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Microfluidic diatomite analytical devices for illicit drug sensing with ppb-Level sensitivity

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ABSTRACT

The escalating research interests in porous media microfluidics, such as microfluidic paper-based analytical devices, have fostered a new spectrum of biomedical devices for point-of-care (POC) diagnosis and biosensing. In this paper, we report microfluidic diatomite analytical devices (μDADs), which consist of highly porous photonic crystal biosilica channels, as an innovative lab-on-a-chip platform to detect illicit drugs. The μDADs in this work are fabricated by spin-coating and tape-stripping diatomaceous earth on regular glass slides with cross section of $400 \times 30\ \mu m^2$. As the most unique feature, our μDADs can simultaneously perform on-chip chromatography to separate small molecules from complex biofluidic samples and acquire the surface-enhanced Raman scattering spectra of the target chemicals with high specificity. Owing to the ultra-small dimension of the diatomite microfluidic channels and the photonic crystal effect from the fossilized diatom frustules, we demonstrate unprecedented sensitivity down to part-per-billion (ppb) level when detecting pyrene (1ppb) from mixed sample with Raman dye and cocaine (10 ppb) from human plasma. This pioneering work proves the exclusive advantage of μDADs as emerging microfluidic devices for chemical and biomedical sensing, especially for POC drug screening.

1. Introduction

In recent years, the escalation of research interests in porous media microfluidics [1,2], especially microfluidic paper-based analytical devices (μPADs) [3–5], have fostered a new spectrum of biomedical devices for point-of-care diagnosis and biosensing. μPADs can be fabricated by simple, low-cost processes using conventional photo- or soft lithographic techniques, utilizing either photoresists [6] or wax printing [7]. Advantages of using μPADs for microfluidic channels include: 1) ubiquitous and extremely cheap cellulosic materials; 2) capillary flow which enables fluid transport without using any external pump; and 3) compatible with many chemical and biomedical applications. Many different chemical and biological assays have been performed using μPADs, including for the detection of glucose [8], protein (albumin) [9], cholesterol [10], and heavy metals [11]. They have also been used as platforms for ELISA [12]. Especially, I. M. White's group used inkjet-printed paper-based surface-enhanced Raman scattering (SERS) substrates for chromatographic separation and detection of target analytes from complex samples [13], which opened a new route for on-chip chemical sensing.

Other than μPADs, porous silica materials and devices also have attracted considerable attention for biosensing due to the use of their large surface area and pore volume to achieve high sensitivity [14,15]. The high porosity, which allows for the immobilization of target molecules not only on the external surface of the substrate but also inside of the pores, enables the loading of large amounts of sensing molecules, giving instant responses and high sensitivity. The optical transparency, on the other hand, permits optical detection through the bulk of the material. In addition, the surface groups and biocompatibility also makes porous silica one of the most potential materials for biosensing. Moon et al. have fabricated polymer and colloidal silica porous composite for nucleic acid biosensing [16]. Yang et al. have synthesized porous SiO_2 material and used it as enzyme immobilization carriers to fabricate glucose biosensors [17]. However, the pores in sol–gel derived silica lack a high degree of order, which results in random paths and consequently non-uniform diffusion of the analytes. A fraction of the sensing molecules might even be unreachable, leading to low response and poor spatial resolution [18].

Diatoms are unicellular, photosynthetic, bio-mineralized marine organisms that possess a biosilica shell, which is called the frustule. The two-dimensional (2-D) periodic pores on diatom

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surface enable it unique optical, physical, and chemical properties [19,20]. In recent decades, a variety of biosensors with ultra-high sensitivity using diatom biosilica have been reported [21]. Zhen et al. developed photoluminescence-based diatom biosensors that have been successfully applied for 2, 4, 6-trinitrotoluene (TNT) sensing [22]. De Stefano et al. have fabricated highly-selective biosensor for immuno-complex detection by modifying diatom frustules (*Coscinodiscus concinnus*) with antibodies [23]. From the optics perspective, the photonic crystal feature of diatoms could provide additional SERS enhancement when hybridized with plasmonic nanostructures [24,25]. Our group has developed an in-situ growth method for depositing silver nanoparticles (Ag NPs) on diatom for ultrasensitive, label-free TNT sensing [26,27]. Other than natural photonic crystal structures from living diatoms, diatomite consists of fossilized remains of ancient diatoms as geological deposits with billions of tons of reserve on earth. Therefore, diatomite is a type of naturally abundant photonic crystal biosilica, which has been widely used in industry as water filters, adsorbents, and medicine [28–30]. Diatomite has similar properties to diatoms such as highly porous structure, excellent adsorption capacity, and photonic crystal effects [31,32].

In this study, we report microfluidic diatomite analytical devices (μDADs), which consist of nano-porous photonic crystal biosilica channels for label-free biosensing of illicit drugs from complex biological samples using on-chip chromatography in conjunction with SERS sensing method. Previously, bio-inspired photonic crystals have been integrated into microfluidic systems as lab-on-a-chip system [33] and SERS has been employed for drug sensing [34]. In this research, Cocaine ($C_{17}H_{21}NO_4$) is chosen as the target analyte in our study, which is an alkaloid derived from coca leaves. Cocaine is one of the most widely used illicit drugs all over the world according to the latest World Drug Report from the United Nations Office on Drugs and Crime (UNODC). Cocaine is a potent stimulant of the central nervous system that leads to a state of increased alertness and euphoria. Its effect is similar to that of amphetamines but with shorter duration. In this study, we report using μDADs for on-chip chromatography-SERS to separate and detect cocaine from real biofluidic samples. The μDADs achieve nearly 1000 times better limit of detection (LOD) than normal chromatography plates to 1–10 ppb level, which is comparable or even higher than that of many laboratory analysis techniques [35], which will be discussed in Section 3.6.

2. Materials and methods

2.1. Materials and reagents

Tetrachloroauric acid ($HAuCl_4$) was purchased from Alfa Aesar (USA). Trisodium citrate ($Na_3C_6H_5O_7$), anhydrous ethanol, hexane and ethyl acetate were purchased from Macron (USA). Celite209 (diatomite), carboxymethyl cellulose, pyrene, 4-mercaptobenzoic acid (MBA), plasma and cocaine were obtained from Sigma-Aldrich(USA). The chemical reagents used were of analytical grade. Water used in all experiments was deionized and further purified by a Millipore Synergy UV Unit (Millipore-Sigma USA) to a resistivity of ~18.2 MΩ cm.

2.2. Preparation and characterization of gold nanoparticles (Au NPs)

The glassware used through the NP synthesis process was cleaned with aqua regia (HNO_3/HCl, 1:3, v/v) followed by rinsing thoroughly with Milli-Q water. Au NPs with an average diameter of 60 nm were prepared using sodium citrate as the reducing and stabilizing agent according to the literature with little modification [36]. Briefly, a total of 100 mL of 1 mM chloroauric acid aqueous solution was heated to boiling under vigorous stirring. After adding 4.1 mL of 1% trisodium citrate, the pale yellow solution turned fuchsia within several minutes. The colloids were kept under reflux for another 15 min to ensure complete reduction of Au^{3+} ions followed by cooling to room temperature. For practical point-of-care (POC) sensing, the Au NPs will be concentrated by centrifuge and stored in refrigerators with expected life time of more than 1 month.

2.3. Fabrication of μDADs

The diatomaceous earth substrates were fabricated by spin coating diatomite on glass slides. The diatomite was dried at 150 °C for 6 h in an oven before spin-coating the glass slides. After cooling to room temperature, 11.55 g of diatomite was first dispersed in 20 mL of 0.4% aqueous solution of carboxymethyl cellulose and then deposited onto the glass slide by spin-coating at 1300 rpm for 20 s. The porous photonic crystal biosilica channels were fabricated by a simple tape-stripping method as shown in Fig. S1: the glass slides were first covered by an adhesive tape; then 400 μm wide channel array was cut by a razor blade through the tape; after spin-coating with diatomite, the tape was removed gently, leaving 400 × 30 μm^2 cross section diatomite channel array on the glass substrate. Last, the μDADs were dried in shade and activated at 110 °C for 3 h to improve the adhesion of diatomite to the glass slide.

2.4. μDADs for on-chip chromatography-SERS biosensing

The on-chip chromatography-SERS sensing method was designed for ultra-sensitive detection of analytes from mixtures or complex biofluid as shown in Scheme 1. First, 0.2 μL liquid sample was spotted onto the reservoir (circular region) of the μDAD. After drying in air, the bottom tips of the μDADs were immersed in the solvent which migrates along the porous channels towards the other end of the μDADs due to capillary forces. After that, the μDADs were taken out from the solvent and dried in air. The separated analyte spots along the porous channels were marked under ultraviolet illumination at 380 nm wavelength and visualized by iodine colorimetry. Then 2 μL of concentrated Au NPs in solution were dropped onto the corresponding spots directly. An alternative method to avoid dispensing the colloid solution is to pre-deposit Au NPs using inkjet printer at the designated spots. However, this process requires precise calibration of the analyte migration rate and will be investigated in our future research. A Horiba Jobin Yvon(USA) Lab Ram HR800 Raman microscope equipped with a CCD detector (uEye cmos, Germany) was used to acquire the Raman spectra, and a 50× objective lens (Olympus Mplan, Japan) was used to focus the laser onto the SERS substrates. A 785 nm laser was chosen as the excitation wavelength and the laser spot size was 2 μm in diameter. The confocal pinhole was set to a diameter of 200 μm. The acquired data was processed with Horiba LabSpec 5 software. Fluorescence spectra were acquired using the previous method [37]. Briefly, we focus light to a diatom surface with the 50× objective lens using the Horiba Jobin Yvon Lab Ram HR800 Raman system with 325 nm UV line.

2.5. Other instruments

UV-vis absorption spectra were recorded on NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific USA) using quartz cells of 1 cm optical path. Scanning electron microscopy (SEM) images were acquired on FEI Quanta 600 FEG SEM (Thermo Scientific, USA) with 15–30 kV accelerating voltage. The microscopy images were obtained using Olympus (Japan) IX73 microscope with 20× objective lens.