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Nanoparticle Probes with Surface Enhanced Raman Spectroscopic Tags for Cellular Cancer Targeting

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We have developed biocompatible, photostable, and multiplexing-compatible surface-enhanced Raman spectroscopic tagging material (SERS dots) composed of silver nanoparticle-embedded silica spheres and organic Raman labels for cellular cancer targeting in living cells. SERS dots showed linear dependency of Raman signatures on their different amounts, allowing their possibility for the quantification of targets. In addition, the antibody-conjugated SERS dots were successfully applied to the targeting of HER2 and CD10 on cellular membranes and exhibited good specificity. SERS dots demonstrate the potential for high-throughput screening of biomolecules using vibrational information.

Nonradioactive tagging technology has gained intense interest in biology for the investigation of the complex spatiotemporal interplay of biomolecules, since it provides safer and more stable alternatives. Up to now, several types of tagging materials have been developed. Fluorescence-based materials have been used most widely in biological applications;[1−6] however, they have intrinsic problems, such as photobleaching, narrow excitation with broad emission profiles, and peak overlapping in multiplex experiments. Semiconductor quantum dots have been under development as a tagging material for both in vivo cellular imaging and in vitro bioassay.[7−12] Despite their potential impact, the practical application of quantum dots is limited by some key problems of surface modification and safety issues associated with semiconductor surface chemistry.[13]

Recently, surface-enhanced Raman scattering (SERS) has attracted considerable interest for its potential applications in sensitive optical detection and spectroscopy.[14−20] To address many problems with fluorophores and quantum dots, Raman tagging materials have been developed by several groups.[21−26] These materials have many advantages in that they can generate large numbers of different Raman tagging signatures for high-throughput screening of various biomolecules; can be excited at any wavelength; do not photobleach; and have narrow peak widths, avoiding spectral overlapping. Nevertheless, the previous Raman tagging materials based on individual gold and silver nanoparticles produced relatively low Raman signatures for small organic compounds used as a label. According to the theoretical calculations, a molecule at nanocrystal junctions can produce several higher orders of magnitude in the intensity of Raman scattering

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(1) Schena, M.; Shalon, D.; Davis, R. W.; Brown, P. O. *Science* **1995**, *270*, 467−470.

(2) Castro, A.; Williams, J. G. K. *Anal. Chem.* **1997**, *69*, 3915−3920.

(3) Pirrung, M. C.; Connors, R. V.; Odenbaugh, A. L.; Montague-Smith, M. P.; Walcott, N. G.; Tollett, J. J. *J. Am. Chem. Soc.* **2000**, *122*, 1873−1882.

(4) Duhachek, S. D.; Kenseth, J. R.; Casale, G. P.; Small, G. J.; Porter, M. D.; Jankowiak, R. *Anal. Chem.* **2000**, *72*, 3709−3716.

(5) He, B.; Burke, B. J.; Zhang, X.; Zhang, R.; Regnier, F. E. *Anal. Chem.* **2001**, *73*, 1942−1947.

(6) Peruski, A. H.; Johnson, L. H.; Peruski, L. F. *J. Immunol. Methods* **2002**, *263*, 35−41.

(7) Bruchez, M., Jr.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013−2016.

(8) Chan, W. C. W.; Nie, S. *Science* **1998**, *281*, 2016−2018.

(9) Yang, C. S.; Kauzlarich, S. M.; Wang, Y. C. *Chem. Mater.* **1999**, *11*, 3666−3670.

(10) Mattoussi, H. J.; Mauro, M.; Goldman, E. R.; Anderson, G. P.; Sundar, V. C.; Mikulec, F. V.; Bawendi, M. G. *J. Am. Chem. Soc.* **2000**, *122*, 12142−12150.

(11) Goldman, E. R.; Balighian, E. D.; Mattoussi, H.; Kuno, M. K.; Mauro, J. M.; Tran, P. T.; Anderson, G. P. *J. Am. Chem. Soc.* **2002**, *124*, 6378−6382.

(12) Gao, X.; Cui, Y.; Levenson, R. M.; Chung, L. W. K.; Nie, S. *Nat. Biotechnol.* **2004**, *22*, 969−976.

(13) Jane, R. K.; Stroh, M. *Nat. Biotechnol.* **2004**, *22*, 959−960.

(14) Jeanmaire, D. L.; Van Duyne, R. P. *J. Electroanal. Chem.* **1977**, *84*, 1−20.

(15) Moskovvits, M. *Rev. Mod. Phys.* **1985**, *3*, 783−826.

(16) Otto, A.; Mrozek, I.; Grabhorn, H.; Akemann, W. *J. Phys.: Condens. Matter* **1992**, *4*, 1143−1212.

(17) Isola, N. R.; Vo-Dinh, D. L. T. *Anal. Chem.* **1998**, *70*, 1352−1356.

(18) Kneipp, K.; Kneipp, H.; Itzkan, I.; Dasari, R. R.; Feld, M. S. *Chem. Rev.* **1999**, *99*, 2957−2976.

(19) Graham, D.; Mallinder, B. J.; Whitcombe, D.; Watson, N. D.; Smith, W. E. *Anal. Chem.* **2002**, *74*, 1069−1074.

(20) Lyandres, O.; Shah, N. C.; Yonzon, C. R.; Walsh, J. T., Jr.; Glucksberg, M. R.; Van Duyne, R. P. *Anal. Chem.* **2005**, *77*, 6134−6139.

(21) Ni, J.; Lipert, R. J.; Dawson, G. B.; Porter, M. D. *Anal. Chem.* **1999**, *71*, 4903−4908.

(22) Cao, Y. W. C.; Jin, R.; Mirkin, C. A. *Science* **2002**, *297*, 1536−1540.

(23) Doering, W. E.; Nie, S. *Anal. Chem.* **2003**, *75*, 6171−6176.

(24) Mulvaney, S. P.; Musick, M. D.; Keating, C. D.; Natan, M. J. *Langmuir* **2003**, *19*, 4784−4790.

(25) Grubisha, D.; Lipert, R. J.; Park, H. Y.; Driskell, J.; Porter, M. D. *Anal. Chem.* **2003**, *75*, 5936−5943.

(26) Kneipp, J.; Kneipp, H.; Rice, W. L.; Kneipp, K. *Anal. Chem.* **2005**, *77*, 2381−2385.

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than one on the surface of a single spherical nanoparticle.[27−29] Therefore, aggregates of silver or gold nanoparticles have emerged as effective SERS substrates.[30−33] Very recently, silver clusters directly coalesced from a destabilized single nanoparticle with organic Raman labels were also developed as a Raman tag.[34] However, precise and reproducible control of the structure of silver clusters has experienced difficulties, which induce a large inhomogeneity in their local structure and the enhancement of Raman scattering.

Herein, we demonstrate a simple and highly reproducible procedure to generate a new type of Raman tag called surface-enhanced Raman scattering dots (SERS dots) using silver nanoparticle-embedded silica spheres, which incorporate a variety of organic Raman label compounds. With the aid of such ultrasensitive SERS dots, we could detect two different targets (HER2 and CD10) with high selectivity on the surface of living cells. These results show that SERS dots can be of practical use for biological cellular detection.

EXPERIMENTAL SECTION

Preparation of Silver Nanoparticle-Embedded Silica Spheres. In the first step, silica nanoparticles were prepared. A 3-mL portion of ammonium hydroxide was added into 40 mL of dry ethanol, and then 1.8 mL of tetraethyl orthosilicate (TEOS) was added to the ethanol solution under vigorous magnetic stirring. The resulting mixture was stirred for 24 h at 25 °C. The final silica nanoparticles were centrifuged and washed with ethanol several times. These silica nanoparticles were then functionalized with 3-mercaptopropyltrimethoxysilane (MPTS). A 100-mg portion of silica nanoparticles was dispersed in 1 mL of ethanol, then 20 μL of MPTS and 50 μL of ammonium hydroxide were added consecutively to the silica dispersion, and the resulting mixture was stirred for 12 h at 25 °C. The MPTS-treated silica nanoparticles were centrifuged and washed with ethanol several times to remove excess reagents. Silver nanoparticle-embedded silica spheres were prepared by using the polyol method. A 100-mg portion of the MPTS-treated silica nanoparticles was redispersed to 3 mL of $AgNO_3$/ethylene glycol solution (30 mM of $AgNO_3$), then 50 μL of ammonium hydroxide was added, and the resulting dispersion was shaken for 10 h at 50 °C. The silica nanoparticles were centrifuged and washed with ethanol and water several times. These silica nanoparticles were redispersed in $AgNO_3$ solution (30 mM) containing ammonium hydroxide and shaken for 12 h at 25 °C. The final particles were centrifuged and washed with ethanol and water. Finally, the silver nanoparticle-embedded silica spheres were analyzed with a high-resolution transmission electron microscope (HR-TEM).

Preparation of SERS Dots. First, a Raman label compound, such as 4-mercaptotoluene (4-MT), 2-naphthalenethiol (2-NT), or thiophenol (TP), and MPTS were self-assembled on the silver nanoparticle-embedded silica spheres. A 250-μL portion of Raman label compound (5 mM in ethanol) and 250 μL of MPTS (50 mM in ethanol) were added to 2 mg of silver nanoparticle-embedded silica spheres, and then the dispersion was shaken for 30 min at 25 °C. The resulting silica spheres were centrifuged and washed with ethanol several times to remove the excess reagents. These Raman-labeled silica spheres were encapsulated by a silica shell in two steps. A precursor silica shell was formed in aqueous sodium silicate solution. Aqueous sodium silicate solution (1. 83 mL, 2.16×10^{-2} wt % SiO_2, pH 9.5) was added dropwise to 15 mL of aqueous solution of the Raman-labeled silica spheres (2 mg of silica spheres) under vigorous magnetic stirring. The resulting dispersion was stirred for 12 h at 25 °C. To complete the formation of a thin silica shell, 60 mL of ethanol was added to the reaction mixture under vigorous magnetic stirring, and the dispersion was stirred an additional 6 h. A thin silica shell around the silver nanoparticle-embedded silica spheres was further grown by the Stöber method. A 250-μL portion of ammonium hydroxide was added to the reaction mixture under vigorous magnetic stirring, followed by addition of 40 μL of TEOS. The mixture was stirred for 24 h at 25 °C. The resulting particles (SERS dots) were centrifuged and washed with ethanol. Finally, the SERS dots were analyzed with HR-TEM and Raman spectroscopy.

Analysis of SERS Dots 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 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 Ar ion laser (Melles Griot, 35-MAP-321) was used as a photoexcitation source with a laser power of 100 μW at the sample. Raman-scattered light was collected with a ×100 microscope objective (Olympus, 0.90 NA) that is also used for focusing the excitation laser light. The strong Rayleigh scattered light was then rejected using a holographic notch filter. Acquisition times for all spectra induced from SERS dots were 10 s.

Immobilization of Antibodies on SERS Dots. A 1-mg portion of SERS dots was added into 1 mL of 3-aminopropyltriethoxysilane (APTS) solution (5 vol % in ethanol), and then 10 μL of ammonium hydroxide was added. The resulting dispersion was stirred for 12 h at 25 °C. The APTS-treated SERS dots were washed with ethanol and then were redispersed in 200 μL of *N*,*N*-dimethylformamide (DMF). A 10-μmol portion of *N*-(9-fluorenylmethoxycarbonyl)-ϵ-aminocaproic acid (Fmoc-ACA-OH) was added into the APTS-treated SERS dot dispersion, followed by 12 μmol of (benzotriazol-1-yloxy) tris(dimethylamino) phosphornium hexafluorophosphate (BOP), 1-hydroxybenzotriazole (HOBt), and *N*,*N*-diisopropyl ethylamine (DIEA). The resulting mixture was stirred for 3 h at 25 °C. After being washed with DMF, the SERS dots were treated with 500 μL of piperidine (20 vol % in DMF) for 40 min to remove the Fmoc protecting group. *N*-(9-Fluorenylmethoxycarbonyl)-β-alanine (Fmoc-β-Ala-OH) was then coupled to the amine on the SERS dots. A 10-μmol portion of Fmoc-β-Ala-OH was added into 200 μL of DMF containing 1 mg of SERS dots. A 12-μmol portion of BOP, HOBt, and DIEA was added to the

(27) Xu, H.; Aizupurua, J.; Käll, M.; Apell, P. *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.* **2000**, *62*, 4318−4324.
(28) Michaels, A. M.; Jiang, J.; Brus, L. *J. Phys. Chem. B* **2000**, *104*, 11965−11971.
(29) Jiang, J.; Bosnick, K.; Maillard, M.; Brus, L. *J. Phys. Chem. B* **2003**, *107*, 9964−9972.
(30) Bonsnick, K. A.; Jiang, J.; Brus, L. *J. Phys. Chem. B* **2002**, *106*, 8096−8099.
(31) Grant, C. D.; Schwartzberg, A. M.; Norman, T. J.; Zhang, J. *J. Am. Chem. Soc.* **2003**, *125*, 549−553.
(32) Koo, T. W.; Chan, S.; Sun, L.; Su, X.; Zhang, J.; Berlin, A. *Appl. Spectrosc.* **2004**, *58*, 1401−1407.
(33) Jeong, D. H.; Zhang, Y. X.; Moskovits, M. *J. Phys. Chem. B* **2004**, *108*, 12724−12728.
(34) Su, X.; Zhang, J.; Sun, L.; Koo, T. W.; Chan, S.; Sundararajan, N.; Yamakawa, M.; Berlin, A. *Nano Lett.* **2005**, *5*, 49−54.

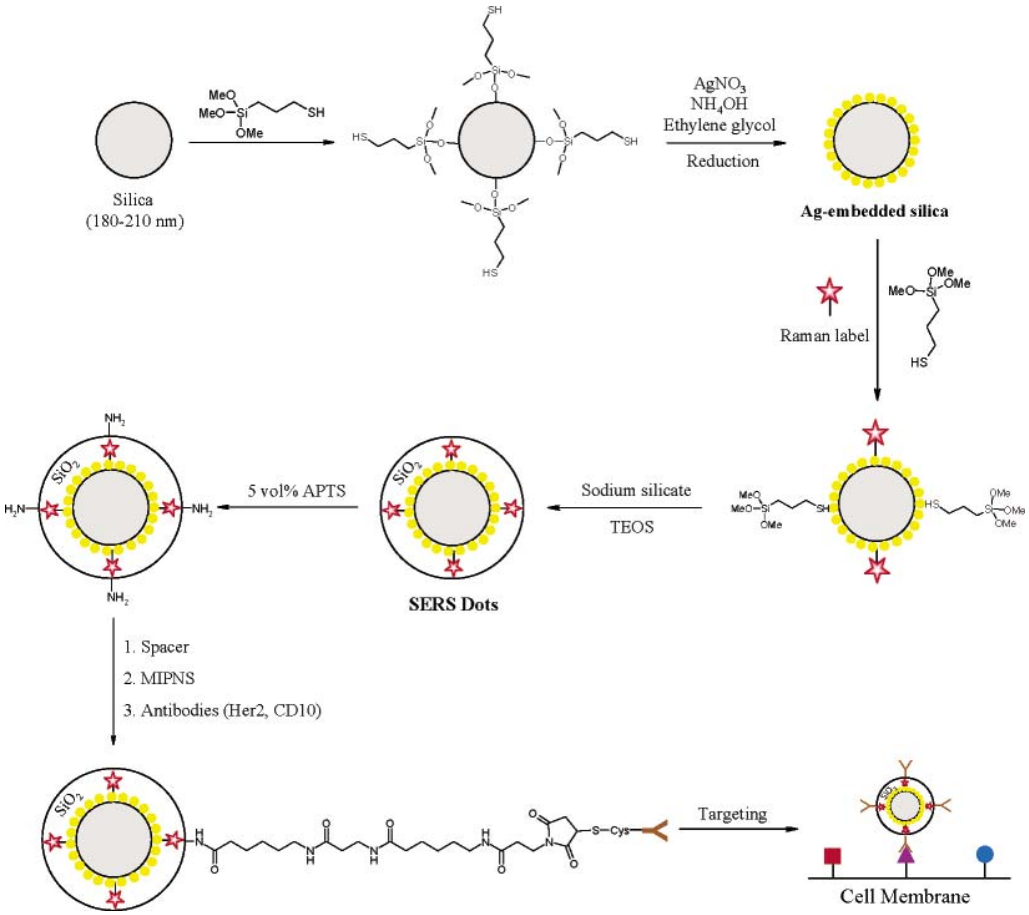

Figure 1. Schematic illustration of SERS dots synthesis and targeting of cellular cancer markers.

reaction mixture, and then the resulting dispersion was stirred for 3 h at 25 °C. After removing the Fmoc protecting group with piperidine, Fmoc-ACA-OH was coupled to amine on SERS dots again by using BOP and HOBt. After the Fmoc protecting group was removed with piperidine, the SERS dots with the free amine group were dispersed in 200 μL of triethanolamine buffer solution (pH 7.0), followed by 12 μmol of 3-maleimidopropionic acid *N*-hydroxysuccinimide ester (MIPNS) dissolved in DMF. The resulting mixture was stirred for 3 h at 25 °C. After being washed with water (1 mL × 10) and DMF (1 mL × 10), the SERS dots were redispersed in 200 μL of phosphate buffered saline (PBS) solution (pH 7.4). A 40-μg portion of antibody was added to the dispersion, and then the resulting mixture was stirred for 2 h at 25 °C. The resulting SERS dots were centrifuged and washed with PBS solution (pH 7.4, 1 mL × 20) containing Tween20 (0.5 wt %). The SERS dots were treated with bovine serum albumin (0.5 wt % in PBS solution, pH 7.4) for 30 min at 25 °C and then were washed with PBS solution containing Tween20 (1 mL × 6).

Cell Culture and Targeting. Breast cancer cells (mammary gland adenocarcinoma, MCF-7), floating leukemia cells (SP2/O), and normal human bronchial epithelial cells (NHBE) were purchased from American Type Culture Collection. Briefly, MCF-7 and SP2/O cells were grown in Dulbecco's Modified Eagle medium (DMEM, Cambrex Bio Science) containing 10% fetal bovine serum (FBS, v/v), 0.5% gentamicin (v/v), and NHBE were grown in RPMI 1640 (Cambrex Bio Science) under the same conditions. After harvest of each cell, the cell suspension was fixed

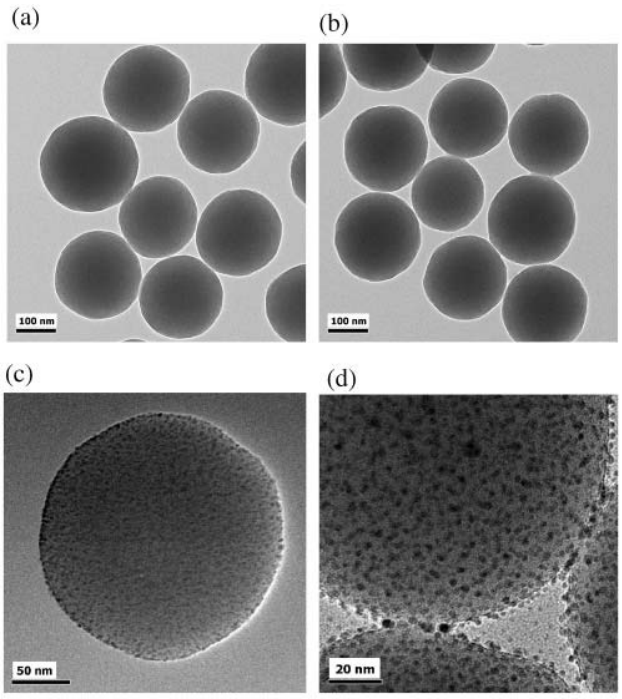

Figure 2. TEM images of silica spheres: (a) original silica sphere, (b) MPTS-treated silica sphere, (c) silver nanoparticle-embedded silica sphere, and (d) the surface of silver nanoparticle-embedded silica sphere after treatment of Raman label chemicals.

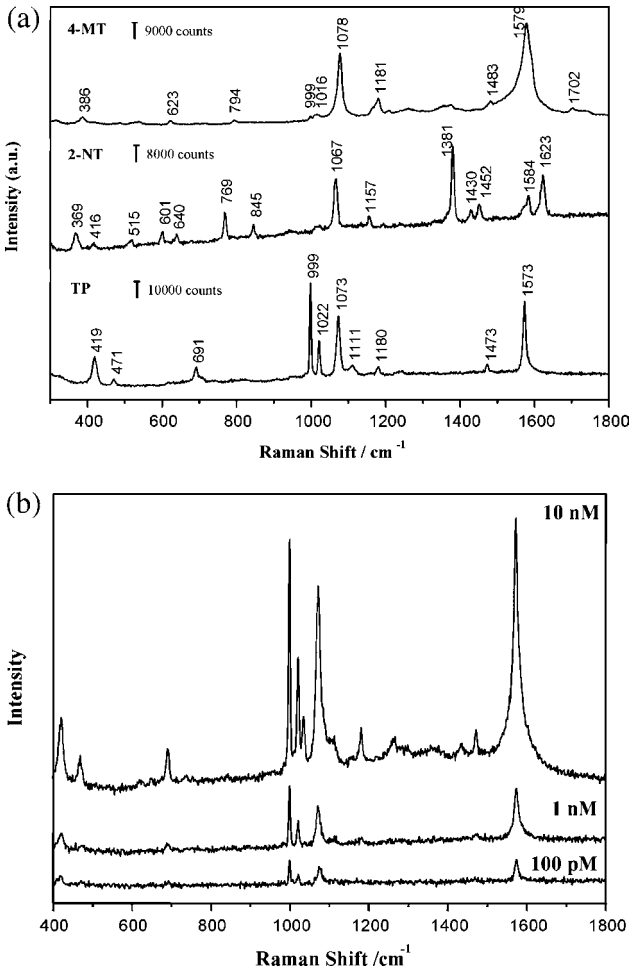

Figure 3. SERS spectra of Raman label compounds on silver nanoparticle-embedded silica sphere: (a) 4-MT, 2-NT, and TP and (b) TP at different concentrations before encapsulation with a silica shell. Acquisition parameters: photoexcitation of 514.5 nm laser line with 100 μW at the sample and integration for 10 s.

with paraformaldehyde (4%) for 2 h at 4 °C. The fixative agent was removed by centrifuge, and then the cells were washed with PBS solution. The cell was incubated with antibody (HER2 or CD10)-conjugated SERS dot (0.03 μg/μL) at 37 °C, 5% CO_2 overnight for the targeting study. However, 1 h of incubation time is sufficient for complete binding of SERS dot−antibody conjugates with target cells. After incubation, cells were washed with PBS solution (pH 7.4, 1 mL × 20) by centrifugation (2000 rpm).

RESULTS AND DISCUSSION

Preparation of Silver Nanoparticle-Embedded Silica Spheres and SERS Experiments. As illustrated in Figure 1, unlike previous Raman tags based on individual gold and silver nanoparticles,[21−26] SERS dots used silver nanoparticle-embedded silica spheres, which have silver nanoparticle junctions and aggregates on their surface, as a SERS-active substrate for the enhancement of Raman signatures. The silver nanoparticle-embedded silica spheres were encapsulated with TEOS again for protection of Raman label compounds and biocompatibility in further bioapplication.

To prepare SERS dots, silica spheres with a diameter of ~180 to 210 nm were prepared using the well-known Stöber procedure, as shown in Figure 2.[35] These spherical silica particles were then functionalized with MPTS to introduce a thiol group that has high affinity to silver nanoparticles. MPTS treatment did not influence the size and the polydispersity of the silica spheres, proved by HR-TEM.

Silver nanoparticles were introduced on the surface of the MPTS-treated silica spheres by using the polyol method.[36−38] Silver nitrate dissolved in ethylene glycol, which plays a role as a reducing agent and a solvent, was added to the MPTS-treated silica spheres. After the resulting dispersion was stirred, silver nanoparticles with a diameter of ~4 to 5 nm, which were analyzed by energy-dispersive X-ray spectroscopy (see Supporting Information), were formed on the surface of silica spheres, as shown in Figure 2. The silver nanoparticles on the surface of silica spheres formed nanocrystal junctions or aggregates (Figure 2d), which can produce intense Raman signatures of organic label compounds, with high reproducibility. Furthermore, the amount and the size of the silver nanoparticles on the surface of the silica spheres could be adjusted by changing the concentration of $AgNO_3$ and the reaction time.

After Raman label compounds such as 4-MT, 2-NT, or TP and MPTS were self-assembled on silver nanoparticle-embedded silica spheres, the labeled silver nanoparticle-embedded silica spheres were analyzed by Raman spectroscopy before being encapsulated by a silica shell. As shown in Figure 3a, the silver nanoparticle-embedded silica spheres gave the intense and reproducible SERS spectra for 4-MT, 2-NT, and TP because of the SERS-active structures of the silver nanoparticles on the surface of silica spheres. In addition, each Raman label compound has its own specific Raman bands without overlapping. In Figure 3b, we illustrate the sensitivity and concentration dependence of silver nanopaticle-embedded silica spheres containing TP after silver nanoparticle-embedded silica spheres were dispersed in 100 μL of each molar solution, 10 nM, 1 nM, and 100 pM, of TP for 30 min and then washed with ethanol thoroughly. The SERS bands of TP were still detectable at a concentration of 100 pM and exhibited a gradual increase in intensity with an increase of TP concentration incorporated. Since silver nanoparticles are attached on the surfaces of silica spheres prior to chemical incorporation, the local structures of silver nanoparticle aggregates are not affected by the concentration of the chemicals incorporated on the silver surfaces. This allows a concentration control in fabricating SERS-tagging materials, which was difficult for the system using destabilization of silver nanoparticles by chemical adsorption. Therefore, many small organic compounds could be used as a Raman label in our case.

Preparation of SERS Dots. Raman label compounds, such as 4-MT, 2-NT, or TP, and MPTS as a silica shell precursor were then self-assembled on the silver nanoparticle-embedded silica spheres. The silver nanoparticle-embedded silica spheres, which incorporated 4-MT and MPTS, were then centrifuged and washed with ethanol several times to remove unadsorbed 4-MT and MPTS. During the treatment of 4-MT and MPTS, the shape and the physical property of silver nanoparticle-embedded silica spheres remained unchanged, reflecting that the silver nanopar-

(35) Stöber, W.; Fink, A.; Bohn, E. *J. Colloid Interface Sci.* **1968**, *26*, 62−69.
(36) Sun, Y.; Xia, Y. *Science* **2002**, *298*, 2176−2179.
(37) Sun, Y.; Xia, Y. *Adv. Mater.* **2002**, *14*, 833−837.
(38) Wiley: B.; Herricks, T.; Sun, Y.; Xia, Y. *Nano Lett.* **2004**, *4*, 1733−1739.

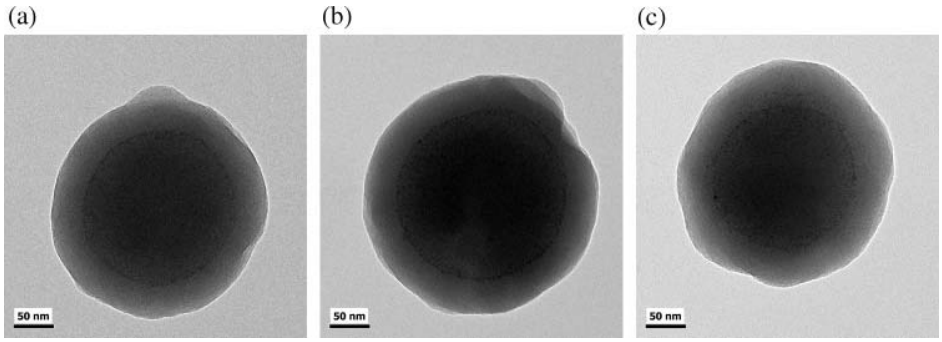

Figure 4. TEM images of SERS dots: (a) SERS dots labeled with 4-MT, (b) SERS dots labeled with 2-NT, and (c) SERS dots labeled with TP.

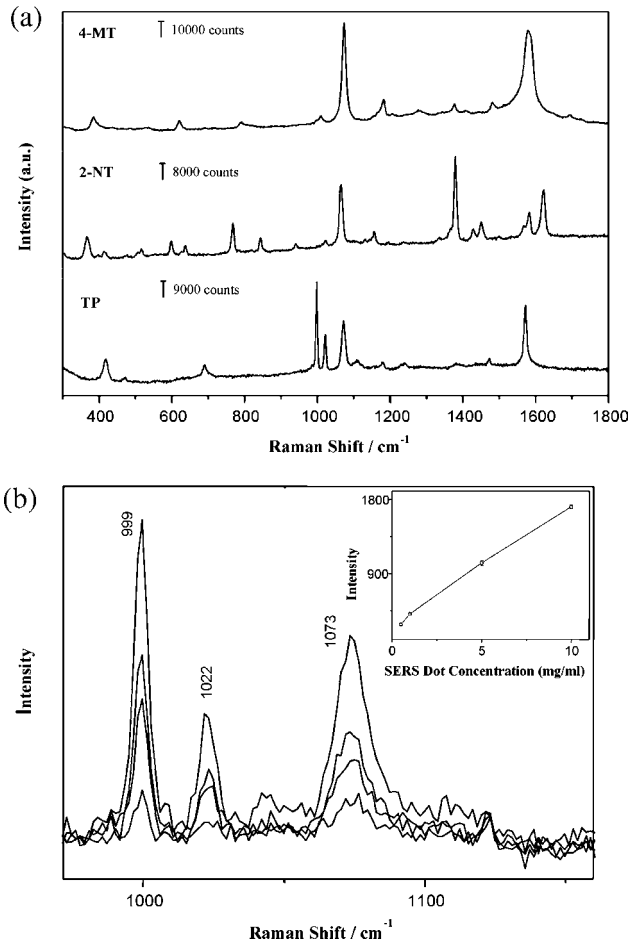

Figure 5. SERS spectra of SERS dots after encapsulation with a silica shell: (a) SERS dots labeled with 4-MT, 2-NT, and TP and (b) SERS spectra at different amounts of SERS dot (labeled with TP) from 10 to 0.5 mg/mL. The inset is SERS dot concentration-dependent graph based on the intensity of the band of TP at 999 cm^{-1}.

ticles were not desorbed from the surface of silica spheres (Figure 2d). Furthermore, the silica spheres did not coagulate. In the case of earlier Raman tags based on individual silver and gold nanoparticles, the control of concentrations of Raman label compounds was critical for preventing coagulation of the nanoparticles, which is a tedious and difficult step to get reproducibility. However, we did not experience any difficulties when we introduced Raman label compounds and MPTS for the preparation of SERS dots. These silver nanoparticle-embedded silica spheres that incorporated Raman label compounds and MPTS were encapsulated by a silica shell in two steps.[39,40] As shown in Figure 4, a silica shell of ~25 to 30 nm was formed around the silver nanoparticle-embedded silica spheres, which have a light brown color. SERS dots were also homogeneous in size and surface morphology irrelevant to Raman label compounds.

To confirm that SERS dots maintain their own Raman signatures, they were analyzed by Raman spectroscopy again. As shown in Figure 5, they revealed their own characteristic SERS spectra of 4-MT, 2-NT, and TP (Figure 5a). SERS intensities of SERS dots were also similar to those before being encapsulated by a silica shell. For application of SERS dots to the quantitative analysis of biomolecules, they should produce consistent Raman signatures of label compounds. In this regard, we measured Raman spectra generated from the different amounts of SERS dot from 10 to 0.5 mg/mL. The intensity of the strongest band at 999 cm^{-1} generated from SERS dot labeled with TP was then compared. As illustrated in Figure 5b, SERS dot at a certain concentration produced consistent Raman signature of TP under the identical measuring condition. Furthermore, the SERS dot concentration-dependent graph revealed linearity based on the intensity of the band of TP at 999 cm^{-1} (the inset in Figure 5b) when the concentration of SERS dot was changed from 10 to 0.5 mg/mL. These data indicate that SERS dot-based assay allows the possibility for quantification of biomolecules in a biological field.

Cellular Cancer Targeting of HER2 and CD10 Using SERS Dot-Antibody Conjugates. We finally examined the possibility of using SERS dots for the cell targeting. The breast cancer cells,[41,42] floating leukemia cells[43,44] and normal human bronchial epithelial cells were chosen as a model system. MCF-7 has been generally used for specific targeting of its surface receptor (HER2), and SP2/O has the CD10 receptor expressed on the outer membrane. NHBE cell was chosen as a control. As shown in Figure 1, SERS dots were modified first with Fmoc-ACA-OH and

(39) Liz-Marzan, L. M.; Giersig, M.; Mulvaney, P. *Langmuir* **1996**, *12*, 4329−4335.
(40) Graf, C.; van Blaaderen, A. *Langmuir* **2002**, *18*, 524−534.
(41) Wu, X.; Liu, H.; Liu, J.; Haley, K. N.; Treadway, J. A.; Larson, J. P.; Ge, N.; Peale, F.; Bruchez, M. P. *Nat. Biotechnol.* **2003**, *21*, 41−46.
(42) Artemov, D.; Mori, N.; Ravi, R.; Bhujwalla, Z. M. *Cancer Res.* **2003**, *63*, 2723−2727.
(43) Vora, A.; Frost, L.; Goodeve, A.; Wilson, G.; Ireland, R. M.; Lilleyman, J.; Eden, T.; Richards, I.; S. *Blood* **1998**, *92*, 2334−2337.
(44) Santra, S.; Zhang, P.; Wang, K.; Tapec, R.; Tan, W. *Anal. Chem.* **2001**, *73*, 4988−4993.

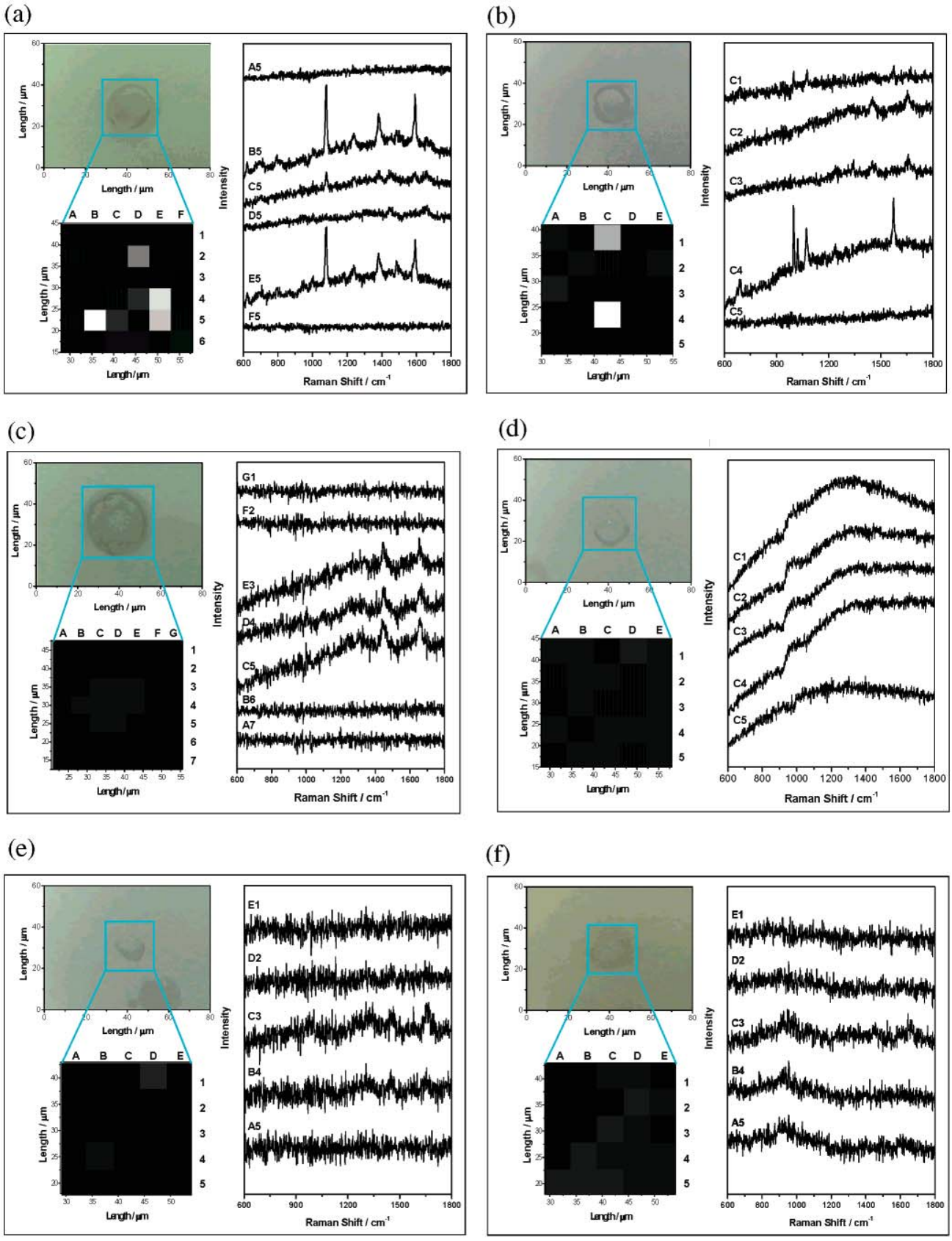

Figure 6. Detection of cancer marker HER2 and CD10 with the antibody-conjugated SERS dots on a single cell; optical microscope pictures denoting the scanning area, spectrally overlaid images indicating cancer marker distribution and SERS spectra. (a) MCF-7 incubated with SERS dot$_{4MT-HER2}$, (b) SP2/O incubated with SERS dot$_{TP-CD10}$, (c) MCF-7 incubated with SERS dot$_{TP-CD10}$, (d) SP2/O incubated with SERS dot$_{4MT-HER2}$, (e) NHBE incubated with SERS dot$_{4MT-HER2}$, and (f) NHBE incubated with SERS dot$_{TP-CD10}$.

then with Fmoc-β-Ala-OH for easy accessibility of bulky antibodies. After removing Fmoc protecting groups, MIPNS was coupled for immobilization of antibodies.[45,46] The HER2 antibody was conjugated to the SERS dot labeled with 4-MT (SERS $dot_{4MT-HER2}$) and CD10 antibody was conjugated to the SERS dot labeled with TP (SERS $dot_{TP-CD10}$). The antibody-conjugated SERS dots were treated with bovine serum albumin for preventing nonspecific adsorption and for blocking unreacted maleimide group. The specific targeting was performed by incubating each cell with the SERS dot probes. The MCF-7, SP2/O, and NHBE cells were incubated with SERS $dots_{4MT-HER2}$ or SERS $dot_{TP-CD10}$ and washed with PBS solution. In Figure 6, optical microscope pictures indicate the scanning area using Raman spectroscopy, and spectrally overlaid images based on the intensity of the band of 4-MT at 1579 cm^{-1} or the band of TP at 1573 cm^{-1} indicate the distribution of cancer markers HER2 and CD10 on the cell membrane. As shown in Figure 6, the Raman signal of 4-MT (Figure 6a) was observed on a MCF-7 cell incubated with SERS $dot_{4MT-HER2}$, and the Raman signal of TP (Figure 6b) was observed on a SP2/O cell incubated with SERS $dot_{TP-CD10}$ when each cell was scanned in two dimensions (5×5 μm, 10 s) by Raman spectroscopy, indicating that the SERS dot probes successfully labeled HER2 and CD10 on the surface of each cell. On the other hand, the Raman signal of 4-MT was not detected on the SP2/O and NHBE cells (Figure 6d, e), which lacked the HER2 receptor, incubated with SERS $dot_{4MT-HER2}$, and the Raman signal of TP was not detected on the MCF-7 and NHBE cells (Figure 6c, f), which lacked the CD10 receptor, incubated with SERS $dot_{TP-CD10}$. This indicated that the SERS dot probes were specific for the targets. The fixative agent did not affect it, and no artifact Raman peaks were observed. Furthermore, under this condition, our antibody-conjugated SERS dots (SERS $dot_{4MT-HER2}$ and SERS $dot_{TP-CD10}$) exhibited nontoxicity in all cell types used in these experiments, which was proved by CCK-8 assay (see Supporting Information). However, under current experimental conditions, the signal collection time is a little longer than in a fluorescent system, which may limit the use of SERS dots in some applications that at present demand a large number of signal collections, such as time-resolved microscopy, laser-scanning confocal microscopy, and flow cytometry.

(45) Jordan, C. E.; Frutos, A. G.; Thiel, A. J.; Corn, R. M. *Anal. Chem.* **1997**, *69*, 4939−4947.

(46) Brockman, J. M.; Frutos, A. G.; Corn, R. M. *J. Am. Chem. Soc.* **1999**, *121*, 8044−8051.

CONCLUSION

In summary, we have prepared the encapsulated surface-enhanced Raman spectroscopic tagging material (SERS dots), composed of silver nanoparticle-embedded silica spheres and small organic compounds as a Raman label, readily and reproducibly. The silver nanoparticle-embedded silica spheres were excellent substrates for producing intense and consistent Raman signatures of the organic label compounds (4-MT, 2-NT, and TP) and maintained their own signatures with similar intensity after encapsulation by the silica shell. In addition, SERS dots revealed linearity at their different concentrations, opening the possibility for quantification of the targets. The antibody-conjugated SERS dots (SERS $dot_{4MT-HER2}$ and SERS $dot_{TP-CD10}$) were successfully applied to the targeting of HER2 and CD10 on cellular membranes and exhibited good specificity for the targets. We expect that the photostable, biocompatible, and multiplexing-compatible SERS dots will be applicable to high-throughput screening of a variety of biomolecules in biological and biomedical studies.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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