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Polysarcosine brush stabilized gold nanorods for *in vivo* near-infrared photothermal tumor therapy

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ABSTRACT

Gold nanorods (AuNRs) are suitable candidates for photothermal therapy *in vivo*, because of their excellent ability to transfer near-infrared (NIR) light into heat. However, appropriate surface should be generated on AuNRs before their *in vivo* application because of the low colloidal stability in complicate biological environment and relatively strong toxicity compared to their pristine stabilizer cetyltrimethylammonium bromide. In the current study, polysarcosine (PS), a non-ionic hydrophilic polypeptoid whose structure is similar to polypeptides, bearing repeating units of natural α-amino acid, was used to stabilize AuNRs due to its excellent hydrophilicity and biocompatibility.

Polysarcosine with optimized molecular weight was synthesized and used to modify AuNRs by traditional ligand exchange. The grafting of PS on AuNRs was evidenced by fourier transform infrared (FTIR) spectroscopy and the alternation of surface zeta potential. The polysarcosine coated AuNRs (Au@PS) showed good stabilities in wide pH range and simulated physiological buffer with the ligand competition of dithiothreitol (DTT). The Au@PS NRs had neglectable cytotoxicity and showed efficient ablation of tumor cells *in vitro*. Moreover, Au@PS NRs had a longer circulation time in body that resulted in a higher accumulation in solid tumors after intravenous injection, compared to AuNRs capped with polyethylene glycol (PEG). Photothermal therapy *in vivo* demonstrated that the tumors were completely destroyed by single-time irradiation of NIR laser after one-time injection of the polysarcosine capped AuNRs. The Au@PS NRs did not cause obvious toxicity *in vivo*, suggesting promising potential in cancer therapy.

Statement of Significance

In current study, polysarcosine (PS), a non-ionic hydrophilic polypeptoid whose structure is similar to polypeptides, bearing repeating units of natural α-amino acid, was used to stabilize AuNRs due to its excellent hydrophilicity and biocompatibility. The polysarcosine coated AuNRs (Au@PS) showed good stabilities in wide pH range and simulated physiological buffer. The Au@PS NRs had very low cytotoxicity and showed high efficacy for the ablation of cancer cells *in vitro*. Moreover, Au@PS NRs had a longer circulation time in blood that led to a higher accumulation in tumors after intravenous injection, compared to AuNRs capped with polyethylene glycol (PEG). *In vivo* photothermal therapy showed that tumors were completely cured without reoccurrence by one-time irradiation of NIR laser after a single injection of the polysarcosine modified AuNRs.

1. Introduction

Recently, inorganic nanomaterials have attracted intensive attention in diseases diagnosis and therapy such as molecular recognition/bioimaging, delivery of therapeutics, and photothermal/photodynamic therapy due to their unique features [1–6].

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However, it remains a big challenge to realize their functions in complicate biological environment. One of the important requests for *in vivo* application is that the NPs must have excellent colloidal stability and biocompatibility [7–10]. Moreover, the NPs need to accumulate in the tumor site to maximize their therapeutic effect and minimize their side effects to healthy tissues. It is well known that small particles have the "passive targeting" ability to tumor sites based on enhanced permeability and retention (EPR) effect in solid tumors that have leaky vascular vessels and damaged lymphatic drainage system [11,12]. The EPR effect requires the systematically injected NPs to stay in blood circulation for a long enough time to have chance to accumulate in solid tumors. Nanoparticles that aggregate in blood or nonspecifically interact with cells/tissues will be easily cleared, subsequently leads to a shorter circulation time and significantly reduce the efficiency of tumor accumulation [10,13–14].

To fulfill this aim, appropriate surface modification is very effective and is widely adopted [15–20]. Hydrophilic polymers such as poly(ethylene glycol) (PEG) [21,22] and zwitterionic molecules such as phosphorylcholine and sulfobetaine [23–24], with low interfacial energy and subsequently high resistance to protein adsorption and cell/tissue recognition, have been used to decorate the surfaces of NPs to endow them with excellent colloidal stability, biocompatibility, and *anti*-fouling ability both *in vitro* and *in vivo* [25]. Alternatively, new hydrophilic polymers are still being explored and expected to overcome the problems of PEG such as low functionality and potential immune reaction after repeated high doses [26–28].

Synthetic polypeptide- and polypeptoid-based materials, are unique biocompatible and biodegradable polymers with structures mimicking natural proteins [29–31]. Polysarcosine (PS), is a non-ionic hydrophilic polypeptoid with similar structure of polypeptides, bearing repeating units of sarcosine, an endogenous but non proteinogenic amino acid [32,33]. Several important groups including Messersmith [34], Kimura [35,36], Whitesides [37], Zuckerman [38], Ling [39–41], and Barz [42–44] have demonstrated the hydrophilicity and protein resistance ability of PS, indicating its potential to functionalize nanomaterials for applications in biomedicine. For example, there have been several kinds of poly (sarcosine)-block-poly(peptide) hybrids bearing the stealth-like properties were used to prepare carrier systems completely based on endogenous amino acids. Recently, Zentel et al. reported the synthesis of polysarcosine polymers with multidental lipoic acid end groups [45]. The obtained multidentate polymeric ligands are successfully used to prepare stable, water-soluble quantum dots.

In last decades, the utilization of gold nanorods (AuNRs) in photothermal therapy (PTT) has shown great promise to combat malignant cancer due to their unique surface plasmon resonance (SPR) which can efficiently and rapidly transfer light into localized heat [46–49]. Thus, when AuNRs were accumulated at cancer cells, irradiation with an optical laser induces enough heat to destroy cancerous cells [50–56]. In particular, NIR light with the wavelength of 650–900 nm has the ability to penetrate in tissues up to 4–10 mm, without severe damaging normal tissues due to comparable low absorption [57,58]. However, the as-prepared AuNRs capped with excess positively charged surfactant cetyltrimethylammonium bromide (CTAB), which are toxic at high concentration and unstable in serum/blood, which are not suitable for clinical use [22,59]. Therefore, a surface coating with excellent biocompatibility and anti-fouling property is necessary for the application of AuNRs. In this study, disulfide functionalized polysarcosine, prepared by ring opening polymerization [39,40], is used to modify the surface of AuNRs to enhance their colloidal stability and applied for *in vivo* cancer treatment (Scheme 1, Au@PS). The colloidal stability, cytotoxicity of the AuNRs@PS and photothermal ablation of tumor cells *in vitro* is systematically studied, with similar molecular weight PEG functionalized AuNRs@PEG as control. Their distribution pattern, toxicity and photothermal therapy effect *in vivo* are further investigated.

2. Experimental section

2.1. Materials

Dithiothreitol (DTT) was obtained from Sangon Biotech (Shanghai) Co., Ltd. Hydrogen tetrachloroaurate hydrate ($HAuCl_4{\cdot}4H_2O$), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$), cetyltrimethylammonium bromide (CTAB), and ascorbic acid (AA) were purchased from Sinopharm Chemical Reagent Co., Ltd. Other common chemicals were of analytical grade and used as received if without specific description. 2,2′-Dithiodiethanol, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), thiol functionalized mPEG (Mn ~2 kD), and fluorescein diacetate (FDA), propidium iodide (PI) and Annexin V-FITC apoptosis detection kit were purchased from Sigma-Aldrich. Milli-Q water was used throughout the experiments.

2.2. Synthesis of disulfide functionalized polysarcosine

The polymer was synthesized according to previous reported method [39,40,60]. In brief, the polymerization was performed using Schlenk technique and all polymerization tubes were predried and purged with argon. Exemplarily, the preparation of polysarcosine was performed as follows. Sarcosine N-thiocarboxyanhydride (Sar-NTA) (1.331 g, 10.15 mmol) was dissolved in 9.0 mL of dry acetonitrile, followed by 1.5 mL of cystamine in acetonitrile solution (0.1126 mol/L). The tube was sealed and kept in a 60 °C oil bath for 48 h. The polymer was precipitated by diethyl ether and dried in vacuum (0.739 g, 98.9%). Degree of polymerization (DP) of PS was 75 (Mn ~5.3 kD), according to ^{1}H NMR and GPC (Fig. S1).

2.3. Preparation of PS coated AuNRs

The AuNRs were prepared according to previous established methods with slight modifications [60–63]. Firstly, the colorless growth solution was prepared in advance by mixing of 5 mL 10 mM $HAuCl_4$, 2.2 mL 20 mM $AgNO_3$, 0.8 mL 1 M HCl, 0.7 mL 78.8 mM AA and 100 mL 100 mM CTAB. The gold seeds were prepared by mixing 0.6 mL 10 mM pre-cooled $NaBH_4$ solution with 10 mL CTAB (100 mM) and 0.25 mL $HAuCl_4$ (2.5 mM), followed with 2 min vigorous stirring and then kept for 2 h at 25 °C. 1.2 mL aged seed solution was added into 100 mL growth solution, stirred for 4 min and then kept still overnight at 27 °C. The concentration of obtained AuNRs was measured by ICP-MS (Xseries II, Thermo Elemental Corporation, USA). The production yield of CTAB capped AuNRs from $HAuCl_4$ is 58.4 %wt, which is defined as the weight ratio of obtained AuNRs compared to the weight of gold element in the $HAuCl_4$.

About 10 mg AuNRs were centrifuged twice at 10000*g* for 15 min to remove free CTAB ligands, and then were incubated in 5 mg/mL thiol PEG or disulfide PS solutions in the presence of 1 mM $NaBH_4$, followed by gentle shaking for 2 days. Au@PEG and Au@PS NRs were collected by centrifugation (10000*g* for 15 min) and washed five times against water to remove free polymers. The production yields for AuNRs@PEG and AuNRs@PS from CTAB capped AuNRs are 72.1%wt and 74.3%wt respectively. The loss of AuNRs is mainly happened during centrifugation process after ligand exchange.