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Lethal drug combination: Arsenic loaded multiple drug mesoporous silica for theranostic applications

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ABSTRACT

Simultaneous delivery of multiple therapeutic agents is of great importance for effective chemotherapy due of its well-known drug synergism and suppression to chemoresistance. We report a new theranostic nanoformulation to shuttle multiple chemotherapeutic agents for successfully exterminating cancer cells. This strategy is based on the fabrication of magnetite doped mesoporous silica nanoparticles (MSNs) in which both internal porous and external surface of MSN are respectively exploited to load two different kinds of cytotoxic cargoes. Notably, an exceptionally high quantity (29%) of poorly hydrophobic drug camptothecin (CPT) is loaded into the nanopores of MSNs; however, in previous reports less than 1% loading efficiency is reported. Following CPT loading in the pores of MSNs, another unconventional but FDA approved arsenic trioxide (ATO) is conjugated onto the surface of nanocomposite via exploiting the thiophilic nature of ATO. Cell inhibition performance of dual drug nanoformulation is significantly higher than single drug formulation, possibly due to additional or synergistic effect, as low as 3 μg/ml of double drug nanocarrier were found effective to exterminate cancer cells. Besides drug delivery, the presence of superparamagnetic magnetite nanocrystals additionally empowers this system to be used as a contrast agent in magnetic resonance (MR) imaging for either monitoring diseased tissues or feedback of chemotherapy. We anticipate that the integration of combination therapy with nanotechnology coupled with versatile magnetic manipulation feature may prove a significant step forward toward the development of effective theranostic agents.

1. Introduction

Over last two decades, Appreciable advancement in nanomedicine has indeed opened numerous new possibilities to improve diagnosis, therapy and monitoring of various debilitating diseases[1–4]. In relation to cancer, chemotherapeutic drugs ubiquitously pose a serious threat to healthy tissues due to their innate ability to target both tumor and normal cells. In order to overcome these obstacles, Nanoparticle-based carriers can provide us lucrative opportunity via specifically targeting cancer cells either through exploiting the leaky vasculature or overexpressed receptors on the surface of cancer cells [5–8]. In addition to drug delivery, nanoparticles also provide an incredible prospect of visualizing and enhancing the images of defected tissues [9]. A great numbers of sophisticated nanobased theranostics platforms, including liposome, polymeric micelle, dendrimers, carbon nanotube, and inorganic nanoparticle have been developed to realize simultaneous diagnosis and therapy [10–16]. Among these nanocarriers, mesoporous silica nanoparticles (MSNs) have been emerged as an effective solid supports in the development of theranostic platform [17–19]. The reason behind its selection lies in its exceptional physicochemical properties, such as enormous surface area, biocompatibility, ready functionalization, controlled and stimuli-responsive drug release. Numerous multifunctional mesoporous silica based systems have, till now, been developed by incorporating diverse kinds of materials in monodispersed silica

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nanoparticles [20]. For instance, Hyeon and coworkers initially reported a facile strategy to obtain multifunctional theranostic nanoparticles by simultaneously incorporating magnetite and quantum dots in the mesoporous shell [21]. The same group later tested the in vivo applicability of improved formulation of MSNs [22]. To achieve targeted specificity, Liong et al. [23] investigated folic acid and dye-doped magnetic MSNs nanocomposite for simultaneous drug delivery, magnetic resonance and fluorescence imaging of cancer cells. Lin Jun group similarly reported a series of mesoporous silica based multifunctional nanoparticles [24]. Despite these noticeable developments in MSNs theranostics, it has rarely been explored to concurrently deliver multiple drugs for combination chemotherapy. Combination chemotherapy has long been an effective regimen in clinics, the biomedical experts nowadays are earnestly contemplating on the option to transport multiple therapeutic agents through a single nanovehicle for potentially enhancing drug efficacy and overpowering the cancer chemoresistance [25]. Pioneering work in this regard was carried out by Agrawal et al. [26] who employed liposomal single nanoparticle to deliver 6-mercaptopurine and daunorubicin against Jurkat and Hut 76 T-cell. In another effort, four times higher therapeutic efficacy was reported, using doxorubicin and verapamil co-encapsulating liposomal nanoparticles [27]. Polymer nanoparticles were also explored for combinatorial chemotherapy to transport verapamil and vincristine [28]. But, mesoporous silica has rarely been used to deliver multiple drugs at the same time.

On another front, apart from typical anticancer drugs, arsenic-based compounds have been garnering a lot of attention due to their well-proven antitumor potential [29]. Arsenic and its methylated species are generally recognized as carcinogen, but on the other hand, they have also been used as therapeutic agents "Delicious Poison" for thousands of years. Specifically, it has been extensively used in Chinese medicine to treat ulcers plague, malaria, cancer, syphilis, and rheumatisms. In recent times, Dr. Thomas Fowler introduced Fowlers solution (As_2O_3 in potassium bicarbonate solution) to treat various malignant diseases such as leukemia, Hodgkin's disease, as well as non-malignant diseases including eczema, asthma, pemphigus, and psoriasis. Recently, A clinical trial was carried based on the principal of traditional Chinese medicine "using a toxic agent against a toxic agent" to treat acute promyelocytic leukemia (APL). Extremely encouraging results (85% remission rate) rekindled the interest in arsenicals and now it is being used as a frontline agent to tackle hematologic malignancies. The therapeutic efficiency of ATO is ascribed to the degradation of an oncogenic protein that drives the growth of APL cells, PML–RAR α [30]. Besides fluid cancers, ATO has also been investigated against several solid tumors, including gastric cancer, esophageal carcinoma, neuroblastoma liver, ovarian, cervical, breast, prostate, and head and neck cancers [31,32]. Due to poor pharmacokinetics and dose-limiting toxicity, much higher As_2O_3 dosages are required for solid tumors which restrict its utility in solid tumors. Various nanoparticulate formulations of ATO have been fabricated to enhance the efficacy and mitigate the toxicity of ATO. For example, ATO has been encapsulated in liposomal or polymeric vesicles but unfortunately large amount of drug is lost within hours at physiological condition [33]. These challenges intensify the need to develop more robust drug nanocarriers for delivering multiple toxic drugs in targeted fashion. In order to meet these challenges, herein, we report a straightforward strategy to fabricate magnetic and dual drug-containing MSNs for simultaneously shuttling drugs cocktail to the site of action. Firstly magnetite nanoparticles were tethered onto the MSNs surface via EDC chemistry. Porous structure of MSNs was then exploited to load significantly lethal dose of campothetacin (CPT) into the nanochannels, afterwards, ATO was tethered onto thiol functionalized exterior surface of MSNs. Prominently, the presence of magnetic particles can enable to achieve not only magnetic targeted delivery of cytotoxic compounds to intended site in the body but also offers a simultaneous opportunity to monitor the therapeutic response of therapy via magnetic resonance imaging (MRI).

2. Experimental

2.1. Materials

Chemical reagents used in this study are of analytical grade and used as received. Cetyltrimethylammonium bromide (CTAB), 3-[4,5-dimethylthialzol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich. 3-Mercaptopropyltriethoxysilane (MPTES), 3-aminopropyltriethoxysilane (APTES), mercaptopropionic acid (MPA), camptothecin (CPT), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), tetraethyl orthosilicate (TEOS, 99.98%), absolute ethanol, dimethyl sulfoxide (DMSO) and toluene were obtained from Aladdin reagent company.

2.2. Synthesis of citrate capped magnetite

Following a literature procedure, citric acid modified magnetite nanocrystals were prepared via co-precipitation method. Typically, 0.86 g $FeCl_2$ and 2.35 g $FeCl_3$ were dissolved in 40 mL distilled water and heated to 80 °C under argon in a three-necked flask. Under vigorous stirring the, 5 mL of NH_4OH was added into reaction mixture by syringe and the heating continued for 30 min. Next, 1 g of citric acid solution (2 mL solution) was introduced and temperature was raised to 95 °C. Stirring was continued for an extra 90 min to complete the coating on the surface of magnetite. The resulting black precipitated magnetite nanoparticles were later washed with excess of ethanol thrice by magnetic separation. Finally, precipitate was re-dispersed in water to obtain transparent solution of well dispersed nanoparticles.

2.3. Synthesis of amine functionalized mesoporous silica

Mesoporous material was prepared by following a previously described method. Typically; 0.5 g of CTAB was first dissolved in 240 mL deionized water. Sodium hydroxide aqueous solution (2 M, 1.8 mL) was added into CTAB solution and then the reaction temperature was raised to 80 °C. After stabilizing the temperature, TEOS (2.5 mL, 11.2 mmol) and 250 μL APTES were consecutively added dropwise to the surfactant solution and held the reaction mixture for 2 h to give a white precipitate. The solid product was filtered, washed with deionized water and ethanol, dried at 60 °C to yield the as-synthesized amine functionalized MSNPs. To maximize the anchoring of amine group, as-synthesized MSNs were refluxed for 12 h in 20 mL anhydrous toluene (300 μL APTES). The product was recovered by centrifugation and washed with ethanol trice. Finally, surfactant (CTAB) was removed by refluxing the nanoparticle in acidic methanolic solution for 6 h.

2.4. Magnetite conjugation and mercapto functionalization of mesoporous silica nanoparticles

Magnetite nanoparticles were anchored onto the surface of MSNs via EDC chemistry. Amine functionalized MSNs (200 mg) were dispersed in 20 mL of water. On the other hand, magnetite NPs (20 mg) were dispersed in 10 mL of water in another beaker and activated by EDC (5 mg). Both solutions were mixed to obtain magnetite conjugated MSNs (Fe_3O_4@MSNs). The solid product was then filtered, washed with deionized water and ethanol. In order to modify the remaining amine group into thiol moiety for ATO