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Label-free and Raman dyes-free surface-enhanced Raman spectroscopy for detection of DNA

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ABSTRACT

In this work, a label-free and Raman dyes-free surface enhanced Raman spectroscopy for DNA detection based on the *in situ* DNA-metallization was developed and studied. A glass slide was employed to immobilize a peptide nucleic acid (PNA) as a recognition probe and to perform the detection procedure. Upon hybridization of the target DNA with the PNA probe, the DNA skeleton could adsorb positively charged silver ions due to the negatively charged DNA target. After chemical reduction by hydroquinone followed by a silver enhancement step, silver nanoparticles could be grown on the surface to reach 10 nm in size. The grown silver nanoparticles along the DNA skeleton induced sufficient interactions between the bases and the substrate to yield a sensitive Raman signal. The results suggested that the SERS spectrum of DNA was strongly dominated by the spectral feature of adenine at 736 cm^{-1}, where adenine served as an endogenous marker for label-free SERS detection of DNA. Using this method, highly reproducible and good quality SERS signals of DNA were obtained with a linear range varied between 1.0×10^{-10} to 1.0×10^{-6} M and detection limit of 34 pM. Overall, this high-performance DNA-metallization based SERS method may offer a versatile platform for label-free SERS detection of DNA at low-cost with promising application in clinical diagnosis.

1. Introduction

The sensitive, rapid and simple determination of sequence-specific DNA plays an important role in many modern fields, including bioterrorism, environmental monitoring, gene therapy, food analysis, and clinical diagnosis [1–4]. As a result, tremendous efforts have been made for developing a wide variety of DNA biosensors aimed for portable and affordable devices. This includes fluorescence [5,6], chemiluminescence [7,8], colorimetry [9,10], surface-enhanced Raman scattering (SERS) [11–13], electric signal [14], and electrochemical based biosensors [15–17]. Among these approaches, SERS is considered as a sensitive, straightforward and inexpensive analytical technique when compared to conventional methods used for DNA detection due to its many inherent properties, such as single molecule level sensitivity, insensitivity to quenching, rich molecular structural information, and the tremendous multiplexing capabilities of Raman spectroscopy [18–22].

These distinct advantages have led to the development of numerous ingenious SERS sensing platforms [23–27], classified as label and label-free strategies. For instance, a heterogeneous SERS method had been developed for label-free detection of DNA using direct electrostatic adsorption of positively charged Ag NPs and subsequent random incorporation of SERS reporter for diagnostic signatures [28]. In addition, Ju group had also reported a label-free SERS method for DNA detection based on DNA-mediated silver nanoparticle for absorbing SERS reporter [29]. These methods detected the signals of the SERS reporter rather than that of the target themselves. However, the attachment of SERS reporter would increase the cost and complexity of analytical assays. Therefore, achieving direct label-free (Raman dyes-free) SERS for DNA detection was of great importance as it could eliminate the complexity associated with the preparation and attachment of appropriate labels.

With the development of highly reproducible substrates, DNA base deoxynucleotides were identified by SERS with high sensitivities. This indicated the possibility of direct or intrinsic SERS (label-free and Raman dyes-free) assays [29–33], but one issue still required solutions. It had to be kept in mind that both target DNA and probe DNA sequences contained the same four DNA bases with different relative contents: adenine (A), thymine (T), cytosine (C), and guanine (G). Thus, distinguishing a specific SERS signal from the target is extremely challenging due to hybridization of the

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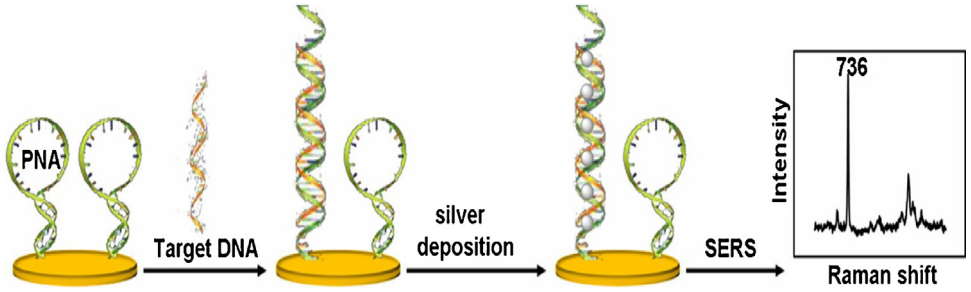

Scheme 1. Schematic illustration of label-free and Raman dyes-free SERS strategy used for DNA detection.

target and probe sequences. To overcome the problem, label-free SERS detection of DNA was proposed by substituting adenine in the probe by 2-aminopurine due to the strong spectral feature of adenine at 736 cm^{-1} [31]. However, this method performed through a sulfur group allows DNA to have a more tilted orientation, which could prevent all the adenine bases from interacting sufficiently with the substrate to provide good Raman signals. The effective SERS detection limit was less than 5 nm from the surface, indicating that the hybridization assay was sensitive to only the first few nucleotides that defined the probe sequence [34].

Recently, DNA metallization had gained considerable interest in functional circuits and certain futuristic nanoscale devices [35,36]. The negatively charged phosphate backbones of DNA molecules were employed to bind with various metal cations (Au, Ag, Pd, Pt, and Cu) through electrostatic interactions to produce metallization [37–39]. The molecular length of DNA metallization could be prepared in a wide range varying from nanometers to micrometers because of the well-defined sequences of the DNA bases. These materials had been demonstrated to be very useful for DNA-based sensors or nanoelectronic devices [40].

In this work, a novel flexible label-free and Raman dyes-free strategy for the sensitive SERS detection of DNA based on *in situ* DNA metallization was developed and studied. A peptide nucleic acid (PNA, the configuration of the PNA was presented in the form of molecular beacon (MB, hairpin structure)) was first modified a glass slide surface as recognition probe. In presence of the target DNA, silver nanoparticles were then formed on the target DNA phosphate skeleton by *in situ* chemical reduction of electrostatically absorbed Ag^+ followed by silver enhancements to act as a SERS-active substrate. This made all the bases to interact sufficiently with the substrate for efficient Raman detection of DNA (Scheme 1). The obtained signals were primarily generated by adenine base of target DNA, which did not require a label conjugation step.

2. Experimental

2.1. Materials and reagents

Hydroquinone and glycidoxypropyltrimethoxysilane (GPTMS) (98%) were obtained from Sigma–Aldrich. Ammonium hydroxide (25%), hydrogen peroxide (30%), sulfuric acid (70%), $AgNO_3$, sodium citrate, toluene and citric acid were purchased from Shanghai Reagent Co. (Shanghai, China). Ultrapure water used in all assays was obtained by a Millipore water purification system (18 MΩ, Milli-Q, Millipore). The DNA hybridization buffer pH 7.4 was prepared from a phosphate-buffered saline solution containing 137 mM NaCl, 2.5 mM Mg^{2+}, 10 mM Na_2HPO_4, and 2.0 mM KH_2PO_4. The citrate buffer was prepared by mixing the stock solutions of sodium citrate and citric acid (0.1 M, pH 3.5). DNA oligonucleotides were synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China) and stored in DNA hybridization buffer. PNA was purchased from Chengdu CP Biochem Co., Ltd. (Chengdu, China). The sequences of these oligonucleotides were as follows:

PNA: 5′-NH_2-AAGTAGTGATTGAGCGTGATGAATGTCACTACTT-3′
Ferrocene (Fc)-PNA: 5′- NH_2-AAGTAGTGATTGAGCGTGATGA-ATGTCACT
ACTT-Fc-3′
Target DNA: 5′-GACATTCATCACGCTCAATCACTACTT-3′
Single-base mismatched: 5′-GACATTCATCACACTCAATCACTACTT-3′
Two-base mismatched: 5′-GACATTAATCACGCTCAATCAGTACTT-3′
Noncomplementary: 5′-ATTCGATGGATTACCCTGATCTACTGC-3′
Control DNA1: 5′- NH_2-CCCAAAAAACCCCCCCCCCCC-3′
Control DNA2: 5′- NH_2-CCCCCCAAAAAACCCCCCCCC-3′
Control DNA3: 5′- NH_2-CCCCCCCCCAAAAAACCCCCC-3′
Control DNA4: 5′- NH_2-CCCCCCCCCCCCAAAAAACCC-3′
Control DNA5: 5′-NH_2-CCCCCCCCCCCCCCCAAAAAA-3′

2.2. Apparatus

The tapping mode atomic force microscopy (AFM) image was acquired under ambient conditions using an Agilent 5500 AFM/SPM system. Cyclic voltammetry (CV) measurements were performed using an electrochemical workstation in the potential range varying from 0 V to 0.6 V at a scan rate 0.05 V s^{-1}. All measurements were performed with four successive cycles to ensure signal stabilization, and the last cycle was kept as the final result. SERS measurements were carried out using a Renishaw in Via-Reflex Raman microscopy system (Renishaw, U.K.). An argon laser at 488 nm was used for excitation and the spectra were acquired using a 10× working objective lens.

2.3. Immobilization of PNA probe

The silanized glass substrate was prepared by immersing the glass substrate in piranha solution containing 70% sulfuric acid and 30% hydrogen peroxide for 0.5 days. The substrate was then washed thoroughly with water and dried under a stream of nitrogen. The obtained glass substrate was silanized by dipping in a 1% GPTMS toluene solution for 24 h at room temperature. The resulting substrate was finally washed with toluene and ethanol to obtain a silanized glass substrate. Afterward, a 10 μL solution containing 1 μM PNA probe was cast onto the silanized glass substrate and left for 2 h at room temperature. The substrate was finally washed with 10 mmol L^{-1} PBS and subsequently used as DNA sensor.

2.4. DNA-metallization

A volume up to 10 μL of various concentrations of target DNA dissolved in PBS was added to the PNA modified substrate and incubated for 1 h. The DNA sensor was then washed with PBS. The absorption of Ag^+ ions on the DNA skeleton was performed by injecting 10 μL of an $AgNO_3$ aqueous solution (0.1 M in ammonium