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Preparation of primary amine-modified gold nanoparticles and their transfection ability into cultivated cells†

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Cationic gold nanoparticles, prepared by reducing $HAuCl_4$ with $NaBH_4$ in the presence of 2-aminoethanethiol, formed a complex with plasmid DNA, and could be transfected into cultivated cells.

Nonviral gene delivery into cells is a key tool for gene therapy.[1] Several kinds of gene carriers, *e.g*., cationic liposomes, polymers and oligopeptides, have been reported.[1,2] Although the nonviral technique circumvents some of the problems occurring with the viral method, such as endogeneous recombination, oncogenic effects and unexpected immune response, the limited efficiency and short duration of transgene expression are major obstacles to the application of nonviral systems to gene therapy. Recently, the use of inorganic nanoparticles such as amino-modified silica nanoparticles and cationic gold nanoparticles[3] was reported. The special character of the nanoparticles differs from that of organic gene carrier molecules, and they are expected to be a novel base material for the next generation of functional gene carriers.

Gold nanoparticles have the advantages of easy preparation and the possibility of chemical modification of the surface, which requires skilled organic synthesis in the case of round gene carrier compounds, *e.g*., polyamidoamine dendrimer[4] and dendritic poly(L-lysine).[5] Yonezawa *et al*. developed cationic gold nanoparticles modified with thiocholine[6]. The gold nanoparticles bound to DNA, and then formed a wire-like structure along the DNA chain by self-fusion of the particles. Sandhu *et al*. demonstrated that gold nanoparticles modified with *N*,*N*,*N*-trimethyl(11-mercaptoundecyl)ammonium chloride and alkylthiol with several chain lengths, named mixed monolayer protected gold clusters (MMPCs), showed DNA-binding and transfection ability into a cultivated cell line. In this study, we developed an easy method for the preparation of cationic gold nanoparticles modified with 2-aminoethanethiol, which have a shorter alkyl chain than the MMPCs, and examined the DNA-binding ability and transfection efficiency of these nanoparticles into cultivated cells.

The gold nanoparticles were prepared by $NaBH_4$ reduction of $HAuCl_4$ in the presence of 2-aminoethanethiol at a Au/$NaBH_4$/2-aminoethanethiol ratio of 56 : 0.1 : 85 (mol/mol/mol).[7] Soon after the $NaBH_4$ reduction, the solution was opaque and brownish. Fifteen minutes stirring changed the brownish solution into clear wine red. It is probable that aggregated gold nanoparticles formed by the $NaBH_4$ reduction are rearranged into larger particles. Transmission electron microscopy (TEM) (Fig. 1A)[8] and zeta potential measurement[9] showed that the sizes and zeta potentials of the gold particles were 33.6 ± 3.0 nm and +36.2 ± 15 mV (Fig. 1B), respectively. Formation of the gold nanoparticle/DNA complex was examined by electrophoretic mobility in an agarose gel at various weight ratios of the gold nanoparticles to plasmid DNA (Fig 2).[5] The cationic gold nanoparticles could completely inhibit the migration of the DNA at a w/w ratio of 8. The DNA complex of the gold nanoparticles showed a negative zeta potential value (−33.2 ± 18 mV), and could stably disperse under biophysical salt codition. At a w/w ratio of 11, highly assembled gold nanoparticles were observed (See ESI†).

Transfection efficiency of the cationic gold nanoparticle into HeLa cells was tested with the plasmid DNA, which contains a luciferase gene (Fig. 3).[10] Gold nanoparticle/DNA complexes at various w/w ratios were added to HeLa cells. Significant gene expression was observed and its level was increased by increasing the charge ratio. At a w/w ratio of 17, the expression was 100-fold

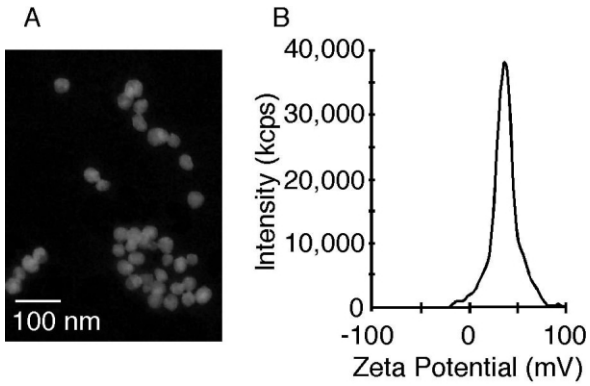

Fig. 1 (A) Transmission electron micrograph, and (B) Zeta potential distribution of cationic gold nanoparticles.

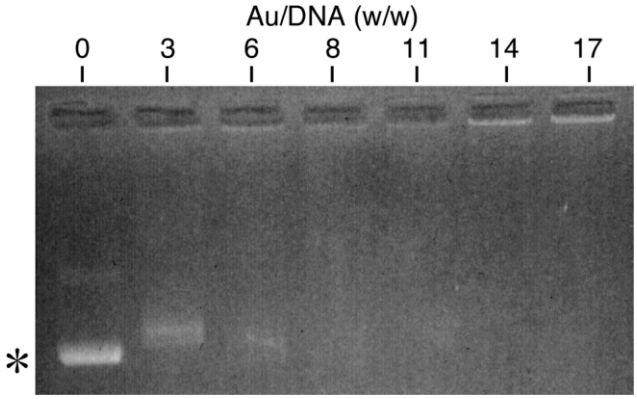

Fig. 2 Agarose gel electrophoresis of the complex of the cationic gold nanoparticles and plasmid DNA. Plasmid DNA (500 ng) and various amounts of the gold nanoparticles were mixed at several w/w ratios given at the top of the gel, electrophoresed in an agarose gel (1% w/v) and stained with ethidium bromide. The asterisk on the left of the gel indicates the position of the supercoiled form of the original plasmid DNA.

† Electronic Supplementary Information (ESI) available: A TEM image of the complex at a w/w ratio of 11. See http://www.rsc.org/suppdata/cc/b4/b406189f/

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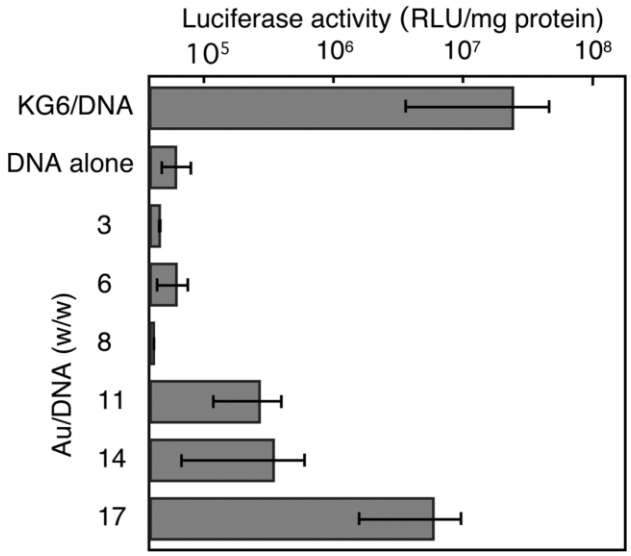

Fig. 3 Transfection efficiency of the cationic gold nanoparticle into HeLa cells.

higher than in the case of DNA alone. Significant expression was also observed with other cell lines (CHO cells).

The amounts of DNA in the cells were evaluated by Southern blotting analysis.[11] The gold nanoparticle/DNA complexes at various w/w ratios were added to HeLa cells. After 3 h incubation, the medium containing the complex was removed and replaced with fresh medium. After 24 h incubation, the cells were washed with PBS, and the plasmid DNA in cells was detected. As a result, intact DNA bands were observed on the blots for the cases with w/w ratios of 11, 14 and 17, at which significant gene transfection was observed. By rough calculation, about 0.5% of total plasmid DNA was present in the cells. The amounts of DNA uptake into cells corresponded to the expression levels. As the addition of excess cationic gold nanoparticles to DNA produces cationic complexes, the cationic surface of the complex would be advantageous for adsorption onto the negatively charged cell surfaces. Microscope observation showed that the complex was present on the cell surface after 3 h of incubation (Fig. 5).

In conclusion, we succeeded in preparing gold nanoparticles coated with 2-aminoethanethiol. The preparation of the cationic nanoparticles was easy, cost effective and reproducible. The cationic gold nanoparticles and the DNA complexes in biophysical condition would be advantageous for functional gene delivery,

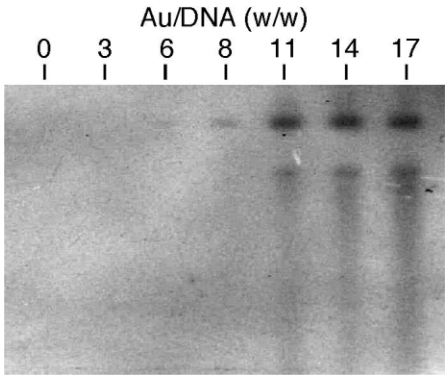

Fig. 4 Southern blotting analysis of plasmid DNA in HeLa cells at several w/w ratios after 24 h incubation.

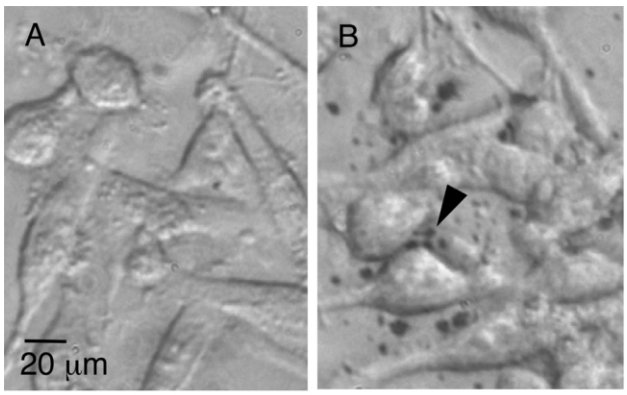

Fig. 5 Microscopic observation of cells treated with (B) or without (A) DNA complex of the cationic gold nanoparticles at a w/w ratio of 17. The complexes were observed as black dots indicated by arrowhead.

which would enable controlled release of DNA at a specific site triggered by exogeneous stimulation such as light irradiation.

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7 Four hundred μL of 213 mM 2-aminoethanethiol was added to 40 mL of 1.42 mM $HAuCl_4$. After stirring for 20 min at r.t., 10 μL of 10 mM $NaBH_4$ was added, and the mixture was vigorously stirred for 10 min at r.t. in the dark. After further mild stirring, the sample was stored in the dark at 5 °C and used within 2 months.
8 Image taken with a JEOL JEM-100CX.
9 Zeta potential was measured by a MALVERN Zetasizer Nano ZS.
10 Immediately prior to the transfection procedure, the cells in the 24-well plate were washed with 1 mL of serum-free medium. Cationic nanoparticle solution (150 μL) was added to 100 μL of serum free medium containing plasmid DNA. After standing for 5 min at room temperature, the mixture was poured gently into the cells. After incubation for 3 h at 37 °C, the medium was replaced with 1 ml of a fresh medium containing FBS, and the cells were incubated for 48 h. Cells were harvested and luciferase activity of the cell lysate was measured by luminometer (Maltibiolumat LB9505, Berthold, Germany). The protein concentrations of the cell lysates were measured by Bradford assay using bovine serum albumin as a standard. The light unit values shown in the figures represent the specific luciferase activity (RLU per mg protein) which is standardized for total protein content of the cell lysate. The measurement of gene transfer efficiency was performed in triplicate.
11 Total DNA of cells was isolated using a DNeasy Tissue Kit (Qiagen). The DNA samples were electrophoresed in a 1% (w/v) agarose gel in TBE buffer. DNA was transferred to Immobilon-NY+ transfer membrane (Millipore Corporation, Bedford, MA) using standard Southern blotting procedures. Hybridization and signal detection were carried out using the ECL Direct Nucleic Acid Labelling and Detection System (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's protocol.