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Optimization of the composition and dosage of PEGylated polyethylenimine-entrapped gold nanoparticles for blood pool, tumor, and lymph node CT imaging

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ABSTRACT

Gold nanoparticles (Au NPs) with a high X-ray attenuation coefficient have a good potential in CT imaging applications. Here, we report the design and synthesis of Au NPs entrapped within polyethylene glycol (PEG)-modified branched polyethyleneimine (PEI) with varying the initial Au salt/PEI molar ratios and with the remaining PEI surface amines being acetylated for blood pool, lung tumor and lymph node CT imaging. The formed unacetylated and acetylated PEGylated PEI-entrapped Au NPs (Au PENPs) were characterized *via* different methods. We show that the PEGylated PEI is an effective template to entrap Au NPs having a uniform size ranging from 1.7 nm to 4.4 nm depending on the Au salt/PEI molar ratio. After optimization of the composition-dependent X-ray attenuation effect, we then selected $\{(Au^0)_{100}$-PEI-NHAc-*m*PEG$\}$ NPs for biological testing and show that the particles have good cytocompatibility in the given concentration range and can be used as a contrast agent for effective CT imaging of the blood pool of rats, lung cancer model of nude mice and lymph node of rabbits after intravenous injection. For each application, the injected dosage of the particles was optimized. In addition, the $\{(Au^0)_{100}$-PEI-NHAc-*m*PEG$\}$ NPs could be excreted out of the body with time. Our results indicate that the formed Au PENPs with an appropriate composition and dosage hold a great promise to be used for CT imaging of various biosystems.

1. Introduction

Computed tomography (CT) has become one of the most popular diagnostic imaging techniques due to its merits such as cost effectiveness, high density and spatial resolution, deep tissue penetration capability, as well as facile three dimensional reconstruction [1,2]. Although the contrast resolution of CT is much higher than that of conventional radiography, it is still difficult to distinguish subtle changes of soft tissues due to the fact that most soft tissues have similar CT values, ranging from 0 to 50 Hounsfield units (HU) [3]. High-quality CT images need the application of CT contrast agents. Traditional contrast agents that used in clinic is mainly iodine-based small molecular contrast agents (*e.g.*, Omnipaque) with serious drawbacks such as renal toxicity at a relatively high concentration, short imaging time and nonspecificity [4–6]. All these shortcomings have limited their wide applications in CT diagnosis.

Recently, nanotechnology has gained considerable attention [7–14]. The formed nanoparticles (NPs) can be used not only as imaging contrast agents [15–18] but also as nanocarriers of drug and gene delivery [19–22]. A range of nanoscale contrast agents have been developed for CT imaging, such as tantalum oxide NPs, bismuth sulfide NPs [23], Er^{3+}-doped Yb_2O_3 up-conversion NPs [24], and gold NPs (Au NPs) [25–28]. Among all these nano-sized CT contrast agents, Au NPs have been given considerable attention because of their better X-ray attenuation property than that of iodinated CT contrast agents, due to the high atomic number (Z = 79) and k-edge value (80.7 keV) of Au element. Au NPs also have good chemical stability and biocompatibility, and can be modified with various functional molecules [15,16,29,30]. Various methods for producing Au NPs have been developed, and reduction of $HAuCl_4$ by citrate is one of the most popular methods [31];

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however the resulting citrate-stabilized Au NPs are not stable in biological media. The traditional way to improve the stability of Au NPs is the modification of thiolated polyethylene glycol (PEG) *via* ligand exchange [32]. Another versatile way is to use template or stabilizer to synthesize Au NPs, such as dendrimers [33,34], micelles [35,36] and proteins [37–39]. The formed Au NPs have extended blood circulation time, better X-ray attenuation property than iodinated small molecular CT contrast agents, and good biocompatibility.

Poly(amidoamine) (PAMAM) dendrimer-entrapped Au NPs have a high X-ray absorption coefficient and the average amount of Au entrapped within each dendrimer is controllable [16]. Polyethyleneimine (PEI) with the branched internal structure and abundant surface primary amines has a structural similarity to PAMAM dendrimers [40–42]. As opposed to PAMAM dendrimers, PEI is cost-effective and widely available due to the easy preparation using an AB-type monomer *via* a simple one-step reaction [43–45]. In our previous study, PEI with surface modified by PEG was proven to be able to entrap Au NPs for CT imaging applications. The synthesized PEI-entrapped Au NPs (Au PENPs) can be tuned to have an Au core size in a range of 1.9–4.6 nm and are water-soluble, stable, and noncytotoxic in a studied concentration range [46]. After the modification of folic acid, the PEGylated Au PENPs can be used for targeted CT imaging of tumors [18]. Furthermore, the PEGylated PEI can be used to simultaneously entrap Au NPs and load gadolinium for CT/MR dual mode imaging [17]. As a drug delivery system, PEI can also be used to load anticancer drug doxorubicin for *in vitro* or *in vivo* targeted cancer therapy [47,48]. Although the potential to use PEGylated Au PENPs has been demonstrated, the effect of the composition and dosage on the X-ray attenuation effect and the CT imaging performance *in vivo* has not been systematically investigated.

In this present study, branched PEI was first modified with PEG monomethyl ether (*m*PEG) and then used as a template to synthesize Au NPs with different Au salt/PEI molar ratios (Fig. 1). The synthesized Au PENPs were then characterized in different ways, and the X-ray attenuation effect of the particles was systematically investigated and optimized based on the composition of the particles. Through cell morphology observation and cell viability assay, the cytocompatibility of the particles was evaluated. The synthesized Au PENPs were then used for blood pool, tumor and lymph node CT imaging under different Au concentrations. To our knowledge, this is the first report related to the investigation of the influence of composition and dosage on the X-ray attenuation effect of the Au PENPs and the performance in CT imaging of different biological systems, respectively.

2. Materials and methods

2.1. Synthesis of PEGylated Au PENPs

PEGylated Au PENPs were synthesized according to our previous study [46]. Briefly, *m*PEG-COOH (60.0 mg, 30.0 μmol, 5.0 mL in water) was activated by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS) (10 mol equiv. of *m*PEG-COOH, 57.5 mg EDC and 34.5 mg NHS) under magnetic stirring for 3 h at room temperature. The activated *m*PEG-COOH was then dropped into a PEI solution (25.0 mg, 1.0 μmol, 10 mL in water) under magnetic stirring at room temperature. The reaction mixture was continuously stirred for 3 days to complete the reaction. Then the reaction mixture was extensively dialyzed against phosphate buffered saline (PBS, three times, 2 L) and water (six times, 2 L) using a dialysis membrane with an MWCO of 14,000 for 3 days, followed by a freeze-drying process to obtain the PEI·NH_2-*m*PEG product.

$NaBH_4$ reduction chemistry was used to prepare PEGylated Au PENPs with initial Au salt/PEI molar ratios of 50:1, 100:1, 200:1, 300:1, and 400:1, respectively. Using the Au salt/PEI molar ratio at 100:1 as an example, a certain amount of $HAuCl_4$ solution (10 mg/mL, in water) was dropped into a water solution of PEI·NH_2-*m*PEG (25 mg, 100 mL) under vigorous magnetic stirring. Thirty minutes later, icy cold $NaBH_4$ solution (5 mL, in water) having 5 molar equiv. to the Au salt was rapidly added into the Au salt/PEI·NH_2-*m*PEG mixture solution under stirring. Within a few seconds, the reaction mixture turned deep red. The reaction mixture was continuously stirred for 3 h, and then subjected to dialysis and lyophilization processes to obtain the product of {$(Au^0)_{100}$-PEI·NH_2-*m*PEG}. The finally formed PEGylated Au PENPs with other compositions were denoted as {$(Au^0)_n$-PEI·NH_2-*m*PEG} NPs (n = 50, 200, 300, and 400, respectively).

Some of the formed {$(Au^0)_n$-PEI·NH_2-*m*PEG} NPs were subjected to acetylation to modify the remaining PEI surface amines according to protocols reported in the literature [46]. Briefly, triethylamine (92.0 μL) was dropped into a water solution of {$(Au^0)_n$-PEI·NH_2-*m*PEG} NPs (the mass of PEI·NH_2-*m*PEG was 25 mg in 100 mL water) under vigorous magnetic stirring for 30 min. Acetic anhydride (76.6 μL) with 5 molar equiv. to the remaining PEI primary amines was then added to the above mixture solution, and the mixture was stirred for 24 h. Then the reaction mixture was dialyzed and lyophilized according to the above protocols to get the {$(Au^0)_n$-PEI·NHAc-*m*PEG} NPs.

2.2. Cytotoxicity and cellular uptake assays

A549 cells were regularly cultured and passaged in Dulbecco modified Eagle's medium (DMEM) containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C and 5% CO_2.

The cytotoxicity of the PEGylated Au PENPs was measured by Cell Counting Kit (CCK-8) assay. Briefly, A549 cells were seeded at a density of 1×10^4 cells per well in a 96-well plate with 100 μL DMEM. After overnight culture to make the cells well attached, the medium was substituted with fresh medium containing {$(Au^0)_{100}$-PEI·NHAc-*m*PEG} NPs with different Au concentrations (0–200 μM). The cells were then incubated at 37 °C and 5% CO_2 for 24 h. After that, the cells were rinsed with PBS for 3 times, and added with CCK-8 (5 μg/mL, 10 μL in PBS) mixed with 90 μL DMEM for each well. The cells were then regularly incubated for another 4 h. The assays were performed according to the manufacturer's instructions. The absorbance at 450 nm in each well was recorded by a Thermo Scientific Multiskan MK3 ELISA reader (Thermo Scientific, Waltham, MA). Triplicate wells for each sample were

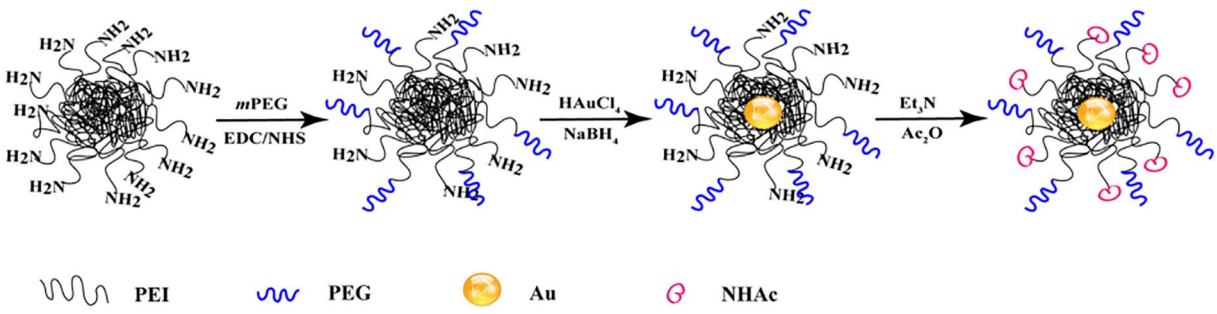

Fig. 1. Schematic illustration of the synthesis of {$(Au^0)_{100}$-PEI·NHAc-*m*PEG}. Et_3N and Ac_2O represent triethylamine and acetic anhydride, respectively.