Biomaterials 34 (2013) 3503–3510

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

The effective nuclear delivery of doxorubicin from dextran-coated gold nanoparticles larger than nuclear pores

Hongje Jang [b], Soo-Ryoon Ryoo [a], Kostas Kostarelos [c], Sang Woo Han [b,d,**], Dal-Hee Min [a,*]

[a] Institute for Basic Science, Department of Chemistry, Seoul National University, Seoul 151-742, Republic of Korea
[b] Department of Chemistry and KI for the NanoCentury, KAIST, Daejeon 305-701, Republic of Korea
[c] Nanomedicine Lab, Centre for Drug Delivery Research, UCL School of Pharmacy, University College London, London WC1N 1AX, UK
[d] Center for Nanomaterials and Chemical Reactions, Institute for Basic Science, Daejeon 305-701, Republic of Korea

ARTICLE INFO

Article history:
Received 4 January 2013
Accepted 22 January 2013
Available online 8 February 2013

Keywords:
Cancer
Dextran
Doxorubicin
Gold nanoparticle
Nuclear delivery

ABSTRACT

To date, gold nanoparticles (AuNPs) have been investigated for diverse bioapplications. Generally, AuNPs are engineered to possess surface coating with organic/inorganic shells to increase colloidal stability in biological solutions and to facilitate chemical conjugation. In the present study, we developed a strategy to prepare dextran-coated AuNPs with control over its size by simply boiling an aqueous solution of Au salt and dextran, in which dextran serves as both reducing agent for AuNP (Au(0)) formation from Au(III) and AuNP surface coating material. The prepared dextran-coated AuNPs (dAuNPs) maintained its colloidal stability under high temperature, high salt concentration, and extreme pH. Importantly, the dAuNP remarkably improved efficacy of an anti-cancer agent, doxorubicin (Dox), when harnessed as a Dox delivery carrier. The half-maximal inhibitory concentration (EC50) of Dox-conjugated dAuNP with diameter of 170 nm was ~9 pM in HeLa cells, which was 1.1×10^5 times lower than that of free Dox and lower than any previously reported values of Dox-nanoparticle complex. Interestingly, smaller AuNPs with 30 and 70 nm showed about 10 times higher EC50 than 170 nm AuNPs when treated to HeLa cells after conjugation with Dox. To achieve high cytotoxicity as cancer therapeutics, Dox should be delivered into nucleus to intercalate with DNA double helix. We show here that Dox-AuNPs was far more efficient as an anti-cancer drug than free Dox by releasing from AuNPs through spontaneous degradation of dextran, allowing free diffusion and nuclear uptake of Dox. We also revealed that larger AuNPs with lower degree of dextran crosslinking promoted faster degradation of dextran shells.

1. Introduction

Many strategies for targeted drug delivery to disease sites and tissues have been developed to enhance drug efficacy and reduce side effects [1–7]. Most of them focus on the selective cellular uptake of drug-loaded carriers by abnormal cells, for example, cancer cells [8–11]. Generally, intracellular localization of nanoparticles showed accumulation mostly in the cytoplasm but hardly in nuclei [12–15]. In fact, nuclear transport of nanoparticles is challenging since the nuclear pores have a specific diameter of 30–40 nm and the diffusion rate through such small size pores is greatly dependent on the nanoparticle size [16]. However, nuclear targeting and transport through the nuclear pore complex are important, especially in order to enhance the efficacy of anti-cancer agents such as doxorubicin (Dox), because their cytotoxic activity depends on its intercalation with DNA and subsequent inhibition of topoisomerase II to block DNA replication inside the nucleus [17]. Therefore, the development of drug delivery platforms with the capacity to translocate drugs to the nucleus is important. Ideal drug delivery system (DDS) should keep anti-cancer agents chemically intact and present long-circulation time until they are delivered into the target cells and its nucleus. For this purpose, several nanoparticle-based systems including silica nanoparticles and quantum dots have been developed with nuclear transport capability by conjugation of nuclear localization signals (NLS) [18–22]. For intranuclear uptake of NLS-drug-nanoparticle complex and for achieving high efficacy, the size of nanoparticles has been restricted below ~30–40 nm, smaller than nuclear pores, which limits types of applicable nanoparticles.

Here, we report the synthesis of dextran-coated gold nanoparticles (dAuNPs) for efficient nuclear delivery of Dox, achieved without any NLS (Scheme 1). Gold nanoparticle (AuNP) is one of the

* Corresponding author. Fax: +82 2 875 6636.
** Corresponding author. Department of Chemistry and KI for the NanoCentury, KAIST, Daejeon 305-701, Republic of Korea. Fax: +82 42 350 2810.
E-mail addresses: sangwoohan@kaist.ac.kr (S.W. Han), dalheemin@snu.ac.kr (D.-H. Min).

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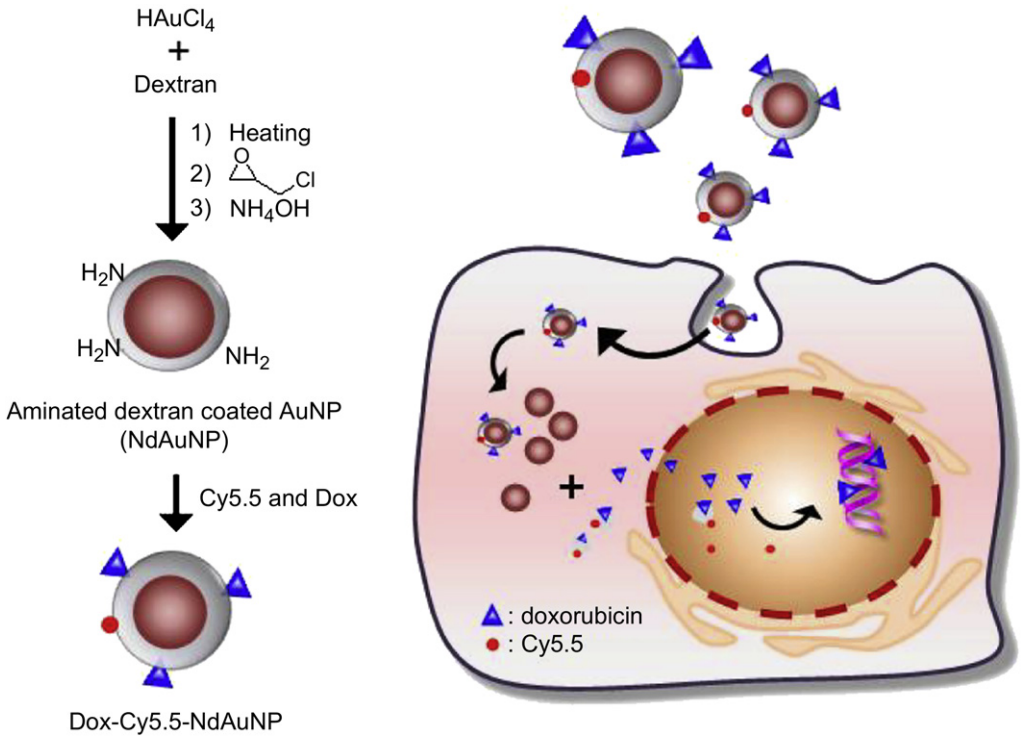
http://dx.doi.org/10.1016/j.biomaterials.2013.01.076



Scheme 1. Synthesis of dextran-coated AuNPs and subsequent conjugation of Cy5.5 and doxorubicin. Dextran plays roles as a reducing agent and a coating material for the AuNPs at the same time. Size of the NdAuNPs can be easily tuned by varying ratio of gold salt to dextran. Bio-degradable dextran coating of the AuNPs might be easily broken into pieces to release doxorubicin from AuNP cores and therefore, diffusion and accumulation of doxorubicin into nucleus could be feasible regardless of the size of original AuNPs.

nanoparticles that have been actively studied in biomedical research in recent years [23,24]. Unique properties including fluorescence quenching [25,26], surface plasmon resonance [27] and relatively low cytotoxicity expedited its application in wide range of researches, demonstrated in immunostaining [28], single particle tracking [29], contrast agents for TEM and X-ray [30,31], vehicle for drug delivery [32,33], hyperthermia [34] and optical trigger [35]. Chemically modified dextrans have been used for Dox delivery. For example, dextran nanoparticles were synthesized by disulfide bond mediated crosslinking and Dox was encapsulated inside the dextran particles [36] and acetal-dextran was self-assembled and stabilized by living radical polymerization [37]. These approaches harnessed dextran only as matrix and Dox was loaded non-covalently during particle preparation. In our strategy, dextran served as a biodegradable polymer that constitutes the shells of the metallic nanoparticle, AuNPs, allowing the release of the covalently-conjugated Dox. The released Dox can then freely diffuse through nuclear pores and accumulate into the nucleus to achieve high apoptotic activity regardless of the size of parent AuNPs. In the present synthesis of the dAuNPs, dextran, a non-cytotoxic carbohydrate polymer, plays roles as a reducing agent for gold nanoparticle core formation and a surface coating for generated gold cores as well [38,39]. We previously reported that the dAuNPs with diameter of ~80 nm exhibit superb biocompatibility and colloidal stability even under harsh conditions such as high temperature, high salt concentrations and extreme pH [40]. In the present study, the dAuNPs were prepared with three different sizes of 30, 70 and 170 nm in diameter by varying relative concentration of gold salt to dextran. We then investigated colloidal stability, biocompatibility and most importantly, efficiency as a Dox delivery vehicle for anti-cancer agents. We hypothesized that although nuclear delivery of the whole Dox–dAuNP complex larger than nuclear pore (30–40 nm) was hardly possible, Dox–dAuNPs could release Dox from AuNPs through spontaneous degradation of dextran, allowing free diffusion of Dox from cytoplasm into nucleus after cellular uptake of Dox–dAuNPs. We also investigated that degradation kinetics of dextran shell could be tuned by varying the size of AuNPs and controlling the degree of dextran crosslinking.

2. Materials and methods

2.1. Materials

Hydrogen tetrachloroaurate(III) hydrate was purchased from Kojima Chemicals Co. (Sayama, Saitama, Japan). Ammonium hydroxide (28–30% in H_2O), Dithiothreitol (DTT, 99%), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphynyl tetrazolium bromide (MTT) were purchased from Sigma (St.Louis, MO, USA). Dextran from leuconostoc ssp. (Mr ~ 15,000–25,000) was purchased from Fluka. (Milwaukee, WI, USA). Epichlorohydrin (99%) and doxorubicin were purchased from Aldrich (Milwaukee, WI, USA). Sodium hydroxide and trisodium citrate dehydrate were purchased from Junsei (Tokyo, Japan). N-Succinimidyl-3-(2-pyridyldithio)propionate (SPDP), di(N-succinimidyl) glutarate (DSG) and cyanine 5.5 (Cy5.5) were purchased from Pierce (Rockford, IL, USA). DAPI was purchased Vector Laboratories (CA, USA). 10× Phosphate-buffered saline (PBS), Dulbecco's modified eagle's medium (DMEM), and fetal bovine serum (FBS) were purchased from WelGENE (Seoul, Korea). Amicon ultra centrifuge filter devices (cutoff:100 kDa) were purchased from Millipore (Billerica, MA, USA). Minisart RC25 syringe filters (0.20 μm and 0.45 μm pore size) were purchased from Sartorius stedim biotech (Goettingen, Germany). All Chemicals were used as received.

2.2. Synthesis of 30, 70, and 170 nm sized dAuNPs

Dextran (12.0 g) was dissolved in distilled water (160 mL) to prepare 7.5 wt% solution. The solution was heated until boiling and 216, 432 or 864 μL of hydrogen tetrachloroaurate(III) hydrate stock solution (0.1 g/mL) was added to make 30, 70 or 170 nm dAuNP, respectively. The reaction mixture was boiled for ~20 min until the colors of the mixtures turned deep-violet (30 nm)/reddish-violet (70 nm)/turbid-orange (170 nm). The reaction mixture was then cooled to room temperature. The product was rinsed with distilled water for 4 times using Amicon filter (cutoff: 100 kDa). The product was further purified by passing the mixtures through 0.45 μm and 0.20 μm pore size syringe filter. Finally, dAuNPs were re-dispersed in 4 mL distilled water, and stored at 4 °C.