Biosensors and Bioelectronics 55 (2014) 180–186

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Facile synthesis of gold nanohexagons on graphene templates in Raman spectroscopy for biosensing cancer and cancer stem cells

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ARTICLE INFO

Article history:
Received 27 August 2013
Received in revised form
8 November 2013
Accepted 11 November 2013
Available online 21 November 2013

Keywords:
Cancer cells
Cancer stem cells
Surface enhanced Raman spectroscopy
Au nanoparticles
Graphene

ABSTRACT

Several surface enhanced Raman spectroscopy (SERS) substrates were prepared based on *in situ* nucleation of gold nanohexagons (Au) on graphene (G) nanosheets (Au@G), G, Au nanoparticles and Au conjugated G nanomaterials. These were applied to enhance Raman scattering and to differentiate human breast normal, cancer and cancer stem cells. These SERS substrates at concentrations of 100 μg/1 × 10^4 cells led to 5.4 fold increase in detecting breast cancer cells (BCCs) and 4.8 fold of sensitivity for detecting breast cancer stem cells (BCSCs) and they were able to identify and differentiate between normal cells, cancer cells and cancer stem cells. These approaches are rapid, simple and reliable for healthy normal cells, cancer cells and cancer stem cell detection which have a huge potential for cancer research for medical or biomedicine applications.

1. Introduction

Several analytical techniques such as mass spectrometry, fluorescence and electrochemical detection (Caldwell and Caprioli, 2005) have been applied for differentiating molecular profiles from healthy and diseased cells. Although these techniques play a significant role in the cell/tissue analysis, they are limited by some disadvantages. For instance, mass spectrometry is destructive and requires complicated sample pretreatments (Griffinand Schnitzer, 2011, Svatos, 2011; Tsuyama et al., 2011; Tsuyama et al., 2012). Fluorescence methods require the involvement of chemically or genetically prepared fluorescent labels for target cells (Cottet-Rousselle et al., 2011, Wombacher and Cornish, 2011). This is tedious, time consuming and requires technical expertise. In electrochemical methods, placing the probe at appropriate locations makes it a major constraint and inaccurate fabrication of electrodes could lead to generating erroneous changes in voltammogram (Ball et al., 2000, Dias et al., 2002) and it is also time consuming due to slow electron transfer (ET).

Raman spectroscopy is a powerful technique that has been widely used in biological analysis due to rapidity, label free and nondestructive analysis (Mahadevan Jansen and Richards Kortum, 1996; Gremlich and Yan, 2001; Thomas, 1999; Harris et al., 2010). Live cell Raman analysis could yield useful biochemical information of cells; the information would be useful to identify diseases, cell death, differentiation and interactions of toxic agents or drugs with cells after treatment (Notingher and Hench, 2006). However, conventional Raman exhibits very weak signals (Gardiner, 1989; Mahadevan-Jansen and Richards-Kortun, 1997). Thus, surface enhanced Raman spectroscopy (SERS) is used, utilizing rough metal surfaces to enhance Raman signals upto 10^{10}–10^{11} folds (Blackie et al., 2007). Various metallic substrates such as electrochemically roughened electrodes (Niaura and Malinauskas, 1993; Roth et al., 2007) metallic nanoparticles, (Chaney et al., 2005; Shanmukh et al., 2006), nanostructured substrates (Willets and Van Duyne, 2007; Mulvihill et al., 2008; Mahajan et al., 2007; Beermann et al., 2009; Gopinath et al., 2009) and black silicon substrate (Deng and Juang, 2014) have been applied in SERS. SERS has single molecular sensitivity and it can be widely applied in chemical molecule/ion detection, clinical discrimination of cancer tissues, food and environmental engineering, art, gas sensing, *etc.* (Colomban et al., 2012; Casadio et al., 2010; Han et al., 2010; Zhai et al., 2011; Rodriguez-Lorenzo et al., 2011; Kim et al., 2012). However, fabrication of SERS substrates to achieve good reproducibility, high sensitivity and cost-efficiency is still a

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http://dx.doi.org/10.1016/j.bios.2013.11.037

challenging task. To date, nanomaterials are highly potential SERS substrates for various bioanalysis (Kim et al., 2012). For SERS, nanostructures such as nanoparticles (bare Au and Ag NPs) and silver nano-dendrites, gold nanorods and gold/silver core-shell or gold nanostructures were prepared either by sol–gel or fabricated by lithography (Lee et al., 2013). Carbon materials (carbon nanotubes) can serve as a platform for Ag nanoparticles that enhance the SERS signal up to 12 orders due to the surface smoothness of Ag nanoparticles (Kneipp et al., 2000).

Compared to metals and other carbon nanomaterials, graphene (G) (Geim and Novoselov, 2007) is an excellent substrate for Raman signal enhancement, due to many advantages such as easier preparation, lower cost, better biocompatibility and high performance to suppress fluorescence in resonance Raman spectroscopy (Ling et al., 2010; Xie et al., 2009). G and their derivatives have been employed in various biosensing and biotechnological applications (Wang Y. et al., 2011; Zhou et al., 2009, Parlak et al., 2013a; Parlak et al., 2013b) such as detection of pathogenic bacteria (Abdelhamid and Wu, 2013b), analysis of various types of molecules such as DNA (Lu et al., 2009), RNA (Yin et al., 2012), ions (Wen et al., 2010), small molecules (He et al., 2011), proteins (Chang et al., 2010; Shiddiky et al., 2012), metallodrugs (Abdelhamid and Wu, 2012) and living cells (Feng et al., 2011; Wang et al., 2010). G combined with Ag (Zhang et al., 2011) and Au is reported to be useful for better SERS effect (He et al., 2012; Huang et al., 2010; Xu et al., 2012) and electrobiosensing sensitivity (Parlak et al., 2013a; Parlak et al., 2013b)

In this paper, we describe the effect of two different SERS substrates on Raman signal enhancement of normal, cancer and cancer stem cells from human breast origin. We prepared SERS substrate-1 'P1' by *in situ* synthesis of Au nanohexagons on G templates (Au@G) and substrate-2 'P2' was synthesized by ornamentation of Au NPs on the G sheets via electrostatic interaction using ultrasonication. SERS effects of both the substrates (P1 and P2) were compared after treating three different intact cells (normal cells, cancer cells and cancer stem cells).

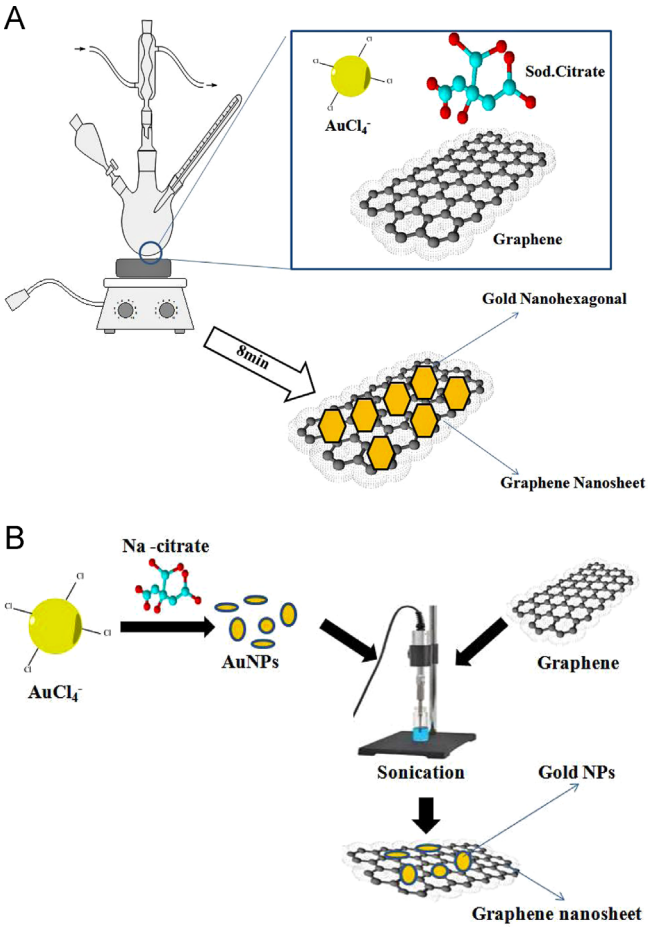

Fig. 1. Schematic representation showing the preparation of SERS substrates: (A) substrate-1 'P1' and (B) substrate-2 'P2'.

2. Materials and methods

Details of the chemicals and the cell lines used in the present study are described in Supplementary material.

2.1. Synthesis of nanomaterials

Au NPs were prepared according to Frens's approach (Frens, 1973). The synthesis method for Au NPs is described in detail in Supplementary material. G and their derivatives were prepared by the Hummers method (Hummers and Offeman, 1958; Abdelhamid and Wu, 2012) as decribed in Supplementary material.

2.2. In situ synthesis of Au on G templates for SERS

The overall scheme for the *in situ* synthesis of Au nanohexagons on G is given in Fig. 1A. The synthesis of nanocomposites on the G templates was based on the reduction of $HAuCl_4$ complex by sodium citrate. In a typical process, 2.5 mL of G aqueous suspension (1 g/50 mL) was added to 50 mL of $HAuCl_4$ solution (0.01%) and heated to 80 °C. Sodium citrate (0.85 mL, 1%) was subsequently added dropwise to the reaction. The resultant nanocomposite was washed with deionised water using centrifugation (3000g). The overall process is shown in Fig. 1A. Conjugation of Au with G using ultrasonication and cell culture are addressed in Supplementary material.

3. Results and discussion

3.1. Characterization of Au, G and their nanocomposite Raman substrates

For the preparation of nanocomposite SERS substrates for enhancing the Raman signals, two different methods were employed. In the first method, the Au NPs were synthesized *in situ* over the G sheet that was used as a template (P1). The synthesis scheme for the P1 is shown in Fig. 1A. In the second method, we prepared G and Au NPs separately and then conjugated them together based on their electrostatic properties via ultrasonication (P2) (Fig. 1B).

The successful synthesis of Au-GSERS substrates by both the methods was confirmed by the UV–vis absorption spectra (Fig. 2A), the changes in the fluorescence emission spectra (Fig. 2B) and the FTIR spectra (Fig. 2C). The prepared Au nanoparticles exhibit a surface plasmon band (SPB), creating a broad absorption band in the visible region around 520 nm showing a characteristic deep-red color (Fig. 2A, Kneipp and Kneipp, 2006). The SPB of P1 shows a shift at the corresponding peak (520 nm) indicating a complete overlap between SPR (Au) and G surface (π electrons). However, the reduced appearance of the absorption peak at 520 nm in the P2 compared to that in Au NPs reflects that the conjugations of Au NPs with G may slightly change of the SPB (Fig. 2A). From the UV absorption results, G shows a continuous absorption in the range of 300–700 nm (Abdelhamid and Wu, 2013a). The weak fluorescence emission of Au was observed at 520 nm while P1 and P2 (500 nm) shows fluorescence emission in the order P1 > P2 > Au (Fig. 2B). The main emission of these