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Antibacterial nanoparticles: enhanced antibacterial efficiency of coral-like crystalline rhodium nanoplates

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Materials.

Silver nitrate, hydrogen peroxide (30%), trisodium citrate dihydrate, and sodium borohydride were purchased from Junsei (Tokyo, Japan). L-ascorbic acid, citric acid, poly(vinylpyrrolidone) (Mw 29 kDa), ethylene glycol, rhodium(III) chloride hydrate, hexamethyldisilazane (>99 %) and Luria Bertani (LB) media (0.2 µm filtered) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

All chemicals were used as received.

Preparation of Ag nanoplates (AgNPs)

Ag nanoplates were manufactured by seed-mediated growth method. For the preparation of Ag nanoseeds, $250 \ \mu$ L of $10 \ m$ M AgNO₃, $300 \ \mu$ L of $30 \ m$ M trisodium citrate dihydrate, $1.5 \ m$ L of $3.5 \ m$ M PVP, and $24.75 \ m$ L of deionized (DI) water were sequentially added into a $50 \ m$ L

clear glass vial. Under the magnetic stirring, 60 μ L of 30% hydrogen peroxide was added then 250 μ L of 100 mM sodium borohydride aqueous solution was rapidly injected followed by additional incubation for 3 h at 30 °C water bath with magnetic stirring. To the formed blue Ag nanoseeds solution, 333 μ L of 75 mM trisodium citrate dihydrate aqueous solution and 1 mL of 100 mM L-ascorbic acid were sequentially added. Meanwhile, Ag growth solution composed of 20 mL of 1 mM AgNO₃, 0.125 mL of 100 mM citric acid, and 10 μ L of 75 mM trisodium citrate dihydrate was separately prepared in conical tube for addition. To the seed solution, 13 mL of growth solution was added by 0.2 mL/s rates. During the growth reaction, solution color became deep-blue in accordance with the edge length elongation by growth. After the additional reaction time of 10 min at ambient condition for stabilizing the growth process, formed AgNPs were used for following galvanic replacement without further purification.

Inverse-directional galvanic replacement for Rh nanoplates (RhNPs) synthesis

To the clear 30 mL glass vial, 4 mL of as-synthesized AgNPs were placed, then 10 mL of ethylene glycol was added and mixed for homogeneous mixture. To the mixture, 900 mL of 4.2 mM Rh(III) aqueous stock solution was added, then heated for 4 h at 190 oC without capping and magnetic stirring. During the galvanic replacement, solution color changed from deep-blue to dark brown. The final product was purified by centrifugation at 9000 rpm for 10 min, then washed with DI water for at least 3 times. After the purification, synthesized RhNPs were re-dispersed in 4 mL of DI water and denoted as 1 eq. In case of Rh nanospheres (RhNSs) synthesis, the procedure was slightly modified by replace the 4 mL of AgNPs for 4 mL of DI water, and additional L-ascorbic acid, trisodium citrate dihydrate and PVP were added in same concentration with AgNPs solution.

Characterizations

Energy-filtering transmission electron microscope LIBRA 120 (Carl Zeiss, Germany) and field-emission scanning electron microscope AURIGA (Carl Zeiss, Germany) were used to characterize the size and morphology of AgNPs, RhNSs, and RhNPs. X-ray diffraction was measured by D8-Advance (Bruker Miller Co., USA). UV-Vis spectra were measured by Lambda 465 (PerkinElmer, USA) for extinction of nanoparticles, and V670 UV/Vis/NIR spectrometer (JASCO, USA). Dynamic light scattering and zeta-potential were measured by Zetasizer Nano ZS (Malvern, UK).

Bacterial cells growth and inhibition

Bacterial cells were cultured in 3 mL of LB media at 37 °C for overnight for primary culture. After the primary culture, cells were relocated to 10 mL of LB media at 37 °C for additional 2 h (1% inoculums), until optical density (OD) became 0.5 to 0.6. Bacterial cells were finally relocated to 5 mL of LB media along with varying the concentrations of RhNPs (10, 15, 30, and 50 μ L of 1 eq. RhNP) and incubated at 37 °C. Control experiments of bacterial cells only

(control), AgNPs, Rh(III) ions, and RhNSs were performed in same manner with RhNPs. For the tracking of bacterial cells growth phase, OD at 600 nm was continuously measured at each 20 min repeatedly by UV-Vis-NIR spectrophotometer.

SEM observation of bacterial cell morphology

After the RhNP mediated bacterial cell growth inhibition test, bacterial cells were gathered by centrifugation at 6000 rpm at 4 °C, then were suspended with 50 μ L of DI water followed by air-drying and fixing with 4% formaldehyde. After the fixation, cells were washed with DI water than dehydrated by using continuous treatment with 20, 25, 50, 75, 90, and 100 % ethanol for 15 min each. Finally, complete dehydration with hexamethyldisilizane was performed and gold sputtered bio-SEM samples were mounted for characterization.



Figure S1. SEM image of RhNP. The scale bar is 100 nm.



Figure S2. HR-TEM and FFT image of RhNP. RhNP exhibited highly crystalline fcc structure.



Figure S3. Characterization of AgNP. (a) TEM image, (b) UV-Vis spectrum and (c) DLS with zeta-potential data represented successful preparation of AgNP as control nanoparticle for antibacterial efficiency test. The scale bar is 100 nm.



Figure S4. Characterization of RhNS. (a) TEM image, (b) UV-Vis spectrum and (c) DLS with zeta-potential data represented successful preparation of RhNS as control nanoparticle for antibacterial efficiency test. The scale bar is 100 nm.