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Synthesis of silk-based microcapsules by desolvation and hybridization

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Experimental section

1. Materials

The extracted silk fibroin was kindly supplied by Huzhou Xintiansi bio-tech Company (P. R. China). Poly[lactic-co-(glycolic acid)] (PLGA) (Mw. 50,000, 50:50) was purchased from the Institute of Medical Instrument of Shandong Province (P. R. China). PVA (degree of polymerization 500, degree of hydrolysis 88 %) was kindly supplied by Sinopec Sichuan Vinylon Works (P. R. China). Thiolated poly-T oligonucleotides (T5: 5' HS-C6-TTTTT) were purchased from Sangon Inc. (P. R. China). FITC-labeled bovine serum albumin (FITC-BSA), FITC-labeled dextrans (FITC-dextran, Mw. 2000 kDa and 250 kDa) were all obtained from Sigma-Aldrich. All other chemical reagents were of analytical grade and obtained from commercial sources. Ultrapure water used in all experiments was produced by Milli-Q synthesis system (Millipore Corp. USA).

2. Synthesis of hybrid capsules

2.1. Silk fibroin purification

The extracted silk fibroin was dissolved in 9.3 M of LiBr solution at 60 °C for 4 h to produce a 20 % (w/v) solution. The solution was dialyzed against distilled water using dialysis bags (Mw. cut off 3500) at room temperature for 72 h to remove the LiBr. The dialysate was centrifuged three times at 8,000 rpm (20 ° C, 20 min) to remove the aggregates and impurities, then filtered using 0.4 µm glass-fiber syringe filters for further purification. The concentration of silk fibroin stock solution was ca. 6 % (w/v) by weighing the residual solid of a known volume of solution after lyophilization. The final product was stored at 4 °C and diluted with ultrapure water before use.

2.2. Preparation of PLGA microspheres by single emulsion-solvent evaporation method

Briefly, 150 mg of PLGA was dissolved in 10 mL of methylene chloride. After that, the resultant polymer solution was injected dropwise into 100 mL of PVA aqueous solution (1 %, w/v) under constant mechanical agitation with the aid of a high-speed homogenizer (Ultra-Turrax T-18 basic, Ika, Germany) to emulsify. High-speed emulsification (5 min) was followed by slow magnetic stirring for about 18 h at room temperature to evaporate organic solvent from the bulk completely. The resulting products were collected with centrifugation and washed with ultrapure water.

2.3. Synthesis of single-component silk microcapsules using desolvation method

Typically, PLGA microspheres were dispersed in the silk fibroin solution (2 mg / mL, in 0.5 M carbonate sodium buffer, pH = 9.16). Then a six time volume of ethanol was added dropwise into the suspension under vigorous shaking. The silk-adsorbed particles were sequentially immersed into glutaraldehyde solutions (2 %, v/v) for 30 min. After centrifugation, 30 mM of sodium borohydride was used to stop the cross-linking reaction for 30 min and was followed by washing with water 3 times to remove the residues of sodium borohydride. Finally, after dissolving the PLGA template by a 1:1 mixture of acetone and 1-methyl-2-pyrrolidinone, the hollow capsules were collected by centrifugation and thoroughly washed with ultrapure water to remove organic solvents.

2.4. Synthesis of gold nanorods (AuNRs) and DNA-modified AuNRs (AuNRs-T5)

AuNRs were synthesized by the seed-mediated method. First, a seed solution was prepared by adding ice-cold NaBH₄ (0.60 mL, 0.01 M) into a mixture composed of

HAuCl₄ (0.05 mL, 2 %, w/v) and CTAB (7.50 mL, 0.1 M) under vigorous mixing. This seed solution was aged 2 h for further use. Second, the AuNRs growth solution was prepared by adding ascorbic acid (0.192 mL, 0.1 M) into a mixture containing HAuCl₄ (0.24 mL, 2 %, w/v), AgNO₃ (0.18 mL, 0.01 M), and CTAB (28.5 mL, 0.1 M). Third, AuNRs were synthesized by adding 0.12 mL of aged seed solution into the above growth solution and left undisturbed for 9 h. The resulting AuNRs were collected by centrifugation at 10,000 rpm for 5 min. DNA-modified AuNRs were synthesized by incubating the mixture of thiol-modified DNA and AuNRs in dark at room temperature for 40 h and eliminating the superfluous DNA by centrifugation.

2.5. Synthesis of hybrid capsules

The synthesized silk microcapsules were mixed with ca. 1 nM of AuNRs-T5 for 2 h under gently shaking. The resulting products were collected by centrifugation.

3. Characterizations

3.1. Fourier transform infrared spectroscopy (FTIR)

The purified silk was mixed with KBr, punched and then IR spectrum was obtained using FTIR spectrometer (Nicolet is-10, USA) in the transmission mode.

3.2. Circular dichroism spectroscopy

CD spectrometer (Jasco J-815, Japan) was used to record the spectrum of purified silk (0.25 mg / mL) from 190 to 250 nm with a 1 mm quartz cuvette at room temperature by averaging three scans at a scan speed of 100 nm / min. The measurement was performed with a bandwidth of 1 nm.

3.3. Scanning electron microscopy (SEM)

SEM (ZEISS, SUPRA 55VP) was employed to examine the morphologies of silk microcapsules and hybrid counterparts which were deposited on a silicon wafer and air-dried prior to observation. The samples were gold-coated in an ion coater (EIKO, IB-3) and measured at an operation voltage of 3.0 kV.

3.4. Transmission electron microscopy (TEM)

TEM (JEM-100CXII) was used to characterize capsules interiors, gold nanorods (AuNRs) and hybrid capsules. For this purpose, a drop of diluted aqueous suspension of sample was deposited on a copper grid and air-dried prior to observation.

3.5. Atomic force microscopy (AFM)

For AFM (Multi Mode8, Veeco), a drop of capsule suspension was placed onto a pre-cleaned silicon wafer and dried in air. AFM images were collected in the contact mode.

3.6. Confocal laser scanning microscopy (CLSM)

CLSM was used to investigate the permeability of silk capsules and their hybrid counterparts. An equal volume of silk capsule suspension and FITC-dextran (2 mg / mL) or FITC-BSA (2 mg / mL) aqueous solution were mixed for 30 min before observation. For hybrid capsules, after incubation with fluorescent probes, AuNRs-T5 were used to plug the pores of silk capsules for additional 4 h prior to observation.

3.7. Size and zeta-potential measurements

The size of unmodified AuNRs and AuNRs-T5 was determined by dynamic light scattering (DLS, Zetasizer Nano-ZS, Malvern). The Zeta-potential values of silk capsules and silk-AuNRs hybrid capsules were measured using the same equipment.

3.8. UV-visible spectroscopy (UV-Vis)

UV-visible spectra of AuNRs, AuNRs-T5 and the hybrid microcapsule solutions were recorded using a microplate reader (Thermo Varioskan Flash3001, Thermo) from 400 nm to 1000 nm.

3.9. Raman Spectroscopy

Raman spectroscopy analysis was performed to assess the SERS property of the hybrid capsules. A drop of diluted aqueous suspension of sample with 5 μ M of crystal violet was placed on a quartz slip. SERS spectra were recorded using 633 nm excitation with acquisition time of 3 s and accumulation for 3 times (DXR Microscope, Thermo). Raman mapping image was obtained based on the signal of the 1619 cm^{-1} with acquisition time of 5 s and accumulation for 3 times (RENISHAW, in Via reflex).

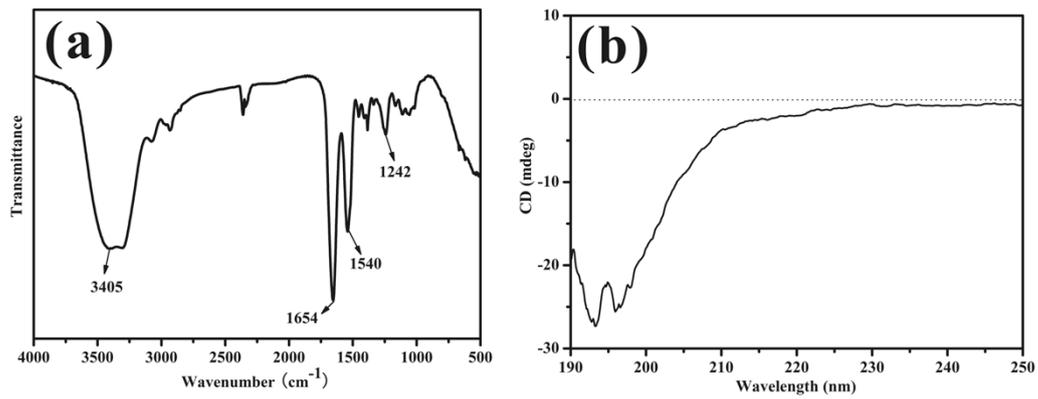


Fig. S1. IR (a) and CD (b) spectra of purified silk fibroin.

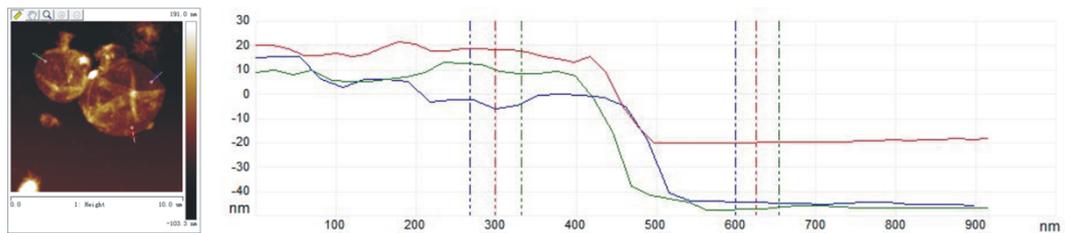


Fig. S2. Typical section analysis profiles for determination of the mean thickness of silk capsules through three parallel measurements recorded in the left AFM image.

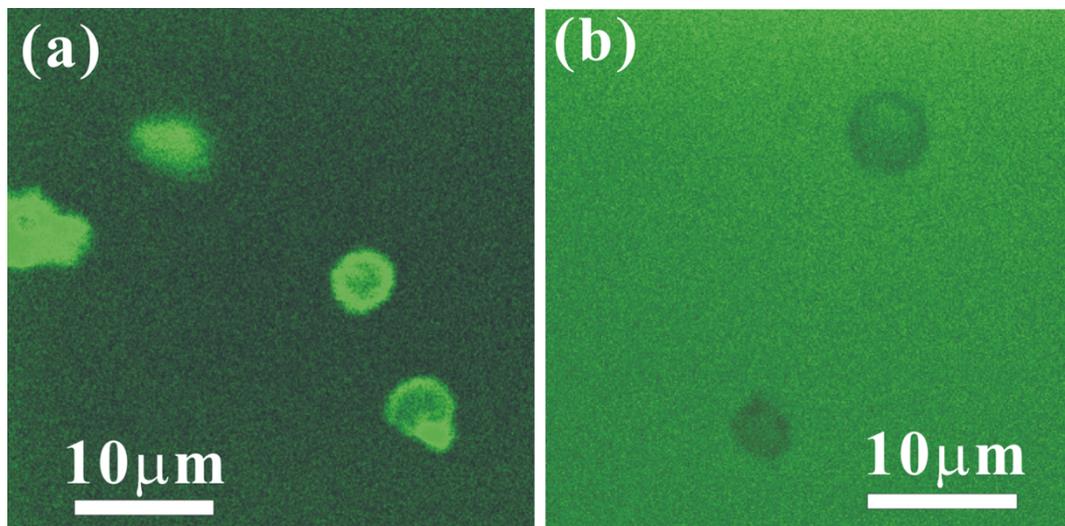


Fig. S3. CLSM images of the permeability of silk capsules when incubation with FITC-dextran with Mw. 2000 kDa (a) and FITC-BSA (b) aqueous solution.

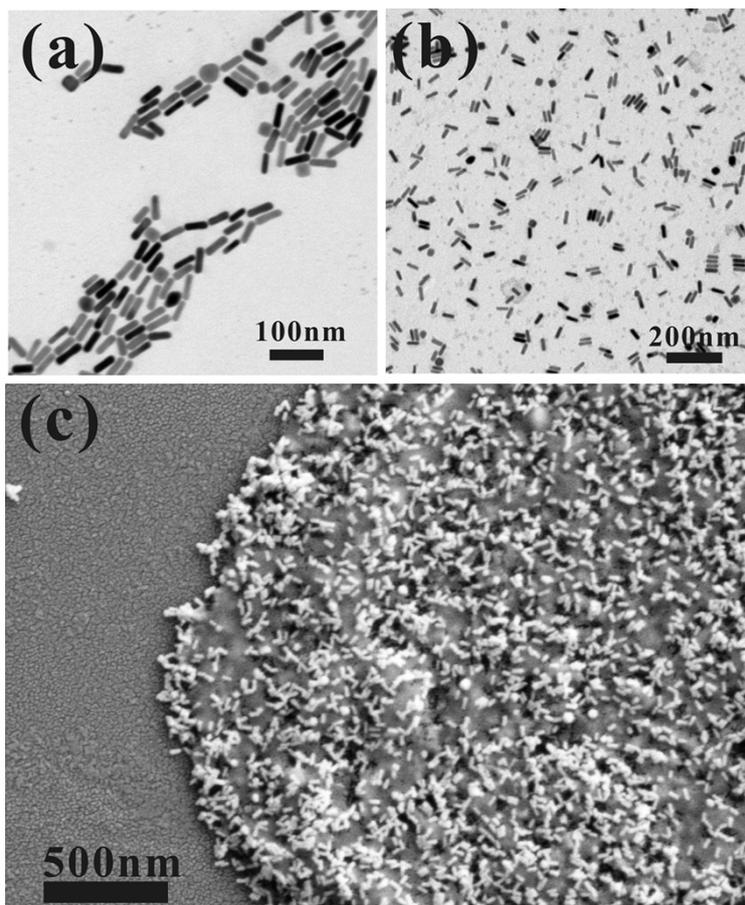


Fig. S4. TEM images of AuNRs (a) and AuNRs-T5. (c) is SEM image of AuNRs-T5 capped silk-based capsules.

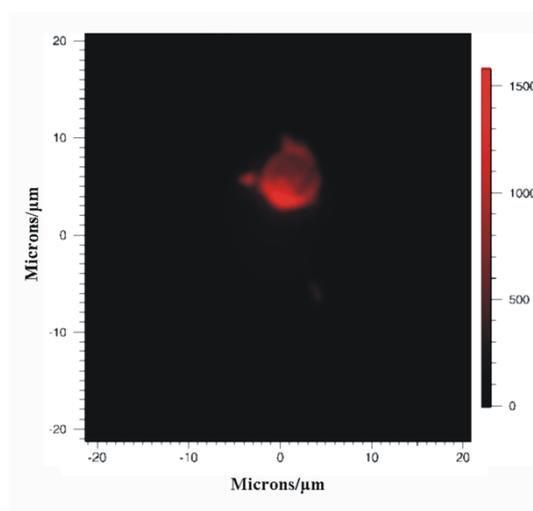


Fig. S5. Raman mapping image of silk-based hybrid capsule, which indicates the enhanced Raman scattering due to AuNRs aggregation compared with the black background near the hybrid capsule. The concentration of crystal violet is 5 μM .