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Research Paper

Redox and pH-responsive gold nanoparticles as a new platform for simultaneous triple anti-cancer drugs targeting☆

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ABSTRACT

Cancer is considered to be one of the leading causes of morbidity and mortality worldwide and nanotechnology was shown to have a unique potential to enhance the therapeutic performance of anti-cancer agents. A novel dual stimuli-responsive polyethylene glycol (PEG) block copolymer was synthesized for the decoration and stabilization of gold nanoparticles (NPs) to carry multiple anti-cancer drugs, doxorubicin (DOX), methotrexate (MTX) and 6-mercaptopurine (MP). DOX, MTX and MP were successfully loaded (the loading capacity of 37%, 12%, and 49%, respectively) into the NPs by ionic interaction (DOX and MTX) and disulphide-covalent bond formation (MP) in the polymeric shell of NPs. Furthermore, the triggered drugs release ability of NPs was shown through the comparison of simulated physiological and tumor tissue environments. The enhanced efficiency of the developed NPs and their targeted performance via MTX (target ligand of folate receptors) decoration were illustrated through the various cell cytotoxicity studies such as MTT assay, DAPI staining, and flow cytometry on various cancer cell lines with different levels of folate receptors. Our proposed idea in simultaneous delivery of three cytotoxic drugs with our newly designed PEGylated gold NPs may provide promising and novel prospect in cancer therapy.

1. Introduction

Nowadays, smart nanoscopic vehicles are designed for bio-medical applications and often are used for encapsulation of drug molecules and releasing them in response to triggers which are important goals in therapeutic applications, especially for cancer treatment (Wu and Wang, 2016; Xu et al., 2016). Nano-scale carriers increase the therapeutic efficiency of drugs against cancer by the accumulation of drugs at the target sites because of a) the passive targeting of anti-cancer agents to the tumor tissues via the enhanced permeability and retention (EPR) effect (Nakamura et al., 2016) and b) enhance the one-way endocytosis of nanoparticles (NPs) which provides promising approach against multi-drug resistance (MDR) (Koushik and Rao, 2016). This drug accumulation in the target tissue decreases the serious side effects associated to the unspecific delivery of cytotoxic anti-cancer drugs to the normal tissues, as well. In order to maximize the targeting ability of NPs, the loaded drugs should be released controllable and rapidly at the site of tumor (Karimi et al., 2016a; Liu et al., 2016). These smart NPs are often decorated with stimuli-responsive polymers containing reactive functional groups to conjugate with targeting ligands and/or drugs (Ghorbani et al., 2016a, 2015a). The decorated intelligent polymers on the surface of NPs are able to control drug biodistribution in response to specific stimuli, either external triggers (e.g. temperature (Karimi et al., 2016b), light (Huang et al., 2016), ultrasound (Boissenot et al., 2016) and magnetic field) or endogenous triggers (e.g. pH (Yi et al., 2016) or redox (Tian et al., 2016)). Among the NPs, gold NPs with stimuli-responsive polymeric shells have been appropriately reputed as a smart bio-platform in therapeutic applications (Paciotti et al., 2016). In spite of many advantages, gold NPs suffer from a) aggregation in physiological conditions and b) rapid elimination from the body by phagocytic activity of the reticuloendothelial system. One of the strategies used to overcome these obstacles is PEGylation of gold NPs. Furthermore, PEGylation helps in shielding the surface charge

☆ This paper is dedicated to the memory of Prof Ali Akbar Entezami, who was the pioneer of Polymer Chemistry of Iran until his untimely death in 2015. Without his knowledge and love of chemistry which he shared so generously, this work would not have been possible.

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of NPs to reduce the non-specific binding in biological environments (Karakoti et al., 2011). The gold NPs can be formed via the autoreduction of auric cations by the tertiary amino groups in polyamines without using any more reducing reagent (Nakamura et al., 2010). Recently, interesting studies have been published on the dual or multi-responsive NPs for application in cancer therapy (Yang et al., 2016). Multi drug therapy of cancers offers noteworthy benefits e.g. increased therapeutics efficiency and simultaneously reduced side effects due to the administration of lower dose than routinely used single drug dose (Qi et al., 2016; Schmiegelow and Nielsen, 2014). Single drug therapy has exhibited a few limitations, primarily due to heterogeneity of cancer cells and a developed resistance to drug. Thus, combination chemotherapy has introduced as a substitute approach to efficient cancer therapy. A few ones focused on the dual delivery of anti-cancer agents via these types of NPs (Cao et al., 2016; Salehi et al., 2015; Surnar and Jayakannan, 2016) and the very limited studies reported the multi-drug delivery by stimuli responsive micellar NPs (Saxena and Jayakannan, 2016; Shin et al., 2009). The micellar nanocarriers suffer from two main drawbacks, a) low drug loading capacity and b) limited to the load of hydrophobic drugs (Kataoka et al., 2001). Both are serious disadvantageous in cancer therapy because most of anticancer-agents are hydrophilic and are administered in high doses. In the reported study, the loading capacity of all of drugs were around 10% and those also limited to the hydrophobic agents. In this work, we designed a novel stimuli-responsive gold NPs that have the capability of stimuli-triggered-controlled release performances by alteration of pH and redox values and was processed for the simultaneous delivery of cationic, anionic, and thiol-containing anti-cancer drugs i.e. doxorubicin (DOX), methotrexate (MTX) and 6-mercaptopurine (MP), respectively. The extra worth of this developed NPs to the micellar structures is potential for efficient cell-uptake due to the folate-mediated endocytosis behavior of MTX. This provides the benefits of active targeting accompanying with the potential passive targeting of NPs (Scheme 1).

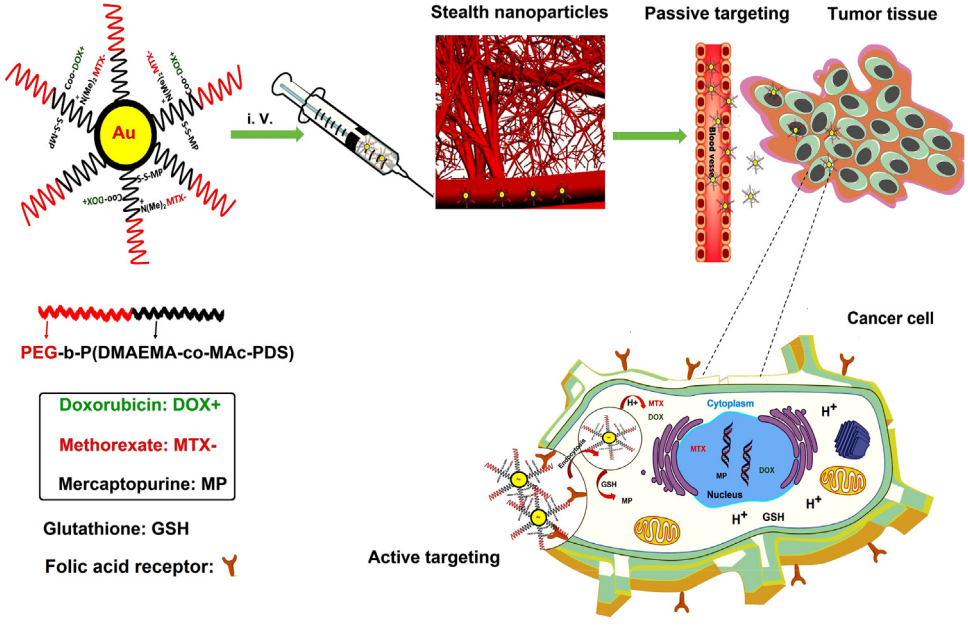
2. Experimental section

2.1. Materials

Methoxy poly(ethylene glycol) (PEG-OH, 5000 Da), hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4.3H_2O$), glutathione (GSH), 6-mercaptopurine (MP) and Copper (I) bromide (CuBr) were purchased from Sigma-Aldrich. Dipyridyl disulfide (DPDS), 2-aminoethanethiol (AET), 2-bromoisobutyrylbromide (BIBB), (*N,N*-dimethylamino)ethyl methacrylate (DMAEMA), maleic anhydride (MAn), N,N,N′,N″,N″-pentamethyldiethylenetriamine (PMDETA) were purchased from Merck Chemicals. MTX was kindly donated from Zahravi Pharmaceutical Company (Iran). DOX was purchased from Exir Nano Sina Company (Iran). CuBr was purified by stirring in acetic acid three times, then washed with ethanol, and dried under vacuum.

2.2. Synthesis of macroinitiator (PEG-Br)

PEG-Br was readily synthesized according to the literature (Zhang et al., 2012a). A solution of PEG5000 (5 g, 1 mmol) and triethylamine (TEA, 2 mL, 14 mmol) in 50 mL of anhydrous THF was cooled in an ice-water bath for 1 h. Then, BIBB (1.6 mL, 7 mmol) was dissolved in 20 mL of THF and added drop-wise to the above solution. The mixture was stirred at 0 °C for another 1 h and then 48 h at room temperature. The insoluble salt of reaction was filtered and then the solvent of the filtrate removed by a rotary evaporator (Hei-VAP Value Digital, Heidolph, Germany). The final product was obtained as a slightly yellow crude. The resulting product was dissolved in 100 mL of CH_2Cl_2 and purified by washing twice with 1 M HCl, 1 M NaOH and saturated aqueous NaCl solution. The solution was dried overnight with $MgSO_4$, filtered, and CH_2Cl_2 was evaporated. The macroinitiator was then precipitated twice in cold ether, followed by drying in a vacuum oven (VO400, Memmert, Germany) at room temperature for 24 h.



Scheme 1. The advantages and capabilities of our newly developed nanocarrier for passive and active multi-drug delivery to the cancerous tissue.