Supporting Information

Chemistry Letters

Antiproliferative Activity of Silver Nanoplates on Human Promyelocytic Leukemia Cell Lines

Tatsuya Sakaguchi, Kenta Mine, Fuki Kudoh, Rui Kamada, and Kazuyasu Sakaguchi*

Department of Chemistry, Faculty of Science, Hokkaido University, N10 W8, Kita-ku, Sapporo, Hokkaido 060-0810

(Received November 26, 2014; CL-141085; E-mail: kazuyasu@sci.hokudai.ac.jp)

Figure S1

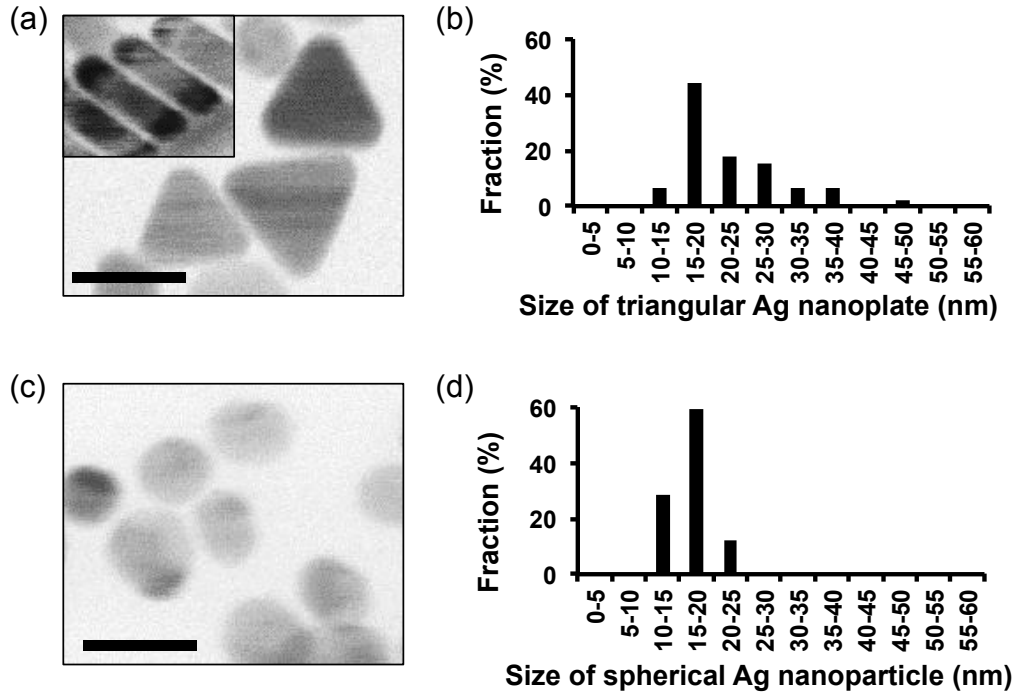

Figure S1. Shape and size distribution of Ag nanostructures. (Left panels) TEM images showing the morphology of (a) triangular Ag nanoplates, and (c) spherical Ag nanoparticles. The inset in (a) shows a TEM image in which Ag nanoplates stand vertically upon their edges. The scale bar is 20 nm. (Right panels) Size distribution of (b) triangular Ag nanoplates, and (d) spherical Ag nanoparticles.

Experimental Section

Materials

Silver nitrate, sodium brohydrate, and trisodium citrate were purchased from Nacalai tesque (Kyoto, Japan). Hydrogen peroxide solution (30 wt %) was purchased from Mitshubishi Gas Chemical (Tokyo, Japan). Alexa Fluor® 555 NHS ester was purchased from Life Technology (Carlsbad, CA, USA). 2-aminoethanethiol was purchased from Tokyo Chemical Industry (Tokyo, Japan).

Silver nanoparticle preparation

Silver Ag nanoplates were obtained according to the method previously reported by Q. Zhang et al.[1] Briefly, a 173.6 ml aqueous solution combining silver nitrate (0.1 M, 180 μl), trisodium citrate (75 mM, 3.6 ml), and H_2O_2 (30 wt %, 864 μl) was prepared. Sodium borohydride ($NaBH_4$, 100 mM, 1.8 ml) was rapidly injected into this mixture, and the mixture was vigorously stirred at room temperature for 10 minutes. Spherical Ag nanoparticles were synthesized by following procedure. 178.9 ml aqueous solution combining silver nitrate (0.1 M, 180 μl) was adjusted. Sodium borohydride ($NaBH_4$, 100 mM, 0.9 ml) was rapidly injected into this mixture, and the mixture was vigorously stirred at room temperature for 10 minutes. Trisodium citrate (750 mM, 360 μl) was added to the nanoparticles to prevent particle aggregation. The prepared Ag nanostructures were concentrated by centrifugation to use experiments.

Cell viability assay

Cell viability of HL-60 cells was determined by using MTS assay with the CellTiter 96® Aqueous One Solution Cell Proliferation Assay (Promega) following the manufacturer's protocol. Briefly, 1×10^4 of cells were re-suspended in 100 μl RPMI 1640 supplemented with 10% Fetal bovine serum (FBS) and placed into 96-well flat-bottom plates. The cells were incubated with various concentrations of the Ag nanostructures at 37°C and 5% CO_2 for 48 hours. Ag nanostructures were removed from the cells by centrifugation and 20 μl of MTS solution was added to the cells. The cells were incubated for another 4 hours and the absorbance at 490 nm was measured using an iMark microplate reader (Bio-rad).

Fluorescent labeling of Ag nanostructures

The fluorescent labeled Ag nanostructures were synthesized as the following procedure: Alexa Fluor® 555 NHS Ester (500 μg/ml in DMSO, 10 μl) and 2-Aminoethanethiol (100 μM, 10 μl) were mixed in 10 mM bicarbonate buffer (pH 8.5, 100 μl) for 3 hours. The mixture (10 μl) was added to the Ag nanostructures solution (150 μg/ml Ag

nanostructures, 1.5 mM trisodium citrate, 1 ml) and incubated for 1 h. After incubation, the fluorescent labelled Ag nanostructures were washed twice by 1.5 mM trisodium citrate. The intracellular behaviors of Ag nanostructures were analyzed using Biorevo BZ-9000 (KEYENCE).

Flow Cytometry

HL-60 cells (2.5×10^5) were seeded onto 24-well plate and were treated with indicated concentrations of triangular Ag nanoplates or spherical Ag nanoparticles for 48 hours. Cell cycle was analyzed by flow cytometry using a Gallios (Beckmann Coulter) with standard PI staining (BD phamingen) in accordance with manufacturer's instructions. Percentages of cells in each phase were analyzed using Flow Jo software (Version 7.5, Tree Star Inc.).

Reference

1. Q. Zhang, N. Li, J. Goebl, Z. D. Lu,Y. D. Yin, *J. Am. Chem. Soc.*, **2011**, *133*, 18931.