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Magnetic mesoporous silica nanoparticles coated with thermo-responsive copolymer for potential chemo- and magnetic hyperthermia therapy

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ABSTRACT

We developed a potential chemo- and magnetic hyperthermia therapeutic platform based on thermo-responsive copolymer coated magnetic mesoporous silica nanoparticles (MMSN@P(NIPAM-co-MAA)). The structure, magnetic heating capacity, drug release behavior, *in vitro* cytotoxicity, cell uptake, and synergistic therapeutic efficacy of MMSN@P(NIPAM-co-MAA) nanoparticles were investigated. The prepared superparamagnetic MMSN@P(NIPAM-co-MAA) nanoparticles had an average particle size of 255 ± 28 nm. The saturation magnetization was 6.2 emu/g and resulted in heat generation to hyperthermia temperature under an alternating magnetic field within a short period. MMSN@P(NIPAM-co-MAA) nanoparticles could load doxorubicin hydrochloride (DOX), and exhibited temperature- and pH-responsive drug release behavior. Importantly, MMSN@P(NIPAM-co-MAA) nanoparticles had low cytotoxicity and were internalized by HeLa cells. The DOX-loaded nanoparticles showed a synergistic effect that combined chemo- and magnetic hyperthermia therapy, resulting in higher efficacy to kill cancer cells. Thus, MMSN@P(NIPAM-co-MAA) nanoparticles have great potential for cancer therapy.

1. Introduction

Magnetic mesoporous silica nanoparticles (MMSNs) are a promising platform for cancer therapy [1–8]. They have attracted much attention because of their potential in chemotherapeutic drug delivery and hyperthermia therapy under an alternating magnetic field (AMF). Gan et al. [3] fabricated a magnetic and reversible pH-responsive drug delivery system by anchoring Fe_3O_4 nanoparticles on the pore outlet of MSNs via a reversible boronate esters linker. By alternately changing the pH from 3 to 7, these Fe_3O_4 cap gates could be switched “on” and “off” to release the entrapped drug in a pulsinate manner [3]. Martín-Saavedra et al. [4] synthesized magnetic γ-Fe_2O_3-encapsulated MMSNs that could conduct magnetic hyperthermia upon exposure to low frequency AMF. MMSNs were efficiently internalized by human A549, Saos-2, and HepG2 cells. Cell viability dropped as a function of the intensity of the heat treatment achieved by MMSNs and AMF exposures [4]. However, chemotherapy alone results in multidrug resistance of tumor cells and magnetic hyperthermia treatment is effective only at localized sites.

Thus, to improve their therapeutic efficacy for cancer therapy, much effort has been made to construct MMSNs-based platforms that combine controlled drug delivery and magnetic hyperthermia [9–17]. This might have synergistic therapeutic effects on tumor cells favoring a reduction of the required effective dose of toxic chemotherapeutic drugs. Benyettou et al. [9] reported a doxorubicin (DOX)-loaded- Pluronic F108-coated system (DOX@F108-mNPs) that was stable at room temperature and physiological pH. This system released DOX slowly under acidic conditions or in a sudden burst with magnetic heating [9]. Furthermore, treatment of cervical cancer cells (HeLa) with both Dox@F108-mNPs and subsequent AMF-induced hyperthermia resulted in significantly

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enhanced reduction of cell viability than either DOX or DOX@F108-mNP treatment alone. Lu et al. [10] prepared magnetic iron oxide-loaded hollow mesoporous silica nanocapsules with a particle size of 100 nm. These magnetic nanocapsules generated heat upon exposure to an AMF and remotely triggered drug release, thus exhibiting great potential for synergistic chemotherapy and magnetic hyperthermia [10]. Additionally, our group prepared MMSNs with controllable magnetization. In an AMF, these MMSNs could controllably generate heat to hyperthermia temperature within a short time, while the DOX-loaded MMSNs exhibited pH-controlled drug release behavior [11–14]. These results suggest the contribution of the magnetic property of MMSNs to the hyperthermia capacity, while the functionalization of MMSNs determined the drug release behavior. Therefore, methods to functionalize MMSNs are key issues for the construction of MMSN-based platforms with better therapeutic efficacy.

Temperature is an ideal stimulus for controlling drug release because the local body temperature can change with ambient conditions [18]. Diverse chemical materials such as phase-change molecules, DNA molecules, and thermo-responsive polymers possess temperature-responsive property and are exploited to regulate the release of drug molecules from the carriers [19–26]. Aznar et al. [19] and Liu et al. [20] designed temperature-controlled drug release systems based on MSNs using phase-change molecules as caps. However, only limited phase-change molecules could be used for constructing controlled drug release systems because of biosafety requirements and phase-change temperature within the range of body temperature. Chang et al. [21] and Li et al. [22] developed NIR light-triggered nanocarriers for controlled drug release. In these nanocarriers, drugs were loaded into mesoporous silica coated Au nanorods and capped with DNA. Au nanorods functioned as nanoheaters by absorbing the NIR laser. Yu et al. [23] studied the relationship between the chain length of DNA and the critical stimuli temperature. MSN-based drug delivery system was capped with different lengths of single-stranded DNA oligomers and drug release was triggered by gate opening in response to an increase in temperature. Jiao et al. [24] prepared a copolymer of 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) and oligo(-ethylene glycol) methacrylate (OEGMA) cross-linked by disulfide bonds (P(MEO_2MA—s—s—OEGMA)) to coat hollow MSNs. The drugs loaded in these nanoparticles were released rapidly at a temperature higher than 37 °C [24]. Thus, temperature-controlled drug release systems have great potential for enhancing the efficiency of drug delivery.

Poly(N-isopropylacrylamide) (PNIPAM) is the most studied thermo-responsive polymer. It shows a sharp phase transition at its lower critical solution temperature (LCST) of about 32 °C in aqueous solution [27]. Below its LCST, PNIPAM exists in a hydrophilic state with a highly hydrated and extended "random coil" conformation. As the temperature increases above its LCST, PNIPAM displays hydrophobic nature with an extensively dehydrated and collapsed "globular" conformation [28]. This is a reversible process and can be used to make the polymer behave as an on-off system when the temperature is changed across the LCST [29–33]. Importantly, the LCST of PNIPAM can be altered by incorporating co-monomer units such as acrylamide and methacrylic acid [34–38]. Shah et al. [34] coated poly(N-isopropylacrylamide-*co*-acrylamide) (PNIPAM-*co*-Am) on $MnFe_2O_4$ magnetic nanoparticles. The LCST of this thermo-responsive copolymer was around 39 °C when the molar ratio of acrylamide and NIPAM was 1:15 [34]. In poly(N-isopropylacrylamide-*co*-methacrylic acid) (P(NIPAM-*co*-MAA)) coated magnetic mesoporous nanoparticles, the LCST of P(NIPAM-co-MAA) shifted from 33.9 °C to 44.4 °C when the MAA content was increased from 0 mol % to 3 mol % [35]. These results suggest the use of PNIPAM-based copolymer coated MMSNs constructs as promising multifunctional platforms favoring temperature controlled drug release and hyperthermia upon exposure to an AMF.

In the present work, we constructed a chemo- and magnetic hyperthermia therapeutic platform comprising of thermo-responsive P(NIPAM-co-MAA) as release "gate-keepers" and MMSNs as drug containers and thermo-seeds. P(NIPAM-co-MAA) copolymer was coated onto the surface of MMSNs via precipitation polymerization of NIPAM and MAA on methacrylate groups modified MMSNs (MMSN-MPS). The model anticancer drug, doxorubicin hydrochloride (DOX), was loaded into MMSN@P(NIPAM-co-MAA) nanoparticles. This was achieved by dispersion of nanoparticles at a greater LCST, causing structural collapse of P(NIPAM-co-MAA) copolymer. When DOX-loaded MMSN@P(NIPAM-co-MAA) (DOX-MMSN@P(NIPAM-co-MAA)) nanoparticles were treated under an AMF, MMSNs could generate heat to hyperthermia temperature, which was higher than the LCST, triggering simultaneous DOX release. Our results indicate the use of MMSN@P(NIPAM-co-MAA) nanoparticles as a promising platform with chemo- and magnetic hyperthermia therapy.

2. Experimental details

2.1. Materials

Magnetic Fe_3O_4 nanoparticles of particle size 15–20 nm were synthesized via co-precipitation method [39]. Tetraethyl orthosilicate (TEOS), triethanolamine (TEA), ethanol, hydrochloric acid (HCl, 36–38%), potassium dihydrogen phosphate (KH_2PO_4), sodium hydroxide (NaOH), ferric chloride ($FeCl_3 \cdot 6H_2O$), ferrous chloride ($FeCl_2 \cdot 4H_2O$), methacrylic acid (MAA), and potassium persulfate (KPS) were obtained from Sinopharm Chemical Reagent Co., Ltd, (Shanghai, China). Doxorubicin hydrochloride (DOX) was purchased from Sangon Biotech Co., Ltd, (Shanghai, China). Hexadecyltrimethylammonium p-toluenesulfonate (CTAT), N-isopropylacrylamide (NIPAM), N, N′-methylenebisacrylamide (MBA), and 3-(trimethoxylsilyl) propyl methacrylate (MPS) were purchased from Sigma-Aldrich. Ultrapure water was obtained from Millipore pure water system (Shanghai, China).

2.2. Preparation of MMSN@P(NIPAM-co-MAA) nanoparticles

MMSNs were prepared as previously described [11]. Briefly, 1.0 g of Fe_3O_4 nanoparticles was dispersed in 90 ml of water. To this, 1.71 g of CTAT and 1.0 g of TEA were added and stirred vigorously at 80 °C until they dissolved completely. Subsequently, 14.0 ml of TEOS was rapidly added to the above solution and the mixture was allowed to react for 2 h. Brown colloidal nanoparticles were separated with a magnet, washed several times with ethanol, and dried in vacuum at 60 °C for 24 h. Finally, MMSNs were obtained after calcination of dried brown colloidal nanoparticles at 540 °C for 7 h.

Before coating P(NIPAM-co-MAA) on MMSNs, MPS was used to modify MMSNs with methacrylate groups. Briefly, 500 mg of MMSNs were suspended in 100 ml of anhydrous ethanol by ultrasonication. Then, 2.0 ml of MPS was added to the mixture and stirred for 24 h at room temperature. The suspension was filtered, washed extensively with ethanol to remove the residual MPS, and was dried in vacuum at 60 °C for 24 h to obtain MPS-modified MMSNs (MMSN-MPS).

Coating of P(NIPAM-co-MAA) on MMSNs was performed by precipitation polymerization method as described previously [35]. Briefly, MMSN-MPS were dispersed in 60 ml of water in a three-neck flask equipped with a reflux condenser. To this, 100 mg of NIPAM, 2.4 mg of MAA, and 7 mg of MBA were added and the mixture was heated to 70 °C under a nitrogen atmosphere. After