Surface-Enhanced Raman Spectroscopic-Encoded Beads for Multiplex Immunoassay

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A new type of encoded bead, which uses surface-enhanced Raman scattering (SERS), is described for multiplex immunoassays. Silver nanoparticles were embedded in sulfonated polystyrene (PS) beads via a polyol method, and they were used as SERS-active substrates. Raman-label organic compounds such as 4-methylbenzenethiol (4-MT), 2-naphthalenethiol (2-NT), and benzenethiol (BT) were then adsorbed onto the silver nanoparticles in the sulfonated PS bead. Although only three kinds of encoding have been demonstrated here, various combinations of these Raman-label organic compounds have the potential to give a large number of tags. The Raman-label-incorporated particles were then coated with a silica shell using tetraethoxyorthosilicate (TEOS) for chemical stability and biocompatibility. The resulting beads showed unique and intense Raman signals for the labeled organic compounds. We demonstrated that SERS-encoded beads could be used for multiplex detection with a model using streptavidin and p53. In our system, the binding event of target molecules and the type of ligand can be simultaneously recognized by Raman spectroscopy using a single laser-line excitation (514.5 nm).

1. Introduction

Multiplex technologies, in which millions of synthetic reactions and biological tests are performed in parallel, are useful for clinical and genetic analysis and in diagnostic fields. One of the most popular methods for the multiplex assay is using micrometer-sized beads encoded with a unique code to identify the attached ligand molecules. For this, several encoding methods have been developed.[1−4]

Among the encoding methods, fluorescence-based encoding methods have been most widely used in biological applications.[5−10] However, photobleaching during multiplexed experiments and a broad emission profiles causing overlap of the emission bands limit the usefulness of this method for broader application. Semiconductor quantum dots (QDs) have also been developed as encoding materials and can circumvent the problems related to fluorescence-based encoding materials.[11−18] Despute their potential impact, the practical application of quantum dots is limited by the key problems of surface modification and safety issues associated with the semiconductor surface chemistry.

Raman and IR spectral features of various molecules have also been used as encoding methods.[19−34] The dual recursive deconvolution (DRED) strategy was introduced with a Raman-encoding method.[31] Mixed styrene derivatives with distinguished Raman and IR signatures at different ratios were used during the polymerization of carrier resins.[32−33] The resins were readily classified using Raman mapping and FT-IR imaging instrumentation.[34] For a reduction of the analysis time or an increase of the S/N ratio, however, a more powerful laser source or surface-enhanced Raman scattering (SERS) technique is needed.

Recently, SERS has attracted considerable interest because of its potential applications in sensitive and selective molecular identification.[35−39] In addition, SERS has several advantages, such as the use of a single laser source, its nonphotobleaching characteristics, its nondestructive nature, and its capacity for structural fingerprinting with high resolution compared with that of fluorescence.

Several groups have reported SERS nanotagging materials based on individual gold nanoparticles that produce relatively low Raman signatures of small organic compounds. Aggregated gold or silver nanoparticles have emerged as SERS-active substrates for high enhancement of Raman signals.[40−54] Recently, an organic−inorganic nanocomposite has been reported as a SERS tag, in which aggregated silver nanoparticles were used.[55] However, precise and reproducible control of the structure of the silver clusters has been difficult. Very recently, the use of silver nanoparticles embedded in a silica nanosphere (so-called SERS dot) has been reported for cellular cancer targeting.[56]

In this study, we report the preparation and properties of SERS-encoded polystyrene (PS) beads (5 μm) composed of

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silver nanoparticles, small organic compounds as Raman labels, and a silica shell. Although tagging with SERS-active nanoparticles is well established, the SERS-encoded beads prepared here take advantage of the polymer backbone for straightforward preparation and reproducible control of the silver nanoparticles and interface it directly with a biological assay platform.

The silver nanoparticle-embedded beads were readily prepared by a modified polyol method and were excellent substrates for producing an intense Raman signature and consistent Raman spectra of the Raman-label compounds such as 4-methylbenzenethiol (4-MT), 2-naphthalenethiol (2-NT), and benzenethiol (BT). Furthermore, the silica-encapsulated silver nanoparticle-embedded sulfonated PS beads containing the Raman-label compounds maintained their own signatures without overlapping. The possibility of a large number of encodings was also shown by combinatorial use of the Raman-label compounds.

We also introduced the new concept that the binding event and the type of ligand could be simultaneously recognized using fluorescence and SERS, respectively, with single laser-line excitation. For this, streptavidin and p53 were chosen as model proteins. They were detected and identified successfully by the SERS-encoded beads coupled with their ligand. This new system could simplify the conventional bioassay formats in terms of cost and time.

2. Experimental Section

2.1. Materials. Silver nitrate ($AgNO_3$), polyvinylpyrrolidone-40 (PVP-40), tetraethylorthosilicate (TEOS), ammonium hydroxide (NH_4OH), sodium silicate, ethylene glycol, 3-aminopropyltriethoxysilane (APTS), 3-mercaptopropyltrimethoxysilane (MPTS), bovine serum albumin (BSA), 4-methylbenzenethiol (4-MT), 2-naphthalenethiol (2-NT), benzenethiol (BT), 3-maleimidopropionic acid *N*-hydroxysuccinimide ester (MIPNS), 1-hydroxybenzotriazole (HOBT), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluoro phosphate (BOP), *N*,*N*-dimethylformamide (DMF), and *N*,*N*-diisopropylethylamine (DIEA) were purchased from Aldrich. Fmoc-ϵ-aminocaproic acid (Fmoc-ACA-OH) and Fmoc-β-alanine (Fmoc-Ala-OH) were purchased from BACHEM. The p53 antibodies, DO-1 C-19 were purchased from Santa Cruz Biotechnology. Fluorescein (FITC)-conjugated affinipure rabbit anti-goat IgG (H + L) was purchased from Jackson Immuno Research and was used without further purification.

2.2. Preparation of Silver Nanoparticle-Embedded Sulfonated PS Beads. Monodisperised PS beads (5 μm, 33% crosslinked with DVB) were prepared by seeded-emulsion polymerization.[57−60] Sulfonation of the PS beads was performed as follows.[61] PS beads (2 g) were added to 5 mL of acetic acid in an ice bath. Sulfuric acid (50 mL) was then added to the beads at 25 °C; the temperature was increased up to 90 °C, and the resin mixture was stirred for 2 h. After they were washed, the sulfonated PS beads (0.1 g) were dispersed in 12 mL of ethylene glycol containing silver nitrate (0.25 M) and PVP-40 (0.35 mM, MW: 40,000), and then the mixture was stirred for 12 h at 100 °C. After the reaction was finished, the silver nanoparticle-embedded sulfonated PS beads were washed with acetone and water several times to remove the ethylene glycol and PVP-40. Finally, the silver nanoparticle-embedded sulfonated PS beads were analyzed by field emission scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM), energy dispersive X-ray (EDX), and Raman spectroscopy.

2.3. Preparation of SERS-Encoded Beads. Raman-label compounds such as 4-MT, 2-NT, or BT and MPTS were adsorbed on the silver nanoparticle-embedded sulfonated PS beads. The Raman-label compound (500 μL, 5 mM in ethanol) and MPTS (500 μL, 5 mM in ethanol) were added to 10 mg of silver nanoparticle-embedded sulfonated PS beads, and then the dispersion was shaken for 30 min at 25 °C. The resulting beads were centrifuged and washed with ethanol several times to remove the excess reagents. These Raman-label-encoded beads were further coated with silica as follows. Ammonium hydroxide (20 μL) was added to 0.1 mg of resin mixture in 1 mL of ethanol under vigorous shaking followed by addition of 10 μL of TEOS. The mixture was shaken for 12 h at 25 °C. The resulting SERS-encoded beads were centrifuged, washed with ethanol three times, and analyzed with FE-SEM, TEM, and Raman spectroscopy.

2.4. Analysis of SERS-Encoded Beads with Raman Spectroscopy. Raman measurements were performed using a confocal Raman system (JY-Horiba, LabRam 300). In this system, the Raman scattering signal is collected in a 180° scattering geometry and detected by a spectrometer equipped with a thermoelectrically cooled CCD detector. A 514.5 nm laser line from a continuous wave (cw) Ar ion laser (Melles Griot, 35-MAP-321) was used as a photoexcitation source with a laser power of ~30 μW at the sample. Raman scattered lights were collected with a ×100 microscope objective (Olympus, 0.90 NA) that was also used for focusing the excitation laser light. The strong Rayleigh scattered lights were then rejected by a holographic notch filter. Acquisition time was 30 s for all spectra of SERS-encoded beads.

2.5. Application of SERS-Encoded Beads to the Detection of the Biotin−Streptavidin Interaction. SERS-encoded beads (1 mg) were added to 1 mL of APTS solution (5% in ethanol, v/v), and then 10 μL of ammonium hydroxide was added. The resulting dispersion was stirred for 12 h at 25°C. The APTS-treated SERS-encoded beads were washed with ethanol and then redispersed in 200 μL of DMF. Fmoc-ACA-OH (10 μmol) was added to the dispersion of APTS-treated SERS-encoded beads followed by the addition of 12 μmol of BOP and HOBT and 18 μmol of DIEA. The resulting mixture was stirred for 2 h at 25 °C. After they were washed with DMF three times, the SERS-encoded beads were treated with 500 μL of piperidine (20% in DMF, v/v) for 50 min to remove the Fmoc-protecting group. Then, Fmoc-β-Ala-OH (10 μmol) was added to 1 mg of the SERS-encoded beads in 200 μL of DMF and coupled to the free amino group of the beads by the method described above. By repetition of the same procedure, Fmoc-ACA-OH was coupled to β-Ala−ACA-coupled SERS-encoded beads.

After the Fmoc protecting group was removed with piperidine, ACA-β-Ala-ACA-coupled SERS-encoded beads (1 mg in 200 μL of DMF) were coupled with 10 μmol of

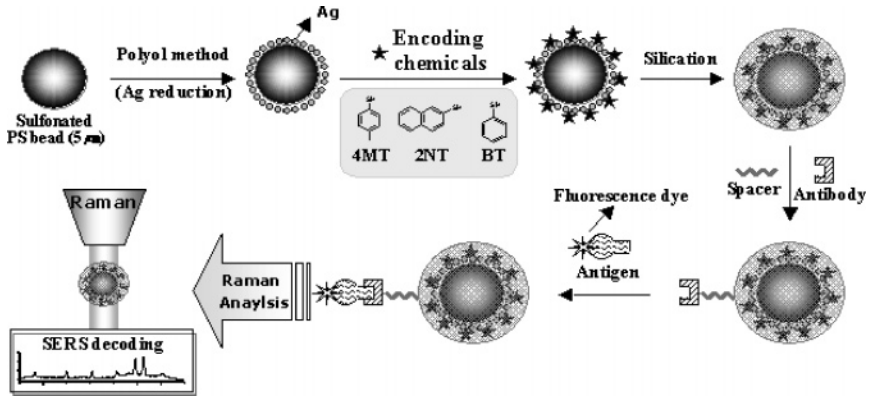

Figure 1. Schematic illustration of SERS-encoded bead synthesis and bioapplication.

biotin, 12 μmol of BOP and HOBT, and 18 μmol of DIEA for 2 h at 2 °C with stirring. After they were washed with DMF and PBS (0.1 M phosphate buffer solution, pH 7.4) three times, the SERS-encoded beads were treated with bovine serum albumin (BSA, 500 μL, 0.5 wt % in PBS) for 1 h at 25 °C and then washed with PBS containing Tween 20 (0.5%, wt) three times. Then, the biotin-coupled SERS-encoded beads were incubated with a steptavidine−FITC solution (500 μL, 1 μg/mL in PBS) for 1 h and washed with PBS containing Tween 20 three times. The beads were analyzed by Raman spectroscopy and confocal laser scanning microscopy (CLSM).

2.6. Application of SERS-Encoded Beads to the Detection of p53. In the same manner as described above, APTS, Fmoc-ACA-OH, Fmoc-β-Ala-OH, and Fmoc-ACA-OH were successively introduced to the SERS-encoded beads (1 mg). After the Fmoc-protecting group was removed with piperidine, the resulting SERS-encoded beads with free amine group were dispersed in 200 μL of DMF and reacted with 12 μmol of MIPNS in 50 μL of DMF. The resulting SERS-encoded bead mixture was stirred for 3 h at 25 °C. After they were washed with DMF, the SERS-encoded beads were redispersed in 100 μL of PBS. Then, 100 μL of p53 (DO-1) antibody (40% in PBS, w/v) was added, and the bead mixture was stirred for 2 h at 25 °C. The resulting SERS-encoded beads were centrifuged, and then they were washed with a solution of PBS containing Tween 20. The SERS-encoded beads were treated with 1 mL of BSA (0.5% in PBS, w/v) for 1 h at 25 °C and washed with 1 mL of PBS containing Tween 20. Next, the SERS-encoded beads were treated with protein extract (liver, ICRmouse 10 weeks, Charles River Laboratories Inc., USA) for 12 h at 4 °C and then washed with PBS containing Tween 20. Then, 200 μL of p53 (C-19) antibody (25% in PBS, w/v) was added, and the resulting bead mixture was stirred for 2 h at 25 °C, centrifuged, and washed with PBS containing Tween 20. Next, 200 μL of FITC-conjugated affinipure rabbit anti-goat IgG (0.2 μg/μL in PBS) was added, and the resulting bead mixture was stirred for 2 h at 25 °C and washed with PBS containing Tween 20 (0.5%, w/v). After they were washed, the beads were analyzed by Raman spectroscopy and CLSM.

3. Results and Discussions

As illustrated in Figure 1, SERS-encoded beads use silver nanoparticle as the SERS-active substrate for the enhancement of Raman signatures. The silver nanoparticle-embedded sulfonated PS beads were coated with tetraethylorthosilicate (TEOS) for further protection of the Raman-label compounds and biocompatibility in further bioapplications.

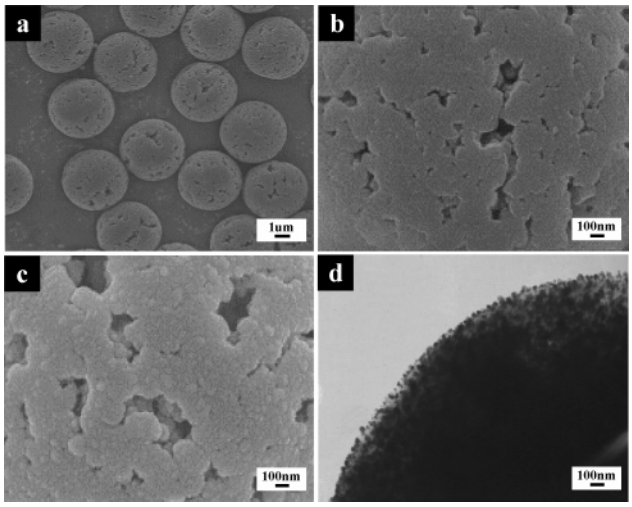

Figure 2. FE-SEM and TEM images of silver nanoparticle-embedded sulfonated PS beads: (a) FE-SEM image of PS beads (5 μm), (b) FE-SEM image of sulfonated PS beads, (c) FE-SEM image of silver nanoparticle-embedded sulfonated PS beads, and (d) TEM image of silver nanoparticle-embedded sulfonated PS beads.

To prepare the SERS-encoded beads, monodisperse PS beads (33% crosslinked with DVB) with a diameter of 5 μm were prepared using a seeded polymerization method. The surface of the PS beads was porous and smooth, as shown in Figure 2. These spherical monodisperse PS beads were then functionalized with a sulfonic acid group using H_2SO_4 for the introduction of silver nanoparticles. The color of the sulfonated PS beads changed from white to a light, muddy yellow after H_2SO_4 treatment. However, the shape and the morphology of the beads were preserved, as shown by FE-SEM (Figure 2).

Silver nanoparticles were then embedded in the sulfonated PS beads using a modified polyol method resulting in a SERS-active substrate.[62] In the process of embedding the silver nanoparticles, silver nitrate ($AgNO_3$) dissolved in ethylene glycol, which acts as a reducing agent and a solvent, was added to the sulfonated PS beads suspended in polyvinylpyrrolidone (PVP). Silver ions are expected to exist inside and outside of the sulfonated beads containing negative

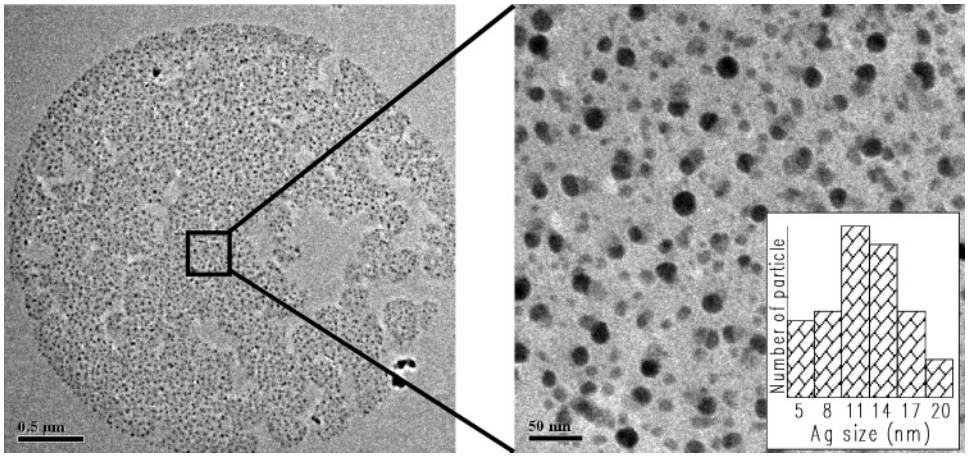

Figure 3. HR-TEM image of cross-sectioned silver nanoparticle-embedded sulfonated PS bead.

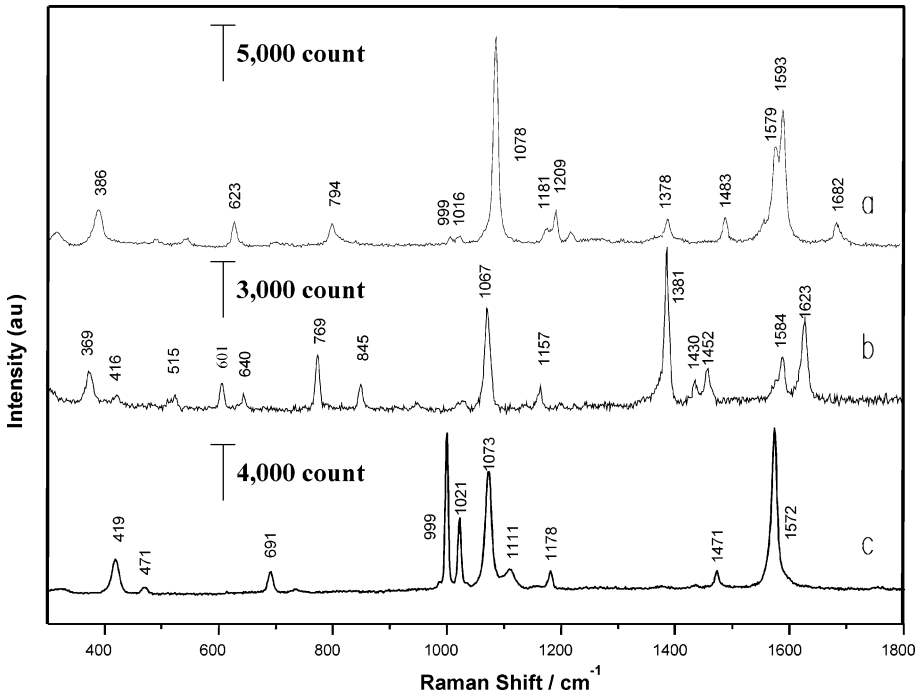

Figure 4. SERS spectra of Raman label compounds on silver nanoparticle-embedded sulfonated PS beads: (a) labeled with 4-MT, (b) labeled with 2-NT, and (c) labeled with BT. The acquisition parameters are photoexcitation by 514.5 nm laser line with 30 μW at the sample and integration for 30 s.

charges. While the resulting dispersion was subsequently stirred for 12 h at 100 °C, silver nanoparticles with a diameter range of 12 nm ± 4.2 nm were formed inside and outside of the porous sulfonated PS beads, as shown in Figure 3. The size of silver nanoparticles could be adjusted and silver nanoparticles could be successfully introduced in the sulfonated beads even at room temperature by changing the amount of PVP and the reaction time, (see Supporting Figures 1 and 2).

Raman-label compounds such as 4-MT, 2-NT, and BT were adsorbed onto the silver nanoparticles embedded in the sulfonated PS beads, together with MPTS, as a silica-shell precursor. During the treatment with 4-MT and MPTS, the shape and the physical properties of silver nanoparticle-embedded sulfonated PS beads remained unchanged.

Raman-labeled beads were analyzed by Raman spectroscopy before being coated by a silica shell (Figure 4). Since each Raman-label chemical has its own unique Raman spectrum that doesn't overlap with the others, these Raman-label chemicals are suitable for encoding. Furthermore, intense Raman signatures with intensity of 237 ± 28 counts for 1 s of acquisition were produced from the organic label compounds, and the Raman spectra showed high reproducibility.

In Figure 5, we illustrated the sensitivity and concentration dependence of the Raman signature intensities of the silver nanoparticle-embedded sulfonated PS beads containing BT, after 1 mg of silver nanoparticle-embedded sulfonated PS bead were dispersed in 1 mL of each of a solution of BT (1 mM, 1 μm, 1 nM, 1 pM, 1 fM, and 0.01 fM) for 10 min and then washed thoroughly with ethanol. As low as a femtomolar concentration, the Raman-label chemicals produced signatures of label chemicals adsorbed onto the silver nanoparticles in the sulfonated PS beads. This illustrates that many small organic compounds could be used as Raman labels and that numerous encoding signatures can be created

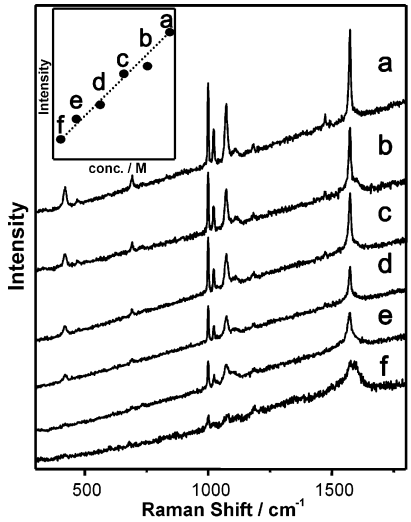

Figure 5. SERS spectra of BT at different concentrations on silver nanoparticle-embedded sulfonated PS beads: (a) 1 mM, (b) 1 uM, (c) 1 nM, (d) 1 pM, (e) 1 fM, and (f) 0.01 fM. The acquisition parameters are photoexcitation by 514.5 nm laser line with 30 μW at the sample and integration for 30 s.

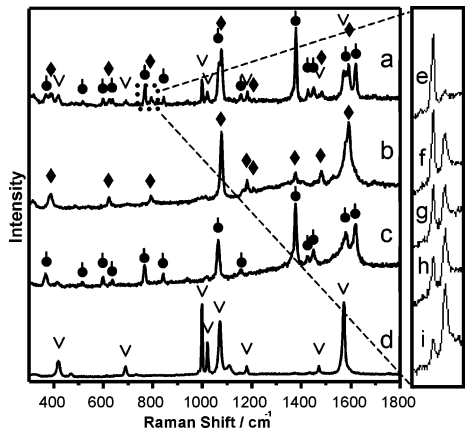

Figure 6. SERS spectra of mixed Raman-label compounds on silver nanoparticle-embedded sulfonated PS beads: (a) a mixture of 4-MT, 2-NT, and BT (4:1:5), (b) 4-MT, (c) 2-NT, and (d) BT. SERS spectra of mixtures of 4-MT and 2-NT at different ratios: (e) 3:1, (f) 7:1, (g) 12:1, (h) 19:1, and (i) 39:1. The acquisition parameters are photoexcitation by 514.5 nm laser line with 30 μW at the sample and integration for 30 s.

by combinations of various label chemicals. When three Raman-label chemicals, 4-MT, 2-NT, and BT, were mixed and introduced to the silver nanoparticle-embedded sulfonated PS beads, their own peaks could be resolved in Raman spectra as shown in Figure 6a−d. When the molar ratio of Raman-label chemicals 4-MT and 2-NT was changed from 3:1 to 39:1, as in Figure 6e−i, we obtained four different tagging signatures. Many other Raman-label chemicals can be used in such combinations with strong SERS signatures of the chemicals. Therefore, the potential number of tagging signatures can be increased in geometric progression simply by increasing the number of Raman-label chemicals.

These silver nanoparticle-embedded sulfonated PS beads incorporating Raman-label compounds and MPTS were then encapsulated by a silica shell with TEOS to maintain chemical stability, biocompatibility, and easy functionalization. As shown in Figure 7, a silica shell of 63 nm ± 39 nm thickness was formed around the silver nanoparticle-embedded sulfonated PS beads. The thickness of the silica shell can be controlled by changing the amount of TEOS, the pH, and the reaction time.[63−64] The morphology of SERS-encoded beads was so rough that it produced a large surface area beneficial for further bioapplication.

SERS-encoded beads were homogeneous in size and surface morphology regardless of the Raman-label compound, and the SERS-encoded beads did not aggregate with each other. To confirm that the SERS-encoded beads maintain their own Raman signatures after silica encapsulation, they were analyzed by Raman spectroscopy again. They maintained their own characteristic SERS spectra of 4-MT, 2-NT, and BT (see Supporting Figure 3). Furthermore, they produced consistent Raman spectra both in organic solvents such as DMF and ethanol and in aqueous buffer solutions (PBS, pH 7.4) (see Supporting Figure 5). This is because the relative refractive index, which the adsorbed molecules experienced, was not significantly affected by the silica shell coating or by a long exposure (1 month) to solvents. As a model study, we applied SERS-encoded beads to a biotin−streptavidin interaction system and to the detection of p53 to evaluate that SERS-encoded beads could be used for multiplex immunoassays (Figure 1). To immobilize biotin on the SERS-encoded beads, the SERS-encoded beads were modified with 3-APTS for the introduction of an amine group. Then, Fmoc-ϵ-aminocaproic acid (Fmoc-ACA-OH), Fmoc-β-alanine (Fmoc-Ala-OH), and Fmoc-ACA-OH were successively coupled to the bead to introduce a spacer for easy accessibility to bulky streptavidin. After removal of the Fmoc protecting groups, biotin was coupled to the SERS-encoded beads, and the biotin-coupled SERS-encoded beads were blocked with bovine serum albumin (BSA). Then, a streptavidin-FITC conjugate was incubated with the biotin-coupled SERS-encoded beads containing 4-MT or with SERS-encoded beads whose amine group was acetylated without biotin as a control group.

After the beads were washed, both the biotin-coupled SERS-encoded beads and the acetylated SERS-encoded beads were analyzed by Raman spectroscopy, as shown in Figure 8. The FITC band at 530 nm was observed on the biotin-coupled SERS-encoded beads, indicating that the target molecule, streptavidin, was bound to the bead. In addition, after laser irradiation of the beads for 110 s, the FITC band was completely photobleached, and the Raman spectra of 4-MT appeared, which denotes the kind of ligand characterizing the specific target. On the other hand, no FITC band was observed on the acetylated SERS-encoded beads, indicating no binding events on the SERS-encoded beads. Of particular note is that the binding events of both the target molecule and the ligand were recognized by a single laser-line excitation, making the biological assay more simple, convenient, and cost-effective. We could also confirm the biotin−streptavidin interaction by CLSM. Figure 9 illustrates that the biotin-coupled SERS-encoded beads had FITC fluorescence, while the other beads did not.

Next, we applied SERS-encoded beads to the detection of p53 (see Supporting Figure 6). To immobilize the p53

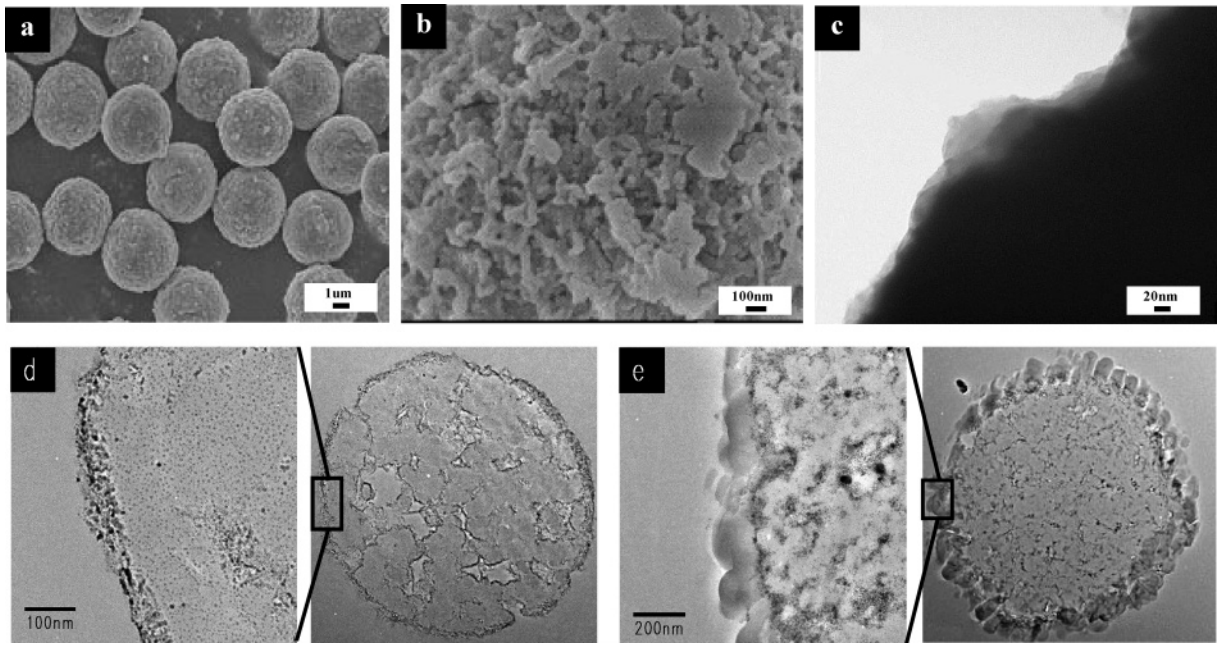

Figure 7. Morphology of SERS-encoded beads: (a) FE-SEM images of silica shell-coated SERS-encoded beads, (b) FE-SEM image of the surface of SERS-encoded bead, (c) TEM images of silica shell of the SERS-encoded bead, (d) cross-sectioned HR-TEM images of the SERS-encoded bead (stirred for 6 h), and (e) cross-sectioned HR-TEM images of the SERS-encoded bead (stirred for 72 h).

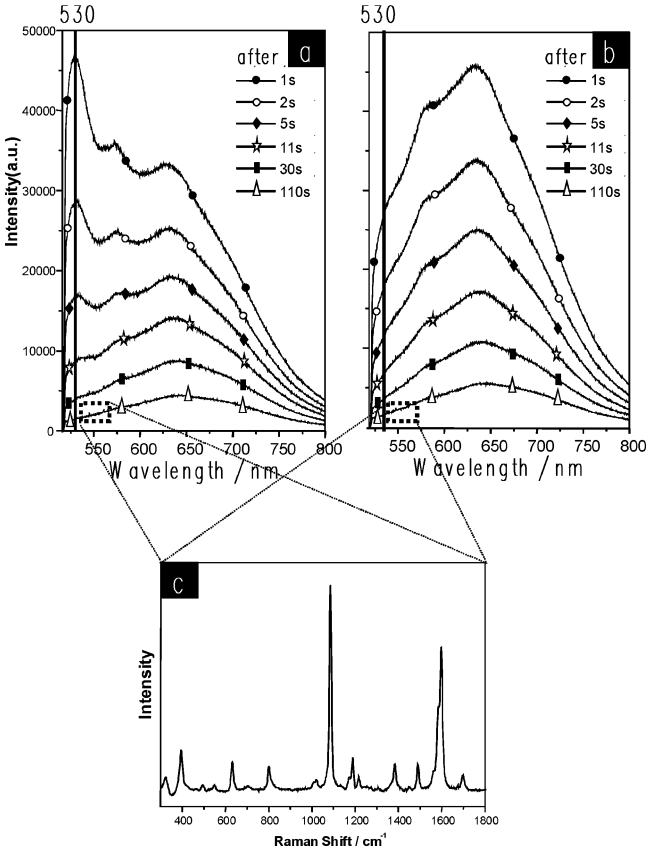

Figure 8. Fluorescence and SERS spectra change after photoexcitation by a 514.5 nm laser line on SERS-encoded beads incubated with the streptavidin−FITC conjugate: (a) biotin-coupled bead, (b) acetylated bead, and (c) SERS spectra after 110 s of irradiation. The acquisition parameters are photoexcitation by 514.5 nm laser line with 30 μW at the sample and integration for 30 s.

(DO-1) antibody on the SERS-encoded beads, 3-APTS, Fmoc-ACA-OH, and Fmoc-β-Ala-OH were introduced successively as before. After removal of the Fmoc protecting

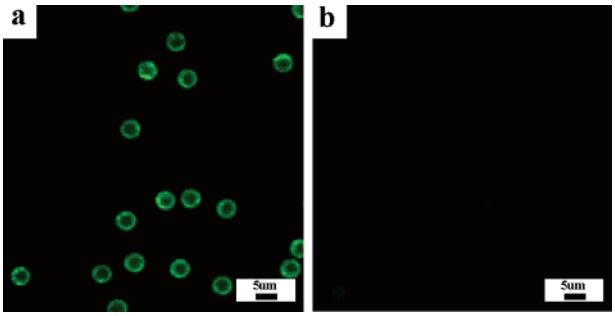

Figure 9. CLSM picture of SERS-encoded beads after incubation with FITC-conjugated streptavidin: (a) biotin-coupled bead and (b) acetylated bead.

groups, 3-maleimidopropionic acid *N*-hydroxysuccinimide ester (MIPNS) was coupled to the SERS-encoded beads containing BT as a encoding chemical for the immobilization of antibodies. Then, p53 (DO-1, isotype mouse IgG) antibody was coupled to the MIPNS-coupled SERS-encoded beads, and the beads were blocked with BSA and incubated with mouse liver protein extract. Then, another antibody (p53 C-19, isotype goat IgG) was incubated with p53 antigen-treated SERS-encoded beads containing BT as the encoding chemical. Finally, a FITC fluorescence-labeled secondary antibody, which can specifically bind with the goat isotype, was incubated with the p53 (C-19) antibody-treated SERS beads.

The other SERS-encoded beads, which contained BT as an encoding chemical but which were acetylated, were tested by the same way as a control.

After the beads were washed, both the p53 antibody-coupled SERS-encoded beads and the control beads were analyzed by Raman spectroscopy, as shown in Figure 10. The FITC band at 530 nm was observed on the p53 (DO-1) antibody coupled SERS-encoded beads, indicating that the target molecule, p53, was recognized. In addition, after laser

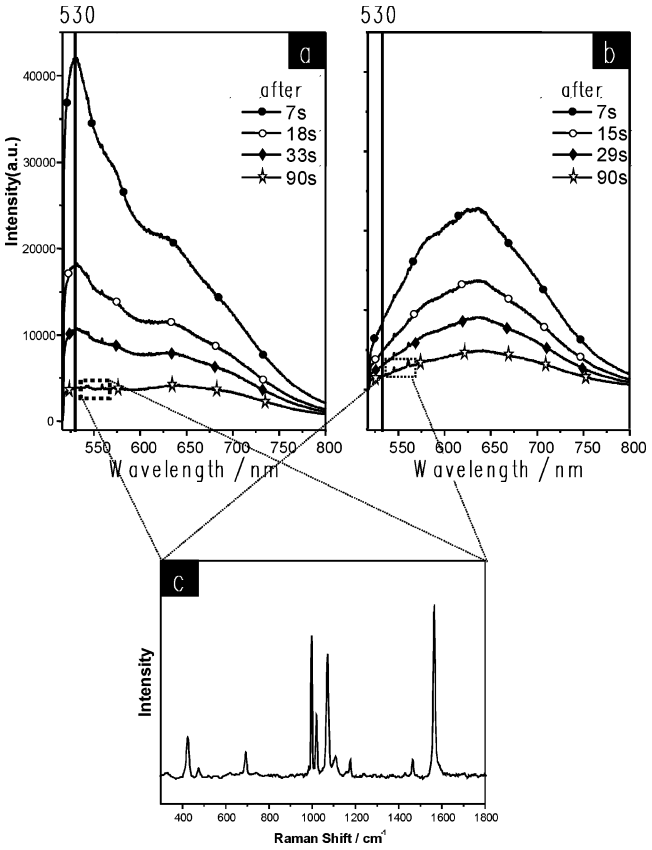

Figure 10. Fluorescence and SERS spectra change after photoexcitation by 514.5 nm laser line on SERS-encoded beads incubated with p53, p53 C-19 antibody, and FITC-labeled secondary antibody: (a) p53 DO-1 antibody-coupled SERS-encoded bead, (b) acetylated SERS-encoded bead, and (c) SERS spectra after 90 s of irradiation. The acquisition parameters are photoexcitation by 514.5 nm laser line with 30 μW at the sample and integration for 30 s.

irradiation of the beads for 90 s, the FITC band was completely photobleached, and the Raman spectrum for BT appeared, which denotes the type of ligand recognizing the specific target. On the other hand, the acetylated SERS-encoded beads did not show any FITC band at 530 nm. Therefore, we could confirm that the SERS-encoded beads were specifically working in this p53 antibody−antigen interaction system. We also confirmed the p53 antibody−antigen interaction event by CLSM (see Supporting Figure 7). We also demonstrated that the p53 antibody−antigen interaction and the kind of antibody could be detected by Raman analysis at the same time and that SERS-encoded beads could be used for multiplex immunoassays.

4. Conclusions

In summary, we have prepared a new type of encoded bead, using surface-enhanced Raman scattering and small organic compounds such as 4-MT, BT, and 2-NT as Raman labels for multiplex immunoassays. As a SERS-active substrate, the silver nanoparticle-embedded sulfonated PS bead, which produced an intense SERS peak without background peaks, was prepared by using the polyol method. The silver nanoparticle-embedded sulfonated PS beads containing Raman-label compounds were coated with silica using tetraethoxysilicate (TEOS). The resulting SERS-encoded beads maintained the Raman signatures of organic label compounds, which were intense and reproducible spectra. Furthermore, we showed the possibility of an unlimited number of encodings by combining the Raman-label organic compounds. Furthermore, we successfully applied the SERS-encoded beads to the biotin−streptavidin system and p53 antibody−antigen interactions, in which both the binding event of the target molecule and the type of ligand were recognized only by Raman spectroscopy using a single Raman laser source.

We expect that SERS-encoded beads, which are photostable and biocompatible will be applicable for multiplex immunoassays and high-throughput screening of a variety of biomolecules in biological and biomedical studies.

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Supporting Information Available. Figures showing FE-SEM and HR-TEM images of the silver nanoparticel-embedded sulfonated PS beads, the loading amounts of silver, the SERS spectra of the SERS-encoded beads, the EDX analysis, schematic illustration of SERS-encoded beads for the immunoassay, and a CLSM picture of the SERS-encoded beads. This material is available free of charge via the Internet at http://pubs.acs.org.

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