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A novel dual-responsive core-crosslinked magnetic-gold nanogel for triggered drug release

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ABSTRACT

A facial approach was reported to prepare a novel dual-responsive core-crosslinked nanogel and investigated for the triggered methotrexate (MTX) release. Nanogels with core-shell architecture were synthesized by decoration of Au/Fe_3O_4 core/shell NPs using poly(ethylene glycol)-b-poly((N,*N*-dimethylamino)ethyl methacrylate-co-2-hydroxyethyl methacrylate)-maleic acid (PEG-b-P(DMAEMA-co-HEMA)-MA) for crosslinking and autoreduction processes. The second block containing amino groups and maleate groups as the inner shell was used for the reduction of $HAuCl_4$ (auric cation) in the presence of Fe_3O_4 NPs and as a crosslinker agent, respectively. Furthermore, to improve the long-term dispersibility of the nanogels, poly(ethylene glycol) was preferred as outer shell even under high ionic strength. After that, NIPAAm was polymerized from the vinyl double bonds for fabricating the thermo and pH-responsive core-crosslinked nanogels. MTX (an *anti*-cancer agent) was successfully loaded (the loading capacity of 37%) into the nanogels by both ionic interaction and entrapment in polymeric network in the inner shell. The triggered MTX release ability of the synthesized nanocarriers was proved through the comparison of in-vitro drug release at simulated physiological condition and tumor tissue environment. MTT assay showed that MTX-loaded nanocarriers revealed high antitumor activity in MCF7 cell line after incubation following 24 and 48 h. It was concluded that the developed nanogels have many promising qualities as an efficient carrier for the targeted MTX delivery to cancer tissues.

1. Introduction

Drug delivery systems (DDSs) using nanosized carriers were considered for targeting tumor tissue because of their enhanced permeability and retention (EPR) effect [1], reduced drug side effects (an extremely important issue for cytotoxic *anti*-cancer agents), prolonged circulation time, and improved drug bioavailability [2,3]. Among the numerous classes of nanoparticles (NPs), polymeric NPs with embedded superparamagnetic iron oxide [4,5] and gold NPs [6] are attractive platforms designed for clinical use or currently in clinical trials. Gold NPs [7,8] and superparamagnetic iron oxide NPs [9,10] are both used as agents for the passive targeting of cancerous tissue and, consequently, imaging [11,12] and cancer therapy via hyperthermia [13]. Fe_3O_4 NPs are also used for targeting with the aid of an external magnetic field. Therefore, it seems that the hybrid nanoparticle system containing gold and Fe_3O_4 would show a synergistic therapeutic effect [14,15]. Moreover, magnetic NPs suffer from rapid oxidation which is responsible for a loss in NP performance in magnetic fields. Therefore, the design of core/shell nanostructures of Au and Fe_3O_4 can be useful in protecting the Fe_3O_4 core from oxidation and providing a platform for surface modification and functionalization [16,17]. The adjustable therapeutic responses of these systems, by changes in size, shape, and surface functionalization with various polymers and targeting agents, propose their use as a promising approach in developing future strategies for cancer therapy. The low drug-loading capacity and lack of sensitivity for controlling drug release are the most common drawbacks of nanoparticulate DDSs which can be overcome by focusing on the design of a stimuli-responsive polymeric shell on the nanocarriers [18,19]. The thermo- and pH-sensitive nanogels [20–22] provide potential advantages for the development of DDSs through their a) interior network structure for the entrapment of drugs, b) controllable release of drug at particular environments, and c) biocompatibility. Polymeric coatings with biocompatible polyamines as polycations such as poly(N,*N*-dimethylaminoethyl methacrylate) (PDMAEMA) may act as pH-sensitive platforms for drug loading [23,24]. In this case, anionic anticancer drugs such as methotrexate (MTX) can be loaded onto the pH-sensitive polymeric shells of nanocarriers by non-covalent interaction (electrostatic interaction) to assist the drug-loading capacity of the nanocarriers and to take advantage of the acidic environment of a tumor for controlled drug release without significant drug release into the blood [25–27].

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Additionally, an amino group is known to show coordination ability on a metal surface. Therefore, the tertiary amino groups in polyamines can assist the autoreduction of auric cations to fabricate gold NPs without using any more reducing reagent [28]. Thus, the tertiary amino groups in PDMAEMA [29–31] can be effectively immobilized on the gold colloid surface to act as: a) a stabilizer of colloidal gold NPs, and b) a platform for drug loading and pH-responsive performance. Poly(*N*-isopropylacrylamide) (PNIPAAm)-based nanogels [32] have been studied as a temperature responsive polymer, because it exhibits thermally reversible collapse above lower critical solution temperatures (LCST) in water [33,34]. To synthesize chemically crosslinked PNIPAAm hydrogels, N,N′-methylenebisacrylamide (BIS) is often used as a chemical crosslinker agent. The lack of BIS biodegradability, however, restricts the clinical applications of the formed PNIPAAm-based hydrogels. This study reports the development of multi-responsive core-crosslinked nanogels based on Fe_3O_4/Au$_{@}$poly(ethylene glycol)-b-poly((N,*N*-dimethylamino)ethyl methacrylate-*co*-2-hydroxyethyl methacrylate)-poly(*N*-isopropylacryamide) (Au/$Fe_3O_{4@}$PEG-b-P(DMAEMA-co-HEMA)-g-PNIPAAm) for triggered MTX release in cancer tissue. In the first step, PEG-b-P(DMAEMA-*co*-HEMA) was synthesized by atom transfer radical polymerization (ATRP) using a PEG macroinitiator. In the second step, the synthesized PEG-b-P(DMAEMA-co-PHEMA)-maleic acid (MA) was used as a crosslinker and autoreduction agent. In the third step, the double bonds of MA, which are synthesized by reacting hydroxyl groups of HEMA with maleic anhydride, were used for the polymerization of PNIPAAm to prepare the novel multi-responsive nanogels (Au/$Fe_3O_{4@}$PEG-b-P(DMAEMA-co-HEMA)-g-PNIPAAm). The newly-developed nanogels were applied for loading MTX and, consequently, *in vitro* cytotoxic performance against cancer cells.

2. Material and methods

2.1. Materials

Ferrous chloride tetrahydrate ($FeCl_2$, $4H_2O$), ferric chloride hexahydrate ($FeCl_3$, $6H_2O$), 2-Bromoisobutyrylbromide (BIBB), 2-Hydroxyethyl methacrylate (HEMA), (N,*N*-dimethylamino)ethyl methacrylate (DMAEMA), N,N,N′,N″,N‴-pentamethyldiethylenetriamine (PMDETA), maleic anhydride (MA), rhodamine B and ammonium peroxy disulfate (APS) were purchased from Merck Chemicals. Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4$) and methoxy poly(ethylene glycol) (MPEG-OH, 2000 Da) were purchased from Sigma-Aldrich. *N*-Isopropylacrylamide (NIPAAm, 99%) was purchased from Acros. MTX was kindly donated from Zahravi Pharmaceutical Company.

2.2. Synthesis of mPEG macroinitiator

mPEG-Br was prepared according to the literature [35]. mPEG2000 (3 g, 1.5 mmol) and triethylamine (TEA, 1 mL, 8.13 mmol) were dissolved in 30 mL of anhydrous tetrahydrofuran (THF), and the solution was cooled in an ice-water bath for 30 min. BIBB (1.1 mL, 8.37 mmol) was dissolved in 15 mL of THF and added in a drop-wise manner to the mPEG/THF. The reaction mixture was warmed to room temperature and stirred for 48 h. The resulting reaction solution was filtered to remove the insoluble salt and then the filtrate was evaporated to remove the solvent under reduced pressure (Edwards RV3 vacuum pump, UK). The resulting crude product was dissolved in water, and the solution was extracted three times with dichloromethane. After that, the solution washed with 1 M HCl, 1 M NaOH and saturated aqueous NaCl solution, dried over anhydrous magnesium sulfate ($MgSO_4$), and finally filtered. The filtrate was precipitated into a large excess of ice-cold diethyl ether. The resulting product was dried in a vacuum oven (VO400, Memmert, Germany) at room temperature for 24 h.

2.3. Synthesis of block copolymer mPEG-b-P(DMAEMA-co-HEMA)

mPEG-Br (1 g, 0.042 mmol) and CuBr (6 mg, 0.042 mmol) were added to a Schlenk reactor equipped with a stir bar. After sealing with a rubber septum, the reactor was evacuated and degassed with nitrogen by three cycles. Deoxygenated cyclohexanone (6 mL) was added to the reactor and stirred for 30 min. Then deoxygenated PMDETA (10 μL, 0.042 mmol) was added to the reactor and the solution changed from cloudy to clear and light green. After that, a deoxygenated mixture of DMAEMA (297 mg, 1.9 mmol) and HEMA (27.2 mg, 0.205 mmol) was added to the mixture of reactor. After 10 min, the reactor was immersed in an oil-bath and the polymerization reaction was performed at 70 °C under nitrogen atmosphere with continuous mechanical stirring for 20 h. For isolation and purification of the copolymer, the reaction solution was diluted with 10 mL of acetone and the solution was transferred into a dialysis bag (molecular weight cut off 1000 Da, Sigma-Aldrich, USA) to remove unreacted monomer and catalyst and dialyzed against deionized water for two days. Finally, the precipitate was obtained after freeze-drying (1–16 LSC, GAMMA, UK). The molecular weight (M_n) of copolymer was calculated according to the result of ^{1}HNMR by comparing the intensities of signals at 4.1 ppm (−COO–C***H***$_2$– of PDMAEMA), 3.9 ppm (−COO–C***H***$_2$– of PHEMA) and a reference peak at 3.65 ppm (−OC***H***$_2$C***H***$_2$– of mPEG) [36].

2.4. Synthesis of PEG-b-P(DMAEMA-co-HEMA)-MA (PEG-copolymer-MA)

PEG-copolymer-MA was prepared according to the literature procedure [37,38]. Briefly, 0.25 g of (PEG-b-P(DMAEMA-co-HEMA) was added into 6 mL of toluene and stirred until the solution was dissolved completely. Then, maleic anhydride (159 mg, 1.59 mmol) was added to the solution under a stream of nitrogen. Upon completion of reaction, the mixture is cooled to room temperature, and the soluble products were isolated by precipitation with 20 mL of diethyl ether at room temperature. Finally, the product was redissolved in 10 mL of dichloromethane and precipitated by addition of 30 mL of diethyl ether to remove excess maleic anhydride (three times). The resulting product dried overnight at 60 °C in a vacuum oven.

2.5. Synthesis of Fe_3O_4 NPs

Iron oxide (Fe_3O_4) NPs were prepared in similar approach as reported [39]. 4 mL of ferric chloride solution (1 M) and 1 mL of ferrous chloride solution (2 M, in HCl 2 M) were mixed and added into 50 mL ammonia solution (0.7 M). After 30 min, the precipitate was isolated by magnetic decantation and agitated with 50 mL diluted $HClO_4$ (2 M). Then, the resulting colloidal suspension was separated by centrifugation and the filtrate was diluted to 50 mL with water.

2.6. Synthesis of Fe_3O_4/Au@PEG-copolymer-MA

By simply mixing the polymer solution with a $HAuCl_4$ solution at 100 °C under specified conditions, Fe_3O_4/Au NP-decorated polymers can be achieved [40]. For the synthesis of the copolymer containing Fe_3O_4/Au NPs at N/Au ratio of 20, an aqueous solution of $HAuCl_4$ (300 μl, 50 mM) was added to 15 mL of DI water and then heated to boiling. N/Au ratio implies on the mole ratio of tertiary amino groups to Au (III) ions. The typical procedure for the synthesis of the copolymer containing AuNPs was introduced at N/Au ratio of 20 [41]. Then, 1 mL of *as*-prepared Fe_3O_4 NPs suspension was added into the reaction mixture followed by the addition of the copolymer solution (100 mg/mL) under stirring and the resulting mixture was adjusted to pH 9. Under vigorous stirring, the color gradually turned from brown to burgundy. This procedure does not require the addition of an external reducing agent and results in stabilized NPs which continue dispersed in aqueous solution upon cooling to room temperature. In order to the purification purify of PEG-copolymer, the free excess polymers in the solution was