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Protocols

On-command controlled drug release by diels-Alder reaction using Bi-magnetic core/shell nano-carriers

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ABSTRACT

A novel bi-functional thermo-responsive system, consisting of core/shell bi-magnetic nanoparticles with furan surface functionality, is bonded with N-(2-Carboxyethyl)maleimide through Diels-Alder reaction. The chemotherapeutics doxorubicin is attached onto the surface, with a high loading efficiency of 92%. This system with high responsiveness to a high frequency external alternating magnetic field shows a very good therapeutic efficiency in hyperthermia and drug release at relatively low temperatures (50 °C). Polyhedron-shaped bi-magnetic nanoparticles ($Zn_{0.4}Co_{0.6}Fe_2O_4$@$Zn_{0.4}Mn_{0.6}Fe_2O_4$) exhibit a significant increase of the specific energy absorption rate up to 455 W/g compared with the core nanoparticles (200 W/g). Real-time florescence spectroscopy studies demonstrate rapid release of doxorubicin up to 50% in 5 min and up to 92% after 15 min upon exposure to high frequency external alternating magnetic field. The stability is evaluated for 8 weeks in phosphate buffer saline with a doxorubicin payload of 85%. In vitro studies using standard MTT cell assays with HeLa and Hep G2 lines prove an excellent biocompatibility with about 90% of cell viability after 24 h of treatment within the highest concentration of functionalized magnetic nanoparticles (200 μg/mL). The results indicate a controlled drug release mediated by thermo-responsive switching under applied alternating magnetic field.

1. Introduction

Remote control of the rate of drug release by using an alternating magnetic field (AMF) is a persistent challenge in medicine. Controlled release formulations of therapeutic molecules from a magnetic platform offer several advantages, such as delivery of the desirable amount of the drug to the target of interest and synergistic effects of hyperthermia to the affected tissue [1]. In order to provide on-demand drug release it is necessary to have a switching material that can modulate the drug release kinetics upon an external triggering electromagnetic field. Cancer cells may confer thermotolerance as a result of continuous heat exposure within 30 min at 43 °C [2]. Inhibition of heat-shock proteins (HSPs) in tumor cells could overcome the thermo-resistance and enhances the apoptosis during magnetic hyperthermia [3]. For optimal efficacy, hyperthermia is combined with chemotherapy, and lower thermal doses could be used by increasing the rate of heat transfer [4]. Otherwise, some authors indicated an uncoupling of thermo-tolerance and Hsp70 expression under slow rate heating conditions [5]. Magnetic hyperthermia is a viable tool for on-demand drug release inducing melting and subsequent capsule rupture on exposure *via* water ingress in vivo [6]. There are very few papers reporting drug release by a Diels-Adler (DA) reaction activated upon high frequency alternating magnetic field (HF-AMF). The DA reaction is known as a thermo-reversible reaction between diene and alkene derivatives (dienophiles) to form cyclic hydrocarbon chains in aqueous conditions [7]. Thermally reversible Diels–Alder reactions (rDA) between various furan and maleimide derivatives have attracted particular attention because they can occur under mild conditions with high chemo-selectivity [8,9]. The speed and reversibility of the DA reaction is dependent on the temperature. At low temperatures the DA adduct is dominant and the cross-linked networks are relatively stable. On the other side, at high temperatures, rDA adduct uncoupling becomes significant. Some reports have focused on the cross-linking mechanism between maleimide and furan groups where DA reaction occurs in a hydrogel medium [10,11]. These materials did not readily degrade by a rDA process as the kinetics of the reaction were too slow under physiologically compatible conditions. However, there are several challenges associated with the self-healing materials based on DA reaction, such as the occurrence at high temperatures (80–120 °C) to trigger the rDA reaction that is not compatible with biological applications [12,13]. Other authors

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reported a new class of functionalized iron oxide nanoparticles initiating the rDA reaction at 70 °C without AMF [14]. Even though those temperatures seem to be very high for a biological medium, recent studies have shown the existence of local heating profiles generated at the magnetic nanoparticles surface upon AMF, leading to high temperatures within a contour only of a few nanometers [15]. Some authors use gold nano-rods to initiate the polyethylene glycol chain release through rDA by the photo-thermal effect [16]. Using the advantage of the exchange coupling between a magnetically hard core and magnetically soft shell, a new type of magnetic nanoparticles with high specific energy absorption rate (SAR) has been reported [17]. The interfacial exchange interaction between hard and soft magnetic phases with reduced surface energy and optimum size of the nanoparticles enhances heating efficiency of the colloidal system. Some authors modified magnetic core/shell nanoparticles with polyethylene glycol and hyaluronic acid to facilitate targeted magnetic resonance imaging guided synergism of magnetothermal and chemo-therapy [18].

We have developed a facile method which is based on functionalized bi-magnetic core/shell nanoparticles (CS-MNPs). Upon HF-AMF exposure the drug would release through rDA reactions at relatively low temperatures of the magnetic fluid (50 °C). The proof-of-concept of our system which consists of shape-anisotropic CS-MNPs, functionalized with doxorubicin (DOX) via a thermo-responsive linker, demonstrated a viable drug release system upon exposure to HF-AMF radiation based on the rDA reaction.

2. Materials and methods

2.1. Material

Zinc (II) acetylacetonate ($Zn(acac)_2$), cobalt (II) acetylacteonate ($Co(acac)_2$), tetramethyl ammonium hydroxide (TMAOH), manganese (II) acetylacteonate ($Mn(acac)_2$), N-hydroxysuccinimide (NHS), and 1,2-Hexadecanediol were purchased from Sigma-Aldrich. Iron (III) acetylacetonate ($Fe(acac)_3$) was provided from ACROS Organics. Absolute ethanol, hexane, toluene and benzyl ether were used as received. Oleic acid and oleylamine were purified under an argon atmosphere. 2-furfuryl isothiocyanate, N-(2-carboxyethyl)maleimide, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and doxorubicin hydrochloride (DOX) were purchased from Fluka. Alendronic acid was acquired from Santa Cruz Biotechnology.

2.2. Synthesis of CS-MNP

The CS-MNPs were synthesized by the seed-mediated growth method [19]. In the first step we synthesized $Zn_{0.4}Co_{0.6}Fe_2O_4$ nanoparticles as seed nanoparticles. Secondly, a $Zn_{0.4}Mn_{0.6}Fe_2O_4$ shell was over-grown by thermal decomposition onto the surface of the seed particle. Synthesis of $Zn_{0.4}Co_{0.6}Fe_2O_4$ nanoparticle seeds: $Zn(acac)_2$ (0.4 mmol), $Co(acac)_2$ (0.6 mmol), and $Fe(acac)_3$ (2 mmol) were placed in a (50 mL) three-neck round-bottom in the presence of oleic acid (6 mmol), oleylamine (6 mmol), and benzyl ether (20 mL). The mixture was heated to reflux for 60 min at 300 °C, with a heating rate of 8 °C/min, under an argon atmosphere and cooled down to room temperature (RT). Under ambient conditions, ethanol was added to the mixture resulting in black precipitate via centrifugation (4500 rpm, 10 min). The black powder was dissolved in hexane.

Synthesis of $Zn_{0.4}Co_{0.6}Fe_2O_4$@$Zn_{0.4}Mn_{0.6}Fe_2O_4$ nanoparticles: $Zn(acac)_2$ (0.4 mmol), $Mn(acac)_2$ (0.6 mmol), and $Fe(acac)_3$ (2 mmol) were placed in a (50 mL) three-neck round-bottom in the presence of oleic acid (6 mmol), oleylamine (6 mmol), and benzyl ether (20 mL). After the addition of the $Zn_{0.4}Co_{0.6}Fe_2O_4$ nanoparticles suspended in hexane, the mixture was first heated to 100 °C for 30 min to remove the hexane, and then heated to reflux 300 °C for 60 min under an argon atmosphere with a heating rate of 8 °C/min. After cooling, the ethanol was added to the mixture resulting in black precipitate. The obtained precipitate was dispersed in toluene after centrifugation. Preparation of Hydrophilic Monodisperse core/shell $Zn_{0.4}Co_{0.6}Fe_2O_4$@$Zn_{0.4}Mn_{0.6}Fe_2O_4$ Nanoparticles: CS-MNPs were transferred from toluene to the aqueous phase by surface modification using TMAOH [20]. The nanoparticles were precipitated and centrifuged, and subsequently TMAOH/butanol (1m) solution was added. The mixture was allowed to react for 30 min under sonication. After the mixture was centrifuged, supernatant was removed and the black precipitate was washed with excess amount of hexane. The nanoparticles were dispersed in water.

2.3. Conjugation of DOX to $Zn_{0.4}Co_{0.6}Fe_2O_4$@$Zn_{0.4}Mn_{0.6}Fe_2O_4$ nano-carriers via thermo-responsive switch

As-synthesized magnetic nanoparticles (50 mg) were mixed with a solution of alendronic acid (100 mg alendronic acid in 25 mL water) and heated for 90 min at 90 °C. The alendronic acid in excess was removed by washing and centrifugation. 2-furfuryl isothiocyanate (100 μL) was added to solution and stirred for 20 h at 45 °C. The excess of 2-furfuryl isothiocyanate was eliminated by washing with distilled water and centrifugation for several times. Afterwards, N-(2-Carboxyethyl)maleimide (0.44 mmol) was added and stirred for 20 h at 45 °C. The excess of N-(2-Carboxyethyl)maleimide was removed by washing with PBS. Functionalized nanoparticles (1 mg/mL) were mixed with EDC (20 mg), NHS (20 mg), and DOX (1 mg) and stirred for 24 h at 37 °C. The DOX-loaded nanoparticles were purified by washing and centrifugation several times with distilled water and stored in a refrigerator in the dark before further experiments.

2.4. Structural characterization of CS-MNPs

A PANanalytical X'Pert Pro MPD X-ray diffractometer was used to determine the crystalline structure of nanoparticles. The diffractograms were recorded using $Cu_{K\alpha}$ radiation (λ=1.54 Å) at room temperature with a step size of 0.02° and a count rate of 2s/step. The mean crystallite size of the nanoparticles was calculated by Scherrerís formula from the (311) Miller plane of the diffraction pattern. TEM analyses were performed on a JEOL JEM-2010 instrument with spatial resolution of 1.4 Å (lattice) and 1.94 Å (point to point). X-ray photoelectron spectroscopy (XPS) Analysis was realized by means of ULVAC PHI 5000 Versa Probe II spectrometer with monochromated Al K_α (1486.7 eV) X-ray radiation. All binding energies were referenced to the carbon 1 s peak at 284.6 eV. The hydrodynamic diameters of CS-MNPs were determined by dynamic light scattering (DLS) using an ALV 5000 spectrometer equipped with a Nd YAG-laser (λ = 532 nm) at 90° scattering angle.

2.5. Magnetic characterization

Room temperature magnetic measurements were done by a vibrating sample magnetometer (MicroMag 3900, Princeton Measurements Corp., U.S.A) which provides a maximum magnetic field of 1 T. Magnetic heating measurements were performed to assay the heating performance of the nano-carriers. The SAR is commonly employed to quantify the heat dissipation rate of a given ferrofluid. SAR measurements of the nanoparticles in water were carried out using two devices: Hüttinger Elektronik, model IG5, which provides an AMF at 1950 kHz and power values up to 7.2 kW; Hüttinger Elektronik, model TIG 20/300, that uses a frequency of 400 kHz and power values up to 20 kW. The ferrofluid sample with a concentra-