Materials Science and Engineering C 80 (2017) 88–92

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Photo-controlled aptamers delivery by dual surface gold-magnetic nanoparticles for targeted cancer therapy

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ARTICLE INFO

Article history:
Received 11 November 2016
Received in revised form 3 April 2017
Accepted 6 April 2017
Available online 29 April 2017

Keywords:
Dumbbell-like gold magnetic nanoparticles
aptamers
VEGF
Photo-control
Ovarian cancer

ABSTRACT

Dual surfaced dumbbell-like gold magnetic nanoparticles (Au-Fe_3O_4) were synthesized for targeted aptamers delivery. Their unique biological properties were characterized as a smart photo-controlled drug carrier. DNA aptamers targeting vascular endothelial growth factor (VEGF) were assembled onto the surface of Au-Fe_3O_4 by electrostatic absorption. The binding capacity of the nanoparticles with VEGF aptamers was confirmed by gel electrophoresis. The targeted recognization of ovarian cancer cells by the aptamers-functionalized Au-Fe_3O_4 nanoparticles (Apt-Au-Fe_3O_4 NPs) was observed by confocal microscopy. Apt-Au-Fe_3O_4 was found to bind with SKOV-3 ovarian cancer cells specifically, leading to marked intracellular release of aptamers upon plasmon-resonant light (605 nm) radiation, and to enhance the in vitro inhibition against tumor cell proliferation. The results show high potential of Apt-Au-Fe_3O_4as a targeted cancer hyperthermia carrier by remote control with high spatial/temporal resolution.

1. Introduction

Aptamers are short, synthetic, single-stranded RNA or DNA molecules identified from randomly synthesized nucleic acid libraries (>10^{14} shapes per library) by using a process termed SELEX (Systematic Evolution of Ligands by Exponential Enrichment) [1,2]. Aptamers can specifically bind to various molecular targets by secondary or tertiary structures for cancer therapy. Their unique characteristics and capabilities include high binding affinity and specificity, small size, non-immunogenicity, and ease of modification compared to the conventional monoclonal antibodies [3,4]. VEGF is associated with neoplastic transformation of cells inside the body, and considered to be the hallmark protein for tumor angiogenesis. VEGF165 is the pre-dominant isoform of VEGF-A protein in the VEGF family, and able to bind to primarily its two tyrosine-kinase receptors: VEGFR-1/Flt-1 and VEGFR-2/KDR/Flk-1 [5]. VEGF aptamers with the VEGF165 protein has been developed for antitumor studies [6].

The major challenges of aptamers for clinical applications include low stability and selectivity that limit them in cell targeting and precise release of aptamers. Various aptamers have been combined with different nanomaterials for cell imaging [7] and targeted drug delivery against tumor cells, in order to prevent DNA degradation and increase therapeutic efficacy while reducing side effects [8,9]. Previous experimental works have shown progress in improved drug bioavailability in target tissue and controlled drug release by using disulfide bond-reducing molecules [10,11] and pH-sensitive polymer carriers [12]. Among all methods of controlled drug-release, photo-control shows great promise for clinical applications due to its remarkable spatial/temporal resolution via remote control.

Plasmonic nanoparticles (NPs) such as gold [13] have been employed for controlled drug release for their high photo energy conversion into heat, which can trigger the release of preloaded drug [14]. Multifunctional nanocomposites with controlled structure and interface interactions are attractive carriers for cancer therapeutics [15]. Magnetically and optically activated nanocomposites such as dumbbell-like nanoparticles containing two different chemical surfaces are particularly suitable for synchronized cell targeting and pH sensitive drug delivery [16,17]. Recently, we developed the dual surface-functionalized Janus nanocomposites of polystyrene/Fe_3O_4@SiO_2 for simultaneous tumor cell targeting and stimulus-induced drug release in acidic intracellular environment [18]. However, a pH-responsive system may not function well in complicated environmental variations where other factors may interfere with the release process. In this study, positively charged dumbbell-like gold magnetic nanoparticles (Au-$Fe_3O_4Fe_3O_4$ NPs) were

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http://dx.doi.org/10.1016/j.msec.2017.04.044
0928-4931/

synthesized for targeted VEGF aptamers' delivery into ovarian cancer cells, and the light-induced antitumor effects were investigated in vitro [19].

2. Materials and methods

2.1. Materials and reagents

Oleic acid (85%), oleylamine (80–90%), 1,2-hexade-candiol, 1-candiol, 1-octadecene (90%), and $Fe(CO)_5$, $HAuCl_4\cdot(H_2O)_3$ were purchased from Aldrich Co. Ltd. All reagents were used without further purification. Poly(maleic anhydride-*alt*-1-decene) modified with dimethylaminopropylamine (PMAL, Mw 18.5 K) was purchased from Anatrace Inc. (Maumee, OH). VEGF aptamers with sequences 5′-TAAT ACGACTCACTATAGGGCGGAATCAGTGAATGCTTATACATCCG-3′ and scrambled VEGF aptamers as 5′-CTAGCTACGACTCACTATATTTTCAA ATCAGTGAATGCTTATAGCTAC-3′ were synthesized and purified by use of HPLC by Takara Co. Ltd.

2.2. Synthesis of Au-Fe_3O_4 nanoparticles

The synthesis process was modified based on the seed-mediated method [20]. Briefly, oleic acid (6 mmol), oleylamine (6 mmol), 1,2-hexadecandiol (10 mmol), and 20 ml 1-octadecene (ODE) were mixed and stirred under a gentle flow of nitrogen at 160 °C for 30 min. Then under a blanket of nitrogen, 0.3 ml Fe $(CO)_5$ was injected into the solution. After 3 min, the deaerated gold precursor solution consisting of $HAuCl_4\cdot(H_2O)_3$ (0.1 mmol), oleylamine (1.5 ml), and 5 ml ODE was dropwised into the hot solution within 10 min to ensure fully mixed. The solution turned to dark red instantly after the injection, indicating the formation of gold nanoparticles. The mixture was slowly heated to 320 °C to reflux for 45 min, cooled down to room temperature, and exposed to air for extra several hours to ensure the formation of Fe_3O_4. 40 ml of isopropanol was added into the solution and centrifuged at 3500 rpm for 10 min to remove large particles. The supernatant was centrifuged at 9000 rpm for another 10 min. The precipitate was re-dispersed into hexane in the presence of 0.05 ml oleylamine and centrifuged again at 7000 rpm to remove any undispersed materials. Ethanol was subsequently added into the solution and centrifuged again, giving a dark red dispersion. The Au-Fe_3O_4 NPs was dissolved in hexane in the presence of oleylamine. A small addition of oleylamine was necessary to ensure long-term stability of the dispersion.

2.3. Synthesis and characterization of water-soluble Au-Fe_3O_4 NPs

Water-soluble Au-Fe_3O_4 NPs were prepared through the amphiphilic polymer modified oil-soluble Au-Fe_3O_4 NPs by use of PMAL polymer, a kind of amphipol [19]. In a typical procedure, 10 mg PMAL dissolved in chloroform was poured into 5 ml oil-soluble Au-Fe_3O_4 nanocrystals solution and the resulting solution was stored in the hood to evaporate the chloroform and hexane. The as-modified Au-Fe_3O_4 NPs were collected with a magnet and washed repeatedly with deionized water to remove excess PMAL polymer. The concentration of PMAL-modified Au-Fe_3O_4 NPs was determined by measurement of dry weight of freeze dried NPs powder from NPs solutions with exact volume.

The morphology of the product was characterized by a JEM-2100 transmission electron microscope (TEM) at an acceleration voltage of 200 kV. The specimens for TEM analyses were prepared by room-temperature deposition of the deionized water of the particles on carbon-coated copper grids. The hydrodynamic diameter and zeta potential of Au-Fe_3O_4 NPs were measured using Nano-ZS90 Malvern laser scattering at 25 °C with a detection angle of 90°. The zeta potential of Au-Fe_3O_4 NPs in PBS was determined at pH 6.5, a similar pH value as intracellular environment. UV–vis absorption spectra were obtained using a VARIAN 50 Conc UV/Vis spectrometer. The magnetic properties of samples were characterized by a JDM-13 sample magnetometer (VSM) with field up to 1.5 Tesla at room temperature. The T2-weighted MR imaging of Au-Fe_3O_4 solution was obtained using a mq60 NMR Analyzer of BRUKER. Confocal fluorescence images were obtained with a confocal microscope (Zeiss LSM 510, Germany) equipped with DPSS, Argon, and He/Ne lasers with lines at 405, 458, 488, 543, and 633 nm.

2.4. Preparation of Apt-Au-Fe_3O_4 NPs and aptamers binding capacity

Upon PMAL modification of the water-soluble Au-Fe_3O_4 NPs, the overall surface charge of the hybrid structure is highly positive, leading to immobilization of the negatively charged biomolecules. VEGF aptamers (2 μl, 4 μg/μl) was added into 10 μl Au-Fe_3O_4 (1 mg/ml) dispersed in DEPC water, and the reaction continued for 15 min at 25 °C to form Apt-Au-Fe_3O_4 NPs. The concentration of VEGF aptamers binded onto the surface of Apt-Au-Fe_3O_4 NPs was determined by NanoDrop 2000 to measure the value of OD260/OD280. The binding capacity of NPs with VEGF aptamers was studied by agarose gel electrophoresis. Various molar ratios of NPs to aptamers at 1:1, 1:3, 1:5, 1:10, 1:20 were mixed and run in 0.8% agarose gel, and photographed by the gel imaging instrument (Bio-Rad, USA).

2.5. Intracellular uptake of Apt-Au-Fe_3O_4NPs by SKOV-3 cells

4 μg/μl Cy-3 labeled VEGF aptamers were assembled with Au-Fe_3O_4 NPs to form Apt-Au-Fe_3O_4 complexes. The final concentration of VEGF aptamers in Apt-Au-Fe_3O_4 NPs is 1 μg/μl. Targeted recognization of Apt-Au-Fe_3O_4 NPs by SKOV-3 cells was observed by confocal microscope. Specially, the effects of laser radiation at 605 nm on intracellular aptamers release were confirmed. The binding capacity of free 1 μg/μl VEGF aptamers was studied as positive control. The intracellular uptake efficiency was quantitatively determined by flow cytometry analysis.

SKOV3 cells were treated with VEGF aptamers, Au-Fe_3O_4 NPs and Apt-Au-Fe_3O_4 complexes respectively. After 24 h incubation, cells were washed with PBS and resuspended in 400 μl DNA binding buffer at a concentration of 1×10^6 cells/ml. The samples were filtered through a cell filter membrane, and then 5 μl Annexin-V and 10 μl propidium iodide (PI) (KeyGEN, China) were added. The experiments were performed in triplicate. After 15 min' incubation at room temperature in the dark, the apoptosis rate of the tumor cells was evaluated by FCM using an Epics-XL-MCL flow cytometer (Beckman Coulter, USA).

2.6. Anti-proliferation of Apt-Au-Fe_3O_4NPs

The cytotoxicities of Apt-Au-Fe_3O_4 NPs and Au-Fe_3O_4 NPs against SKOV-3 cells were evaluated by measuring the inhibition of cell growth using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [21]. SKOV-3 cells (3×10^5 cells) were plated in 96-well plates, incubated in MEM containing 10% fetal bovine serum and 1‰ antibiotics at 37 °C in a humidified atmosphere with 5% CO_2, and treated with Au-Fe_3O_4 NPs and Apt-Au-Fe_3O_4 NPs at various concentrations for 4 h. The medium was then aspirated with new one added. The samples were subsequently irradiated with a 605 nm diode laser (2 W cm^2, 3 cm beam diameter) for 10 min to evaluate their photothermal effects against cells. An MTT assay was performed with the relative percentage of cell viability, calculated as the ratio of formazan intensity in cells treated with Au-Fe_3O_4 and Apt-Au-Fe_3O_4 NPs. The experiment was carried out with/without plasmon-resonant light (605 nm) radiation. The experiment was also carried out on the control cells without treatment.

3. Results and discussion

The synthesized dumbbell-like Au-Fe3O4 nanoparticles were water insoluble due to the presence of oleic acid and oleylamine. They were modified using PMAL polymer to ensure its stability in biological