., 2012; Kuila et al., 2011; Mandal et al., 2012). Material immobilized on exposed surface of electrodes determines its specificity, which include enzymes, antibodies, proteins, aptamers or nucleotides depending upon the nature of the target. Biosensors have been widely designed against molecules like glucose, ascorbic acid, uric acid, proteins, DNA etc. (Kanyong et al., 2012; Luppa et al., 2001; Oliver

Abbreviations: AMI, acute myocardial infarction; FITC, fluorescein isothiocyanate; GO, graphene oxide; PE, phycoerythrin; PMP, platelet-derived microparticle; PPP, platelet-poor plasma; PRP, platelet-rich plasma

* Corresponding author.
E-mail address: ddash.biochem@gmail.com (D. Dash).

http://dx.doi.org/10.1016/j.bios.2014.10.056
0956-5663/

et al., 2009; Patel et al., 2010; Wen et al., 2012), but with advancement of fabrication techniques electrochemical sensing of individual cells like cervical cancer cells and microorganisms has been reported (Chandra et al., 2011; Deisingh and Thompson, 2004; Mandal et al., 2012; Yanik et al., 2010). Although there has been attempt to detect microparticles using electrochemistry, the method does not differentiate between their cells of origin and types (Lvovich et al., 2010) and thus has limited medical application. Here we describe a simple, quick, sensitive and cost-effective method to detect PMPs circulating in blood of individuals with potential for point-of-care diagnostic at peripheral health care system. The novelty of this product lies in its ease of fabrication and detection method, as no electrochemical sensor has yet been reported which could facilitate quick screening and diagnosis of individuals at 'high-risk' for developing cardiovascular diseases, eliminating requirement of high end lab facilities and experienced technicians as needed in current PMP detection procedures.

2. Materials and methods

2.1. Materials and reagents

Graphene oxide (GO) was purchased from Graphene Supermarket. Ethyl (dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), bovine serum albumin (BSA) and calcium ionophore A23187 were from Sigma. FITC-PAC1 and PE-annexin V and Trucount tubes were obtained from BD Biosciences. Ethanol, sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Merck. Heparin was procured from Gland Pharma limited. Glassy carbon electrodes (GCE) (2 mm diameter) were the products of CH instruments.

2.2. Electrode surface modification

GCE were polished with alumina slurry and thoroughly cleansed. Colloidal GO was diluted with a mixture of Milli-Q grade (type 1 deionized, 18.2 MΩ cm, Millipore) water and ethanol (at ratio 2:5:1:: GO:water:ethanol, v/v/v). Four microliter of above mixture was dropped on each electrode and dried in desiccators. Dried GO layer was gently rinsed with phosphate-buffered saline (PBS, 0.1 M phosphate buffer, 0.9% NaCl, pH 7.2). For activation of carboxyl groups, 10 μL each of 100 mM (EDC) and 100 mM (NHS) were deposited over GO layer and incubated for 30 min. Following washing, 5 μL PAC1 antibody (stock concentration, 25 μg/mL; diluted 1:10 with PBS) was dropped on each GO-fabricated electrode and incubated for 1 h. Electrodes were gently rinsed with PBS, blocked with 2% BSA for 30 min (Sanchez et al., 2007; Tran et al., 2012; Wang et al., 2013) inside humid chambers at room temperature, and finally carefully rinsed with Milli-Q water. Electrodes were stored at 4 °C till further use. All incubations were carried out at room temperature under normal ambient illumination in desiccators.

2.3. Sample preparation

Antecubital venous blood was collected from healthy volunteers, as well as patients diagnosed with acute myocardial infarction (AMI) characterized with ST-segment elevation in electrocardiogram, under informed consent as per the recommendations of the Institutional Ethical Committee. Blood was anticoagulated with heparin

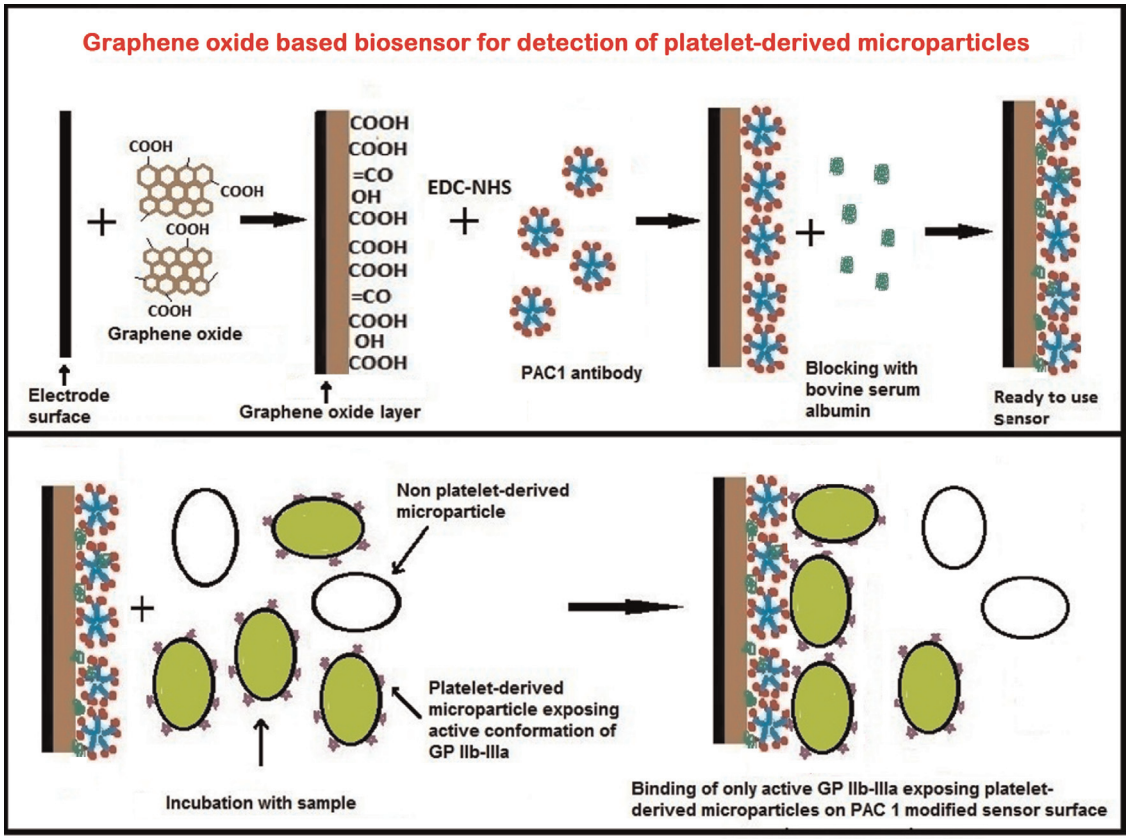

Fig. 1. Schematic design of nano-biosensor for detection of PMPs depicting stepwise immobilization of GO and PAC1 antibody on electrode surface. Subsequent incubation of coated electrode with sample resulted in binding of platelet-derived microparticles, bearing active conformation of integrins $\alpha_{IIb}\beta_3$, to the sensor surface, which can be detected by impedance analysis.