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Enhancing photothermal cancer therapy by clustering gold nanoparticles into spherical polymeric nanoconstructs

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ABSTRACT

Gold nanoparticles (AuNPs) have been proposed as agents for enhancing photothermal therapy in cancer and cardiovascular diseases. Different geometrical configurations have been used, ranging from spheres to rods and more complex star shapes, to modulate optical and ablating properties. In this work, multiple, ultra-small 6 nm AuNPs are encapsulated into larger spherical polymeric nanoconstructs (SPNs), made out of a poly(lactic acid-co-glycol acid) (PLGA) core stabilized by a superficial lipid-PEG monolayer. The optical and photothermal properties of the resulting nanoconstructs (Au-SPNs) are modulated by varying the initial loading input of AuNPs, ranging between 25 and 150 μg_{Au}. Au-SPNs exhibit a hydrodynamic diameter varying from ~100 to 180 nm, growing with the gold content, and manifest up to 2-fold increase in thermal energy production per unit mass of gold for an initial input of 100 μg_{Au}. Au-SPNs are stable under physiological conditions up to 7 days and have direct cytotoxic effect on tumor cells. The superior photothermal performance of Au-SPNs is assessed in vitro on monolayers of breast cancer cells (SUM-159) and tumor spheroids of glioblastoma multiforme cells (U87-MG). The encapsulation of small AuNPs into larger spherical nanoconstructs enhances photothermal ablation and could favor tumor accumulation.

1. Introduction

Photothermal therapy represents a promising strategy for the localized ablation of malignant tissues that cannot be removed via surgical resection [1–3]. Different energy sources have been employed to induce the thermal ablation of biological tissues including laser-based techniques [3], radiofrequency systems [4], high-intensity focused ultrasound [5], and alternating magnetic field generators [6]. Some of these approaches have been successfully translated into a clinical setting. For instance, laser induced thermal therapies are routinely used for treating prostate cancer, varicose veins, and cardiac arrhythmias [7]; radiofrequency ablation is applied for the treatment of bulky tumors developing in the kidneys, liver, and lungs [8]; high-intensity focused ultrasound is suggested in the treatment of uterine fibroids [9]; and magnetic ablation via alternating magnetic field has been recently approved for the treatment of deadly brain tumors [10]. The efficacy of these ablation therapies can be improved by combining the energy source with agents that could both specifically accumulate within the diseased tissue and enhance the energy conversion into heat [3,4,11,12].

Gold nanoparticles (AuNPs), iron oxide nanoparticles and echogenic nano/micro-particles have been proposed as injectable agents to improve the localized delivery of heat in ablation therapies. In particular, AuNPs have been shown to be an ideal agent for enhancing laser-based ablation therapies mostly because of their tunable optical properties and surface plasmon resonance (SPR) effect [3,13]. In nanoparticles, a SPR effect takes place at the moment the electric field of an incident light wave induces polarization of the conduction electrons in respect to the positive ionic core. As the wave propagates on the particle surface, a dipolar oscillation is generated over the particle body. For nanometric particles the collective in-phase oscillation of all the free electrons in the conductive band is achieved at ordinary plane-wavelengths. The characteristic resonance frequency for SPR

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strongly depends on AuNPs size, shape, interparticle interactions, dielectric properties, and local environment [14]. As examples, an increase of the particle size brings the SPR to redshift, whiles has been calculated that clustered aggregates of several nanoparticles can manifest wide broadening of the peaks in the absorption spectrum [15].Therefore by modulating all these factors, the efficacy of the ablation therapy can be significantly enhanced. Furthermore, AuNPs can be easily synthesized, chemically modified and have shown favorable toxicity profiles in vivo.

In order to improve the biodistribution performance of AuNPs and their ablation properties, in this work multiple 6 nm spherical gold nanoparticles have been encapsulated into a larger polymeric nanoconstruct (Fig. 1). The resulting gold-loaded spherical polymeric nanoconstructs (Au-SPNs) are constituted by a polymeric core made out of poly(lactic acid-co-glycol acid) (PLGA), in which 6 nm AuNPs are encapsulated, and a surface lipid monolayer used to stabilize the system and provide long circulation capability. The optical and ablation properties of AuNPs are compared and characterized as a function of the initial input of AuNPs into the Au-SPNs.

2. Materials and methods

2.1. Materials

All experiments were performed with spherical hydrophobic gold nanoparticles (AuNPs) of 6 nm diameter, purchased from Ocean NanoTech LLC and used as recommended by the manufacturer. Egg PG (L-α-Phosphatidyl-DL-Glycerol (Egg and Chicken) and DSPE-PEG-OCH$_3$ (1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine-N-Carboxy(Polyethylene glycol)-2000, were purchased from Avanti Polar Lipid, Inc. and used as received. Size and morphology of the gold nanoparticles were verified by Transmission Electron Microscopy (TEM) (JEM-2100F TEM by JEOL Ltd.). Purified samples were diluted in DI water 10 times and 10 µl of the solution was deposited onto the surface of a TEM grid (Ted Pella, Inc., Formvar/Carbon 400 mesh, Copper, approx. grid hole size: 42 µm) and left to dry for 1 h. The size distribution was estimated from the analysis of the TEM images considering more than 150 AuNPs. Extinction measurements were performed on a spectrophotometer (Beckman Coulter, Life Science UV/vis Spectrophotometer, DU^{R}730) confirming the extinction spectrum calculated by the company. The particle weight was determined using a high sensitive laboratory microbalance.

2.2. Synthesis of Au-SPNs

Au-SPNs were prepared through a single step nanoprecipitation method [11]. A hydrophobic mixture of AuNPs (25, 50, 100 and 150 µg) in chloroform (Fisher Sci., HPLC grade) and PLGA (1 mg, Mn ~50 kDa) (poly (lactic acid-co-glycolic acid), Lactate Absorbable Polymers—DURECT Corporation) was left dry. The dried composite film was dissolved in 1.0 ml acetonitrile (Fisher Sci., HPLC grade). On the other hand, 200 µg of EGG-PG and 260 µg DSPE-PEG-OCH$_3$ were dissolved in 4% ethanol at 64 °C. To this solution, the composite film solution in acetonitrile was added drop wise while heating and stirring, followed by the addition of DI water (1 ml). The solution mixture was stirred at room temperature for 2 h, centrifuged at 3500 rpm and washed several times using an Amicon Ultra centrifugal filter (Millipore, Billerica, MA) with a molecular-weight cutoff of 10 kDa to remove residues and not encapsulated AuNPs. Finally, the purified

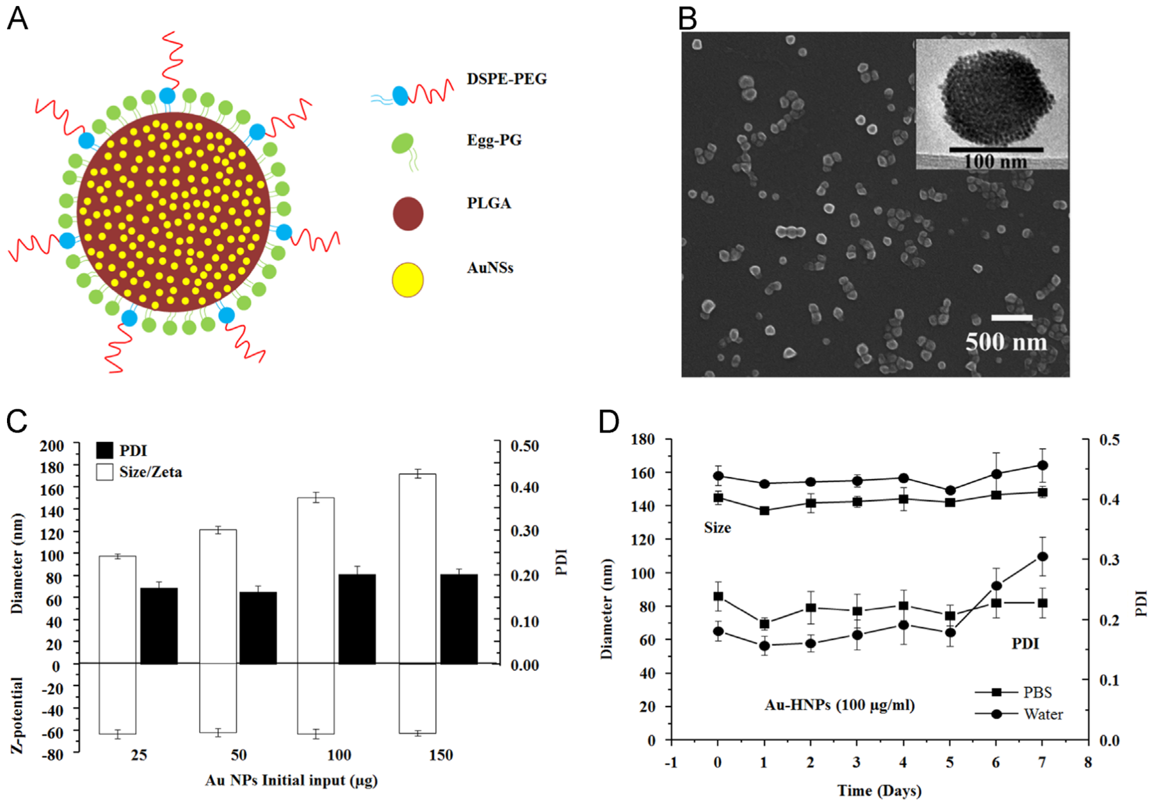

Fig. 1. Spherical plymeric nanoconstructs encapsulating gold nanoparticles. (A) Schematic represenation of a spherical polymeric nanoconstruct (SPN) encapsualting thaousands of ultra-small gold nanoparticles (AuNPs). (B) Scanning electron micrograph of Au-SPNs. The upper right inset shows a transmission electron micrograph of a Au-SPN encapsulating a myriad of small AuNPs (round, black dots). (C) Variation of the hydrodynamic size and Z-potential of Au-SPNs as a function of the initial gold loading. (D) Colloidal stability of Au-SPNs in water and PBS at 37 °C over a 7 day observation period.