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Tunable Surface Plasmon Resonance–Based Remote Actuation of Bimetallic Core-Shell Nanoparticle-Coated Stimuli Responsive Polymer for Switchable Chemo-Photothermal Synergistic Cancer Therapy

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ABSTRACT

New dual light/temperature-responsive nanocarriers were synthesized using bimetallic plasmonic Au-Ag and Ag-Au nanoparticles (NPs) as cores of vehicles which subsequently functionalized with an upper critical solubility temperature–based poly acrylamide-co-acrylonitrile using reversible addition-fragmentation chain transfer for spatiotemporally controlled chemo-photothermal synergistic cancer therapy. The bimetallic cores were assigned to sense wavelengths close to the localized surface plasmon resonance of monometallic NP shell to produce heat which not only can increase the surrounding temperature over the upper critical solubility temperature of polymer to open its valves and promote drug diffusion but also can kill cancerous cells through photothermal effects with increase in environment temperature by nearly 18°C after about 5 min radiation. The bimetallic NPs were shown good reusability even after 5 heating/cooling cycles, and the efficiency of both photothermal/chemotherapic procedures can be modulated by manipulating carrier's concentration and radiation time. In addition, the cytotoxicity of drug-free nanocarriers on normal L929 fibroblast and letrozole-loaded nanocarriers on MDAMB 231 breast-cancer cell lines were investigated in the absence/presence of laser radiation. Finally, the prepared nanocomposites were exhibited switchable on/off drug release in 2 buffered solutions (pH 5.5 and 7.4) with light actuation. The results revealed that the prepared nanocarriers can be served as efficient delivery platforms for remote-control chemophotothermal synergistic cancer therapy.

Introduction

Nowadays, metal-based nanoparticles (NPs) have prevalent applications in medicine and pharmaceutical industries,[1] and among them, noble metal-based NPs were revealed promising antitumor activities capable of reinforcement of photothermal therapy (PTT), hyperthermia, and chemotherapeutic-based cancer manipulation.[2] This extensive usage arises from their inherent properties including nanometer size, large surface to volume ratio, optical properties, and high stability.[3,4] One of the promising optical properties used in cancer treatment is localized surface plasmon resonance (SPR), which comes from the collective oscillation of free electrons produced by radiation at special wave lengths[5,6] and is responsible for many plasmon-modulated optical applications.[7] These wavelengths are strongly depended to the nanostructure's morphology, composition, and local dielectric microenvironment. Among noble-metal NPs, gold (Au) and silver (Ag) were demonstrated as strong antitumor activity in many *in vitro* and *in vivo* applications.[8-11] However, in comparison with monometallic NPs, bimetallic ones have gathered more attention because they amplify the physical properties of each individual NPs.[12] The witness is considerable applications of bimetallic core-shell/alloy structures of Au-Ag or Ag-Au NPs in bioimaging,[13] biosensing,[14] cancer treatment,[15] and catalysis[16] in the past few years. Similar lattice constants and face-centered crystal structure of both NPs simplify producing their bimetallic structures.[17] Resulting plasmonic characteristic produces more improvement in local optical field at particle-particle interface and is one of the most interesting optical properties yield due to such a coupling process.[18] Furthermore, these plasmonic characteristics are tunable due to the ability of tuning the size, shape, and composition of the prepared alloy/core-shell structure, which greatly influence the biological

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activity of them.[19] It is noticeable that different optical responses can be observed based on the type of core-shell/alloy compositions (i.e., Au-Ag or Ag-Au structure) even when they have the same NP content.[20] Such characterizations make them candidates for cancer treatments especially by PTT. The PTT has important role in tumor treatment due to its special temporal/spatial selectivity and minimal aggression. Ideal PTT materials must be biocompatible and nontoxic and should have wide absorbance in the 400-1100 nm region along with the ability of aggregating in the cancerous cells following systemic administration. When combined with light radiation in special wave length, bimetallic NPs as PTT materials can efficiently convert photon energy to heat to destroy cancerous cells with minimum damage to the nontargeted health tissues,[21] and Au-Ag/Ag-Au NPs already have most of these properties that making them superior materials for PTT.

However, complete destroying of cancerous cells by PTT alone is tough because of light scattering. Therefore, it is usually accompanied by chemotherapic treatment referred as chemo-PTT. In this combined technique, additional release of chemotherapeutic drugs in a controlled manner increases the PTT effects using light stimulus and reveals remarkable therapeutic efficiency due to the increased amount of chemotherapeutic materials in elevated temperatures.[22] Contrariwise, photoresponse-induced PTT can compensate the reduced drug efficacy caused by drug resistance in cancerous cells. Because in conventional chemotherapy, anticancer drugs have cytotoxicity effects not only to cancer cells but also to healthy tissues, microenvironment-sensitive drug carriers that can adjust their volume and properties in response to environmental stimuli are needed. To the best of our knowledge, no dual-responsive polymer-coated bimetallic NPs have yet been discussed in terms of PPT and chemotherapic aspects, and the idea can enlarge the spectrum of the nanocomposites applications, such as multidelivering and releasing of biological moieties. In addition, by preparing various bimetallic NPs with different SPR, the vehicles can absorb the light in wider wave length ranges, which means that applications like PTT can occur for a wider range.

In this study, 2 core-shell bimetallic NPs namely Au-Ag and Ag-Au NPs were synthesized and functionalized with poly (acrylamide-co-acrylonitrile), abbreviated as [poly (AAm-co-AN)], with upper critical solubility temperature (UCST) property using reversible addition-fragmentation chain transfer technique. The polymeric part is responsible for chemotherapic properties such as anticancer drug loading and release. Letrozole was used as a model drug, and the vehicle's phase transitions, swelling/deswelling ability, thermal sensitivity, and switching properties (both thermal and optical) were investigated. The bimetallic NP cores are responsible for both PTT and inducing volume-phase transition of polymeric shell by actuating with light for triggering drug release. Their photothermal properties were evaluated in 2 different concentrations. The cytotoxicity of both nonloaded and drug-loaded nanocomposites was also evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test on normal and cancerous cells. Furthermore, drug loading and release behaviors of the carriers were studied in 2 buffer solutions with pH = 5.5 and 7.4 to simulate cancerous and healthy-cells environment, respectively. The results reveal that the prepared nanocomposites have the properties of dual light- and temperature-responsiveness and thereby making them superior agents for chemo-PTTs.

Experimental

Chemicals and Reagents

All reagents and chemicals were of analytical grade and used as received without further purification. Millipore water was used throughout the experiments. 2-(Dodecylthiocarbonothioylthio)-2methylpropionic acid, Roswell Park Memorial Institute (RPMI) 1640 medium and MTT were purchased from Sigma-Aldrich. Acrylamide (AAm), acrylonitrile (AN), 2,2′-Azobis(2-methylpropionitrile), hydrogen tetrachloroaureate (III) hydrate ($HAuCl_4$), silver nitrate, trisodium citrate, polyvinyl pyrrolidone, sodium dihydrogen phosphate (Na_2HPO_4), potassium chloride, sodium hydroxide, sodium borohydride ($NaBH_4$), trisodium citrate, hydrochloric acid, dimethylsulfoxide, and methanol were purchased from Merck. Letrozole standard was received from faculty of pharmacy, Tehran University, Iran.

Instrumentation and Optical Set-up

Digital IR thermometer model DT-8811 from CEM Company (Shenzhen, China) was used to measure temperature produced by the nanocarriers in photothermal studies. Two visible lasers with central wavelengths of 405 and 532 nm with the intensities of 630 and 820 kW cm^{-2}, respectively, were applied to irradiate the carriers by focusing them on the top of a 3.8-mL quartz cell containing nanocarriers. An objective lens was mounted in front of each laser to focus the light beams with about 5-mm diameter spots. A wave length calibrated CCD-based AvaSpec3648 spectrometer from Avantes Company (Apeldoorn, Netherlands) equipped with two 200-μm optical fibers, a CUV-VAR-UV/VIS cuvette holder and an AvaLight-DHS deuterium/halogen light source were applied in the range of 200-1100 nm for measuring UV-Vis spectra. A Zeiss EM900 Transmission Electron Microscope (TEM) (Oberkochen, Germany) was used to determine the size and morphology of bimetallic NPs, and a Hitachi S-4160 field emission scanning electron microscope (SEM) (Tokyo, Japan) was applied to evaluate the morphology of the prepared nanocomposites. Phase characterization of the bimetallic NPs was performed using a PANalytical X'Pert PRO MPD X-ray diffractometer (XRD) (Eindhoven, Netherlands) equipped with X'Pert High Score Plus (V. 3) software.

Synthesis of Bimetallic NPs

Successive reduction is the most favorable procedure for generating core-shell bimetallic NPs.[23] The method involves the deposition of second metallic NPs on the previously synthesized monometallic NPs of another type. Following this procedure for synthesis of Au-Ag NPs, an amount of 5-mL chloroauric acid (5 mM) was transferred to a 100-mL beaker containing 30 mL of deionized water on a magnetic stirrer and heated to boil with continuous stirring. Then, 15 mL of citric acid (5 mM) was immediately added to the solution where the color was changed from light yellow to red. The reaction continued for 5 min, and then, the beaker was placed in an ice bath to cool down to the room temperature. The prepared Au NPs was stored in a refrigerator in dark, and it was stable at room temperature for several months. In the next step, the as-prepared Au NPs were transferred to a 250-mL beaker, and 5 mL of $AgNO_3$ (5 mM) containing 1% (w/v) of polyvinyl pyrrolidone was added to the solution with vigorous stirring at 60°C. Then, 15 mL of $NaBH_4$ (5.0 mM) was added to the solution where the color turned to orange. Finally, the resulting Au-Ag NPs were stored at the concentration of 50 mg L^{-1} in a dark flask at 4°C until being used.

The Ag-Au NPs were prepared with the same procedure by changing the order of adding reagents.

Synthesis of Plain Polymer and Nanocomposites

Plain polymer was synthesized via reversible addition-fragmentation chain transfer polymerization technique as described previously in the literatures[24] with some modifications.