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Synthesis and Application of Streptavidin Functionalized Organosilica Microparticles

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ABSTRACT: This article reports on the chemical synthesis, functionalization and application of streptavidin (SA)-coated organosilica microparticles. Thiol-functionalized organosilica microparticles are synthesized via a two-step approach involving acid-catalyzed hydrolysis and condensation of 3-mercaptopropyl trimethoxysilane (MPS), followed by base-catalyzed condensation. The surfaces of these particles are modified with 3-aminopropyltriethoxysilane (APS), and characterized by FTIR, XPS, and TGA. Then, APS-functionalized microparticles are covalently coated with SAs by using glutaraldehyde as a coupling agent, and characterized by FTIR, SEM, and AFM. Immobilization of biotinylated λ DNA onto microparticles via SA-biotin immuoreaction is confirmed by confocal microscopy. The results show that these SA-functionalized organosilica microparticles are useful carriers for DNA manipulation at the single molecule level. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41560.

KEYWORDS: bioengineering; functionalization of polymers; synthesis and processing

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INTRODUCTION

Solid-state surfaces are commonly used as solid supports for the immobilization of DNA, which has a series of biological and technological applications in DNA sequencing,[1–5] computing,[6,7] and biosensors.[8,9] Microparticles and nanoparticles, such as gold, magnetic, latex, polystyrene, and silica particles, are also used as carriers for single molecular manipulation with optical[10–13] or magnetic tweezers[14] in combination with microfluidics. These systems have been used in single-molecule DNA stretching[14,15] and behavior observation,[16–18] speed control and electrophoretic dynamics of DNA translocation through nanopore,[19,20] and DNA–protein interactions.[10–14,21,22] Majority of these experiments rely on attachment of DNA molecules onto carrier microparticles. There are many approaches to attach DNA onto particles. SA-biotin system is one of the most common methods.[23–26]

In recent years, a series of microparticle species have been synthesized followed by SA coating, some of which have been commercialized, e.g., Dynal, Bangs Labs, Spherotech, and Miltenyi. Although the commercial microparticles have very narrow size distributions, they are not proper for large volumes of usage because of their high cost and short shelf life. Moreover, commercial inorganic silica microparticles can't covalently bind organic fluorescent dyes throughout the interior of particles.[27] And commercial polymer microparticles are typically optically encoded by physically entrapping, which are not unsuitable for organic solvent-based applications.[28,29] Therefore, home-made microparticles with uniform structure, good monodispersity of size, suitability for optical encoding, and availability in bulk, would be an ideal alternative. Organosilica microparticles achieves all of these objectives, which have been successfully synthesized and utilized in biomolecular screening,[28] DNA biosensors,[30] combinatorial synthesis, and microsphere-based flow cytometric immunoassays.[29]

Glutaraldehyde has been one of the most potent and versatile tools for immobilizing of enzymes on a support.[31–33] Supports activated with glutaraldehyde are expected to react mainly with nonionized primary amino groups. The final structure of a support may give three different kinds of interactions with a protein: hydrophobic, anionic exchange and covalent, one of which can be dominant under some experimental conditions. These different ways of immobilizing enzyme may lead to different orientations of enzyme on the support. Covalent immobilization may prevent enzyme desorption, improve enzyme stability, but result a loss of binding capability due to orientation and conformational change of enzyme. Nevertheless, SA is a non-glycosylated protein with a lower non-specific absorption in some biotechnologies.[34,35] Moreover, SA is a tetramer and each subunit binds biotin with equal affinity, which makes it possible to permit efficient SA-biotin interactions. Therefore, SAs can be immobilized with a well-defined orientation mainly by the terminal amino groups on the protein surfaces.

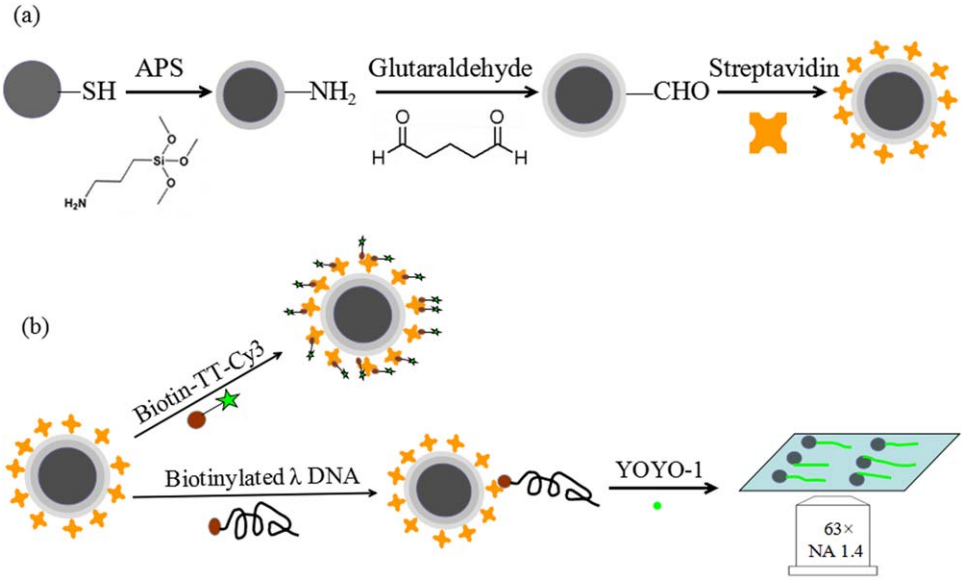

Figure 1. Preparation of SA-functionalized microparticles (a), detection of fluorescence-labeled microparticles and their conjugation with single-molecule λ DNA (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Herein, we reported the synthesis of SA-coated organosilica microparticles using bifunctional linker molecule glutaraldehyde. Characterization of their physical and chemical properaties was performed using FTIR, TGA, SEM, and AFM. λ DNA molecules modified with biotin at one end were immobilized onto the SA-coated organosilica microparticles. Ionic exchange was one possibility between DNA and a cationic support. However, organosilica microparticles had an isoelectric point (pI) at pH = 4.4,[36] and an pI of SA was pH 6.[26] Once SAs were immobilized on the particles under basic conditions (pH 7.4), the particles were negatively charged, as the pH of the buffer was higher than pI of SA. And DNA was obviously negatively charged. Therefore, the electric repulsion between DNA and the particles could prevent exchange adsorption at some extent. Moreover, the radius of gyration (R_g) of λ DNA (48,502 base pairs, 16.2 μm with a B-form contour length[37]) was ~670 nm,[38,39] which was large relative to the distance between SA molecules, preventing DNA from interacting with the cationic support. The steric constrains would therefore limit the number of DNA per particle and minimize DNA entangling or wrapping around the particles. Immobilization of single DNA molecules onto the particles demonstrated that they were useful biomacromolecule carriers in biotechnological study.

MATERIALS AND METHODS

Materials

The 3-mercaptopropyl trimethoxysilane (MPS, 95%), 3-aminopropyltriethoxysilane (APS, 98%), hydrochloric acid (HCl, 37%), triethylamine (TEA, 99%), streptavidin (SA), ammonium hydroxide (NH_4OH, 25%), glutaraldehyde ($C_5H_8O_2$, 25%), sulfuric acid (H_2SO_4, 70%), hydrogen peroxide (H_2O_2, 30%), and AR grade ethanol were purchased from Sigma–Aldrich. Bacteriophage λ-DNA and T_4 DNA Ligase were obtained from New England Biolabs. The 3′-biotinylated 12-mer oligonucleotides (5′-P-AGGTCGCCGCCC-biotin-C6-3′) and biotin-coupled Cy3 (5′-biotin-C3-TT-Cy3-3′) was synthesized by Sangon Biotech (Shanghai). Cover glasses (22 × 50 mm^2, 0.13–0.17 mm thick) were obtained from Thermo Fisher. Amicon® Ultra 0.5 mL filters were purchased from Millipore. YOYO-1 (1 mM in DMSO) was obtained from Invitrogen. All other solvents were AR grade or better, and were used as received without any further purification. Water was purified using a Milli-Q water purification system (Millipore).

Methods

Synthesis of Organosilica Microparticles. Organosilica microparticles were prepared according to previously reported procedures with some minor modifications.[27,36] Briefly, the thiol-functionalized microspheres were synthesized via a two-step process involving acid-catalyzed hydrolysis and condensation of MPS, followed by base-catalyzed condensation. Finally, the microparticles were dispersed in ethanol with a concentration of 10 mg mL^{-1} and stored at 4°C before use.

Preparation of Amino-Functionalized Microparticles

Particle suspensions were washed three times in ethanol before APS coating.[40] Four milligrams of washed microspheres were suspended in a mixture of 940 μL of ethanol, 50 μL of ammonia, and 20 μL of APS. The suspension was briefly sonicated, followed by incubation in a shaker at room temperature for 3 h. The microparticles were then washed several times in ethanol to remove excess ammonia and APS, resuspended in ethanol (10 mg mL^{-1}), and stored at 4°C before use.

Preparation of SA-Modified Microparticles

A two-step procedure was chosen to immobilize SAs onto amino-functionalized organosilica microparticles using glutaraldehyde as the coupling agent[40] [as shown in Figure 1(a)]. Firstly, 2 mg of amino-functionalized organosilica microparticles were immersed in 2 mL of ethanol and sonicated for 5 min. Then, 1 mL of glutaraldehyde (25% in H_2O) and 0.25 mL of PBS (0.01M, pH 8.0) were added into the suspension in

nitrogen atmosphere at room temperature, followed by constant stirring for 3 h. To remove excess glutaraldehyde, glutaraldehyde-modified microparticles were washed with ethanol and PBS (0.01M, pH 7.4) for three times, respectively. Then, aldehyde-modified microparticles were mixed with 0.5 mg of 1 mg mL^{-1} SA (in 0.01 M PBS, pH 7.4), and incubated at 4°C for 24 h.[41] Unreacted SAs were washed with PBS (0.01M, pH 7.4) and the UV spectra of the supernatant determined at 282 nm. The amount of SAs coated onto aldehyde-modified microparticles could be calculated according to the method reported by literature.[42] Finally, the SA-coated microparticles were washed three times and dispersed in PBS (0.01M, pH 7.4) with a final concentration of 10 mg mL^{-1} and stored at 4°C before use.

Fluorescence Image of SA-Modified Microparticles by Confocal Laser Scanning Microscopy

Biotin-coupled Cy3 (5′-biotin-C3-TT-Cy3-3′) was used to verify that SAs had been covalently bound onto organosilicate microparticles [as shown in Figure 1(a)]. Ten microliters of fresh SA-modified microparticles (10 mg mL^{-1}) and 0.5 μL of biotin-coupled Cy3 (100 μM) were mixed on a mixed instrument for 3 h in the dark at room temperature. Then, the stained particles were thoroughly washed with PBS (0.01M, pH 7.4) in order to remove unbound biotin-coupled Cy3 from particles due to non-specific absorption. The stained particles were diluted 10 times in TE buffer (10 mM Tris-HCl and 1 mM EDTA). One microliter of particles suspension was pipetted onto a dried coverslip cleaned with piranha solution. The coverslip was examined under a confocal laser scanning microscopy (CLSM, Leica SP5), and false color images were acquired at the excitation and emission wavelengths at 550 and 570 nm, respectively.

Preparation of Biotinylated λ DNA

To specifically couple DNA molecule onto an SA-coated organosilica particle, one end of λ DNA was functionalized using a 5′-phosphorylated and 3′-biotinylated 12-mer oligonucleotide (5′-P-AGGTCGCCGCCC-biotin-C12-3′) complementary to one of the cohesive end of λ DNA.[21]

The modified protocol was adopted from previous references.[11,37,43] Forty-four microliters of λ DNA solution (0.5 μg μL^{-1}) and 5 μL of the oligonucleotide (100 μM) were added into 36 μL of TE buffer (10 mM Tris-HCl and 1 mM EDTA). The mixture was incubated at 65°C for 10 min and cooled down to room temperature. After 5 min, 10 μL of 20 × T_4 DNA ligase buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, 100 mM DTT, 10 mM ATP, pH 7.5) and 5 μL of T_4 ligase stock solution (4000 U) were added into the resultant construct, and incubated at 16°C for 2 h or overnight. Then T_4 ligase was inactivated by heating at 65°C for 10 min. The mixture was purified and concentrated to about 1.1 ng μL^{-1} by using Amicon® Ultra 0.5 mL filters in TE buffer.

Binding of Biotinylated λ DNA onto SA-Modified Microparticles

Immobilization of biotinylated λ DNA onto microparticles was illustrated in Figure 1(b). Two microliters of SA-modified particles in PBS (0.01M, PH 7.4) (10 mg mL^{-1}) were washed three times in washing buffer (10 mM Tris-HCl, 1 mM EDTA, 2.0 mM NaCl, pH 7.5). Then DNA–particle complexes were prepared by incubating mixture of the washed particles, 2 μL of biotinylated λ DNA (1.1 ng μL^{-1}), and 2 μL of binding buffer (5 mM Tris-HCl, 0.5 mM EDTA, 1.0 mM NaCl, pH 7.5) on a mixing instrument at room temperature for 3 h. Then the mixture was washed three times in washing buffer and stored at 4°C before use.

Two milliliters of SA-modified particles (10 mg mL^{-1}) and 2 mL of biotinylated λ DNA (1.1 ng μL^{-1}) were mixed in the same way as above. The DNA–particle complexes were then examined by electrophoresis using 0.8% agarose gels running in 1 × TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.0) at 100 V for 2 h. The ~0.2 μm pore size of such gels was small enough to block migration of the particles but not individual DNA chains on the particles by physical adsorption.[44]

Preparation of Aminated Coverslips

Clean coverslips were prepared through several washing steps.[45,46] First, each coverslip was thoroughly washed in acetone and ethanol in an ultrasonic bath for 30 min, respectively. Then, the coverslips were cleaned in piranha solution (3 : 1 H_2SO_4/30% H_2O_2) at 90°C for 1 h. Afterward, the coverslips were placed in a solution of 5 : 1 : 1 H_2O/NH_4OH/30% H_2O_2, and sonicated for 1.5 h. The coverslips were washed thoroughly and sonicated in plenty of deionized water for several times. Finally they were stored in deionized water for no longer than 1 day before use.

The procedure for aminosilane modification of coverslip surface had been described previously.[47] Clean coverslips were heated in an oven at 200°C for 5 min to evaporate surface water layer. Then, they were submerged in 1% APS in aqueous solution for 10 min followed by thorough washing with water, dried under a stream of nitrogen gas, and heated for 30 min at 120°C. The prepared glasses were stored in vacuum desiccators for no longer than 1 day before use.

Single DNA Manipulation by CLSM

DNA-particle complexes were stained with fluorescent dye YOYO-1 at a ratio of five to ten base pairs per dye molecule (bp/dye = 5–10 : 1) in the dark at room temperature for at least half an hour. The sample solution was washed three times and diluted 100 times in PBS (0.01M, pH 7.4).

The clean coverslips from deionized water were dried under a stream of nitrogen gas. A droplet (1 μL) of the stained DNA-particle complexes was pipetted onto an aminosilane-modificated coverslip. A clean coverslip was used to cover the droplet, and DNA molecules were stretched. The sample was observed under a Leica SP5 CLSM (in false color). Immobilization of DNA without biotinylation onto SA-coated particles, and biotinylated DNA onto aldehyde-modified particles was set as controls using the same protocol described above.

Scanning Electron Microscopy

Size and morphology of the as-synthesized microparticles were characterized by scanning electron microscopy (SEM, FEI NOVA NanoSEM 230, Phenom ProX). Platinum coated samples were collected on FEI NOVA NanoSEM 230 (at 5 kV accelerating voltage), equipped with an Oxford Energy Dispersive

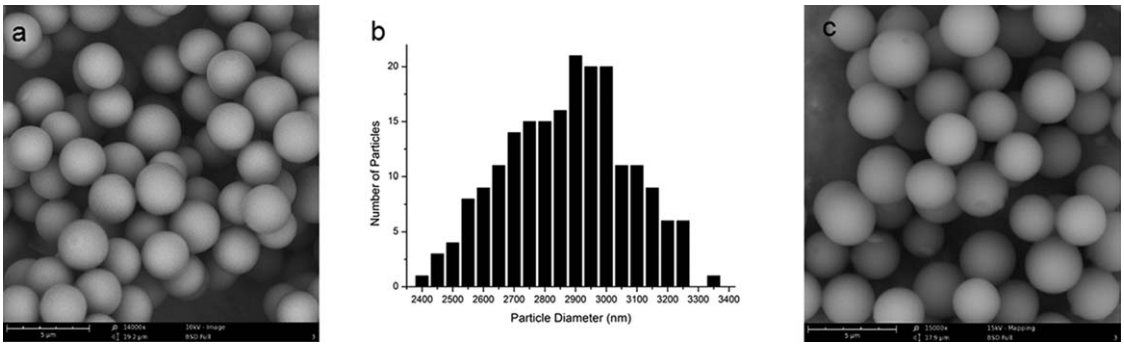

Figure 2. SEM micrographs of MPS particles (a), the particle size distribution (b), and SEM image of APS-coated particles (c).

Spectrometer (EDS). Uncoated samples were imaged with FEI NOVA NanoSEM 230 and Phenom ProX (at 5 kV accelerating voltage). Samples were prepared for SEM by placing a drop of sample (diluted with ethanol) on silicon wafer/aluminum foil and left to dry. Images were analyzed with Image-J software to determine size distributions by counting at least 200 particles.

The ζ Potential Analysis

Microparticles were suspended in ethanol at a low-enough dilution (0.1–0.01 mg mL^{-1}) to prevent aggregation. The ζ potential was measured on a Zetasizer Nano ZS 90 (Malvern Instruments). Each sample was measured at least three times.

Fourier Transform Infrared Spectrometer Analysis

To characterize the surfaces of microparticles, Fourier transform infrared spectrometer (FTIR) spectra were measured by a Nicolet 6700 spectrometer (Thermo Fisher Scientific Inc.) in the wavelength range from 400 to 4000 cm^{-1}.

X-ray Photoelectron Spectroscopy Analysis

To determine functionalized surfaces of organosilica microparticles, atomic percentages of certain elements were obtained according to previous reports.[30,48] Each sample was washed thoroughly with ethanol, dried in an oven at 80°C under a nitrogen atmosphere, and then degassed overnight before analysis. Data were acquired using a Kratos AXIS UltRA^{DLD} X-ray photoelectron spectrometer incorporating a 165 mm hemispherical electron energy analyzer. Al X-rays (1486.6 eV) at 75 W were used for sample irradiation. Wide scans were taken at an analyzer pass energy of 160 eV, and narrow high-resolution scans were taken at 40 eV. Wide scans were accomplished over a 1200–0 eV binding energy range with 1.0 eV steps.

Thermal Gravimetric Analysis

Each sample was dried for 24 h in a vacuum. Thermal gravimetric analysis (TGA) was performed with a thermal analyzer (Pyris 1 TGA, PerkinElmer). The initial mass of the samples was 10 mg. The experiments were carried out under a nitrogen atmosphere at a heating rate of 10°C min^{-1} from 25 to 900°C.

RESULTS AND DISCUSSION

Characterization of Amino-Functionalized Organosilicate Microparticles

Using the two-step synthesis method described above, particles with diameters from nanometers to microns were synthesized by varying the initial volume of added MPS precursor. The size distribution of microparticles was calculated from SEM images with Image-J software by counting at least 200 particles.

Organosilicate particles with mean size of 2.87 μm and standard deviation of 205.44 nm were shown in Figure 2(a,b), respectively. Clearly, organosilicate microparticles (the CV of size was 9.4%) had smooth and continuous spherical surfaces, and narrow size distributions according with Gaussian fit, validating good monodispersity (with polydispersity index below 2%). APS-coated particles were shown in Figure 2(c). There were no observable size increases after APS modifications, which may be due to microparticle permeability according to references.[29,30]

The EDS element analysis of the particles was performed as shown in Figure 3. C, O, Si, and S peaks were clearly recorded, demonstrating that organosilicate microspheres synthesized by MPS had plenty of thiol groups.

Organosilicate microparticles made from MPS could easily aggregate in water, which was in accordance with previous report.[48] However, they would readily disperse and form stable suspension in ethanol. The ζ potential of particles was -52 ± 3 mV in ethanol, and -49 ± 2 mV after APS coating, respectively. This confirmed that APS modification did not impact stability of the particles. Therefore, ethanol was used as dispersant solvent for MPS particles in this report.

The FTIR spectra of MPS and APS-coated particles were analyzed as shown in Figure 4(a,b). The bands at 2930 and

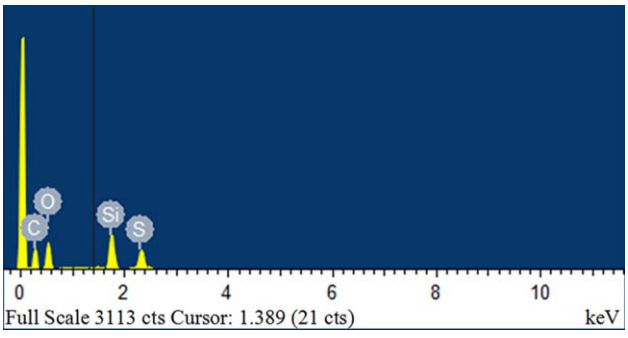

Figure 3. EDS spectrum of MPS organosilicate microparticles. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

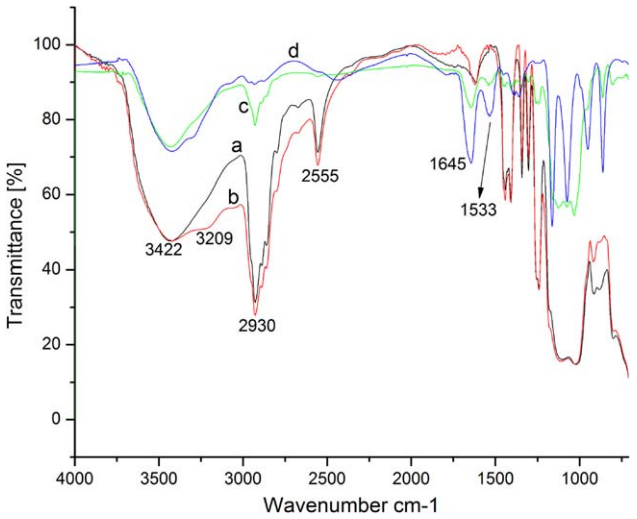

Figure 4. FTIR analysis of uncoated (a), APS-coated (b), SA-coated (c) particles, and SA (d). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

2555 cm^{-1} (—SH) represented the characteristic bonds of MPS (curve a). There was a weak absorption band at 3209 cm^{-1}, which was characteristic peak of APS-modified silica.[30] At 3422 cm^{-1}, the band on curve b was wider than that of curve a. This was due to stretching vibration band of $—NH_2$ on APS and overlap of surface silanol groups of MPS. Therefore, the surfaces of MPS microparticles were successfully modified by APS.

Figure 5(a) showed the XPS spectra from unmodified MPS (curve a) and APS-coated particles (curve b). In comparison with trace a, the C 1s peak intensity in trace b at 284.8 eV was considerably increased, suggesting that more carbon chains of APS were added onto the surfaces of MPS particles. There was no N 1s peak at 399.5 eV in curve a, but observable peak in curve b [Figure 5(b)], suggesting the presence of nitrogen atoms on the surfaces of APS-coated particles. The S 2p peak decreased obviously at 163.6 eV in curve b compared with a [as shown in Figure 5(c)]. These results indicated that the acidic surface-silanol groups of organosilicate microparticles and the basic APS-silanols experienced condensation reaction.[49–51] MPS particles had been successfully functionalized via APS. Therefore, amino-functionalized particles could be further coated with SAs using glutaraldehyde as a crosslinker.

The TG curves of unmodified MPS particles and APS-coated particles under a nitrogen atmosphere were shown in Figure 6. It was observed that, as temperature increased, curve a declined faster than curve b. Curve a decreased to 48.1% at about 850°C, while curve b to 57.4% at about 850°C. Previous studies proved that ammonia-treated microparticles were more condensed and less permeable than untreated ones.[29] So, APS-coated particles showed a slower weight loss than their unmodified counterparts while temperature increased from 25 to 900°C. The final weight

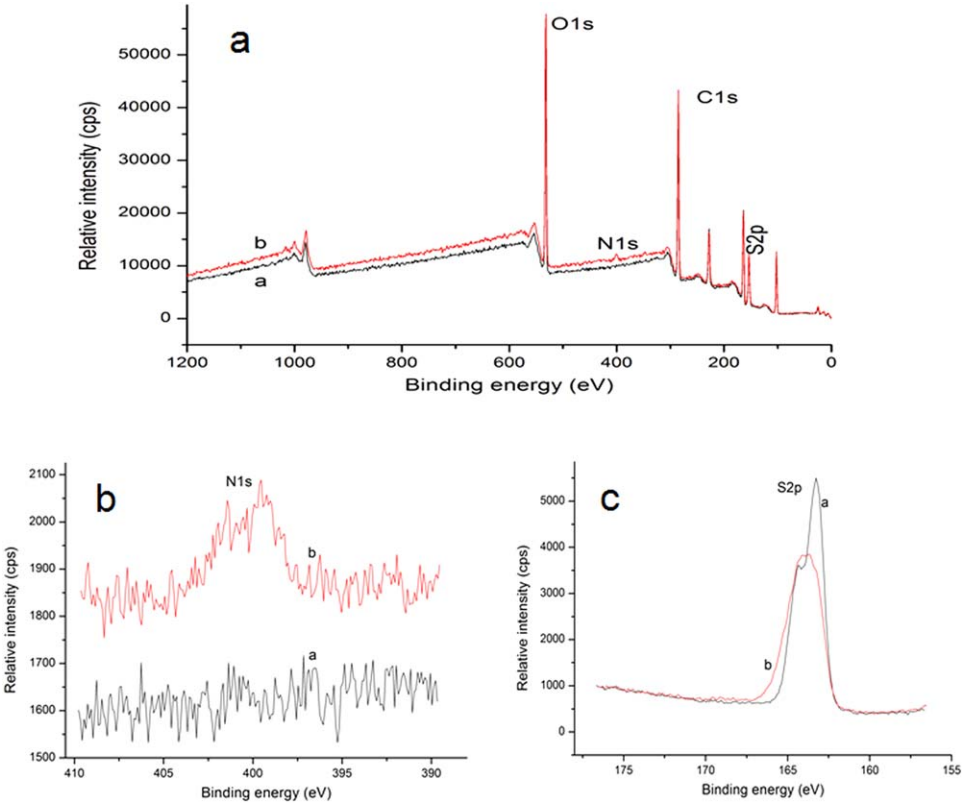

Figure 5. XPS spectra for MPS and APS-coated particles (a), in N (1s) and S (2p) regions for APS-coated particles (b, c). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

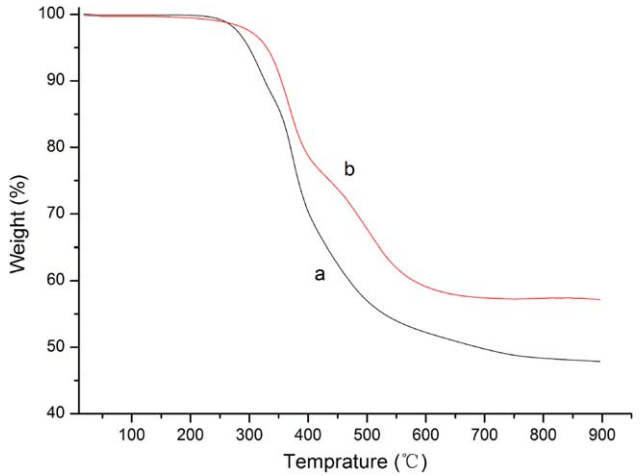

Figure 6. TG curves for MPS (a) and APS-coated particles (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

of APS-coated particles (57.1%) was higher than unmodified MPS particles (47.8%), indicating that MPS particles had been successfully modified with APS.

Immobilization of SAs onto Amino-Functionalized Organosilicate Microspheres

To immobilize biotinylated DNA onto organosilica microparticles via streptavidin-biotin system, SA was used to coat APS-modified particles using glutaraldehyde as a crosslinking agent, which was different from previous report.[30,48] UV–vis spectrometer showed that the amount of immobilized SA on the surfaces of organosilica microparticles was 152 μg mg^{-1}. FTIR spectra of SA-coated particles and SA were presented in Figure 4(c,d). It was evident that the characteristic bands of SA at 1645 and 1533 cm^{-1} (curve d) existed in curve c. This indicated that aldimine condensation reactions had taken place between amino groups of SA and aldehyde groups on the surfaces of the microparticles. These results demonstrated the conjugation of SA onto glutaraldehyde-modified particles.

As shown in Figures 7 and 8, SA-coated particles possessed rougher surfaces than those coated with APS. There were lots of protuberances [Figures 7(b) and 8(b)] on the surfaces of SA-coated particles, which, again, suggested that SAs were covalently bound onto organosilicate microparticles.

To further validate that SAs had been covalently bound onto APS-coated particles, biotin-coupled Cy3 was used to stain SA-

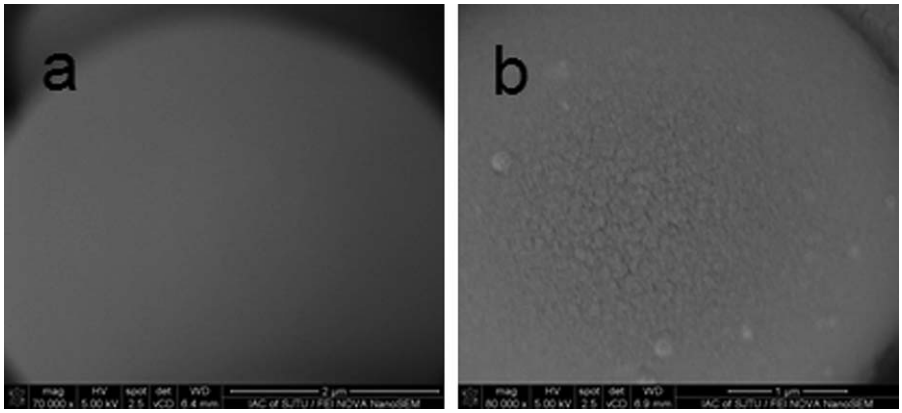

Figure 7. SEM images of APS-coated (a) and SA-coated particles (b).

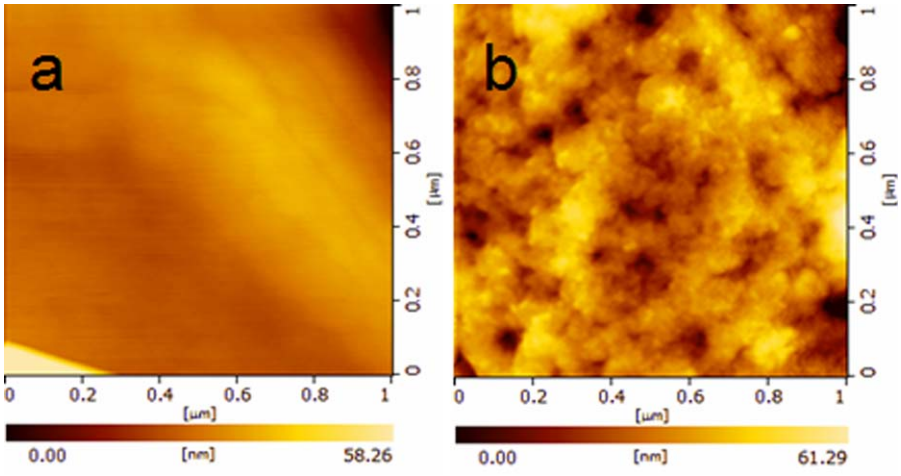

Figure 8. AFM images of APS-coated (a) and SA-coated particles (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

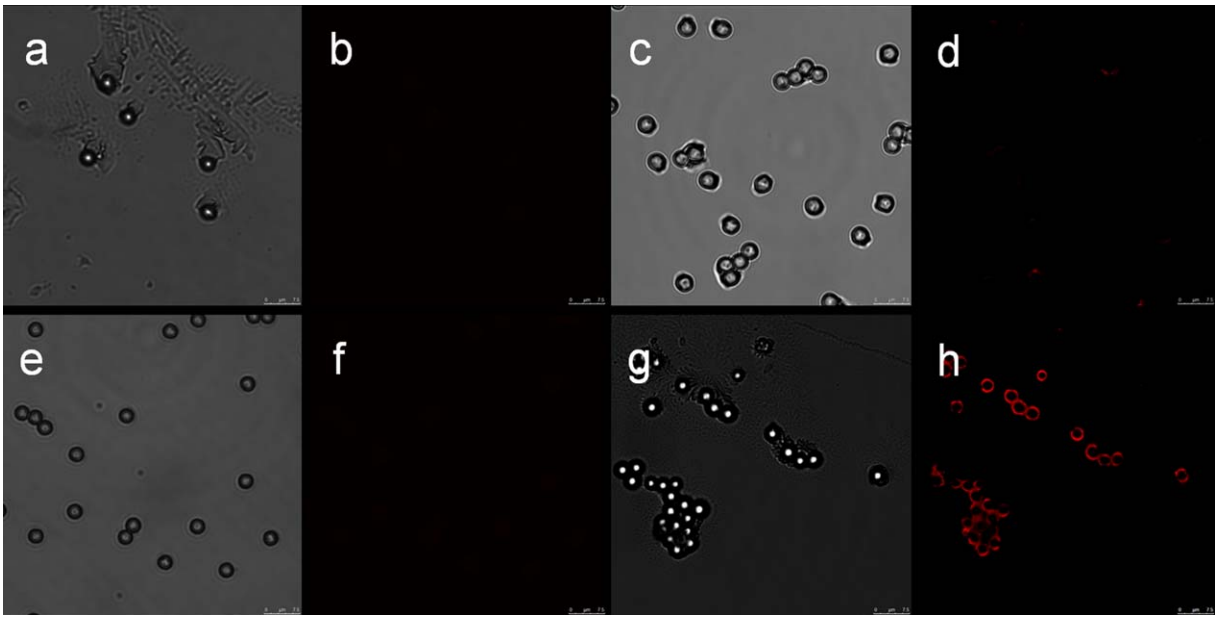

Figure 9. CLSM images of MPS (a, b), APS-coated (c, d), aldehyde-modified (e, f) and SA-coated microparticles (g, h). a, c, e, and g showing bright field micrographs, and b, d, f, and h showing fluorescence micrographs. The scale bars were 7.5 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

coated particles through immunoreaction between biotin and SA. In Figure 9, it could been seen that SA-coated particles [Figure 9(h)] showed strong fluorescence on the surfaces of the particles, while the controls, MPS particles [Figure 9(b)], APS-coated particles [Figure 9(d)], and aldehyde-modified particles [Figure 9(f)], showed little/no fluorescence. These results confirmed that SAs had been bound onto the surfaces of the microparticles.

Application of SA-Coated Organosilicate Microspheres in Single-Molecule DNA Manipulation

To verify whether or not the nonspecific adsorption occurred between biotinylated λ DNA and the surfaces of SA-coated organosilicate microparticles, the DNA-particle complexes were detected by electrophoresis using 0.8% agarose gels (Figure 10). It could be seen that there was no observable electrophoretic band in lane 2 compared with the control, indicating that nonspecific adsorption (ion exchange) of biotinylated λ DNA on SA-coated organosilicate microparticles was negligible.

Conventional far-field microscopy is limited in its resolving power by the Rayleigh criterion to length scales on the order of 200 nm.[52] As one of the highest binding affinity dyes used in nucleic acid staining, YOYO-1 molecules make fluorescence intensity of stained dsDNA increase at least a thousand times, so they are commonly used in single molecule imaging. To observe single λ DNA molecule, YOYO-1 was used to insert into dsDNA molecules.

Figure 11(a) showed that single λ DNA molecules were stretched on the surface of APS-modified coverslip. In this work, SA-coated organosilicate microparticles were also exploited to bind biotinylated λ DNA in a particle-DNA molar ratio of 1 : 1. In this case, the resultant mixture included particles without DNA and those with one or more DNA molecules. Figure 11(b) showed that most of λ DNA molecules on particles could be stretched well on aminosilane coverslips. Stretching of λ DNA molecules and DNA-particle complexes were also performed on coverslips without APS coating. It was found that DNA molecules (free or attached onto particles)

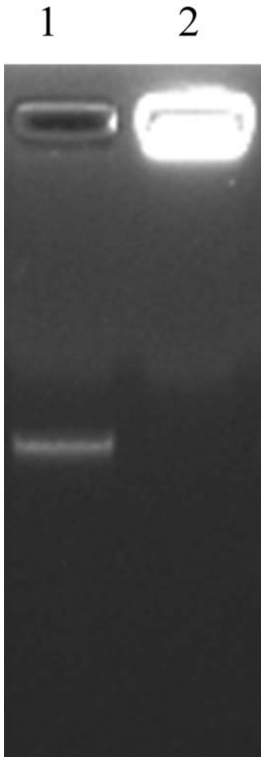

Figure 10. The 0.8% agarose gel showing biotinylated λ DNA (lane 1) and its attachment to SA-coated organosilicate microparticles (lane 2).

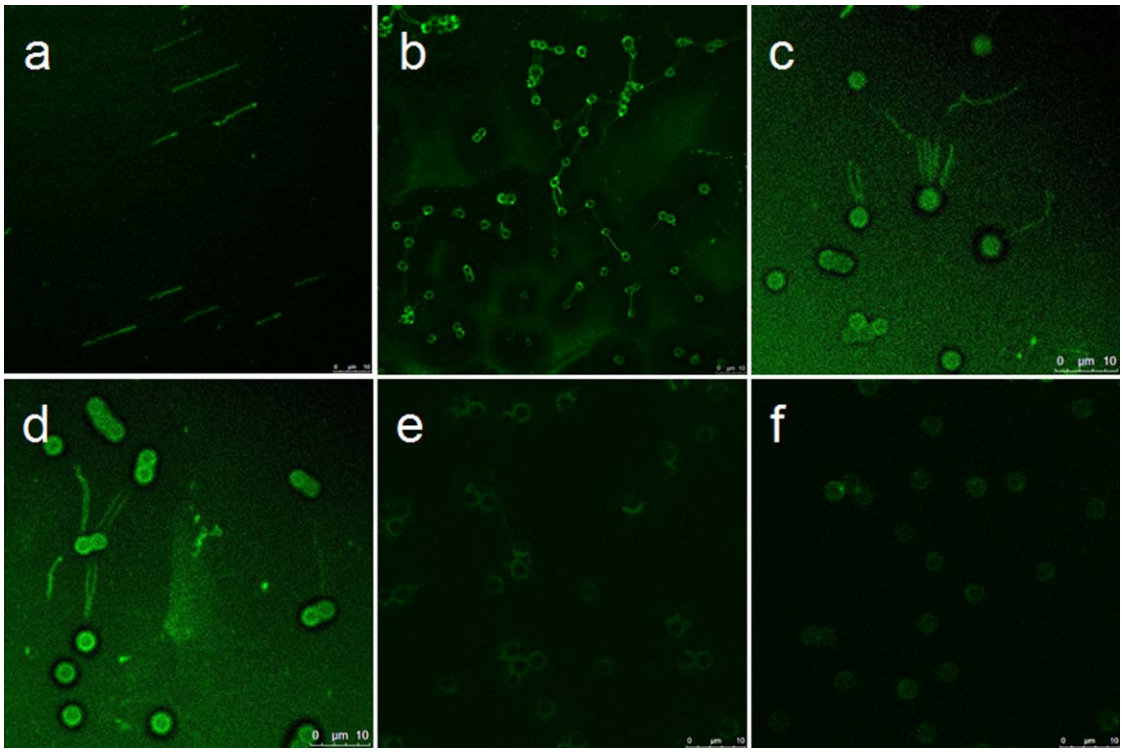

Figure 11. CLSM images of λ DNA and DNA-particle complexes stretched on APS-modified coverslip (a, b). DNA-particle complexes stretched directly on cleaned coverslip (c, d). Non of λ DNA without biotin was bound onto SA-coated particles (e), and non of biotinylated λ DNA was bound onto aldehyde-coated particles (f). The scale bars were 10 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

could be stretched very well on coverslips cleaned in piranha solution. If the ratio of particles to DNA was <1, a particle could bind several DNA molecules. When DNA molecules were stretched well, the number of DNA molecules bound to a particle could be precisely counted [Figure 11(c,d)]. The controlled experiments [Figure 11(e,f)] showed that λ DNA without biotin was not bounded onto SA-coated particles, and there was also no biotinylated λ DNA on the aldehyde-modified particles. These hence further suggested that SAs had been coated onto the surfaces of organosilicate microparticles. We concluded that these particles could be used as carriers in single-molecule DNA manipulation.

CONCLUSIONS

In this study, organosilicate microparticles were successfully synthesized, and amino-functionalized organosilica microparticles were further covalently coated with SAs using glutaraldehyde as a coupling agent. These microparticles had a narrow size distribution, good monodispersity, stability, and usability as solid carriers for single-molecule λ DNA manipulation.

Although SAs modified microspheres were often exploited in single molecule manipulation using optical or magnetic tweezers,[10–14] it was a time-consuming process to obtain particles carrying only one DNA molecule.[53] According to Poisson distribution, one can calculate the proportion of one DNA-one particle complexes by controlling the DNA-to-particle ratio, which depends on the size of the particles and the radius of gyration of (R_g) of the DNA. According to Poisson distribution, the proportion of particles with 0, 1, and 2 DNA per particles should be 33, 50, and 15%, respectively. However, our experiments showed that, when the ratio of DNA molecules to particles was small (typically <1 : 1), most particles bound DNA had only one DNA molecule, which might be explained by lower reaction kinetics in practice than in theory. Therefore, this fact is practically helpful to enhance efficiency and easiness in such single-DNA manipulation studies.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: CLH, ZMW. Performed the experiments: CLH, LXZ. Analyzed the data: CLH, YW. Drafted the paper: CLH. Revised the paper: CLH, ZMW.

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