Biosensors and Bioelectronics 68 (2015) 350–357

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Magnetically optimized SERS assay for rapid detection of trace drug-related biomarkers in saliva and fingerprints

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ARTICLE INFO

Article history:
Received 22 October 2014
Received in revised form
24 December 2014
Accepted 9 January 2015
Available online 10 January 2015

Keywords:
Magnetic nanocomposites
Drug-biomarker
Saliva
Fingerprint
Portable Raman

ABSTRACT

New developments in the fields of human healthcare and social security call for the exploration of an easy and on-field method to detect drug-related biomarkers. In this paper, Au nanoparticles dotted magnetic nanocomposites (AMN) modified with inositol hexakisphosphate (IP_6) were used as surface-enhanced Raman scattering (SERS) substrate to quickly monitor trace drug-related biomarkers in saliva and to on-site screen a trace drug biomarker in fingerprints. Due to inducing with an external magnet, such substrate presented a huge SERS activity, which has met the sensitivity requirement for assay to detect the drug biomarkers in saliva from the U.S. Substance Abuse and Mental Health Services Administration, and also the limit of detection for drug biomarker in fingerprint reached 100 nM. In addition, this AMN-based SERS assay was successfully conducted using a portable Raman spectrometer, which could be used to on-site and accurately differentiate between the smokers and drug addicts in near future.

1. Introduction

A quick, cost-effective protocol for detection of drugs in biological fluids would be of great application and value in healthcare, law enforcement, and home testing. Among such substances, nicotine is the primary neuroactive alkaloid in tobacco products (Griffiths, 1996). This alkaloid is easily absorbed in humans, leading to a variety of negative physiological effects including cancer, pulmonary and cardiovascular disease (Thorgeirsson et al., 2008; Haley et al., 1985). Despite its high toxicity, nicotine has some therapeutic effects (Sopori, 2002) in neurodegenerative diseases such as Alzheimer's and Parkinson's (Quik and Kulak, 2002). Therefore, it is important to monitor nicotine in the human body. The determination of nicotine quantities in living organisms allows for the evaluation of passive and active smoking effects on human health. In order to facilitate in the quantification of nicotine, the detection of a metabolite of the parent drug is necessary and advantageous. For example, the amount of cotinine from the metabolite of nicotine (Leggett et al., 2007) and benzoylecgonine from the metabolite of cocaine (Hazarika et al., 2008) can be analyzed in saliva or other biological fluids to indicate the amount of nicotine or cocaine present in the original sample. Saliva, 99.5% water, is the relatively preferred biological fluid for noninvasively determining the use of drugs due to the ease of sample collection in comparison with sampling blood or urine, which often requires supervision (Strano-Rossi et al., 2008). Additionally, concentrations of drugs in saliva are similar to the concentrations found in blood plasma (Drummer, 2005). Alternatively, sweat is also a preferred biological fluid, and through the touch of a finger the components within the sweat are transferred to a surface, thus leaving a latent fingerprint. It is important to develop new methods for the detection of narcotic drugs through drug metabolites excreted in the sweat and deposited with the latent fingerprints of drug users.

Currently, drug tests are usually carried out in laboratories by ELISA (Laloup et al., 2005; Pujol et al., 2007), gas chromatography (GC) (Paterson et al., 2001; Es'haghi, 2009; Dunn et al., 2011), capillary electrophoresis (CE) (Phan et al., 2013; Kohler et al., 2013), or HPLC (Chiuminatto et al., 2010; Bjørk et al., 2010), which are time-consuming and require costly equipment and trained operators. In addition, the routine method of ELISA prevailingly experiences low enzyme stability. For testing narcotics at home, commercial drug-screening products are available using the Marquis reagent and a similar colorimetric reaction. However, this method requires specialized reactants, large sample volumes and is open to misinterpretation due to subjective color perception (Andreou et al., 2013). These disadvantages prompt the investigation of rapid and on-site assays for monitoring trace level drugs in biological fluids.

SERS is a label-free technique which has extreme sensitivity

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http://dx.doi.org/10.1016/j.bios.2015.01.021
0956-5663/

down to trace concentration levels, which allows for the measurement of tiny sample amounts (Michaels et al., 1999; Lombardi and Birke, 2009; Kneipp et al., 2008). Furthermore, SERS technique can be used to identify molecular structures of drugs using the vibrational information generated to isolate signature peaks (Li et al., 2010; Nie and Emory, 1997). SERS technique also could provide a real-time signal, which has been successfully used to detect narcotics (Yang et al., 2012), explosives (Dasary et al., 2009) and molecules of biological interest (Wang et al., 2013; Qian et al., 2008; Kong et al., 2012). The electromagnetic (EM) mechanism of SERS shows that SERS signal diminishes exponentially from the metal surface and surface plasmon resonance induces strong Raman scattering of a species within an optimal spatial zone of 0–4 nm (Szuromi, 2005). Especially enormous SERS enhancements arise from dislocations or sharp discontinuities on a metal surface (Yang et al., 2004; Michota and Bukowska, 2003), or at the junction of two or more metal nanoparticles (Wustholz et al., 2010; Rycenga et al., 2011; Ma et al., 2014), regarded as "hot spots" (Yang et al., 2010). Consequently, the controllable aggregation of the noble metal nanomaterials is the better process to produce the junction sites for gaining SERS sensitivity. SERS applications in previous literature needed to induce nanoparticle aggregation by a chemical method (Li et al., 2000, 2001), however, the aggregation was an irreversible process.

In the presence of inositol hexakisphosphate (IP_6) as a stabilizing reagent, Au NPs dotted magnetic nanocomposites (shortly named as AMN) was successfully used to detect trace pesticide residues on vegetable surfaces by SERS (Yang et al., 2014). This work expands upon the idea that the aggregation of superparamagnetic metallic nanocomposites can be controlled by a magnet (Timonen et al., 2013). Additionally, the detection sensitivity of some methods in literature was optimized by the magnetic effect (Yin et al., 2014; Ma et al., 2013; Kuang et al., 2013). In this paper, the utilization of a magnet to reversibly control AMN for generating a favorable density of hot spots via the optimization of aggregation is investigated, and the direct determination of trace amounts of drug-related biomarkers in saliva and fingerprints is studied using a portable Raman analyzer-AMN-based SERS technique. Additionally, in the case of detection of drug residues in saliva, the magnetic separation and preconcentration of the given trace analytes could overcome several difficulties. Such challenges include matrix interference and a lower sensitivity from using small laser power to avoid sample damage. The limit of detection of cotinine and benzoylecgonine in saliva are 8.8 ppb and 29 ppb respectively, which are lower than the current accepted cut-off threshold for detection of most drugs in saliva at 50 ppb (50 ng/mL) as defined by the U.S. Substance Abuse and Mental Health Services Administration (SAMHSA). While examining drugs and drug metabolites in fingerprints, the application of the proposed magnetic SERS protocol is attractive, as SERS signal can provide evidence of drug use. Since the 1960's, a routine collection method for fingerprints seen in the field of forensic investigation involves identifying an individual from his fingerprint dusted with magnetic powders (Choi et al., 2007; Seah et al., 2005). The SERS technique proposed in this work builds upon this previous procedure, yet the application of SERS for analysis of fingerprints has never been seen before. A detection limit of 17.6 ng/mL cotinine in fingerprints was reached, which is lower than a previously reported mean combined concentration (780.8 ng/mL) of nicotine and its metabolites in eccrine sweat using a radioimmunoassay for active smokers (Balabanova et al., 1992). As a perspective, magnetic field inducing improvement of SERS protocol could be developed into a possible routine analytical tool with accuracy, versatility, specificity, and sensitivity for monitoring drugs of abuse.

2. Experimental section

2.1. Materials

Sodium salt of phytic acid (90%) was purchased from Sigma-Aldrich. $FeCl_3 \cdot 6H_2O$ (99%), $FeCl_2 \cdot 4H_2O$ (99%), NaOH ($\geq$96.0%), sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$, 99.8%), chloroauric acid ($HAuCl_4 \cdot 4H_2O$, 99.9%), Rhodamine 6G (R6G), crystal violet (CV), 2-amino-5-(4-pyridinyl)-1, 3, 4-thiadiazole (APTD), nicotine, cotinine and benzoylecgonine were used without further purification. Ultrapure water (18 MΩ cm) was produced using a Millipore water purification system and used for all solution preparations.

2.2. Instruments

The morphologies of nanocomposites were measured with a JEOL JEM-2000 FX transmission electron microscopy (TEM) operating at 200 kV. X-Ray photoelectron spectroscopy (XPS; PHI 5000 Versa Probe) was performed to identify the chemical composition of the surface of the observed nanocomposites. A Jobin Yvon micro-Raman spectroscope (Super LabRam II) with a mode of 50 × objective (8 mm), a holographic grating (1800 g mm^{-1}), 1024 × 256 pixels charge-coupled device detector and 5 mW He–Ne laser at 632.8 nm as an excitation line was employed for Raman measurements. A Portable Stabilized R. Laser Analyzer (Enwave) with a narrow line width diode laser at 785 nm with an adjustable power of the maximum at 300 mW was also used for online detection purpose.

2.3. Fabrication of the Au dotted magnetic nanocomposites (AMN)

Fe_3O_4 nanoparticles (NPs) were made via the chemical co-precipitation method in the presence of IP_6 according to previously reported methods after some modifications (Yang et al., 2014). The resulting magnetic NPs protected by IP_6 were black brown. The Fe_3O_4 nanoparticles were rinsed multiple times with ultrapure water through several collections from the solution by applying an external magnetic field. 2 mL of 1.9 mg/mL collected magnetic NPs were dispersed in 150 mL of ultrapure water and then heated to boiling. After slowly injecting 2.5 mL of 1% chloroauric acid under vigorous stirring, the mixed solution was refluxed for 15 min and 5 mL of 1% sodium citrate was rapidly added into the solution. The final color of the mixture presented was reddish brown after heating for an additional 45 min. The mixture was cooled to room temperature and the resulting NPs were collected by a magnet, washed several times with ultrapure water and dried to form magnetic powder.

2.4. Magnetic separation of AMN and magnetically induced improvement of SERS

Rhodamine 6G (R6G), crystal violet (CV) and 2-amino-5-(4-pyridinyl)-1, 3, 4-thiadiazole (APTD) were used as model molecules and each prepared as 1 μM solutions. A volume of 300 μL of each solution was then added to 300 μL of 0.32 mg mL^{-1} AMN. Each dispersion was then shaken for 3 min at 25 °C. AMN with the Raman probe molecules were collected from the solution by an external magnet and the magnetic adsorption and magnetic separation capabilities of AMN were evaluated with Jobin Yvon micro-Raman spectroscope. Each Raman spectrum was obtained using three accumulations and the acquisition time in each case was typically 10 s.

2.5. Saliva sample preparation

Saliva was obtained from healthy and drug-free volunteers.