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Multifunctional magnetic cargo-complexes with radical scavenging properties

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ABSTRACT

Core-shell magnetic nanoparticle synthesis offers the opportunity to engineering their physical properties for specific applications when the intrinsic magnetic properties can be associated with other interesting ones.

The purpose of this study was to design, synthesize, and characterize core-shell magnetic nanoparticles that mimic superoxide dismutase activity offering the possibility of guidance and therapeutic action. We proposed, for the first time, the synthesis and characterization of the nanocarriers comprised of magnetite nanoparticles functionalized with branched polyethyleneimine of low molecular weight (1.8 kDa) permitting the loading of the protocatechuic acid or its inclusion complex with anionic sulfobutylether-β-cyclodextrin for active drug delivery, in order to combine the useful properties of the magnetite and the protocatechuic acid antioxidant effect. NMR and DSC analyses confirmed the formation of the inclusion complex between sulfobutylether-β-cyclodextrin and protocatechuic acid, while structural and compositional analyses (FT-IR, TEM, XRD) revealed the synthesis of the multifunctional magnetic systems. Due to the possibility of being formulated as blood system injectable suspensions, antioxidant activity (using DPPH test) and cytotoxicity (using MTS assay on normal human dermal fibroblasts cells) were also measured, showing adequate properties to be used in biomedical applications. Moreover, we proposed a nanocarrier that would be able to load unstable active principles and with very low solubility in biological fluids to increase their biological ability.

1. Introduction

In the last decade, the knowledge about reactive oxygen species (ROS) and their importance in human health have increased considerably. ROS are chemically interesting and biologically ambivalent, extremely reactive species due to their unpaired electrons. They can come from natural metabolic processes (phagocytosis, mitochondrial activity, hepatic detoxification) or may derive from environment (cigarette smoke, pollution, processed food and therapeutic agents). The pathway from molecular oxygen to superoxide anion, hydrogen peroxide and finally hydroxyl radicals goes through enzymes, such as superoxide dismutase and glutathione peroxidase. Their effects in biological media can be both beneficial and damaging. In low quantities they can act as signalling molecules, alerting the immune function, whereas the high levels induce oxidative stress, which can damage macromolecules and trigger the pathogenesis of many diseases [1]. The involved systems are respiratory, renal, vascular (endothelial dysfunctions) and the pathologies can be diabetes, hypertension, Parkinson's disease and ischemia. The oxygen in ROS can initiate aggressive oxidation reactions inside cells or at the surface of cell membranes, damaging DNA, oxidizing unsaturated fatty acids from lipids or amino acids from proteins [2]. Antioxidants (enzymatic or non-enzymatic) can inhibit these dangerous oxidative processes by maintaining a redox balance.

When a therapeutic approach is under consideration, two important things must be taken into account: one is the importance to maintain an antioxidants adequate level on the interest site, like the endothelial vessel affected by atherosclerosis; the second important think is the long term stability and constant release of the therapeutic agent.

Taking into account the combined properties of the free radical scavengers of the natural antioxidants [3] and the thermic effect of the magnetite nanoparticles (MNP) in alternating magnetic field, one interesting proposal is to implement a magnetic nano-entity able to intravascularly deliver anti-oxidants in a pharmacologically controlled

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and spatially guided manner. In this way it is possible to simultaneously reduce ROS and eradicate modified cells from cancer tumours by hyperthermia, which relies on the properties of ferromagnetic iron oxide core of the nanoparticles. To attain this aim a nano-sized cargo-complex having a magnetic core and polyelectrolyte - based shell able to load and to deliver precise amounts of natural antioxidants as free radical scavengers can be taken into consideration.

The fact that a wide range of antioxidants are organic acids, suggested the idea that they could be charged in layers of organic cationic polymers, allowing easy attachment of the antioxidant (bearing carboxylic groups) based on electrostatic interactions or by metal complexation [4]. As many synthetic and natural antioxidants have low water solubility, they are encapsulated in water soluble macrocyclic molecules such as cyclodextrins (CD) [5]. Currently, native and modified cyclodextrins are required in biomedical applications due to their properties, such as good biocompatibility, water solubility and, as the demand is increasingly high, have become low-priced in a large variety of chemical modifications [6]. In this context, modified cyclodextrins with anionic groups may be selectively absorbed in cationic polyelectrolyte shell of the nanoparticles based on electrostatic driven forces, in which case the cyclodextrin may play the role of host for low soluble antioxidant by forming host-guest inclusion compounds.

As antioxidant compound can be used a natural phenolic acid as 3,4-dihydroxybenzoic acid, also named protocatechuic acid (PCA), originated usually in traditional Chinese herbal medicines. Apparently, many of the pharmacological effects of PCA are in close relation with the antioxidant properties. In this context, the antioxidant activity and antioxidant mechanism of PCA was recently highlighted by the Xican Li et al. [7] using *in vitro* antioxidant assays. Therefore, they reported that PCA shows more antioxidant activity *in vitro* than Trolox. The authors suggested that PCA can act as a scavenger of free radicals by mechanisms such as donating a hydrogen atom or an electron.

PCA was used, also, as a stabilizer of magnetic nanoparticles in water, when the functionalization of the magnetic nanoparticles was done by a ligand exchange reaction [8]. The resulted nanoparticles presented a good biocompatibility, high saturation magnetization and a good potential as contrast agent in Magnetic Resonance Imaging (MRI). Later, the PCA was used as a template for polymeric coating of magnetite nanoparticles, increasing stability in physiological media and improving pharmacokinetic properties by tailoring drug loading capacity and release behavior [9].

Most of the time, the success of magnetite nanoparticles was closely linked to their physical properties, generated by the presence of a permanent magnetic field, which make them useful in applications as contrast agents in MRI [10] or as therapeutic agents in hyperthermia treatment [11]. Along with their covering with polymers to increase their colloidal stability in solution, it has opened a wide range of applications as in targeted and controlled drug delivery and a variety of other theranostic applications [12].

Moreover, core-shell magnetic nanoparticle synthesis offers the opportunity to engineering their physical properties for specific applications when the intrinsic magnetic properties can be associated with other interesting ones (ex. chemical or biological purposes) when the surface allows the presence of drugs, active chemical agents (like polymer initiators) or redox substances [13–15]. Hence, the loading of core-shell magnetic nanoparticles with natural antioxidants could combine the magnetic properties of the core with the radical scavenging characteristics of the drugs, offering both the possibility of guidance and therapeutic action. In this context, a potential example would be an atherosclerotic artery, where restenosis after percutaneous coronary intervention can be avoided by reducing local oxidative stress levels.

The purpose of this study was to design, synthesize, and characterize core-shell magnetic nanoparticles that mimic superoxide dismutase activity; therefore they are working as a free radical scavenger in a desired place. Specifically, we proposed, for the first time, the synthesis and characterization of the nanocarriers comprised of ferrite nanoparticles functionalized with branched polyethylenimines (PEI, 1.8 kDa) permitting the loading of the protocatechuic acid (PCA) or its inclusion complex with sulfobutylether-β-cyclodextrin (SBEβCD/PCA) for active drug delivery in order to combine the useful properties of the magnetite and the PCA antioxidant effect. It should be pointed out that PEIs of low molecular weight, due to their primary, secondary and tertiary amino groups, ionically interact with anionic polymers and ampholytes at neutral or mildly alkaline values of pH and are accepted for biological interest [16,17].

2. Materials and methods

2.1. Materials

Materials were bought as follows: ferric chloride ($FeCl_3{\cdot}6H_2O$), ferrous chloride ($FeCl_2{\cdot}4H_2O$), 25% ammonium solution, polyethylenimine of 1.8 kDa (PEI), protocatechuic acid (PCA), and 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS diammonium salt), and potassium persulfate from Sigma Aldrich; sulfobutylether-β-cyclodextrin sodium salt (SBEβCD) was purchased from Ligand Pharmaceuticals, Inc.; normal human dermal fibroblasts (NHDF) from PromoCell; CellTiter 96® AQ_{ueous} One Solution Reagent, containing a novel tetrazolium compound [3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] and an electron coupling reagent (phenazine ethosulfate; PES) from Promega; alpha-MEM medium and 1% Penicillin-Streptomycin-Amphotericin B mixture from Lonza; 10% fetal bovine serum FBS from Gibco.

2.2. Syntheses

Synthesis of loaded conjugates with PCA antioxidant was performed in three steps:

(a) synthesis of MNP by co-precipitation method of Fe^{2+} and Fe^{3+} salts in a molar ratio of 0.5, in water solution at pH of 11–12 [18–20,23];
(b) MNPs were coated with PEI polymer (MPEI) by mixing the components in deionized water at room temperature;
(c) loading of MPEI nanoparticles with PCA and SBEβCD/PCA inclusion complex, named MPEI-PCA and MPEI-SBEβCD/PCA, respectively when PEI cationic polymer is able to easily embed acidic-type natural polyphenol antioxidants.

When SBEβCD/PCA inclusion complex is used, the drug is released in two stages: in the first step the inclusion complex is released from the PEI polymeric coating of the nanoparticle, and in the second step the antioxidant will be released from the SBEβCD cavity in a desired place due to the magnetic properties of the core. It should be mentioned that PCA was included in SBEβCDs cavity to favour its solubility and bioavailability and, also, the absorption of the inclusion complex into the polymeric shell of nanoparticles creates the premises for the possibility of embedding other natural antioxidants in the PEI shell.

2.2.1. Inclusion complexes synthesis (SBEβCD/PCA)

1.84 g (1.29 mmol) of SBEβCD were dissolved in 50 ml deionized water and the solution was kept under stirring, at room temperature, until the solution became clear, than 0.2 g (1.29 mmol) of PCA were added and the solution was stirred until became clear when it was submitted for lyophilization [21,22].

2.2.2. Magnetite nanoparticles (MNP) synthesis

MNP were synthesized by the classical method of co-precipitation [23]. In brief: 10 ml ammonium aqueous solution (25%) were added onto a solution mixture of ferrous and ferric salts, in a molar ratio of 0.5 under nitrogen protection and mechanical stirring at 70 °C and the