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Magnetic gold-nanorod/ PNIPAAmMA nanoparticles for dual magnetic resonance and photoacoustic imaging and targeted photothermal therapy

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ABSTRACT

Nanomedicine can provide a multi-functional platform for image-guided diagnosis and treatment of cancer. Although gold nanorods (GNRs) have been developed for photoacoustic (PA) imaging and near infra-red (NIR) photothermal applications, their efficiency has remained limited by low thermal stability. Here we present the synthesis, characterization, and functional evaluation of non-cytotoxic magnetic polymer-modified gold nanorods (MPGNRs), designed to act as dual magnetic resonance imaging (MRI) and PA imaging contrast agents. In addition, their high magnetization allowed MPGNRs to be actively localized and concentrated by targeting with an external magnet. Finally, MPGNRs significantly enhanced the NIR-laser-induced photothermal effect due to their increased thermal stability. MPGNRs thus provide a promising new theranostic platform for cancer diagnosis and treatment by combining dual MR/PA imaging with highly effective targeted photothermal therapy.

1. Introduction

Gold nanoparticles are commonly used as contrast and therapeutic agents based on their superior optical properties, non-cytotoxicty, and ease of bioconjugation with biomarkers for targeted delivery [1–5]. Gold nanorods (GNRs) are particularly interesting because of their small size (10–40 nm), ease of synthesis, and tunable resonance in the red and near infra-red (NIR) spectrum where the optical window permits photon penetration into biological tissues with relatively high transmittivity. Moreover, GNRs possess an extremely high absorption cross-section and nanoparticle-facilitated absorption of pulsed light leads to the production of sound waves, thus allowing GNRs to serve as an effective contrast agent for photoacoustic (PA) imaging [3,6,7].

During PA imaging, GNRs are exposed to high-energy nanosecond laser pulses. GNRs absorb a portion of the light but also generate substantial heat that leads to nanorod reshaping and geometrical changes in the absorption cross-section [8]. PA imaging contrast depends on the optical-to-acoustic conversion efficiency, i.e. how many incident photons can be absorbed and converted to heat, and on how fast the generated heat can diffuse out from the target during thermoelastic expansion and wave generation.

GNRs also have the potential to serve as photothermal therapeutic agents since they are capable of converting over 90% of the absorbed photons into heat through non-radiative processes [9]. Photothermal therapy relies on the resonant absorption of light by nanoparticles and the conversion of electromagnetic energy into heat to destroy malignant tissue, and the geometrical change of GNRs causes significant heat absorption decay [10–14].

Recent studies have also investigated the enhancement of NIR photo-activated cancer therapy by gold nanoparticles [15,16], gold nanoshells [17,18], gold nanocages [19], GNRs [20,21], carbon nanotubes [22], and graphene oxide [23]. GNRs are again particularly suited to this application due to their interesting optical

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properties, including: (1) a strong surface plasmon absorption band in the NIR region which could be exploited for ultra-efficient energy quenching due to the extremely high absorption coefficients (10^6-fold higher than those of conventional organic dyes or quantum dots [24–26]); (2) compared to other nanostructures, the peak absorption band of GNRs in the NIR region can be easily tuned by adjusting the aspect ratio [27], while the absorption maximum can be shifted to NIR regions to obtain better light penetration into soft tissues.

GNRs are treated with cetyltrimethylammonium bromide (CTAB) to allow future surface modification, but this leaves them slightly cytotoxic if they are not further modified [28]. Surface modification also prolongs the half-life of GNRs in blood and prevents them from changing shape after laser irradiation. Surface-modified GNRs have included poly(4-styrenesulfonic acid) (PSS)-coated nanorods [29], PEG-modified nanorods [30], or PAA-derived nanorods [31].

Another key reason to perform surface modification of GNRs is to increase their thermal stability. Plasmonic nanoabsorbers with more geometrically stable characteristics are highly desirable for PA imaging or photothermal applications. Typically, GNR melting has been shown to occur at significantly lower temperatures than bulk melting of the metal, in part because of the domination of surface reorganization processes [32–34]. Nanorods held at fixed temperatures have been shown to melt and form spheres below 100–250 °C depending on the surface coating and the environment in which they are embedded [34,35]. Several studies have found that embedding nanorods in a solid environment such as carbon or PMMA significantly increases their photothermal stability; in the latter case to just below the glass transition temperature [36]. Silica coating of nanorods has been achieved by several strategies, and silica-coated GNRs possess good photothermal stability [6,11].

In this study, we present the synthesis of MGNRs by carboxylic poly(N-isopropylacrylamide-co-methacrylic acid) (PNIPAAmMA) coating and highly-magnetic nanoparticles (HMNPs) conjugation, and demonstrate their versatile and multiple functions resulting from stabilization of their shape and increased thermal tolerance. Therefore, this system can be considered as important therapeutic and diagnostic targets for the treatment and detection of cancers.

2. Materials and methods

2.1. Synthesis of GNRs

A seed solution was prepared by addition of 0.6 mL of freshly-prepared, ice-cold 0.01 M $NaBH_4$ to a mixture of 0.025 mL 0.1 M $HAuCl_4$ and 10 mL 0.1 M cetyltrimethylammonium bromide (CTAB). To synthesize GNRs of aspect ratio 5, a separate growth solution was prepared by mixing 0.5 mL of 0.1 M $HAuCl_4$, 0.08 mL of 0.1 M $AgNO_3$, and 100 mL of a CTAB/benzyldimethylhexadecylammonium chloride (BDAC) mixture (CTAB 0.1 M, BDAC 0.125 M) at room temperature. Next, 0.55 mL 0.1 M ascorbic acid was added to the growth solution as a mild reducing agent. The seed solution (0.1 mL) was slowly added into the growth solution. The color of the growth solution slowly changed from clear to violet indicating the growth of GNRs. The solution was then centrifuged twice at 10,000 rpm for 20 min. The sediment of each centrifugation step was recovered by redispersion in deionized (DI) water.

2.2. Surface modification of GNRs

The surface of GNRs was coated with poly(N-isopropylacrylamide-co-methacrylic acid) (PNIPAAmMA; M_w = 60,000). Briefly, a 2 μg/mL solution of PNIPAAmMA was prepared by sonication for 30 min in 6 mM NaCl, and GNRs were diluted to 10 mL with DI water. The PNIPAAmMA solution was then added drop-wise to 1 mL of the GNRs, followed by vigorous stirring for 6 h. PNIPAAmMA-coated GNRs (PGNRs) were obtained in the sediment after two centrifugation steps to remove excess polyelectrolyte, and were finally dispersed in 2 mL of phosphate-buffered saline (PBS) (pH = 7.4).

2.3. Synthesis of magnetic PGNRs (MPGNRs)

Detailed procedures for the synthesis of high-magnetization Fe_3O_4 nanoparticles (HMNPs) were reported previously [37]. The surface of HMNPs was activated in 1 mmol/L NaOH. Activated HMNPs were suspended in 50 mL of toluene followed by the addition 1.0 g of (3-aminopropyl)triethoxysilane and reflux for 24 h. The solid phase was separated with a magnet, washed with 10 mL ethanol and DI water and dried at room temperature to obtain NH_2-functionalized HMNPs. PGNRs were mixed with excess 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (sulfo-NHS) in 0.5 M 2-morpholinoethanesulfonic acid (MES) by vortexing for 30 min in the dark at 25 °C. After separation, PGNRs were washed with 0.8 mL of 0.1 M MES buffer, resuspended in 0.2 mL of the same buffer, and added to 0.1 mL of NH_2-functionalized HMNPs at 25 °C. The solution was mixed by vortexing for 1 h followed by sonication for another hour. MPGNRs were separated from the solution with a magnet, washed with DI water to remove unreacted PGNRs, and then dispersed in 0.2 mL DI water.

2.4. Photothermal irradiation

Two milliliters of water, PGNRs, or MPGNRs were stored in eppendorf vials at 37 °C. The vials were irradiated for 1 min with an 808 nm laser at a power of 2 W/cm^2 (0.6 cm^2 laser area). Thermal images were captured with a thermo camera (Thermo Gear G100, NEC, Japan) to measure the temperature increase and depth of heat penetration.

2.5. In vitro cytotoxicity assay

Glioma C6 cells were cultured in RPMI 1640 medium supplemented with 2.2 mg/mL sodium carbonate, 10% fetal bovine serum, 50 μg/mL gentamicin, 50 μg/mL penicillin, and 50 μg/mL streptomycin at 37 °C and 5% CO_2. Approximately 10,000 cells (i.e. 150 μL of a suspension of 6.67×10^4 cells/mL) were placed in each well of a 96-well culture plate and were incubated in a humidified chamber at 37 °C and 5% CO_2 for 24 h. Different concentrations of PGNRs and MPGNRs (50 μL each) were added to the medium, and incubation was continued for 24 h. Cells were irradiated with an 808 nm continuous-wave NIR laser at a power density of 2 W/cm^2 for 1 min, or not irradiated as controls, and were then incubated at 37 °C for a further 24 h. The culture medium was removed, and the cells were incubated in 120 μL of XTT solution for 3 h. Subsequently, 100 μL of the XTT solution was removed from each well and transferred to a 96-well counting dish. In vitro cytotoxicity of PGNRs and MPGNRs toward C6 cells was evaluated by measuring the OD at 490 nm using an ELISA reader.

2.6. Photoacoustic imaging (PAI) system setup

We used high-frequency (>20 MHz) dark-field confocal photoacoustic microscopy (PAM) to demonstrate the characteristics of our samples as photoacoustic imaging (PAI) contrast agents [38]. Laser pulses were provided at different wavelengths using an optical parametric oscillator (Surlite OPO Plus, Continuum) pumped by a frequency-doubled Nd:YAG Q-switched laser (Surlite II-10, Continuum) with pulse width of 6.5 ns and pulse repetition frequency (PRF) of 10 Hz. The 50-MHz ultrasonic transducer of the PAM system was custom-made by the Resource Center for Medical Ultrasonic Transducer Technology at the University of Southern California. It had a 6-dB fractional bandwidth of 57.5%, a focal length of 9 mm, and a 6-mm active element, offering axial resolution of 36 μm and lateral resolution of 65 μm [39]. During the imaging process, the transducer was immersed in a water tank with a hole in the bottom that was sealed with a piece of 15-μm-thick polyethylene film. The wavelength of the OPO laser was tuned starting at 808 nm to expose different samples at the particular peak value of their optical absorption. Blood displays strong optical absorption at both 808 and 760 nm, thus producing a strong PA signal compared to our particle samples. We therefore chose a wavelength of 808 nm for blood. Laser exposure was adapted to 10 mJ/cm^2 for all the experiments.

2.7. In vitro and in vivo photoacoustic imaging

We investigated five different concentrations of our PGNRs and MPGNRs: 40 μg/mL, 312.5 μg/mL, 625 μg/mL, 1250 μg/mL and 2500 μg/mL. The strong PA signal from blood was used as comparison. Particle samples were injected into polyvinyl chloride tubes (S-54-HL, 720994, Tygon® Microbore Tubing; 0.5 and 1.5 mm inner and outer diameters, respectively) located in a small water tank below the transducer of the PAM system to capture PA images. Regions of interests (ROIs) were selected and the PA signal was analyzed using MATLAB software.

The use of MPGNRs as a PA imaging contrast agent was also investigated in tumor-bearing nude mice. A series of sequential PA images were acquired to cover the subcutaneous tumor regions. For magnetic targeting, an external magnet was attached to the skin over the tumor at the time of a single intravenous injection of MPGNRs (2 mg/mL, 100 μL). To compare local retention of MPGNRs, enhanced PA imaging and photothermal efficacy, magnetic targeting was carried out for 36 h and a second set of PA images were acquired 36 h after the first scan.