Magnetic Hybrid Colloid Decorated with Ag Nanoparticles; Bites Away Bacteria and Chemisorbs Virus

Supporting Information

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1. Experimental Part

Materials: Sodium acetate was purchased from Yakuri Pure Chemicals. Calcium chloride (CaCl$_2$, anhydrous, 95.0%) and ethylene glycol (EG) were purchased from Kanto Chemical and J. T. Baker, respectively. Sodium hydroxide (NaOH, > 93.0%) was purchased from SHOWA. Ammonium hydroxide aqueous solution (28~30%) and magnesium sulfate (MgSO$_4$, anhydrous, 99.5%) were purchased from Junsei Chemical. Sodium citrate tribasic dihydrate (Na$_3$Cit·2H$_2$O, > 99.0%), tetraethyl orthosilicate (TEOS, 98%), hydrogen tetrachloroaurate(III) trihydrate (HAuCl$_4$·3H$_2$O, 99.9+%), silver nitrate (AgNO$_3$, > 99.0%), iron (III) chloride hexahydrate (FeCl$_3$·6H$_2$O, 97%), formaldehyde solution (37% in water), 3-aminopropyl-trimethoxysilan (APS, 97%), tetrakis(hydroxymethyl) phosphonium chloride (THPC, 80% solution in water), N-Acetyl-Cysteine (NAC, 99%), and cesium chloride (CsCl, 98%) were purchased from Sigma-Aldrich. 5-(and-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (carboxy-H$_2$DCFDA, mixed isomer) was obtained from Invitrogen Molecular Probes™, USA.

Synthesis of Ag$_{07}$@MHC and Ag$_{15}$@MHC: Ag$_{07}$@MHC and Ag$_{15}$@MHC were synthesized as reported in our previous study.$^{[1]}$ Firstly, the superparamagnetic Fe$_3$O$_4$ core was synthesized in a 3 times larger scale than our previous work by a solvothermal reaction at 200 °C from the mixture of 1.95 g (12.0 mmol) FeCl$_3$, 0.60 g (2.04 mmol) Na$_3$Cit·2H$_2$O, and 3.6 g (26.4 mmol) sodium acetate·3H$_2$O in 60 mL of ethylene glycol. Compared to our previous work, the synthesized Fe$_3$O$_4$ core in a large scale showed broad size ranges, generally about 300 ~ 500 nm in diameter. The resultant Fe$_3$O$_4$ core was dispersed in 60 mL of ethanol and encapsulated with silica using Stöber process. Typically, 5 mL of the Fe$_3$O$_4$ core in ethanol was diluted to 500 mL and then, 50 mL of de-ionized water (DW), 15 mL of NH$_4$OH (30% in water), and 0.225 g of Na$_3$Cit·2H$_2$O were added. After stirring the reaction...
mixture for 1 hr with a mechanical stirrer, 22.5 mL of TEOS was injected, stirred for 12 h at room temperature, and the product was magnetically rinsed with DW and ethanol. The obtained Fe₃O₄/SiO₂ core/shell-structured magnetic hybrid colloid was dispersed in 20 mL of ethanol (MHC solution, roughly 600 nm in diameter). To the solution, 80 mL of ethanol, 3 mL of NH₄OH (30% in water), 3 mL of DW, and 0.011 mL of APS were added in turn and the mixture was stirred for 12 hr with a mechanical stirrer at room temperature. The product was magnetically rinsed with ethanol and DW 5 times each and dispersed in 20 mL of DW, yielding the AP-functionalized Fe₃O₄/SiO₂ (AP-MHC stock solution containing 3.7 × 10¹⁰ AP-MHC/mL, detailed calculation can be refered to our previous report). As the seeding step, 1 mL of AP-MHC was mixed with 5 mL of Au seed solution prepared according to the previous report. Au-seeded MHC was separated from the reaction mixture by a magnet and dispersed in 1 mL of DW. For the synthesis of Ag07@MHC (or Ag15@MHC), the mixture of 10 mL (or 20 mL) of AgNO₃ (0.01 wt/v % in water) and 0.001 mL (or 0.002 mL) of NH₄OH (30% in water) were added to 1 mL of Au-seeded MHC solution with stirring. After 5 min, 0.02 mL of formaldehyde (37% in water) was added slowly as a reducing reagent to each reaction mixture, and the mixture was stirred with a mechanical stirrer for 30 min and left for 1.5 h without perturbation. The final products were purified with DW 3 times using magnetic separation and dispersed in 4 mL of DW, resulting in 9.2 × 10⁹ particles/mL of AgNP@MHC solution, which was used for further analysis and antimicrobial test.

**Preparation of Target Microorganisms:** Two strains of *Escherichia coli* (*E. coli*), CN13 (ATCC No. 700609) and C3000 (ATCC No. 15597), were cultured in tryptic soy broth (TSB, BD Bacto™, USA) as previously described. After overnight culturing at 37 °C, the bacterial stocks were aliquoted and stored at 4 °C. For each experiment, the concentration of the *E.*
coli stocks was measured using serial dilution and cultivation. Bacteriophage MS2 (ATCC No. 15597-B1) was propagated using the single agar layer (SAL) method and E. coli C3000 as the host bacteria, and the viral stock was prepared as described previously.\[3,4\] Briefly, after overnight culturing at 37 \(^\circ\)C, the bacteriophage MS2 was purified from phosphate-buffered saline (PBS)-washed E. coli lysates.\[4\] An equal volume of chloroform was added to the lysates followed by centrifugation at 4000 rpm for 30 min at 4 \(^\circ\)C. Then, the supernatant was recovered for viral stock and stored at -80 \(^\circ\)C until use.

**Statistical Analysis:** The data were analyzed with one-way analysis of variance (ANOVA) or Kruskal-Wallis one-way ANOVA with Dunnett’s test for multiple comparisons. P value less than <0.05 was considered statically significant. SPSS\(^\circ\) statistics for Windows ver. 19.0 (IBM\(^\circ\), USA) and SigmaPlot for Windows ver. 12.0 (Systat software Inc, USA) were used for the statistical analysis.

**Interaction Between Ag\(^+\) Released from Ag30@MHC and E.coli CN13:** Freshly synthesized Ag30@MHC was washed 5 times with DW using magnetic separation to remove any residual silver ions and re-suspended in DW. After 1 h, Ag30@MHC was separated by magnetic bar and the supernatant containing the Ag\(^+\) released from Ag30@MHC was collected. Equal volumes of E.coli CN13 (2 \(\times\) 10\(^6\) CFU/mL) and Ag\(^+\) solution (~ 0.3 ppm) were mixed and treated in a shaking incubator (1 hr, 25\(^\circ\)C, 150 rpm). One drop of the reaction mixture was deposited on a clean silicon wafer to analyze the morphology of E.coli using SEM.
Purification of Bacteriophage MS2 for SEM and TEM Images: To purify bacteriophage MS2, cesium chloride (CsCl) gradient ultracentrifugation was applied to viral suspension as previously described with some modification.\textsuperscript{[5,6]} After overnight culturing at 37 °C and crude purification using chloroform, as mentioned above, the phages were separated from the supernatant by CsCl gradient ultracentrifugation at 35,000 rpm for 24 hr at 4 °C using Optima™ L-100 XP ultracentrifuge and Beckman SW55Ti rotor (Beckman Coulter Korea Ltd.). The band of bacteriophage MS2 particles were collected with a syringe and dissolved in PBS. Then, bacteriophage MS2 suspended in PBS was centrifuged at 40,000 rpm for 3 hr at 4 °C for further purification of phage particles. After the second round of ultracentrifugation, the supernatant was discarded and bacteriophages MS2 were suspended in PBS at 4 °C and used for following analysis.

1 mL of bacteriophage MS2 (2 \times 10^6 PFU/mL) was reacted with 1 mL of Ag30@MHC (9.2 \times 10^9 particles/mL) for 5 min with shaking. One drop of the reaction mixture was collected and deposited on a clean silicon wafer to investigate the surface of Ag30@MHC using SEM. For TEM image, a drop of the reaction mixture was placed on a copper grid and dried. This was stained for 10 sec by adding one drop of the uranyl acetate solution (1 wt/v % in methanol) and then, fully dried before TEM analysis.
2. TEM Images

**Fig. S1** TEM images of MHC decorated with nearly continuous silver layer.

**Fig. S2** TEM images of MHC (A) with self-assembled Ag seeds by chemical affinity, (B) after addition of Ag$^+$ solution to A, and (C) after addition of reducing agent to B. Upper image shows single MHC composite and the lower one shows a magnified part of single MHC composite (A and B) or many MHC composites (C).
Fig. S3 TEM images of (A) Ag-seeded MHC, (B) after addition of only Ag\(^+\) to A. Upper image shows single MHC composite and the lower one shows a magnified part of single MHC composite.

Fig. S4 TEM images of MHC decorated with broadly sized AgNPs by reduction of a small amount of Ag\(^+\) ions.
3. XPS analysis

Fig. S5 XPS spectra of survey scan (a) and the corresponding specific scan of Ag (b) and N with curve fitting (c) for AP-MHC, complex between Ag\(^+\) and AP-MHC, and Ag30@MHC.
4. Magnetization

**Fig. S6** Magnetic properties of MHC, Ag07@MHC, Ag15@MHC, and Ag30@MHC.
5. UV-Vis Spectra

![UV-Vis Spectra](image)

**Fig. S7** Absorption spectra of the AgNP solutions at 1 h after addition of different concentration of A) Na\(^+\), B) Ca\(^{2+}\), and C) Mg\(^{2+}\).
6. TEM Images and UV-Vis Spectra

Fig. S8 TEM images of (A) AgNPs only and (B) AgNPs with Mg\(^{2+}\) (0.5 mM) after 1 day and the corresponding UV-Vis spectra.
7. SEM Images

Figure S9. SEM images of (A) *E. coli* CN13 treated with Ag⁺ released from Ag30@MHC for 1h, (B) enlarged view of A.
8. References


