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

Supporting Information

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Contents

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 ($Na_3Cit{\cdot}2H_2O$, > 99.0%), tetraethyl orthosilicate (TEOS, 98%), hydrogen tetrachloroaurate(Ⅲ) trihydrate ($HAuCl_4{\cdot}3H_2O$, 99.9+%), silver nitrate ($AgNO_3$, > 99.0%), iron (Ⅲ) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$, 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_2DCFDA, mixed isomer) was obtained from Invitrogen Molecular Probes™, USA.

Synthesis of Ag07@MHC and Ag15@MHC: Ag07@MHC and Ag15@MHC were synthesized as reported in our previous study.[1] Firstly, the superparamagnetic Fe_3O_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) $Na_3Cit{\cdot}2H_2O$, and 3.6 g (26.4 mmol) sodium acetate$\cdot 3H_2O$ in 60 mL of ethylene glycol. Compared to our previous work, the synthesized Fe_3O_4 core in a large scale showed broad size ranges, generally about 300 ~ 500 nm in dimeter. The resultant Fe_3O_4 core was dispersed in 60 mL of ethanol and encapsulated with silica using Stöber process. Typically, 5 mL of the Fe_3O_4 core in ethanol was diluted to 500 mL and then, 50 mL of de-ionized water (DW), 15 mL of NH_4OH (30% in water), and 0.225 g of $Na_3Cit{\cdot}2H_2O$ 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 Fe_3O_4/SiO_2 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 NH_4OH (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 Fe_3O_4/SiO_2 (AP − MHC stock solution containing 3.7×10^{10} AP − MHC/mL, detailed calculation can be refered to our previous report).[1] As the seeding step, l mL of AP − MHC was mixed with 5 mL of Au seed solution prepared according to the previous report.[1] 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 10mL (or 20 mL) of $AgNO_3$ (0.01 wt/v % in water) and 0.001 mL (or 0.002 mL) of NH_4OH (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 9.2×10^9 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.[2] 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 °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 °C. Then, the supernatant was recovered for viral stock and stored at -80 °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® statistics for Windows ver. 19.0 (IBM®, 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 supernantent containing the Ag^+ released from Ag30@MHC was collected. Equal volumes of *E.coli* CN13 (2×10^6 CFU/mL) and Ag^+ solution (~ 0.3 ppm) were mixed and treated in a shaking incubator (1 hr, 25°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.[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 (2×10^6 PFU/mL) was reacted with 1 mL of Ag30@MHC (9.2×10^9 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

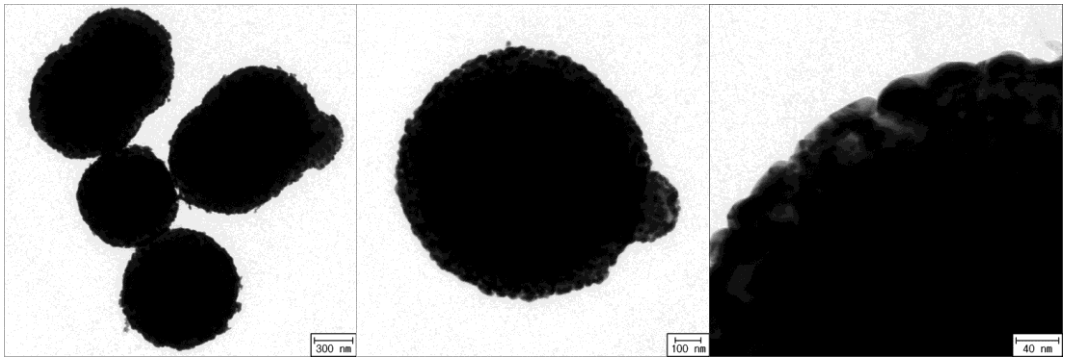

Fig. S1 TEM images of MHC decorated with nearly continuous silver layer.

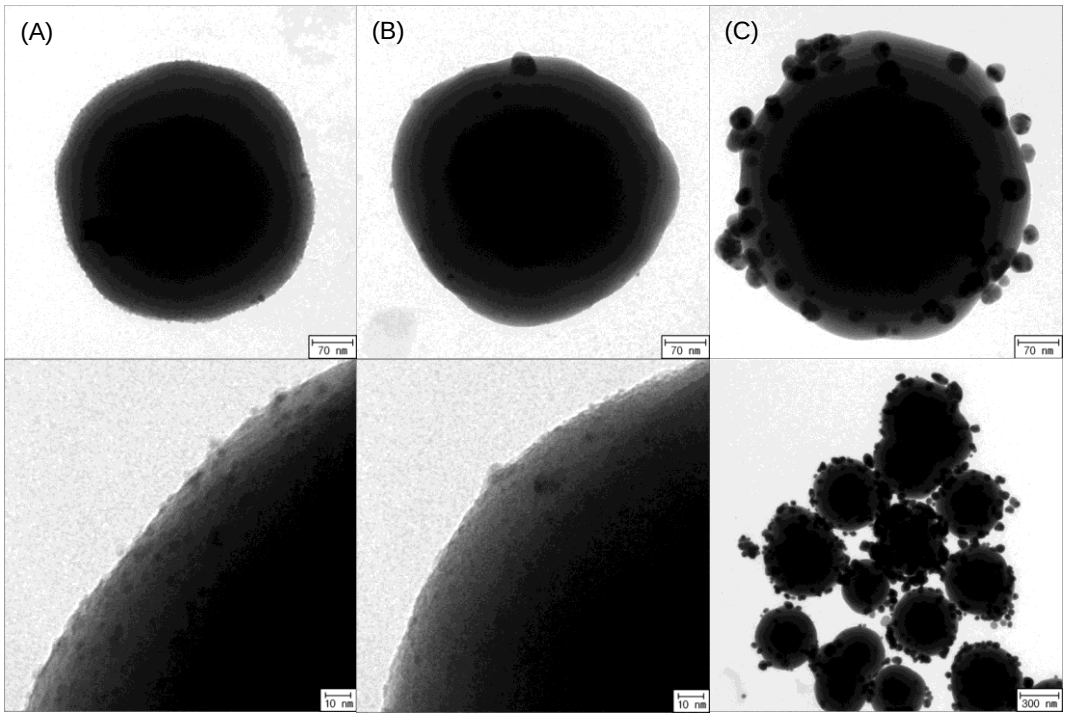

Fig. S2 TEM images of MHC (A) with self-assembled Ag seeds by chemical affinity, (B) after addition of Ag^{+} solution to A, and (C) after addition of reducing agent to B. Upper image shows single MHC composite and the lower one shows a magnified part of single MHC composite (A and B) or many MHC composites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

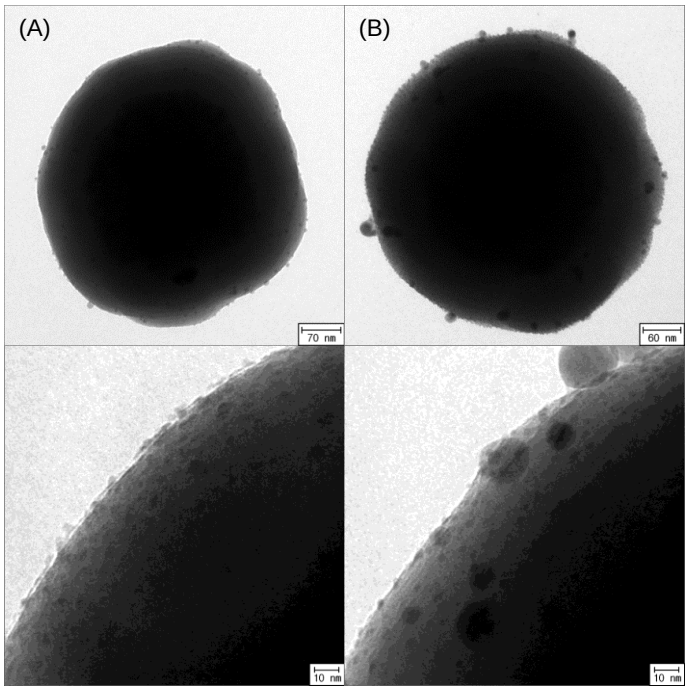

Fig. S3 TEM images of (A) Ag-seeded MHC, (B) after addition of only Ag^+ to A. Upper image shows single MHC composite and the lower one shows a magnified part of single MHC composite.

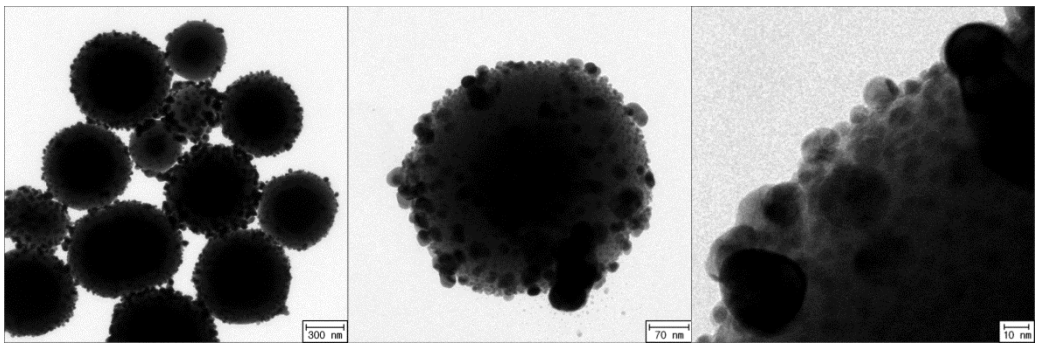

Fig. S4 TEM images of MHC decorated with broadly sized AgNPs by reduction of a small amount of Ag^+ ions.

3. XPS analysis

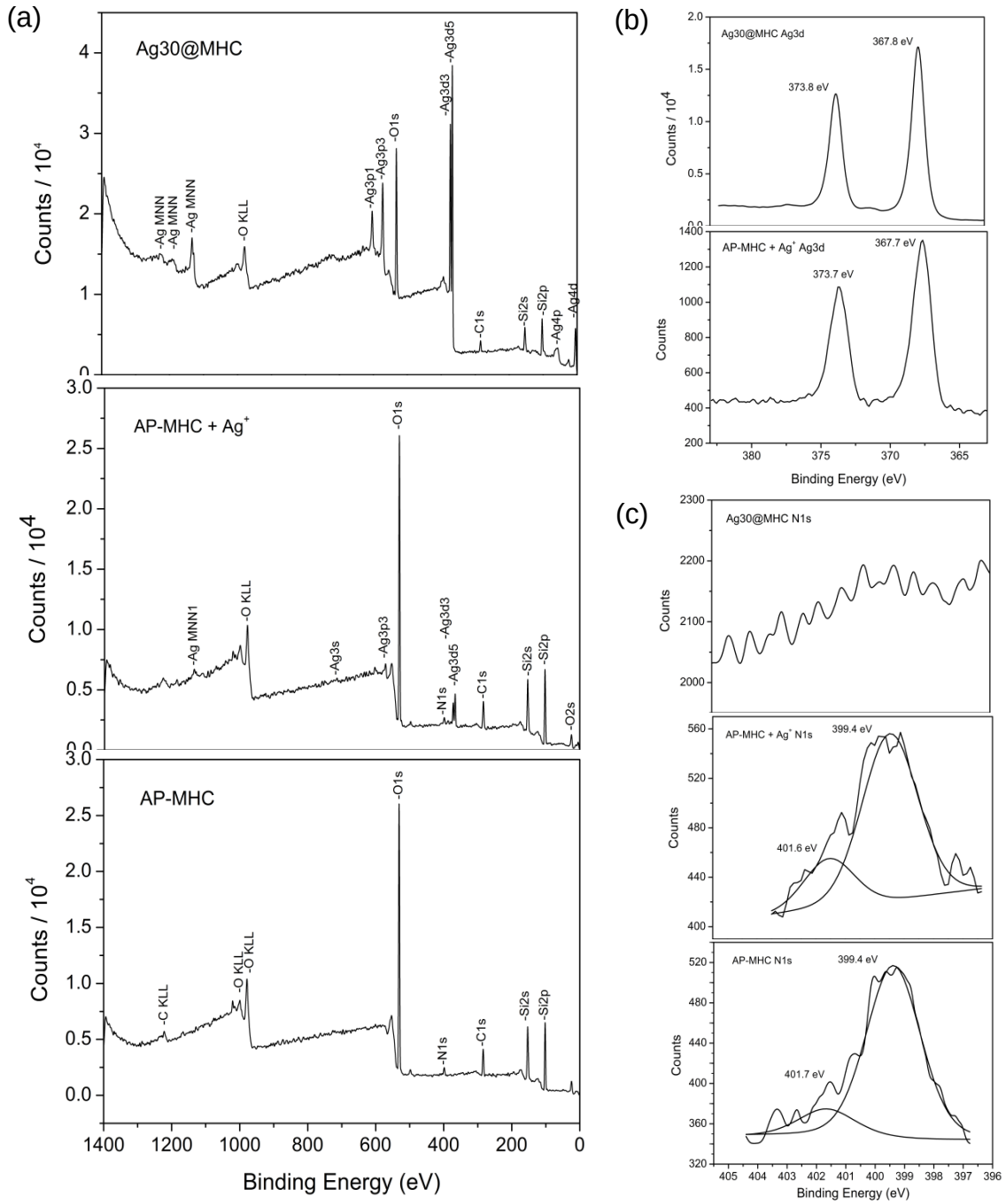

Fig. S5 XPS spectra of survey scan (a) and the corresponding specific scan of Ag (b) and N with curve fitting (c) for AP-MHC, complex between Ag^+ and AP-MHC, and Ag30@MHC.

4. Magnetization

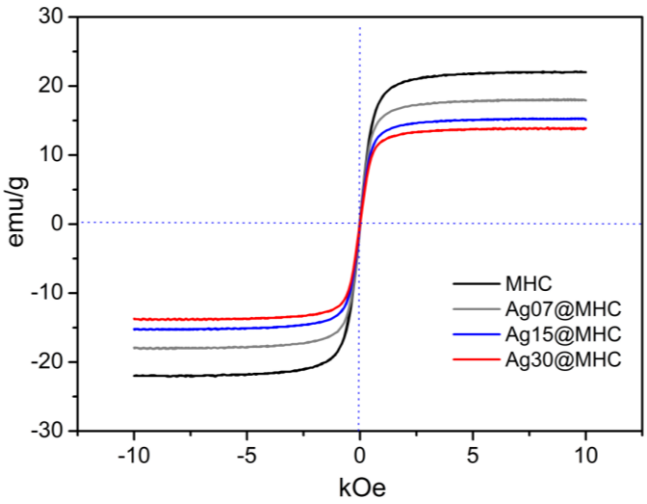

Fig. S6 Magnetic properties of MHC, Ag07@MHC, Ag15@MHC, and Ag30@MHC.

5. UV-Vis Spectra

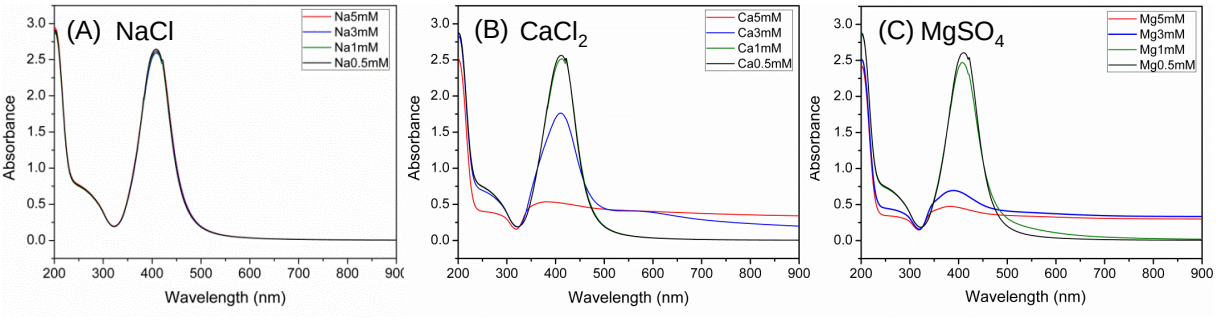

Fig. S7 Absorption spectra of the AgNP solutions at 1 h after addition of different concentration of A) Na^{+}, B) Ca^{2+}, and C) Mg^{2+}.

6. TEM Images and UV-Vis Spectra

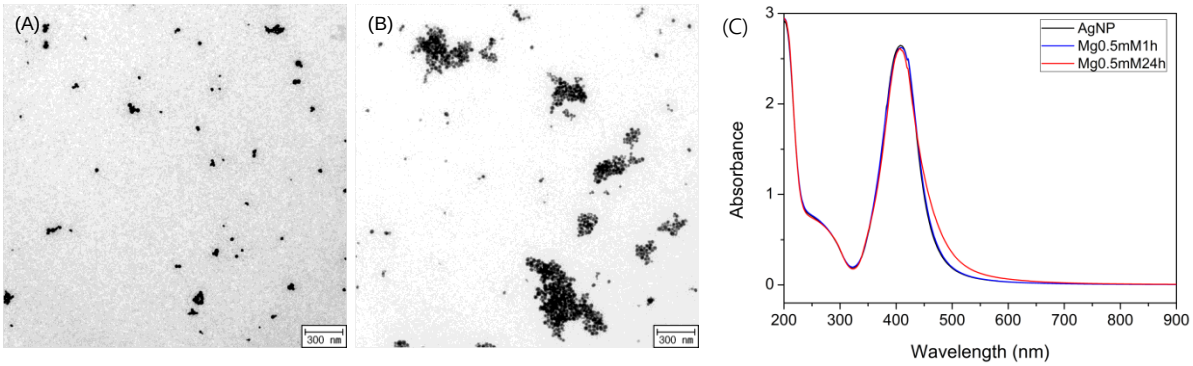

Fig. S8 TEM images of (A) AgNPs only and (B) AgNPs with Mg^{2+} (0.5 mM) after 1 day and the corresponding UV-Vis spectra.

7. SEM Images

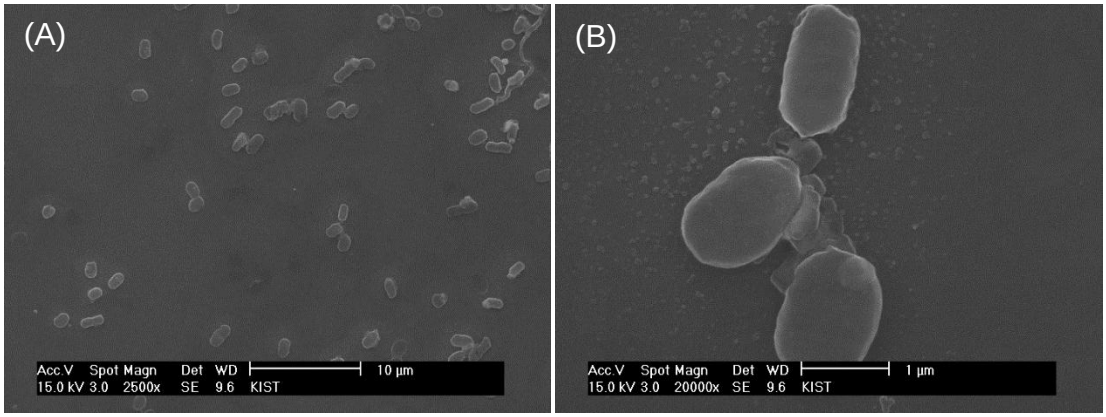

Figure S9. SEM images of (A) *E.coli* CN13 treated with Ag^{+} released from Ag30@MHC for 1h, (B) enlarged view of A.

8. References

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