Synthesis and characterization of gold-deposited red, green and blue fluorescent silica nanoparticles for biosensor application†

Kyoung G. Lee, Rinbok Wi, Tae Jung Park, Sun Hong Yoon, Jaebeom Lee, Seok Jae Lee and Do Hyun Kim* a

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Fluorescent silica nanoparticles deposited with highly mono-disperse gold nanoparticles (1–2 nm) were synthesized via the W/O method and intensive ultrasound irradiation. A large surface area of gold-doped fluorescent silica nanoparticle serves as a platform to immobilize a specific binding protein for biomolecules interaction in bioimaging applications.

Research on hybrid fluorescent inorganic nanoparticles (NPs) has brought great interest in chemistry, biology, medical science, as well as biotechnology, because of the high biocompatibility and photostability of the nanoparticles. For biotechnological and biomedical applications, various types of inorganic materials, including quantum dots (QD), gold, silica, and europium oxide, have been developed.1–4 However, only a limited number of inorganic materials are suitable for biological applications due to the potential toxicity of the nanoparticles and low photostability. Considering these limitations, fluorescent silica-based NPs have been found to be one of the best candidates among the inorganic materials to be used as biostaining and labeling tools.5,6 Silica NPs (SiNPs) have been known to be a support material to decorate other NPs and to be highly biocompatible for the biomolecular immobilization. The surface of silica, including nanorods and NPs, has been modified with a thiol or an amine group using numerous types of silane compounds to deposit metal NPs or to immobilize biomolecules so far.7–10 However, the employed amine group can cause cytotoxicity and limit the performance of silica nanomaterials in biological applications.11 Therefore, it would be worthwhile to develop a new way to modify the surface of silica nanomaterials without employing other chemical components. To overcome these problems, a new synthetic method has been developed to deposit metal NPs onto the silica surface using ultrasound without surface modification. Among various kinds of metal NPs, gold NPs have been employed due to their large surface area, biocompatibility, easy bioconjugation and biomodification.12–14 In order to maximize the advantages of gold-deposited SiNPs, gold binding polypeptide (GBP) has been adopted for further applications. Recently, Brown and other researchers found GBPs which selectively immobilize onto gold NPs and are used in various biosensors and bioseparation applications.15–17

In this work, a water-in-oil (W/O) microemulsion was employed to synthesize gold-deposited fluorescent SiNPs with three different colors, red (R), green (G) and blue (B), using ultrasound irradiation. Specific target protein was also adopted for the biomolecular separation using a magnet. GBP-fused avian influenza viral surface antigen (Ala) protein was employed and immobilized onto the gold NPs to detect its specific antibody and a magnet, which is a portable tool for bio-molecular separation, was used to separate the conjugated fluorescent NPs.

Depicted in Scheme 1, the synthesis strategy for gold-deposited SiNPs is based on a two-part process as follows: (a) encapsulation or covalent conjugation of organic dye precursors to a silica matrix through the W/O method, and (b) deposition of gold NPs on the surface of fabricated SiNPs under ultrasound irradiation. The detailed procedure for the preparation of fluorescent SiNPs is described in the ESI.

These overall experimental procedures are schematically illustrated in Scheme 1. As TEOS is hydrolyzed and silica precursors are formed, dissolved dye molecules are incorporated into the silica matrix and then fluorescent SiNPs are created. After dispersing the RGB fluorescent SiNPs, which act as support materials, the gold NPs can be directly deposited over the silica surface under highly intensive ultrasound irradiation. The ultrasound would promote the generation of gold NPs in the silica mixture solutions and gold NPs are collapsed with SiNPs and deposited onto the silica surface. The detailed experimental procedures are explained in ESI.

Morphologies of both silica and gold NPs were characterized by field-enhanced scanning electron microscopy (FE-SEM), field-enhanced transmission electron microscopy (FE-TEM), photoluminescence, and X-ray diffraction (XRD). Fig. 1a–c show FE-SEM images of R-SiNPs, G-SiNPs, and B-SiNPs, respectively. The fabricated RGB-SiNPs show a regular spherical monodisperse shape and its diameter is in the range of 50–60 nm with amorphous structures (see Table S1 and Fig. S1–S2). FE-TEM images of gold doped on R-SiNPs,
energy to convert water into H and OH radicals. Au(III) ions process, the applied ultrasound provides a highly intensive and cooling rate (\( \sim 5000 \text{K} \)) and pressure (\( \sim 1000 \text{atm} \)) with enormous heating and cooling rate (\( \sim 10^9 \text{K s}^{-1} \)) in a short period of time. These unique conditions can enhance the chemical reaction.18 In our process, the applied ultrasound provides a highly intensive energy to convert water into H and OH radicals. Au(III) ions from HAuCl\(_4\) are reduced with H radicals and transformed into gold NPs (See Scheme S1).19 In addition, the ultrasound applied to the mixture of NPs solution can generate a shock wave to cause strong interparticle collision between gold NPs and SiNPs as reported before.20 In a similar way, the generated intensive energy can cause the synthesized gold NPs to collide into SiNPs with enormous force and gold NPs are instantly melted as they collapse onto SiNPs and are cooled down. In these sequences of reactions, gold NPs can be decorated on the surface of SiNPs. However, the gold NPs without the presence of SiNPs have a strong tendency to agglomerate with each other, which may result in the increased diameter of the gold NPs compared to those on the surface of RGB-SiNPs (see Fig. S7). These results could provide a clue to understanding the role of SiNPs in the reaction. Once the gold NPs are synthesized, the energy from ultrasound made gold NPs collapse onto SiNPs and prevents the agglomeration between gold NPs. APTS has been only employed to conjugate with FITC fluorescent dye for the synthesis of G-SiNPs. The FITC–APTS conjugation was encapsulated inside of a silica matrix and it has been confirmed using FT-IR analysis as shown in Fig. S8. The peaks in the range of 1000 and 1100 cm\(^{-1}\) are assigned due to Si–O–Si from silica. The common amine group from APTS should be observed in the two ranges as follows: (1) 2800 and 3000 cm\(^{-1}\) (indicating –C–NH\(_2\) stretch vibration), (2) 3000 and 3600 cm\(^{-1}\) (NH\(_2\) stretch vibration). During the analysis, only one peak in the range of 3000 and 3600 cm\(^{-1}\) was observed during FT-IR analysis. However, the relative intensity of the peak from Si–O–Si is much larger than peaks from NH\(_2\) stretch. This analysis indicated that APTS is not the major cause of deposition of gold NPs on the surface of SiNPs during ultrasound irradiation. In addition, the two doublet peaks with a maximum at (84.0, 87.6 eV) and (85.9, 89.5 eV) from XPS analysis were assigned to the 4f\(_{7/2}\) and 4f\(_{5/2}\) of Au\(^0\) and Au\(^{3+}\) in Au\(_2\)O\(_3\). This analysis demonstrated the presence of Au–O species based on the XPS spectra. However, it is difficult to distinguish the origination of Au–O species from chemisorbed oxygen, bulk gold oxide or the employed substrates (SiO\(_2\)). Therefore, the employed APTS has no effects on the deposition of gold NPs on the surface of SiNPs. It may prove that the gold NPs are physically attached to the surface of SiNPs without any chemical binding.

The RGB-SiNPs produce strong and bright photoluminescence (PL) due to the incorporation of fluorescent dyes in the silica matrix as shown in Fig. 2. The emission wavelengths of red and green fluorescent SiNPs are similar to those of the parent fluorescent dyes. However, the blue fluorescent SiNPs show different emission characteristics compared to the parent dyes after incorporation in the silica matrix. This phenomenon was assumed to be caused by the large Stokes-shift of SiNPs and excitation energy transfer between the dyes in the confined space (i.e. SiNP) which leads to

**Scheme 1** Schematic illustration for the preparation of fluorescent NPs and gold-deposited R-/G-/B-SiNPs and biosensor application using GBP-based protein immobilization on the surface of gold NPs.

**Fig. 1** FE-SEM images of (a) R-SiNPs, (b) G-SiNPs, and (c) B-SiNPs (scale bars represent 1 \( \mu \)m). FE-TEM images of gold deposited on the surface of (d) R-SiNPs, (e) G-SiNPs, and (f) B-SiNPs (scale bars represent 20 nm).

**Fig. 2** Photoluminescence spectra of (a) R-SiNPs, (b) G-SiNPs and (c) B-SiNPs. (d) Photographs of dye-incorporated SiNPs as solid powders and (e) fluorescent image of RGB-SiNPs.
increased PL intensity and different PL characteristics from the parent dyes. PL studies for maximum excitation and emission wavelengths of fabricated SiNPs in water were undertaken. The fluorescent intensity of SiNPs was decreased after gold NPs doping due to the gold nanoparticles acting as quenchers (see Fig. S9).

To illustrate the potential application of gold-deposited RGB-SiNPs as biosensor tools, first, gold-deposited R-SiNPs (GR-SiNPs) were selected for the binding affinity test between GBP-GFP fusion proteins and gold NPs. Fig. 3a shows each confocal fluorescent image of GBP-GFP fusion proteins (green color) and GR-SiNPs (red color). The fluorescence images of GBP-GFP and GR-SiNPs overlapped with each other, which indicates the successful immobilization of GBP-GFP on the gold NPs. This method was then tested using anti-GFP antibodies in place of fluorescent proteins.

The GBP-AIa fusion protein is covalently linked to the surface of gold-deposited SiNPs (Fig. 3b). The immobilized GBP-AIa fusion protein can specifically interact and bind with its specific anti-AI polyclonal antibody. In this experiment, the presented GBP-AIa-immobilized GR-SiNPs reacted with anti-AI antibodies immobilized with iron oxide and were easily separated from the mixture solution as shown in Fig. 3c. The GBP-AIa can be separated under magnetic field, due to the strong interaction between GBP-AIa and target anti-AI which was conjugated to GR-SiNPs-iron oxide particles and shows strong fluorescent properties, which can be easily detected through a confocal microscope as shown in Fig. 3d. The magnetically separated iron oxide particles and GR-SiNPs were also observed using FE-TEM as shown in Fig. 3e.

In conclusion, we developed a new method for preparing gold-doped fluorescent silica nanoparticles without any surface modifications using a combination of the W/O method and ultrasound irradiation. For the synthesis and modification of silica-based NPs, we demonstrated three major processes: (1) formation of fluorescent SiNPs, (2) gold doping on the silica using intensive ultrasound irradiation and (3) biomolecular immobilization based on GBP-fusion protein on the surface of gold NPs. Silica and gold NPs with uniform size and shape were successfully fabricated and produced hybrid nanostructured material. The fluorescent dyes were encapsulated and protected by a silica matrix from the outer environment to maintain the fluorescent properties. One biosensor application of gold-doped RGB-SiNPs was demonstrated through the attachment of GBP-AIa and the subsequent interaction with anti-AI antibody. These results indicated that the gold-deposited RGB-SiNPs with a large surface area for binding can be an important material in the development of the new detection tool for biomolecules, such as viruses, with high selectivity and reproducibility.

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Notes and references


Fig. 3 (a) Specific binding of GBP-GFP fusion protein and anti-GFP antibody to the GR-SiNPs and its confocal fluorescent images. (b) Scheme for AI detection using GR-SiNPs. (c) Magnetic separation image of MNPs via specific biomolecular interaction between anti-AI antibody and GBP-AIa-immobilized GR-SiNPs, and its (d) fluorescent image (Scale bar is 10 μm) and (e) FE-TEM image (Scale bar is 100 nm).