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Potential use of SERS-assisted theranostic strategy based on Fe_3O_4/Au cluster/shell nanocomposites for bio-detection, MRI, and magnetic hyperthermia

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ABSTRACT

A surface-enhanced Raman scattering (SERS)-assisted theranostic strategy was designed based on a synthesized multifunctional Fe_3O_4/Au cluster/shell nanocomposite. This theranostic strategy was used for free prostate specific antigen (free-PSA) detection, magnetic resonance imaging (MRI), and magnetic hyperthermia. The lowest protein concentration detected was 1 ng mL^{-1}, and the limit of detection (LOD) of the calculated PSA was 0.75 ng mL^{-1}. Then, MRI was carried out to visualize the tumor cell. Lastly, magnetic hyperthermia was employed and revealed a favorable killing effect for the tumor cells. Thus, this SERS-assisted strategy based on a Fe_3O_4/Au cluster/shell nanocomposite showed great advantages in theranostic treatment.

1. Introduction

Recently, theranostic nanomedicine incorporating both diagnosis and therapy into one nanomatrix has been the focus of researchers due to its ability to carry various purposes in biomedical application [1]. The integration of imaging, detecting, and therapeutic functions into a single platform provides the possibility of combining disease diagnosis, tissue imaging, and real-time monitoring of drug delivery [2–4]. It is important for visualizing biodistribution [5], optimizing strategies [6], monitoring drug release [7], and assessing therapeutic effect [8]. In this inevitable trend, different types of nanoparticles have been proposed for simultaneous diagnosis and treatment [9,10].

Achieving simultaneous observation and treatment is of utmost importance in the new generation of cancer drugs [11]. Considerable effort has been directed toward synthesizing composite materials with different functions [1]. For example, the method of imaging and therapy has already been combined, and a certain curative effect showed up well [12]. However, the majority of such strategies lack the ability to make a critical difference in the specific detection of the disease. Thus, other imaging agents should be taken before therapy treatment to make a definite diagnosis, a step that leads to low efficiency and high cost [13]. In recent years, a number of specific detection approaches have taken advantages of physical or chemical methods, such as fluorescence [14], enzyme-linked immunosorbent assay (ELISA) [15], electrochemistry [16], and chemiluminescence assay [17]. Among these approaches, surface-enhanced Raman scattering (SERS) is considered desirable because of its high sensitivity, significant specificity, and excellent efficiency [18]. Given these advantages, we expected the nanomedicine coupled with SERS method to be capable of overcoming the limitation of current assays and have a better ability of realizing theranostic treatment.

PSA ($M_r \approx 34{,}000$) is a species- and tissue-specific glycoprotein produced only by human prostate epithelial cells. Confirming the existence of PSA is critical in the early diagnosis of prostate cancer. With the help of the SERS method, the limit of detection (LOD) of PSA decreased and the testing time was greatly reduced. Noble metals, such as Au and Ag, Cu, are known to provide a SERS-active surface. After combining with other nanomaterials, the metal composite can be further used for multifunctional application [19]. Numerous papers have reported the use

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of metal composites (e.g., Fe_3O_4@Au, SiO_2@Au, polymer@Au) for theranostics [20]. However, Fe_3O_4/Au nanoparticles with theranostics and SERS for early diagnosis of PSA have not been reported so far.

In this study, a SERS-assisted theranostic strategy was designed based on multifunctional nanocomposites using gold shelled Fe_3O_4 clusters [21,22]. Fe_3O_4 nanoparticles exhibit fairly strong saturated magnetization (M_s) and high r_2 relaxivity, which can be used in both magnetic resonance imaging (MRI) and magnetic hyperthermia [23–27]. Compared with non-cluster Fe_3O_4 nanoparticles, same-sized cluster Fe_3O_4 nanoparticles had spin–spin (T_2) contrast enhancement and increased magnetic response [28]. On the other hand, gold nanoshells grow on the surface of Fe_3O_4 clusters as coverage, which can supply excellent chemical stability, biocompatibility, better heating property for magnetic hyperthermia, and well-established chemistry for functionalization, as well as act as an appropriate SERS substrate [29–33]. The present study used the SERS method to confirm the existence of PSA in detecting prostate cancer. The LOD of PSA is 0.75 ng mL^{-1}, which is far below the 4–10 ng mL^{-1} detection range in human serum, demonstrating the potential of this approach in complex matrices. Then, MRI was carried out to visualize the tumor cell. Lastly, magnetic hyperthermia was employed to achieve the goal of treatment; this technique exhibited efficient performance and could be considered a desirable magnetic hyperthermia agent in future clinical applications. Our work demonstrated that this strategy possesses great advantage in the theranostic treatment of prostate cancer.

2. Experimental

2.1. Chemicals

Polyethyleneimine (PEI, Mw ≈ 10,000), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), chloroauric acid ($HAuCl_4 \cdot 4H_2O$), sodium borohydride ($NaBH_4$), urea, sodium citrate, polyacrylamide (PAM), hydroxylamine hydrochloride ($NH_2OH \cdot HCl$), phenylmethanesulfonyl fluoride (PMSF) and dimethylsulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co. Ltd. Malachite green isothiocyanate (MGITC) was purchased from Invitrogen. Free prostate specific antigen (free-PSA), and its capture antibody as well as detector antibody were obtained from Shanghai Linc-BioScience Co. Ltd. LNCaP (androgen-sensitive human prostate adenocarcinoma) cells line was purchased from Cobioer Bioscience Co. Ltd. Annexin V-FITC/PI purchased from Alexis (USA). All chemicals were of analytical grade and used as received directly without further purification.

2.2. Characterization techniques

TEM images were obtained using a JEOL JEM-2100 (Tokyo, Japan) operated at a 200 kV accelerating voltage. Scanning electron microscope (SEM) images were performed on a field emission scanning electron microscope (FESEM, Leo 1530) with an accelerating voltage of 20 kV. Extinction spectra were performed on a DU 800 UV/vis spectrophotometer. The X-ray powder diffraction patterns of the as-prepared samples were collected on an X'pert PRO X-ray diffraction system (Almelo, Netherlands) with a graphite monochromator and Cu Kα radiation ($\lambda = 0.15406$ nm) between 10 and 90 (2θ) degrees. Raman spectra were collected on a Deltanu Inspector Raman using a 785 nm excitation source with a 100 mW peak power. Field-dependent magnetization (M–H curves) was performed using a vibrating sample magnetometer (VSM-7400 magnetometer, Lakeshore, America) at room temperature. Flow cytometry was taken on the Becton Dickinson C6 system.

2.3. Preparation of cluster/shell Fe_3O_4/Au nanoparticles

Fe_3O_4 nanoparticles were synthesized by a hydrothermal method [34]. The Fe_3O_4 nanoparticles synthesized by this method will form clusters and exhibit well water-dispersibility. In a typical procedure, $FeCl_3 \cdot 6H_2O$ (1 mmol) was dissolved in 20 mL water, followed by the addition of urea (3 mmol) and sodium citrate (2 mmol). The resulting mixture was stirred until forming a clear solution and then 0.15 g PAM (7.5 $g \cdot L^{-1}$) was added under continuous stirring. The solution was transferred to a Teflon-lined autoclave (25 mL) and sealed in air. Following this, the autoclave was kept at 200 °C for 12 h. After that, the black precipitation was collected under the assistance of a magnet and washed several times with distilled water and absolute ethanol. The resulting Fe_3O_4 nanoparticles were freezing-dried for further use. Fe_3O_4 nanoparticles with size of 27 nm, 80 nm, 200 nm, and 270 nm were obtained at different concentration of PAM with 25 $g \cdot L^{-1}$, 15 $g \cdot L^{-1}$, 7.5 $g \cdot L^{-1}$ and 2.5 $g \cdot L^{-1}$, respectively.

The 3 nm gold nanoparticles colloidal solution was prepared according to the Jana et al. [35] Briefly, 1 mL $HAuCl_4 \cdot 4H_2O$ (1 wt%) and 2 mL sodium citrate (38.8 mM) were added to ultrapure water (90 mL) with magnetic stirring. Then, 1 mL 0.075 wt% $NaBH_4$ in sodium citrate (38.8 mM) was added, and the reaction mixture was stirred for 5 min.

The typical steps of synthesis of cluster/shell Fe_3O_4/Au nanoparticles are shown as following: First, the as-prepared Fe_3O_4 nanoparticles (10 mg) were dissolved in 20 mL PEI (5 $g \cdot L^{-1}$) and stirred for 2 h. The products were rinsed with water three times and collected with magnetic separation. Next, the Fe_3O_4-PEI nanoparticles were mixed with the as-prepared Au seed colloidal solution (90 mL) to mechanically stir for 2 h and magnetically separated 3 times and dispersed in ultrapure water to remove dissociated Au seeds. Then, the as-synthesized Fe_3O_4/Au seeds product was added to NaOH aqueous solution (110 mL, 0.01 M) with mechanically stirring (400 rpm), and added 0.25 mL $NH_2OH \cdot HCl$ (0.2 M) and 0.5 mL $HAuCl_4$ (wt 1%) into the mixture, 10 min later, the product was rinsed with water three times by magnetic separation. A total of up to five iterations were made, followed by freezing-drying for further use. The above section is the strategy of synthesis of 20 nm Au shell, while the volume of $HAuCl_4$ (wt 1%) changes to 0.25 mL and 1 mL, the thickness of the Au shell changes to 5 nm and 25 nm, respectively.

2.4. Detection of free-PSA with Fe_3O_4/Au nanoparticles via a SERS method

Typically, Fe_3O_4/Au nanoparticles was dispersed in 1 mL ultrapure water with a concentration of 2.4 pM, then HS-PEG-COOH (7.5×10^{-11} mol, Mw: 500) and HS-PEG (4.25×10^{-10} mol, Mw: 2000) were added to the Fe_3O_4/Au aqueous solution (1 mL, 2.4 pM), after the mixture was stirred for 30 min, EDC-NHS (0.2 μmol–0.5 μmol) was added with mechanically stirring at room temperature. 30 min later, the detector antibody (146.5 pmol) was added and incubated with the mixture at 37 °C for 3 h. After magnetic separation to remove the supernatant, the resulting antibody-conjugated nanoparticles were resuspended in PBS (200 μL) and stored at 4 °C.

Raman reporter molecules MGITC were conjugated to the 30 nm Au by Au—S bond. The capture antibody was conjugated to the 30 nm Au by ester bond activated by EDC and NHS. MGITC (80 pmol) used as the Raman label and was added to the 30 nm Au colloidal solution (1 mL). With the same procedure as that of the Fe_3O_4/Au nanoprobe, the capture antibody was conjugated to Au-dye nanoparticles as the SERS labels.

The free-PSA connected the nanoprobes with SERS Labels as a sandwich mode. Free-PSA (53.1 pmol) was added to the mixture of as-prepared Au-MGITC-cAb and Fe_3O_4/Au-dAb and incubated at 37 °C for 30 min. The control sample was prepared in the same manner without free-PSA. Then the samples were collected by magnetically separation and resuspended in ultrapure water for SERS measurement.

2.5. MRI relaxation properties

The as-prepared Fe_3O_4 and Fe_3O_4/Au nanoparticles with different iron concentrations were dispersed in 1% agarose solution. MR relaxivities of the as-synthesized nanoparticles were measured with a