Supporting Information

SERS-based immunoassay of anti-cyclic citrullinated peptide for early diagnosis of rheumatoid arthritis†

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Experimental details

CCP-conjugated magnetic beads were prepared in the following way: 500 μL of 300 nM biotinylated CCP (biotin-HQCHQEST-Cit-GRSRGRCGRSGS-COOH with disulfide linkage between residues Cys3 and Cys16, 2.6 kDa, Peptron Inc., Daejeon, South Korea) reacted with 500 μL of 0.5 mg/mL streptavidin-bound magnetic beads (1 μm in size, Dynabeads® MyOne™; Invitrogen, Eugene, OR, USA) by the streptavidin-biotin interaction. The mixtures were allowed to react for 2 h at room temperature, and then unreacted streptavidin was blocked with 500 μL of 300 nM biotin. Unreacted reagents were rinsed out twice with PBS buffer solution.

The process of antibody conjugation to the surface of HGNs was reported elsewhere. In total, 1 μL of 50 nM MGITC (Invitrogen) was added to 1.0 mL of 0.7 nM HGN colloids. The surface of the HGNs was then modified using 10 μL of 3.0 μM poly(ethylene glycol) 2-mercaptoethyl ether acetic acid (PEG linker, Aldrich) for 2 h at room temperature, followed by removal of unreacted chemical reagents by centrifugation at 6000 rpm for 10 min. Next, 1.0 mL PBS buffer (pH 7.4) was added to disperse HGNs. The carboxyl groups on the surface of the HGNs were activated with 10 μL of 0.1 μM EDC/NHS solution for 10 min at room temperature. After gentle shaking, the solution was reacted with 10 μL of 10 μM rabbit anti-IgG (or human anti-IgG) for 2 h at room temperature. Subsequently, unreacted succinimidyl groups were deactivated using 10 μL of 0.01 M ethanol amine for 3 h. Finally, unreacted reagents were removed by centrifugation at 5000 rpm for 10 min, and the solution was washed twice with PBS buffer.

Raman measurements were performed using a Renishaw 2000 Raman microscope system (Renishaw, UK). A Melles Griot HeNe laser, operating at $\lambda = 632.8$ nm, was used as the excitation source, with a laser power of approximately 15 mW. The Rayleigh line was removed from the collected Raman scattering using a holographic notch filter located in the collection path. Raman scattering was collected using a charge-coupled device (CCD) camera at a spectral resolution of 4 cm^{-1}. All spectra were calibrated to the 520 cm^{-1} silicon line. An additional CCD camera was fitted to an optical microscope to obtain optical images. A 20× objective lens was used to focus a laser spot on the glass tube. All the Raman spectra reported here were collected over 10 exposure times in the range of 750-1270 cm^{-1}.

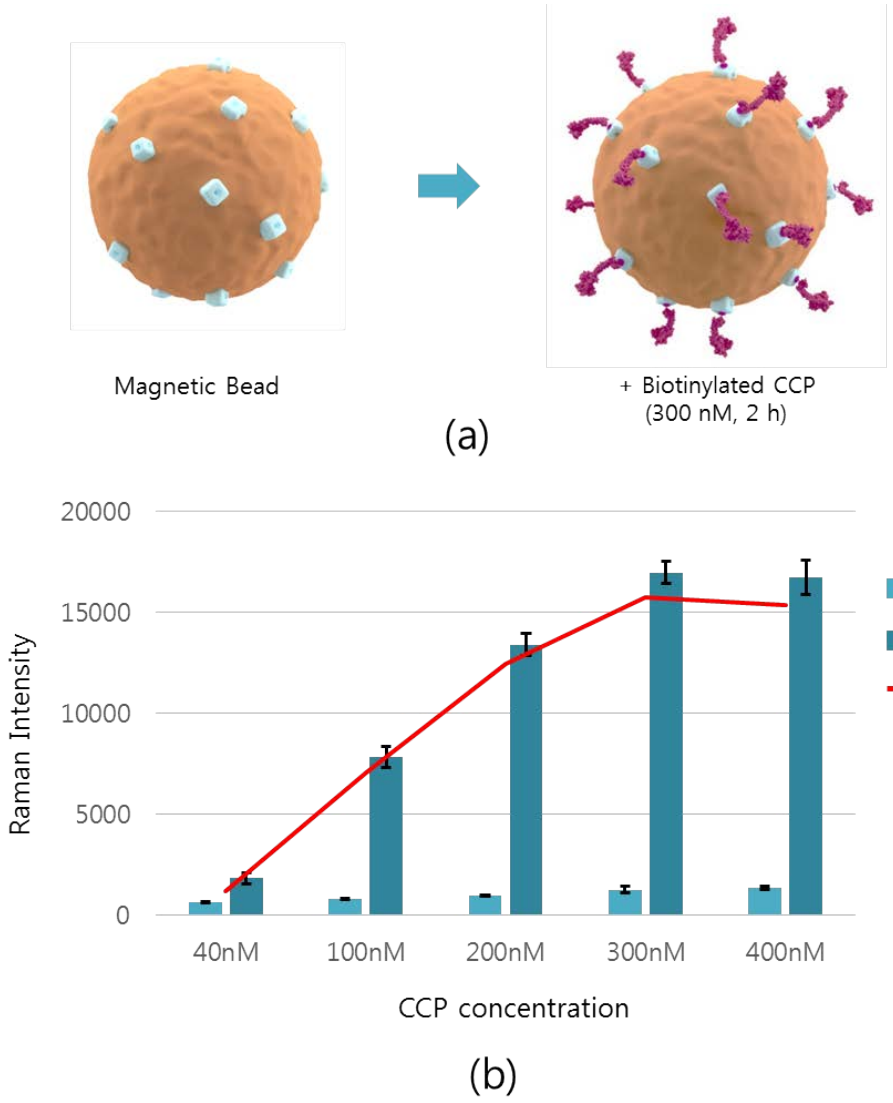

Fig. S1. (a) Immobilization of biotinylated CCPs on the surface of a streptavidin-bound magnetic bead. (b) Monitoring of changes in SERS intensity to determine the optimal CCP concentration for the assay. Here, the SERS intensity change at 1617 cm^{-1} was monitored in the 40~400 nM range of CCP for 100 ng/mL of anti-CCP autoantibody.

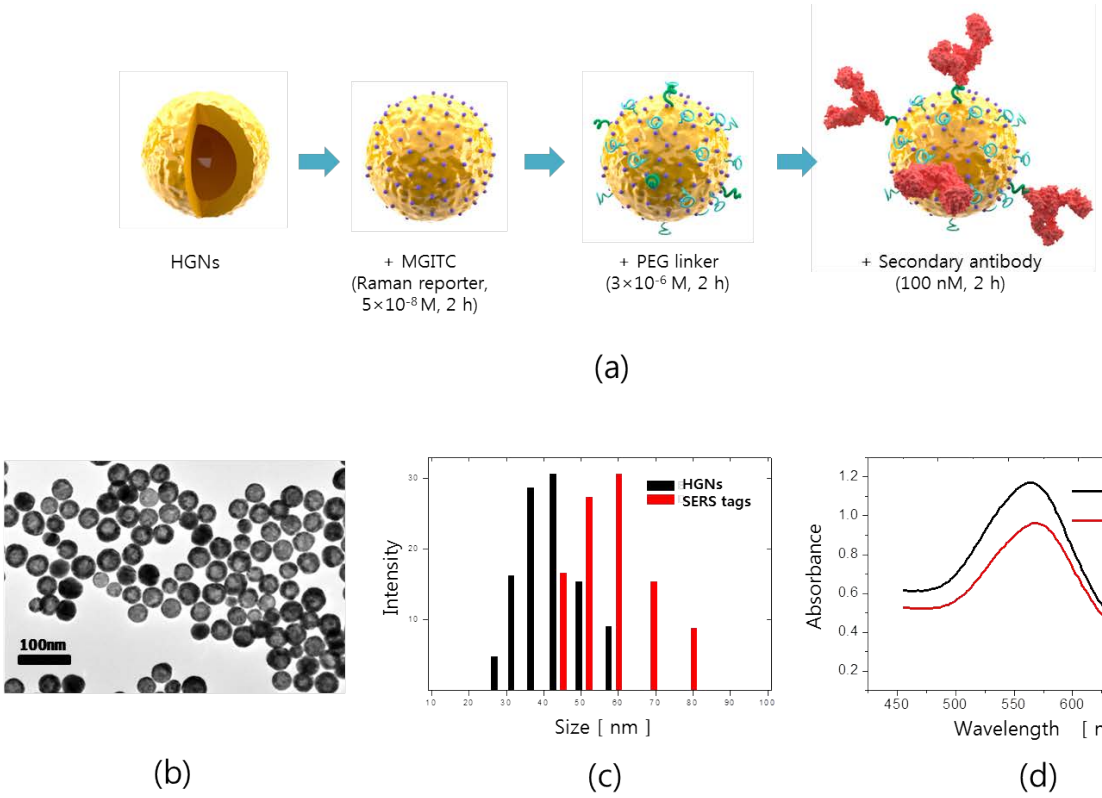

Fig. S2. (a) Sequential procedure for fabrication of SERS nano-tags. MGITC was used as a Raman reporter molecule. (b) Transmission electron microscope (TEM) images of HGNs. (c) Dynamic light scattering distributions of HGNs (black bars) and antibody-conjugated SERS nano-tags (red bars). (d) UV/Vis absorption spectra of HGNs (black line) and antibody-conjugated SERS nano-tags (red line).

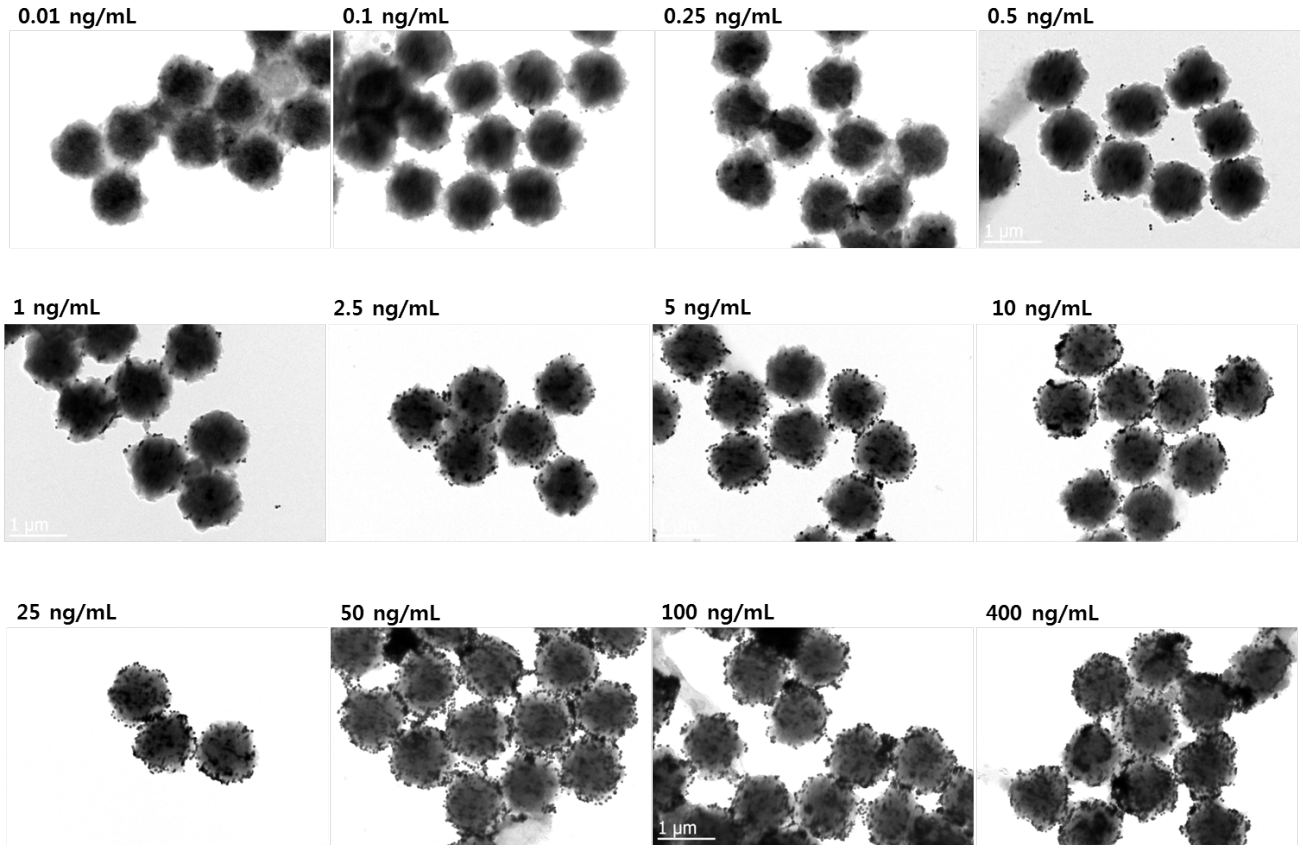

Fig. S3. TEM images of sandwich immunocomplexes for 12 different concentrations of rabbit anti-CCP in the 10~400 ng/mL range.