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Carbon encapsulated iron oxide nanoparticles surface engineered with polyethylene glycol-folic acid to induce selective hyperthermia in folate over expressed cancer cells

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ABSTRACT

Carbon encapsulated iron oxide nanoparticles (CEIO-NPs) prepared by carbon arc method were successfully applied for in vitro magnetic hyperthermia. The CEIO-NPs were chemically oxidized and surface modified with PEG–FA for selective tumor localization in cancer cells that over expresses the folate receptors (FR^+). The size, morphology, heating efficiency, biocompatibility and in vitro cell uptake of CEIO–PEG–FA NPs are extensively characterized. The as-prepared nanoparticles have generated quick heating (43–45 °C) upon exposure to an alternating magnetic field (AMF) with the saturation magnetization of 25 emu/g. The LDH cytotoxic assay demonstrated that the nanoparticle did not affect the viability of normal human fibroblast. The quantitative and cellular uptake studies by TEM confirmed the selective and increased uptake of CEIO–PEG–FA NPs when compared to the CEIO-nanoparticles. In conclusion, CEIO–PEG–FA NPs have the potential to induce magnetic hyperthermia in FR^+ cells via the receptor mediated endocytosis uptake mechanism.

1. Introduction

Magnetic iron oxide nanoparticles (MIONs) are widely applied in biological, electrical and biomedical fields owing to their unique magnetic and tunable physical and chemical properties (Hsiao et al., 2008). The maghemite (γ-Fe_2O_3) and magnetite (Fe_3O_4) are the most probed types of iron oxides. The potential application of superparamagnetic nanoparticles includes cell labeling and targeting, drug delivery, magnetic resonance imaging (MRI), hyperthermia and magnetofection etc. (Ladj et al., 2013). Unmodified iron oxide nanoparticles generally possess high surface energies due to larger surface to volume ratio leading to particle aggregation. Besides, they tend to be easily oxidized in air resulting in the loss of magnetic property as well dispersability (Wu et al., 2008; Hamley, 2003). It greatly affects the biomedical application of magnetic iron oxide nanoparticles especially in magnetic resonance imaging (MRI) and hyperthermia. Hence, a suitable surface coating with surfactants or polymers is often needed to ensure the stability of the nanoparticles and it could be achieved with inorganic and polymeric materials (Chen and Lin, 2009). The carbon or graphene coating would significantly reduce the magnetic agglomeration and protect the particles from oxidation (Bystrzejewski et al., 2006). Furthermore, the carbon encapsulation offers excellent chemical and thermal stability and facilitates smooth functionalization.

The development of functionalized or multifunctionalized nanoparticles is expected to offer more benefits in targeted drug delivery, tissue engineering, MRI and hyperthermia. It was also reported that the magnetic NPs combined with polymers enhance the surface chemistry of nanocomposites and act as a linker to couple functional molecules on their surface (Tassa et al., 2011). Magnetic nanoparticles (MNPs) with appropriate surface modifications have also been successfully applied to deliver therapeutic biomolecules, such as anticancer drugs, antibodies, and siRNAs, to target tumor cells. Similarly, various molecules such as antibodies, proteins, targeting ligands, etc., are conjugated to the nanoparticle surface by chemical coupling via amide or ester bonds. Hyperthermia is an active cancer therapy, in which certain body tissues are

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exposed to the temperature range of 41–46 °C to damage and kill cancer cells. The conventional hyperthermia treatments have several limitations like damage to healthy tissue, limited heat penetration and under-dosage of heat etc. (Issels, 2008). Further, their efficacy is greatly challenged by deep seated tumors because it lacks the ability to penetrate the surrounding tissue to achieve desired temperature in tumor tissues. Magnetic fluid hyperthermia (MFH) can possibly resolve all the limitations of conventional hyperthermia, in which the heat is generated by magnetic iron oxide nanoparticles with the help of an alternating magnetic field (AMF) that ensures the direct delivery of heat to the tumor site and limit the damage to surrounding tissues (Zhang et al., 2010a). Despite the numerous achievements, the prolonged blood circulation time of the nanomaterials and high targeting efficiency at tumor sites should be improved to avoid nonspecific accumulation in healthy tissues and improve the treatment efficacy.

The most common limitation of nanoparticle based therapeutic applications is due to the rapid elimination of nanoparticles from the blood stream by macrophage mediated mononuclear phagocytic system (MPS). To reduce the macrophage recognition, the circulation time of the particles should be considerably protracted. Modification of particle surface with polymers like poly(ethylene glycol) (PEG) is a popular strategy in nanomedicine to improve longer circulation time. It is hypothesized that PEG anchored surface attracts a water shell that resulted in reduced adsorption of opsonins and recognition by MPS (Senior et al., 1991). The prolonged circulation time alone is not sufficient for the improvement of treatment efficacy. The targeting moieties are important for the cellular uptake process. Active targeting is a kind of strategy, where functional targeting moieties are peripherally conjugated with the nanoparticle system to permit preferential accumulation of particles at the tumor site. In active targeting based cancer treatment, the common molecular targets include carbohydrate, antibody and receptor targeting. The folate receptor (38 kDa glycosylphosphatidylinositol-anchored glycoprotein) is overexpressed in many cancer tissues such as ovarian, epithelial, breast, lung, etc., and its expression is found to be limited in healthy tissues (Yoo and Park, 2004). Hence, folate receptor targeted treatment appears to be a most promising option in cancer therapeutics. Folic acid conjugated nanoparticles or drugs will selectively bind to the folate receptor and internalize through endocytosis (Dai et al., 2014).

The synthesis of magnetic nanoparticles (MNPs) with an iron oxide core and a graphite carbon shell as an effective magnetic fluid hyperthermia to kill folate overexpressed cancer cells is proposed. The CEIO nanoparticle surface was modified with PEG and folic acid for prolonged blood circulation and specific targeting. The CEIO–PEG–FA nanoparticle system was extensively characterized by XRD, TEM, EDS, FTIR and SQUID. The biocompatibility and hyperthermia effect of CEIO–PEG–FA NPs along with in vitro cell uptake was also demonstrated.

2. Materials and methods

2.1. Synthesis of carbon encapsulated iron nanoparticles (CEIO-NPs)

The carbon encapsulated iron oxide nanoparticle was prepared according to the modified Krätschmer–Huffman carbon arc plasma method (Bystrzejewski et al., 2006). The synthesis is based on sublimation of the heterogeneous anode containing iron (Fe) and graphite (C). The materials were transformed to the vapor phase due to the very high temperature in the carbon arc (5000–6000 K). The as-produced metal–carbon gas undergoes rapid cooling, which leads to nucleation and solidification of iron nanoparticles encapsulated in carbon cages. In brief, the carbon arc discharge was ignited between the graphite cathode and graphite anode, which was doped with Fe (45 wt%). The discharge was maintained under Ar–H_2 atmosphere (1:1) at a total pressure of 60.0 kPa. The final product contains both carbon encapsulated iron oxide nanoparticles (CEIO) and non-encapsulated iron oxide (IO) NPs. The non-encapsulated Fe particles (Fe) were removed from the C–Fe mixture by refluxing in boiling 3 M HCl for 24 h, followed by washing with water and ethanol, and drying in air at 70 °C.

2.2. Surface modification of carbon encapsulated iron (CEIO) nanoparticles

The purified CEIO nanoparticle (2.0 g) was treated with the mixture of concentrated H_2SO_4 (80 mL) and HNO_3 (26 mL) under sonication at 25 °C for 3 h to introduce the surface acidic groups over the carbon coating. The suspension was diluted with 1 L distilled water and allowed to cool for 2 h. The suspended particles were recovered by a membrane filtration under reduced pressure and washed with excess of water and ethanol.

2.3. Synthesis of PEG–FA

25 mg folic acid (FA) was conjugated to the 250 mg PEG-bis-amine (NH_2–PEG–NH_2 MW 3000) (1.5:1.0 molar ratio) in the presence of 300 mg EDC and 100 mg NHS. The reaction was further accelerated by adding 0.5 mL HCl (2%) and stirred for 5 h at room temperature. A centrifugal concentrator Vivaspin 20 was employed for the removal of unreacted molecules (<3000 MW) such as NHS, EDC, HCl, unreacted FA and parts of unreacted PEG, settled at the bottom of the tube. The top layer containing FA conjugated PEG (PEG–FA) was dialyzed by cellulose membrane (3500 MW) and freeze-dried. The as-produced PEG–FA was engaged for the preparation of CEIO–PEG–FA nanoparticles.

2.4. Surface modification of CEIO with PEG–FA

In brief, 15 mg CEIO nanoparticles was dispersed in 30 mL deionized water containing 0.5 mL HCl (2%), 200 mg EDC and 100 mg NHS. Then the solution was sonicated for 2 h at 55 °C and then treated with 10 mL PEG–FA solution. The resulting mixture was again sonicated for 1 h and vortex for 72 h. The unreacted molecules viz., NHS, EDC, HCl and unreacted FA–PEG were removed by dialysis membrane (6000–8000 MW). The CEIO–PEG–FA nanoparticles were collected and washed with water and the grafting of PEG–FA on to the CEIO NPs was confirmed by UV–vis absorption spectroscopy (Jasco V550, Japan) at 200–400 nm.

2.5. Characterization of nanoparticles

The X-ray diffraction (XRD) patterns of CEIO nanoparticles were performed using a diffractometer (PANalytical X'Pert Pro) in continuous scanning mode with Cu Kα radiation (λ = 1.541 E). FTIR spectra were recorded in the transmittance mode in the range of 400–4000 cm^{-1} at a resolution of 1 cm^{-1} using a Jasco FTIR 410 series spectrometer (Jasco, Tokyo, Japan). The nanoparticles were thoroughly milled with KBr into a pellet before the measurement. Energy dispersive X-ray spectrum (EDS) was carried out using a Philips-EDAX/DX4 energy-dispersive spectroscope. The TEM images of CEIO particles were obtained by a transmission electron microscopy (H-7500 TEM, Hitachi). Magnetic characterization was examined using an MPMS-7 magnetometer system (Quantum Design Company, San Diego, CA, USA) under the magnetic field of ±10,000 Oe at 300 K. The magnetic heating of CEIO–PEG–FA NPs (500 μg/mL) was carried out under a high-frequency generator (Power cube 64/900, 750–1150 kHz, Ceia, Italy) with an alternating magnetic field frequency (*f*) 750 KHz and