Supplementary Information for

An operationally simple colorimetric assay of hyaluronidase activity using cationic gold nanoparticles

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Experimental Details

Materials

Hydrogen tetrachloroaurate (III) trihydrate, cysteamine, sodium borohydride, and hyaluronidase (type IV-S) were purchased from Sigma-Aldrich. Hyaluronic acid (M.W ≥ 100,000) was supplied by Hansonbiotech (South Korea). Purified water from the Millipore Milli-Q water purification system was used for all experiments.

Synthesis of positive charged gold nanoparticle.

The gold nanoparticles were prepared by sodium borohydride reduction of hydrogen tetrachloroaurate (III) trihydrate (Sigma-Aldrich, USA) in the presence of cysteamine (Sigma- Aldrich, USA) [1]. 400 μl of 213 mM cysteamine was added to 40 ml of 1.42 mM $HAuCl_4$. Sodium borohydride (Sigma-Aldrich, USA) was dissolved in cold DW immediately before use. After stirring for 20 min at room temperature, 10 μl of 10 mM $NaBH_4$ was added to the mixture solution with vigorous stirring for 10 min in the dark condition. The color of mixture solution was changed from yellow to brownish. After further mild stirring, the gold nanoparticle solution was stored in the dark condition at 4°C. The size and monodispersity of the gold particles were confirmed by performing Transmission Electron Microscopy, a Jeol JEM-2100F and surface charge of the gold nanoparticles was measured using Light Scattering Photometer on an Otsuka electronics ELS-Z2. See Figure S1.

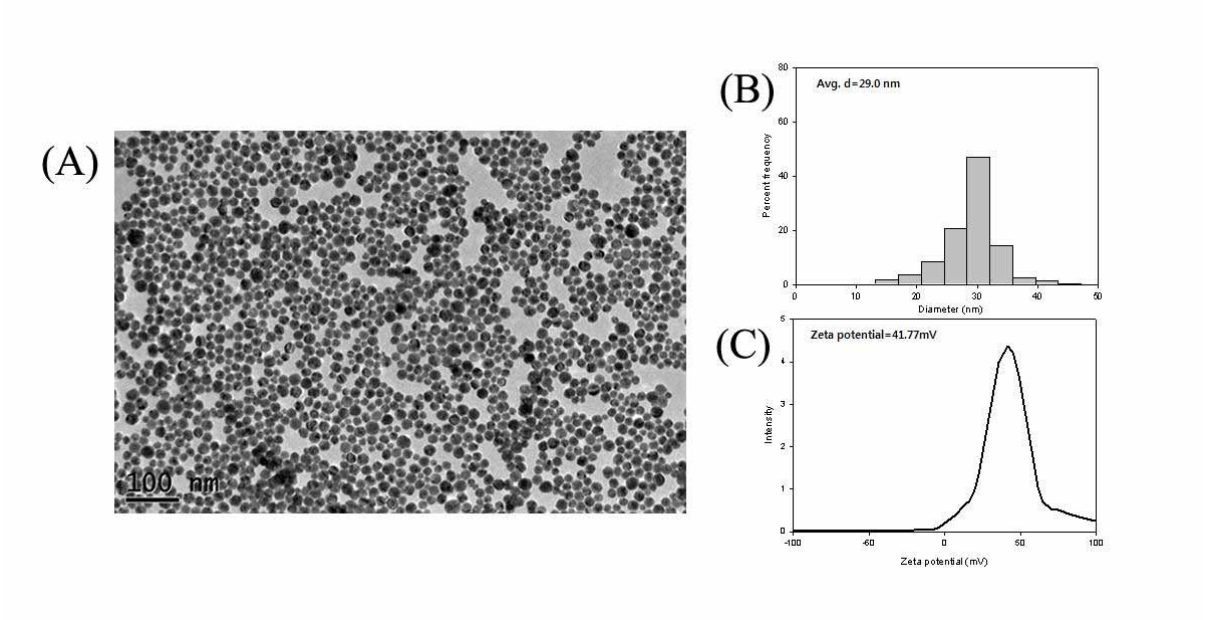

Figure S1: FE-TEM image (A), size distribution (B) and zeta potential distribution (C) of gold nanoparticle prepared using cysteamine. The gold nanoparticle showed about 29 nm in size and a positive zeta potential value (41.77 mV).

Measurement of HAase activity

The optical density of the gold nanoparticle solution was matched to 0.9 at 525 nm. HAase was dissolved in 0.1 M MES buffer (pH 6.0). 20 µl HAase solution was added to 180 µl HA solution and mixture was incubated at 37 ^{o}C. After incubation for an appropriate time, the enzymatic reaction was stopped by heating the mixture in a boiling water for 5min. After cooling the mixture to room temperature, 10 µl of the sample was mixed with 0.4 ml of the gold nanoparticle (optical density 0.9 at 525nm) with inverting for 3 min. Then absorbance of the final sample was measured by UV/Vis spectrophotometer (DU® 800, Beck Man Coulter, USA). For Morgan-Elson method[2], mixture of 2.7 ml of 0.003% HA (w/v) and 0.3 ml of HAase (5.3 units/ml) was incubated at 37 ^{o}C for an appropriate time. After stopping the enzyme reaction in boiling water for 5 minutes, 150 µl of the incubated mixture was combined with 30 µl of 0.8 M potassium tetraborate (Sigma-Aldrich, USA) in water, heated at 100 ^{o}C for 3 minutes and mixed with 0.75ml of p-dimethylaminobezaldehdye solution. For the preparation of this reagent, 2 g of p-dimethylaminobezaldehyde solution was dissolved in a mixture of 2.2 ml of concentrated HCl (Junsei, Japan) and 17.5 ml of acetic acid (Junse, Japan) in 17.5 ml of water, and the mixture was diluted in 10 volume of acetic acid

immediately before use. After incubation of the mixture at 37 ^{o}C for 10 minutes and centrifugation at 4 ^{o}C and 14000 rpm for 10 minutes, absorbance of the sample at 588 nm was measured by the spectrophotometer.

References

1. T. Niidome, K. Nakashima, H. Takahashi and Y. Niidome, *Chem. Comm*., 2004, **17**, 1978-1979.
2. K. P. Vercruysse, A. R. Lauwers, and J. M. Demeester, *Biochem, J*. 1995, **310**, 55-59