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Influence of ligands property and particle size of gold nanoparticles on the protein adsorption and corresponding targeting ability

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ABSTRACT

Nanoparticulated vesicles were widely used for carriers of drugs and imaging probes. To improve the targeting delivery efficiency of these vesicles, ligands were often functionalized onto their surfaces. However, the interaction between vesicles and plasma proteins may cover the ligands and hinder the targeting delivery. It is important to address the potential influence of ligands modification on plasma protein adsorption and the following targeting delivery. In this study, two common used ligands were chosen as the model: transferrin and RGD peptide. Gold nanoparticles were utilized as model particles. Sodium dodecyl sulfate polyacrylamide gel electrophoresis data demonstrated that higher PEG modification and smaller particle size could reduce the plasma protein adsorption, while ligand modification could increase. The cellular uptake results showed that the targeting ability of smaller ligand RGD peptide would be more easily influenced by the proteins corona.

1. Introduction

Owing to the fast development of nanotechnology, many kinds of nanoparticles have been constructed for drug delivery in the past several decades (Chen et al., 2016; Wang et al., 2017b). However, the *in vivo* distribution of these nanoparticles is determined by their intrinsic properties, for example, particle size, and the physiological conditions of tissues (Perry et al., 2017; Wang et al., 2017b). To improve the distribution of nanoparticles in the diseased tissues and cells, active targeting delivery systems are developed (Gao, 2016; Gao et al., 2013; Sun et al., 2017). Generally, these delivery systems are constructed by modification of active targeting ligands onto nanoparticles. The ligands can be recognized by specific receptors or carriers that highly expressed on diseased cells (Kang et al., 2014). In different kinds diseased cells, many receptors and carriers have been proved to be over expressed (Peer et al., 2007; Quadir et al., 2017). Therefore, ligands for these receptors and carriers are widely used in constructing active targeting delivery systems (Cun et al., 2015; Luo et al., 2017; Ruan et al., 2017). Until now, several active targeting nanomedicines are under clinical evaluation (van der Meel et al., 2013).

However, as soon as the nanoparticles are injected into the blood, the nanoparticles would immediately interact with blood proteins, and the protein corona will form onto the surface of nanoparticles (Gao and He, 2014; Nel et al., 2009). Protein corona is consisted by over 50 kinds of proteins, which would influence the *in vivo* distribution of the nanoparticles (Schottler et al., 2016). Normally, the proteins can be divided into two categories: opsonin and dysopsonin (Thiele et al., 2003). The adsorption of opsonin can elevate the recognization by reticuloendothelial system (RES) and lead to rapid elimination by the body and the adsorption of dysopsonin can improve the blood circulation time of these nanoparticles (Aggarwal et al., 2009; Nel et al., 2009). There are also special kinds of protein in the corona that could be recognized by specific receptors (Gao and He, 2014). For example, Tween 80 modified nanoparticles can adsorb apolipoprotein E (ApoE) and be used for brain targeting delivery because ApoE could be recognized by the receptor on blood brain barrier (Aggarwal et al., 2009; Goppert and Muller, 2005). The formation of protein corona is affect by many factors, including the particle size, shape, surface property, etc (Lundqvist et al., 2008; Wang et al., 2016; Zhang et al., 2017). Thus protein corona must be taken into consideration when constructing targeting delivery systems (Corbo et al., 2016; Mahmoudi et al., 2011).

The targeting efficiency of ligands is also affected by the protein adsorption. Several studies showed the adsorption of proteins could lead to the off-target effect of the ligand modified nanoparticles (Salvati et al., 2013; Xu et al., 2016). Mountain of papers demonstrated that the ligands modification indeed improved the targeting delivery efficiency

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of nanoparticles (Hong et al., 2016; Wei et al., 2015), however, several studies showed that modification with ligands could not elevate the distribution of nanoparticles in the target site (Pearson et al., 2014; Salvati et al., 2013). Due to the contradiction in the literatures, it is important to clearly elucidate the potential effect of protein corona formation on the targeting delivery efficiency of the ligands modified nanoparticles.

Two famous ligands, transferrin (Tf) and RGD peptide (c(RGDyC)) were used to evaluate the interaction between ligands modified nanoparticles and blood proteins, and the influence of the ligands on the composition of protein corona. Tf could bind with Tf receptor and RGD could bind with integrin. Both receptors were highly expressed on the tumor cells, thus U87, a glioma cell line, was selected as model cells (Gao, 2016). Gold nanoparticles (AuNPs) were widely used as model nanoparticles on account of its advantages, including easy controlled particle size, convenient to surface modification and well tolerance (Dreaden et al., 2012; Ruan et al., 2015b). Thus we utilized AuNPs as the model particles.

In this study, AuNPs with three different particle sizes were prepared. Then the ligands were functionalized onto the three kinds of AuNPs. The effect of particle size and ligands modification on the amount and types of proteins in corona was determined by gel electrophoresis. At last, cellular uptake by U87 cell line of ligands modified AuNPs before and after protein corona formation was evaluated.

2. Materials and methods

2.1. Materials

Methoxy-polyethylene glycol-thiol (SH-PEG, MW = 5000) and carboxymethyl-polyethylene glycol-thiol (SH-PEG-COOH, MW = 5000) were purchased from Laysan Bio Inc. (Arab, USA). Chlorauric acid was purchased from Sinopharm Chemical Reagent (Shanghai, China). Transferrin was purchased from Sigma Aldrich (St. Louis, USA). RGD peptide was synthesized by Chinapeptide (Shanghai, China). The U87 cell line was purchased from Chinese Academy of Sciences Cells Bank (Shanghai, China).

2.2. The synthesis of gold nanoparticles

The seed growth method was used for the synthesis of the AuNPs with different particle size (Huang et al., 2007; Perrault and Chan, 2009). 1.25 mL 0.22% (W/V) of chloroauric acid ($HAuCl_4$) solution was add to 25 mL water in three mouth flask. After boiling the solution, 1 mL 1% (W/V) sodium citrate (NaAc) and 0.05% (W/V) citric acid mixture were quickly added and continued boiled for 5 min to obtain gold nanocrystal solution (AuNP1). Then 1.5 mL AuNP1 solution was added into three mouth flask that contained 8.5 mL water. A solution (0.22% $HAuCl_4$ solution 1 mL dilute to 5 mL) and B solution (1% vitamin C solution 0.25 mL, 1% sodium citrate 0.125 mL, dilute to 5 mL) were added dropwisely and separately at room temperature to the flask and stirred violently for 45 min. After heating the mixture to boil and keeping for 30 min, the first growing step nanoparticles solution (AuNP2) was obtained. Furthermore, 2.25 mL AuNP2 solution was add to 7.75 mL water, and then operated in accordance with the method of forming AuNP2 (adding A solution and B liquid, stirring, boiling, and cooling), the second step growth of gold nanoparticles (AuNP3) can be obtained.

2.3. Synthesis of RGD-AuNPs and Tf-AuNPs

2 mL solution from AuNP1, AuNP2 and AuNP3 separately was added to 20 μL 1 mg/mL mixture of mPEG-SH and CM-PEG-SH (mPEG-SH:CM-PEG-SH = 9:1) (Ruan et al., 2015a), stirred mildly for 24 h at room temperature and then centrifuged at 18,000*g*, 12,000*g* and 8000*g* respectively for 15 min. The mixture was then added with EDC (75 mg) and NHS (25 mg) respectively, followed with reaction for 30 min in dark. After centrifugation and 3 times PBS washing, the particles were resuspended in 0.5 mL PBS solution. Finally, Tf (700 μg/mg gold) and RGD (5.6 μg/mg gold) were added respectively and stirred slowly for 4 h to get Tf modified AuNPs (Tf-AuNPs) and RGD modified AuNPs (RGD-AuNPs).

2.4. Characterization of different gold nanoparticles

The particle size and zeta potential of the AuNPs were characterized by laser dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS (Malvern, UK). The morphology of particles was evaluated by transmission electronic microscopy (TEM) (JEM-100CX, JEOL, Japan).

To determine the modification efficiency of Tf, the Tf-AuNPs solution with unmodified Tf was centrifuged and the content of protein in the supernatant was determined by BCA protein kit. The modification efficiency of RGD was determined by high-performance liquid chromatography (HPLC). The supernatant was centrifuged as above, and then 20 μL supernatant was sampled for the HPLC test. Chromatographic condition was as follows: chromatographic column 300SB-C18 (5 μm, 4.6 × 250 mm); mobile phase A liquid was acetonitrile: water: three trifluoroacetic acid (2:98:0.05), B liquid was acetonitrile: water: trifluoroacetic acid (90:10:0.05); 100% A liquid was gradient eluted to 70%; flow rate was 1.2 mL/min; detection wavelength of 210 nm; column temperature was 35 °C.

2.5. Incubation of gold nanoparticles with plasma proteins

1 mL FBS with different concentration (10%, 50% and 100%) was added to the AuNPs mixture, and then incubated for 10 min and 2 h at 37 °C respectively to investigate the effect of incubation time on plasma protein adsorption. After 20 min centrifugation at 15,000*g*, the precipitate was suspended with PBS containing 5 mM EDTA and 0.05% (v/v) Tween 20. After repeated washing for three times, the precipitate was enriched to 50 μL.

2.6. Determination of the plasma proteins on different AuNPs

The total amount of adsorbed protein was determined by BCA method. 20 μL enriched liquid and 200 μL BCA reaction liquid were added to a 96 well plate, and incubated for 30 min at 37 °C. The left 30 μL enrichment solution was used to determine the different adsorption proteins by SDS-PAGE gel electrophoresis. 200 μL SDS sample buffer was added into the 30 μL enrichment solution, denatured at 90 °C for 5 min, and then changed the temperature to 4 °C to stop the reaction. After 15 min centrifugation at 15,000*g*, 5 μL supernatant was used for SDS-PAGE gel electrophoresis. After electrophoresis, rapid silver staining method was used to stain the protein bands on the gel.

To determine the effect of particle size and serum concentration on protein adsorption, AuNPs were centrifuged and the precipitation was added with 1 mL of 10% or 100% of FBS, after 2 h incubation, the mixture was treated as described above to analyze the amount and types of adsorbed proteins.

2.7. Preparation of FITC labeled AuNPs

After adding PEG for 12 h, the thiol-FITC was added into AuNPs solution at the concentration of 10^5 thiol-FITC molecules per particle. 2.7 nM FITC fluorescent probe was added every 0.05 mg NPs, and then stirred at room temperature for another 12 h, the other steps were the same as unlabeled nanoparticles.

2.8. Culture of cells

Human brain glioma U87 cells were used as model cells. The cells were cultured in DMEM medium containing 10% FBS. The density of