Aerosol based fabrication of thiol-capped gold nanoparticles and their application for gene transfection

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ABSTRACT

In this study, an ambient–spark–produced–Au–nanoparticle–laden nitrogen gas was mixed with an atomized solution of 1–hexanethiol [$CH_3(CH_2)_5SH$] and ethanol (EtOH). By increasing the thiol concentration from 0.1% to 1.0% (*v*/*v*), the size distribution of merged particles (Au–thiol) was changed from bimodal to unimodal configuration. The latter phenomenon is attributed to quantitative incorporation of Au nanoparticles into atomized particles. Measurements of cell viability and transfection revealed that even though the merged particles had a higher cytotoxicity (~78% in cell viability > ~49% for polyethyleneimine, PEI) than that did chitosan (~96%), the transfection (2.56×10^6 in RLU mg^{-1}) of gene was higher than those for chitosan (7.63×10^4) and PEI (6.84×10^5).

Keywords: aerosol fabrication; thiol–capped; Au nanoparticle; gene transfection

1 INTRODUCTION

The usual route to functionalize gold nanoparticles is wet chemistry. In contrast to classical wet chemical methods, aerosol processing involves a much more limited number of preparation steps [1]. It also produces materials continuously, allowing for a straightforward collection of particles and generating low waste. The combination of aerosol processing and more conventional chemical routes has the potential to bring a "wind of change" to the synthesis of advanced nanomaterials [2]. In this work, functionalized gold nanoparticles were synthesized "on the fly" in a serial aerosol reactor (Fig. 1). Spark generated Au nanoparticles passed over a collison atomizer (1.00% (*v*/*v*) 1-hexanethiol solution, EtOH based) orifice where they mixed with atomized particles to form hybrid droplets. The droplets then passed through a heated tubular flow reactor operating at a 90°C wall temperature to drive ethanol from the droplets, resulting in thiol–capped nanoparticle gold.

2 METHODS

The size distributions of the aerosol particles are measured using a scanning mobility particle sizer (SMPS). The SMPS system, which measures the mobility equivalent diameter, is operated at a sample flow of 0.3 L min^{-1}, a sheath flow of 3.0 L min^{-1} (measurement range: 4.61–156.8 nm). TEM (CM-100, FEI/Philips, US) images were obtained at an accelerating voltage of 19-180 kV. The zeta potential of sample/pDNA complexes was determined using a zeta potential analyzer (Nano ZS-90, Malvern Instruments, UK).

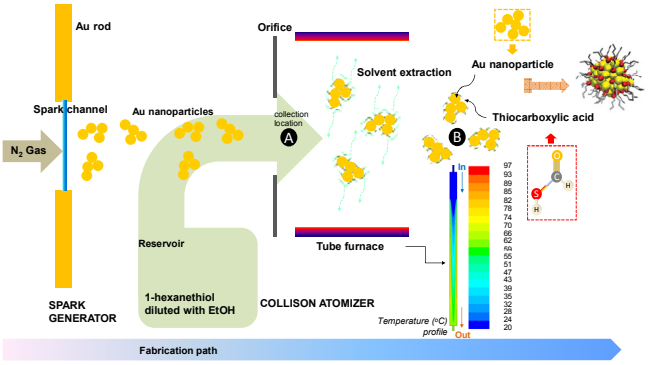


Figure 1: Aerosol based fabrication of thiol–capped Au nanoparticles.

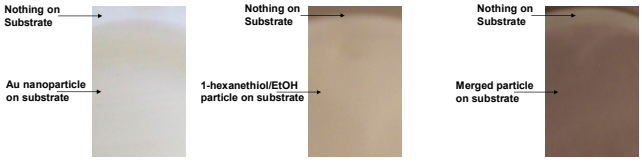


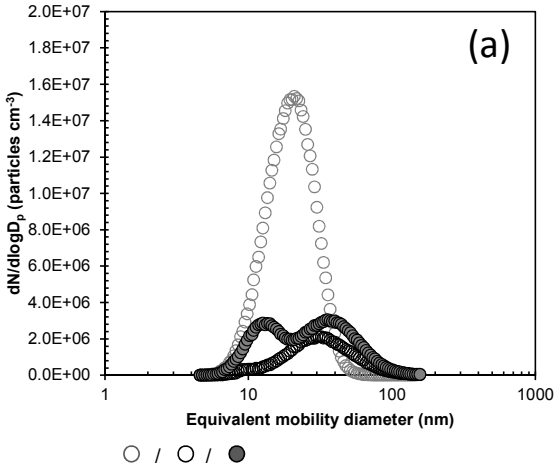
Figure 2: Photographs of sampled substrates for Au, 1–hexanethiol/EtOH, and merged particles.

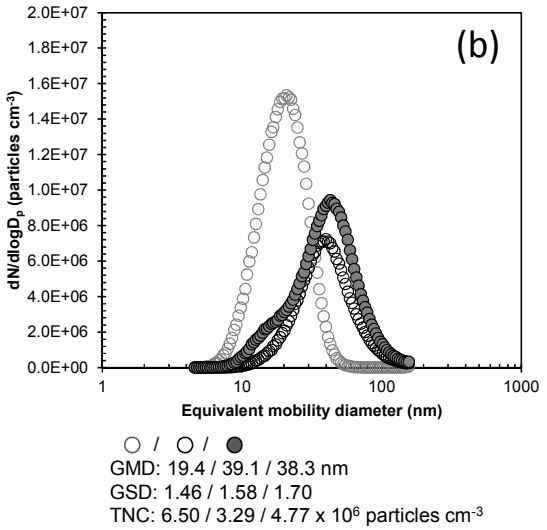
Before *in vitro* definitions, the sampled particles (Fig. 2) on a polytetrafluoroethylene substrate (0.2 µm pore size, 47 mm diameter, 11807–47–N, Sartorius, Germany) were detached in an ultrasound bath for 10 s. 1×10^6 HEK 293 cells pre-incubation in a 24-well culture plate for 24 h were replaced separately with the merged particle and control samples. After challenged with the different samples, cells were replaced with 2 mL culture medium containing MTT assay reagent (4 mg mL^{-1}) and incubated for additional 4 h. The formed purple crystals were dissolved by 2 mL dimethyl sulfoxide (DMSO). 250 µL of the DMSO solutions from the culture wells were loaded into a 96-well plate and had the absorbance measured at 570 nm by an ELISA plate reader (Thermo Multiskan Spectrum, US). The percentage cell viability was related to untreated control cells.

HEK 293 cells were seeded at a density of 1×10^5 cells well^{-1} in 24-well plate. The cells were treated with polyplex solution containing 2 mg of pDNA at various weight ratios for 4 h at 37°C. Luciferase activity was measured with a luminometer (TD-20/20, Promega, US). The final luciferase activity was expressed as RLU mg^{-1} of protein. Inverted fluorescent microscope (DMI 4000 B, Leica, Germany) was used to observe the EGFP expression of the polyplexes in the 293 cells.

3 RESULTS AND DISCUSSION

Fig. 3 summarizes results of the size distributions of Au, the atomized hexanethiol/EtOH solution, and the merged particles. The total number concentration (TNC), geometric mean diameter (GMD), and geometric standard deviation (GSD) of the merged particles are 2.35×10^6 particles cm^{-3}, 25.3 nm, and 1.93, respectively, as shown in Fig. 3a. The same data for the Au nanoparticles are 6.50×10^6 particles cm^{-3}, 19.4 nm, and 1.46, respectively, and for the atomized hexanethiol/EtOH particles are 1.17×10^6 particles cm^{-3}, 30.7 nm, and 1.71, respectively. For merged particles (at thiol 0.1%), although a large proportion of the Au nanoparticles were incorporated into larger hexanethiol/EtOH particles, some of the Au nanoparticles remain in the initial state. This implies that the number concentration of hexanethiol/EtOH particles was not sufficient to collect all the Au nanoparticles at the orifice outlet. The size distributions after the merging of the Au and atomized hexanethiol/EtOH particles changed when the thiol concentration was increased to 1.0%, as shown in Fig. 3b–3c. In Fig. 3c, the Au nanoparticles are shown to be nearly quantitatively incorporated into atomized hexanethiol/EtOH particles. The size distribution of the merged particles (at thiol 1.0%) is similar to the atomized particles. This implies that nearly quantitative incorporation of the Au nanoparticles into the hexanethiol/EtOH particles may be possible at ~1.0% thiol concentration.





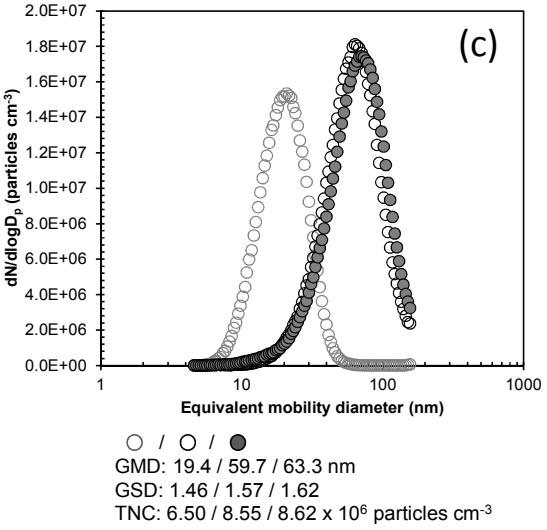


Figure 3: Size distributions of the spark generated Au, collison atomized hexanethiol/EtOH, and merged particles. The fabrication was applied using (a) 0.1% (*v*/*v*), (b) 0.5% (*v*/*v*), and (c) 1.0% (*v*/*v*) thiol concentrations (in EtOH). The spark generated Au, hexanethiol/EtOH, and merged particles are gray (unfilled), black (unfilled), and black (gray filled) circles, respectively.

Fig. 4 shows representative TEM images of collected Au, hexanethiol/EtOH, and merged particles. Merged particles were collected both before and after passing through the tube furnace. The mean mode diameters of Au and the droplet particles are 16 ± 4.1 nm and 52 ± 11.2 nm, respectively. The center image shows that Au nanoparticles collected at the orifice outlet were entirely incorporated into atomized droplet particles. After thermal treatment in the tube reactor, the size of the embedded Au nanoparticles increased from ~16 to ~28 nm, whereas the overall sizes of

the merged particles shrank due to evaporation of volatile material.

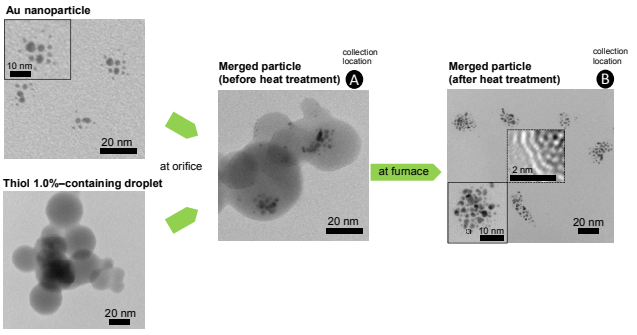


Figure 4: TEM images of spark generated Au and collison atomized hexanethiol/EtOH (left), and merged (center, before passing through the tube furnace; right, after passing through the tube furnace) particles.

Specifically, the spherical shapes of primary particles evolved to more irregular shapes, and the particle surfaces became more corrugated (see inset). This may originate from the adsorption of thiol molecules, resulting in an interaction between S and Au [3-6]. The morphologies of the merged particles is consistent with thiol–capped Au nanoparticles reported in previous reports [7-10].

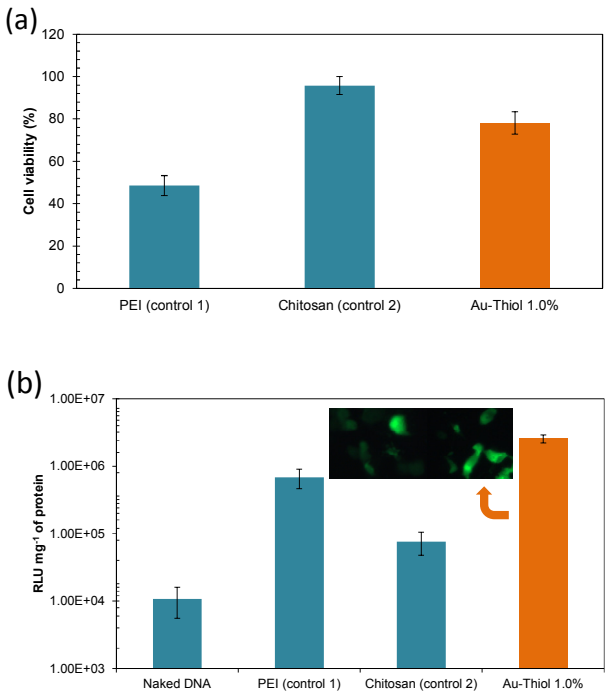


Figure 5: *In vitro* definitions of (a) cell viability and (b) gene transfection efficiency for merged particles.

We tested the cytotoxicity and gene transfection properties of merged particles as a potential material for biomedical applications. Human embryonic kidney (HEK) 293 cells were incubated with merged particles (at thiol 1.0%) for 24 h, and cell viability was determined through a standard MTT [3–(4,5–dimethylthiazol–2–yl)–diphenyltetrazolium bromide] assay (Fig. 5a). The results show that cell viability was ~78% for the merged particles, while the measured viabilities of the PEI and chitosan control systems were ~49% and ~96%, respectively. This implies that the merged particles have a biocompatibility that may be suitable in a clinical context. PEI and chitosan have been widely applied in biomedical applications because of their excellent properties such as biocompatibility, non–toxicity, or biodegradability [11,12]. We next examined the ability of the merged particles to transfect HEK 293 cells using plasmid deoxyribonucleic acid (pDNA) that contain the luciferase and enhanced green fluorescent protein (EGFP) gene. The transfection efficiencies of sample/pDNA complexes in the HEK 293 cell line were higher than that of naked DNA (Fig. 5b). The efficiency for the merged particles was the highest, even a higher than those of chitosan and PEI. Inset of Fig. 5b shows fluorescence of HEK 293 cells for the merged particles derived from EGFP expression, which further comfirmed the transfection. The higher efficiency of the merged particles could relate zeta potential (19.7 ± 4.85: Au–thiol > 13.3 ± 1.91: PEI > -1.59 ± 0.60: chitosan) of sample/pDNA complexes, and might also ascribe to the combination of high affinity between the luciferase and merged particles and relatively small size (~60 nm) of the merged particles (*cf.* ~120 nm for PEI and ~165 nm for chitosan).

4 CONCLUSIONS

For the first time, an aerosol–based method has been used to construct thiol–capped Au nanoparticles. These results further establish aerosol processing as an efficient, scalable, and generalizable method for designing and fabricating an extraordinary broad range of functionalized nanobiomaterials.

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