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Hybrid folic acid-conjugated gold nanorods-loaded human serum albumin nanoparticles for simultaneous photothermal and chemotherapeutic therapy

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ABSTRACT

Hybrid nanoparticles containing both structural and functional nanocomponents might result in higher success and increased quality of life for patients suffering a disease such as cancer. In this study, we combine chemotherapy of conventional drug doxorubicin (Dox) with gold nanorods (AuNR) for photothermal therapy using multifunctional human serum albumin nanoparticles (HSA NP's) fabricated via desolvation technique with high efficiency. Folic acid (FA) was conjugated to HSA NP's trough an amidation via carbodiimide reaction for a more specific nanoplataform to HeLa cancer cells. The loading efficiency of Dox into AuNR loaded-HSA NP reached up to 2 μg Dox/mg HSA. The HSA-AuNR-Dox NP experienced photothermal heating varying laser potency (1, 0.5 and 0.2 W); reaching the bulk particle solution an increment of 16, 8 and 6 °C after 10 min of near-IR laser exposure respectively. When HeLa cells were treated with this multifunctional nanoplataform containing only AuNR, cancer cells experienced 96% cell viability without irradiation and 55% cell viability after just one irradiation session. When Dox is present in the nanoplataform, viability were 60% and 24% for non-irradiated and irradiated nanoplataforms, respectively. This study demonstrates that HSA-AuNR-Dox nanoparticles are suitable systems allowing a synergic chemo and phothothermal therapy.

1. Introduction

The emergence of nanotechnology and its application to the biomedical field has originated the development of new biocompatible nanoscale drug carriers as liposomes and polymeric nanoparticles, which improve the therapeutic efficacy of multiple drugs [1]. Drug-loaded nanocarriers have many advantages in comparison with the administration of free drugs, particularly at the systemic level such as a longer circulation half-life times, improved pharmacokinetics due to the enhanced permeability and retention effect (EPR) and diminished adverse side effects [2–4]. Despite their success, nevertheless there is a current need for multi-functional, temporally active cancer treatments which maximize therapeutic effects through less invasive techniques. Hybrid nanoparticles containing both structural and functional nanocomponents might result in higher success rates and increased life quality of patients suffering a disease as cancer. The structural components of hybrid nanoparticles can be mainly classified based on the therapeutic function they possess. For example, structural nanocomponents such as liposomes, polymeric micelles, mesoporous silica, gels, or viruses can carry mainly a drug cargo; while functional nanocomponents such as gold nanoparticles or carbon nanotubes enable the application of photoablation therapy as a result of their outstanding optical properties. In this way, hybrid nanoparticles can retain the beneficial features of both type of nanomaterials and, at the same time, allow the systematic fine-tuning of their properties through the combination of functional components [5]. Dou et al. [6] combine up conversion NPs for delivery of doxorubicin and imaging for bioapplications against cancer cells. Ellis et al. [7] combined an biocompatible pH-responsive polymer anchored to gold NPs surface for enhancing cancer therapy.

In particular, plasmonic noble metal nanoparticles (NPs) are distinguished for their unique surface plasmon resonance (SPR) properties [8–10], noble metals commonly used are silver, gold, titanium and cooper for different application such as: antimicrobial agents, solar cells, 3D printing and biomedical applications, catalytic applications, among others [11–13]. Specifically, gold nanorods (Au NRs) of suitable dimensions and aspect ratio have the ability to absorb light in the near-infrared region of the electromagnetic spectrum (the so-called biological window), giving rise to a subsequent release of the adsorbed energy through localized heat emission, which can be harnessed to be

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used in plasmonic photothermal therapy (PPTT) [14, 15], this nanostructure has been synthetized by many different methods as multi-stage microfluidic reactor [16]. However, PPTT faces significant limitations. For instance, the complete eradication of cancer cells under laser irradiation is difficult because of the uneven heat distribution within the tumor caused by the heterogeneous distribution of NPs and the Gaussian distribution of energy of the laser beam. One of the most promising approaches is to combine PPTT with current chemotherapy biocompatible nanocarries.

At this respect, human serum albumin (HSA) nanoparticles have been demonstrated to be i) biocompatible in different in vitro assays with several cell lines over a wide range of concentrations; ii) easy to be internalized into cells via membrane receptor gp60, iii) non-immunogenic, and iv) to exhibit a long half-life time in the blood circulation [17–19]. Current FDA-approved nanoplataforms for drug delivery use HSA NPs as safe medical carriers for drugs such as Abraxane™, a paclitaxel-loaded HSA NP formulation, or Albunex™, an echocardiographic contrast agent made of sonicated, air-filled HSA microspheres. Besides, HSA NPs have been shown to be capable of encapsulating and delivering small-molecular drugs along with inorganic particles [20–22]. For example, Yang et al. [20] developed a hybrid bovine serum albumin (BSA) nanoparticle formulation, in which magnetic iron oxide NPs and the chemodrug doxorubicin (DOX) were loaded in the NPs core. In this way, upon administration, lower side effects and an improve therapeutic outcome was noted by the combination of the antitumor drug and hyperthermia therapy. In another study using gold particles, Peralta et al. [22] encapsulated Au NRs and paclitaxel into HSA NPs for application of a combined therapy against breast cancer; which results in cell mortalities ca. 90% in comparison with single therapy based on PPTT cell, that only 40% of cell death was achieved. However, there are still significant issues related to the passive accumulation and subsequent unspecific biodistribution of hybrid nanocarriers to exert their therapeutic activity so there is an urgent need to develop targeted hybrid nanocarriers to allow the development of a localized dual therapy, enhancing effectiveness and reducing damage to healthy cells. Thus, in the present study we synthesized a hybrid nanoplataform using inorganic Au NRs and DOX entrapped inside HSA NPs, which were subsequently functionalized with folic acid (FA) as a targeting agent ligand to overexpressed folate receptors in several cancer cells such as cervical ones. The irradiation of HSA NPs with a near-infrared (NIR) laser beam allowed the release of DOX in a controlled manner inside the cells to perform a dual chemo- and PPTT therapies; as a consequence, lower light irradiation doses/times and lower drug concentrations were needed for eliminating tumoral cells than the individual treatments alone. In the present study, we used Human serum albumin NPs containing AuNR and doxorubicin conjugated with folic acid (HSA-AuNR-FA NPs) to investigate whether targeted delivery is effective for dual therapy combining chemotherapy with phototherapy. Our goal was then to optimize NPs fabrication and their psycho-chemical characterization, with special attention to both loading and irradiation triggered release of the chemodrug, and to the synergistic effects of combinatorial therapy, facilitated by an active targeting strategy through the surface functionalization of the hybrid protein NPs.

2. Materials

Human serum albumin as a lyophilized powder ≥97% purity, Corning Costar cell culture plates (flat bottom, 6, 12 and 96 well), and 50% aqueous glutaraldehyde stock solution were obtained from Sigma-Aldrich, USA. Dialysis membrane tubing (molecular weight cutoff ~3500) was purchased from Spectrum Laboratories, Inc. (Rancho Dominguez, California). Absolute ethanol for analysis EMPARTA® ACS and sodium chloride were obtained from Merck Millipore. DOXO HCl was obtained from Thermofisher Scientific (Pittsburgh, PA, USA). ProLong® Gold antifade reagent with DAPI and Dulbecco's modified eagle medium, fetal bovine serum (FBS), L-glutamine, penicillin/streptomycin, sodium pyruvate, and MEM non-essential amino acids (NEAA) were purchased from Invitrogen (Thermo Fisher Scientific Inc. Carlsbad, USA). HeLa cervical and HTB-26 breast cancer cells were obtained from Cell Biolabs (San Diego, CA, USA).

2.1. Synthesis of Au NRs

Au NRs were synthesized by the well-known seed-mediated method [23] with some modifications in our work group [24, 25]. Briefly, gold seeds were prepared by adding 0.6 mL of ice-cold $NaBH_4$ (10 mM) to 7.5 mL of hexadecyltrimethylammonium bromide (CTAB) (0.2 M) and 0.25 mL of $HAuCl_4$ (10 mM) solution, under magnetic stirring. After 5 min, the magnetic stirring was turned off and the seeds solution was kept undisturbed at 33 °C for 60 min. Au NR growth solution was prepared by mixing CTAB, $AgNO_3$, $HAuCl_4$ at final concentrations 0.2 M, 0.01 M and 0.01 M, respectively, and mix for 5 min at 500 rpm. Thereafter, 68 μL of a solution 0.1 M of ascorbic acid was added followed by the disappearance of the typical orange color of the gold salt; then 110 μL of Au seeds solution were added and mixed for 2 min and stopped. The color of this solution slowly changed to purple in a period of 16 h. Then, the particle solution was centrifuged and redispersed in 3 mL of water.

2.2. Synthesis of HSA, HSA-DOX and HSA-DOX-Au NR NPs

HSA, HSA-DOX and HSA-DOX-Au NR NPs were prepared using a desolvation technique using absolute ethanol [26, 27]. For preparation of empty NPs, 5 mg/mL HSA in 10 mM NaCl solution was titrated to pH 8.2 and transformed into nanoparticles by the continuous addition of ca. three mL of the desolvating agent ethanol at a rate of 1 mL min^{-1} under magnetic stirring (500 rpm) at room temperature until the solution became just turbid. After the desolvation process, 3 μL of 8% glutaraldehyde in water was added to induce 100% crosslinking between lysine ε-amino groups in HSA molecules of the particle followed by stirring overnight. The resulting NPs were purified by three centrifugation cycles (13,500 rpm, 10 min) followed by resuspension of the pellet to the original volume in distilled water. Each resuspension step was performed in an ultrasonication bath for 5 min. For HSA-DOX NPs, DOX was added to the albumin solution after pH adjustment under magnetic agitation for 2 h to absorb the drug to the protein before the desolvation process; for HSA-DOX-Au NR NPs, 150 μL of the Au NR stock solution (OD = 1) were added after 2 h of HSA-DOX stirring, and the resulting NPs performed as describe above.

DOXO concentration entrapped inside HSA NPs was quantified by a direct method by Uv-Vis spectroscopy using a calibration curve as previously reported [28]. Briefly, all formulated NPs were dispersed in 0.5% pepsin solution, digested in a water bath at 37 ± 1 °C for 2 h and, then, centrifuged at 13,500 rpm for 10 min and supernatant was measured at 480 nm.

2.3. Determination of nanoparticle yield

Nanoparticle yield was determined by BCA assay (Pierce, Thermo Scientific, IL. Briefly, HSA NP's supernatant was read at 562 nm in a microplate reader, a standard curve was generated by dissolving HSA in water (0.5 to 5 mg mL^{-1}, $r^2 = 0.999$). The particle yield was calculated using the following formula where M_a is the actual amount of HSA in the supernatant and M_t is the amount of HSA used for the preparation of nanoparticles.

$$Yield = \frac{Ma}{Mt} \times 100$$