Talanta 179 (2018) 200–206

Contents lists available at ScienceDirect

Talanta

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

# Glucose-bridged silver nanoparticle assemblies for highly sensitive molecular recognition of sialic acid on cancer cells *via* surface-enhanced raman scattering spectroscopy

Rong Deng[a,1], Jing Yue[a,1], Huixin Qu[b], Lijia Liang[a], Dan Sun[a], Jing Zhang[a], Chongyang Liang[b,*], Weiqing Xu[a], Shuping Xu[a,*]

[a] State Key Laboratory of Supramolecular Structure and Materials, Institute of Theoretical Chemistry, Jilin University, 2699 Qianjin Ave, Changchun 130012, People's Republic of China

[b] Institute of Frontier Medical Science, Jilin University, Changchun 130021, People's Republic of China

ARTICLE INFO

*Keywords:*
Cancer cell
Sialic acid
MPBA
Molecular recognition
SERS

ABSTRACT

The expression levels of glycans on the surfaces of cancer and normal cells show different, however, this difference is not noticeable enough to distinguish them directly. So, herein, based on the targeted molecular recognition of the glycans on cell surfaces by 4-mercaptophenyl boronic acid (MPBA), a novel surface-enhanced Raman scattering (SERS) nanoprobe (glucose-MPBA@AgNPs) was prepared by inducing controllable assembly of MPBA decorated Ag nanoparticles (MPBA@AgNPs) in a certain level *via* the bridge of glucose to amplify such a limited difference in SERS measurements. On the basis of the aggregation-induced 3D SERS hot spot effect, this multi-particle nanoprobe possesses over 10 times stronger SERS enhancement ability than the individual MPBA@AgNPs. As the different sialic acid (SA) expression on the surfaces of cancer and normal cells led to the different accumulation of glucose-MPBA@AgNPs, the results we obtained (mean intensities recorded from five cells) indicate the SA amounts on two kinds of cells can provide 5–7 times signal contrast grade in SERS band intensities ($P < 0.001$). Compared with the monodispersed nanoprobe, our developed nanoprobe amplifies the SA expression difference on cell surfaces and supports high sensitivity for cancer cell recognition, which might be useful in providing highly effective recognition of the edges of tumor tissues in clinic field.

## 1. Introduction

The targeting of cancer cell is usually based on the specific molecular recognition toward antigens, overexpressed proteins on cell surface *via* aptamers, antibodies, biotin and peptides [1–3]. Nowadays, another targeting mechanism, the unique glycosylation expressive level on cell surface, has drawn great attention due to the close relationship with many diseases and lesions in the evolution or deterioration process of cells, and it has become an important marker for disease diagnosis. Among these saccharides expressed on cell surfaces, the most prominent target is the specific expression of sialic acid (SA) residues [4,5]. As a kind of anionic oligosaccharide, the amount of SA has a certain difference in the normal cells and tumours and unnormal SA expression is closely associated with various disease progression. Therefore, highly sensitive and selective determination of SA on cell surfaces becomes a critical foundation for the distinction of cancer cells and normal ones.

Up to know, the targeted binding of SA is mainly based on the special molecular recognition. One of the primary studies has adopted the lectin for specific binding of SA [6]. And bioorthogonal reactions and metabolic labelling methods based on click chemistry gain a success in the fluorescence sensing of SA [3,4,7,8]. While as a unique lewis acid, boronic acid and its derivatives have been widely applied in the cancer cell recognition owing to its targeted binding with SA [9–11]. In an alkaline solution, they can react with the cis adjacent dihydroxyl group contained molecules, such as glucose and glycoproteins, to form stable cyclic esters. This affinity interaction has become one of the common-used mechanism for saccharide detections [11–15]. However, at the physiological condition (or under the condition of weak acid), boronic acid has higher affinity with SA on the surface of the cells compared with other saccharides to form a different, stable triangle-shaped compound [16,17]. Wang et al.[18] reported this dual response process of a phenylboronic acid-containing polymer brush in reversible

*Abbreviations:* SERS, surface-enhanced Raman scattering; MPBA, 4-mercaptophenyl boronic acid; SA, sialic acid; AgNPs, Ag nanoparticles

* Corresponding authors.
*E-mail addresses:* liang@jlu.edu.cn (C. Liang), xusp@jlu.edu.cn (S. Xu).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.talanta.2017.11.006
Received 3 April 2017; Received in revised form 25 October 2017; Accepted 3 November 2017
Available online 04 November 2017
0039-9140/ 

capture and release cell according to the binding with SA on the cell surface with the competition of high concentration glucose. Owing to its special recognition feature, many boronic acid-functionalized materials have been developed as unique targeted probes for saccharide identification, sensing, cancer cell recognition and drug delivery [19–25]. However, these extra fluorescent and chemiluminescent labelled molecules might be susceptible to disturbances, causing poisonous side effect on cells.

The plasmonic-based surface-enhanced Raman scattering (SERS) spectroscopy as an ultrasensitive vibrational fingerprinting technique to single molecule detection has shown promising applications in bio-/chem-sensing and assays [26–28]. In these SERS sensing methods, SERS label/tag methods based on the integration of Raman-active molecules and plasmonic nanoparticles have been frequently exploited due to their advantages of identifiable Raman signals and high sensitivity relative to label-free SERS methods [29–33]. 4-Mercaptophenylboronic acid (MPBA) displays distinguishable Raman peaks when it binds with plasmonic nanoparticles or metal surface due to the high affinity of its mercapto group to metal (e.g. Au and Ag) [34]. A more important thing is that it can selectively recognize SA among other saccharides. Owing to the dual functions of MPBA in molecular recognition and Raman activity, the MPBA-based SERS probe has been used for SERS sensing of saccharides [26,35,36] and also provides future potential for the testing of cells and tissues *in situ* and *in vivo* [37]. However, by reviewing previous work, the individual metal NPs with MPBA have been tried. The SERS enhancement from individual metal NPs is much weaker compared with the aggregation ones, which maybe provide limited sensitivity in tracing SA on cell surface and allow for further improvements.

In this study, we aimed to identify and discriminate the cancer cells from normal ones by a controllable and ultrasensitive SERS nanoprobe (glucose-MPBA@AgNPs), which synthesized by the glucose-induced aggregation of MPBA decorated Ag nanoparticles (MPBA@AgNPs) under the alkaline condition (pH = 9.18), combined with SERS technique. Since the combination of MPBA and glucose is as a ratio of 2:1, the produced glucose-MPBA@AgNPs generates a certain extent of aggregation of AgNPs, which significantly amplifies the SERS signal of MPBA and provides more remarkable contrast degree when using them as a SERS signal reporter. While under the physiological conditions, the MPBA can target recognize the SA, so different SA expressions on the surfaces of cancer and normal cells result in different accumulations of glucose-MPBA@AgNPs, the SERS intensity of MPBA on two cells are also great different accordingly. It obviously amplifies the difference in SA expression of cells, which possesses significant meaning in in situ diagnosis and targeting study of tumor tissues instead of excisional biopsy.

## 2. Experimental procedures

### 2.1. Materials and instrument

Silver nitrate ($AgNO_3$, 99.99%), phosphate-buffered-saline solution (PBS, pH = 6.86 and 9.18), glucose and sodium citrate were purchased from the Beijing Chemicals Reagent Company. 4-mercaptophenylboronic acid (MPBA) was obtained from Aladdin Industrial Corporation. HepG2 (human liver cancer cell carcinoma) and BNL.CL (a noncancer liver cell line) were both bought from Shanghai ATCC cell bank. Culture media (DMEM) and fetal bovine serum (FBS) were from JIBCO.

UV–vis spectrometer (Ocean Optics, USB4000), DLS (MALVERN Zetasizer Nano ZS), TEM (JEM-2100F), portable B&WTek Raman spectrometer, UV–vis spectroscopy (Ocean Optics, USB4000) and confocal Raman spectrometer (LabRAM ARAMIS, HORIBA JobinYvon, USA) were used for the characterizations of the prepared nanoprobes.

The bright-field, dark-field/fluorescence co-imaging were carried out on a self-built Raman detection platform. The configuration and working way can be found in our previous work [38]. The detection system is mainly composed of an inverted fluorescence microscope (IX71, Olympus), a dark-field microscope (Olympus), and a Raman detection system, including a He-Ne laser (20 mW, 633 nm, UNIPHASE), a set of Raman filters (Semrock) and a spectrometer (IHR 320, Horiba Jobin Yvon, France). In our experiments, the dark-field microscope (20×, NA = 0.5) was used to locate AgNPs through collecting their scattering light. Fluorescence imaging taken by fluorescence microscope (20×, NA = 0.5) was to identify the location of cell nuclei. Based on the dark-field and fluorescence co-imaging, the measured positions would be co-located accurately.

### 2.2. The preparation of SERS nanoprobe (Glucose-MPBA@AgNPs)

The synthetic process of AgNPs is according to the traditional method proposed by Lee and Meisel [39]. Firstly, 0.018 g of $AgNO_3$ was added in the 100 mL deionized water, which is almost boiling, then 2 mL of 1% sodium citrate aqueous solution was added rapidly in. The mixture was under continuously heating and stirring until the color became grayish green and stabled it for ten minutes. The produced Ag colloid was cooled to the room temperature before further use.

The concentration of the obtained Ag colloid is approximate $2.5 \times 10^{-11}$ M according a molar extinction coefficient of $3 \times 10^{11}$ particles $M^{-1}$ $cm^{-1}$ [40]. In order to fully cover the surface of silver nanoparticle (coverage, $\theta$), about 8 μL of MPBA (1.0 mM) aqueous solution was added to 10 mL of the AgNPs solution according the formula (1),

$$\theta = \frac{0.25nN_a}{S} = \frac{0.25n}{C_{Ag}V\pi d^2} \quad (1)$$

where *Na* is Avogadro's number, $C_{Ag}$ the concentration of AgNPs, *V* the volume of AgNP colloidal solution, and *d* the average diameter of AgNPs. The mixture was stirred for 12 min and then stewed for one night. The redundant MPBA were removed by centrifugation (7000 rpm, 10 min). The precipitates were dissolved in a PBS buffer with the pH of 9.18 for a short time to activate the MPBA to form cyclic boronate esters with diols.

The activated MPBA@AgNPs ($2.5 \times 10^{-11}$ M, 0.9 mL) was further cultured with different concentrations of glucose solutions (0, 1.0, 5.0, 10, 20 and 50 mM, 0.1 mL) for 1 h. We used the resulted glucose concentrations, 0, 0.1, 0.5, 1.0, 2.0 and 5.0 mM to annotate different nanoprobes. As the MPBA/glucose bonding ratio is 2:1, the aggregation of AgNPs happened. The obtained glucose-MPBA@AgNPs were purified again by centrifugation and then redispersed in a phosphate buffer (pH = 6.86) before use. Owing to the excess molar ratio of MPBA, a great number of unbonded boronic acid groups were still left for SA identification even parts of them had been linked with glucose.

The glass slides were rinsed by water and dried. Then, the relevant glucose-MPBA@AgNPs solutions were dripped on these glass slides, respectively. When the liquid drops were dried in the air, the SERS signals of the MPBA were measured by a confocal Raman system (LabRAM ARAMIS, HORIBA JobinYvon, USA) with a 633 nm laser.

### 2.3. Cell culture and cell viability assays

HepG2 (human liver cancer cell carcinoma) and BNL.CL (a noncancer liver cell line) were both bought from Shanghai ATCC cell bank who have been issued the permission of the Human Research Ethics Committee of the country for manupulations of human's cells. The cells were grown in the Iscove's modified Dubecc's medium (IMDM, Gibcos) supplemented with 4.5 g/L of glucose and sodium pyruvate, 10% v/v of fetal bovine serum (FBS, Mediatech), and 1% of antimycotic solution (Mediatech). Cell cultures were maintained at 37 °C in a 5% CO2 humidified cubator.

The toxicity of AgNPs, MPBA@AgNPs and glucose(0.5 mM)-MPBA@AgNPs probes to HepG2 cells were tested by the WST-1 (2-(4-