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Study on iron oxide nanoparticles coated with glucose-derived polymers for biomedical applications

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ABSTRACT

This study reports an approach for a facile one-step synthesis of magnetic nanoparticles (MNPs) coated with glucose-derived polymers (GDP) through a mechanochemical hydrothermal process for biomedical applications. Polymer-coated magnetic nanoparticles (Fe_2O_3/Fe_3O_4), with sizes below 10 nm, exhibited superparamagnetic behavior, with a specific magnetization saturation value of about 40 emu/g, and a maximum specific absorption rate (SAR) of 30 W/g in AC magnetic fields. Depending on the intensity of the applied AC magnetic field, a temperature of 42 °C can be achieved in 4–17 min. The surface polymerized layer affords functional hydroxyl groups for binding to biomolecules containing carboxyl, thiol, or amino groups, thereby making the coated nanoparticles feasible for bio-conjugation. In vitro cytotoxicity evaluation pointed out that a relatively high concentration of polymer-coated magnetic nanoparticles (GDP-MNPs) did not induce severe cell alteration, suggesting a good biocompatibility.

1. Introduction

The nanostructured materials are largely considered as ones of the most suitable building blocks for new emerging technologies and innovative applications. Endowed with distinctive optical, chemical, electronic, or magnetic properties, they have become keystones in many central domains, comprising medicine, food industry, communications, or military area.

Amid nanomaterials, metal oxide nanoparticles, such as ferrites (e.g., Fe_2O_3, Fe_3O_4), MgO, CaO, Al_2O_3, coated with carbohydrates or polymers, have started to attract consistent research since their application potential has been evaluated as promising [1–6].

The advantages provided by the inclusion of hydrophilic coatings onto metal oxide nanoparticles reflect into increase of the surface area, hindering of the oxidative processes at the surface of air-sensitive metal oxides, and decreasing of the magnetic coupling induced by reciprocal magnetic attraction, in the case of magnetic oxides nanoparticles.

On the other hand, the intrinsic magnetic properties of the magnetic oxides nanoparticles along with their intrinsic biocompatibility and low toxicity afford them high potential to be exploited for specific biomedical applications. Thus, they can be used in molecular detection (e.g., protein and DNA separation, bacteria detection and sequestration, stem cells harvesting), contrast agents for magnetic resonance (e.g., molecular imaging with targeted contrast agents, in vivo MRI of enzyme activity, in vivo tracking of labeled dendritic cells, monitoring stem cell migration), as drug/gene delivery systems (e.g., targeted delivery of cytotoxic agents, magnetic field-assisted drug transport and magnetofection for gene therapy), or even in cancer treatment via magnetic hyperthermia, the latter taking advantage from the biodegradation mechanisms developed inside heated cancer cells [7–13].

To date, the strategies developed to synthesize magnetic nanoparticles functionalized with carbohydrate-derived structures comprise arc discharge methods [14,15], magnetron and ion-beam co-sputtering [16], chemical vapor condensation [17], solid-state pyrolysis [18], catalytic methods [19], explosion [20], polyol-thermal synthesis method [21], or sonication [2].

However, many of these methods are complicated, energy consuming, and require special equipment [21]. By making use of inherently high energy consumption and intensive exploitation of hardware, these techniques lead to high cost of manufacturing [3]. Therefore, different routes with improved characteristics in terms of simplicity and low cost equipments were alternatively considered. For instance, chemical hydrothermal methods were found to respect the imposed criteria, being suitable for synthesis of carbon spheres or core-shell metal-carbon nanoparticles starting from glucose – a biocompatible natural monosaccharide – as carbon source

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[3,22,23]. In the carbon-coating method, glucose has been selected as primary carbon source following previous results focused on glucose aromatization and carbonization/caramelization processes generated by heating aqueous glucose solutions that led to carbon spheres [24].

Subsequently, carbon-metal nanoparticles were obtained either in one or two-steps by using autoclave-based hydrothermal reactions at temperatures ranging from 160 to 200 °C for 2–24 h in air or protective atmosphere [3,21,24–30].

However, this hydrothermal route needs increased reaction time and important exogenous heat supply, leading generally to agglomerated and large size carbohydrate/polymer-coated metal oxide compounds. Therefore, decreasing the reaction time and even lowering the exogenous heat required by the chemical precursors, along with a convenient synthesis process in terms of simplicity to obtain low-size compounds are needed. In this context, it is known that the chemical reaction rate for ferrous ion oxidation strongly depends on time and temperature [31]. Therefore, an increased temperature should be as much as possible coupled with a short reaction time, at least in the framework of a non-protective atmosphere. On the other hand, a short reaction time creates the premises for getting nanoparticles with small dimensions and narrow size distributions [32].

A strategy that affords both short reaction time and, to some extent, endogenous heat to prepare magnetic iron oxides functionalized with glucose-derived polymers can be based on a hydrothermal mechanochemical method.

This work describes a suitable approach based on a modified mechanochemical hydrothermal reaction at temperatures slightly below the melting point of glucose to simultaneously obtain, in one-step fast reaction, MNPs coated with glucose-derived polymerization products, starting from glucose and iron salts, under alkaline conditions. The physical properties, biocompatibility and potential of the GDP-MNPs to be used in magnetic hyperthermia were investigated.

To the best of our knowledge, there have not yet been reports on the synthesis of biocompatible GDP-MNPs through one-step hydrothermal mechanochemical polymerization-precipitation method at temperatures close to the melting point of glucose monosaccharide, and on the potential application of such biocompatible magnetic composites in magnetic hyperthermia for cancer therapy.

2. Materials and methods

All chemicals used in polymerization–precipitation reactions were purchased from commercial companies and used without any further purification. Iron chlorides (reagent grade 99.5%), β-D-glucose (reagent grade 99.5%), sodium hydroxide (reagent grade 99.5%), hydrochloric acid (37% concentration), and sodium chloride were purchased from Sigma–Aldrich.

2.1. Preparation of GDP, MNPs, and GDP-MNPs

2.1.1. GDP

Considered for comparison, GDP were prepared thermally by heating glucose in air at 140 °C, 200 °C, and 400 °C, respectively, the samples being noted as aGDP, bGDP, and cGDP, respectively.

2.1.2. MNPs

The synthesis of MNPs followed a slightly modified mechanochemical hydrothermal approach showed in our previous study [33]. The mechanochemical synthesis is essentially based on a heterogeneous chemical reaction activated by mechanical treatment of solid chemical precursors [34]. Thus, 1.1 g $FeCl_2{\cdot}4H_2O$ and 3 g $FeCl_3{\cdot}6H_2O$ (Fe^{2+}/Fe^{3+} molar ratio 1:2) were mechanically blended in a round-bottom glass vessel and heated at about 50 °C few minutes. 5 g of slightly heated NaOH (solid-state precipitation agent) was then transferred over iron salts solution. The reactants were initially gently blended, allowed to boil for a short time, and quickly blended again inside a fume hood. During the blending, a significant amount of heat and vapor was released. The output of the reaction was a hot black magnetic paste made up of magnetic nanoparticles. After natural cooling, the magnetic paste was dispersed in distilled water and washed several times with NaCl solution until neutral pH was reached. For physical characterization, the magnetic nanoparticles were preserved in distilled water at 4 °C.

2.1.3. GDP-MNPs

The synthesis of MNPs followed in principle the same procedure as for MNPs. Thus, a freshly prepared glucose solution, i.e. 0.3 g glucose in 1.5 ml HCl (37%), was added over iron salts, and mechanically mixed few minutes at 50–55 °C. After adding 5 g of NaOH over glucose-iron salts solution and quick mixing, a hot magnetic paste consisting mainly in coated magnetic nanoparticles was obtained. After cooling and washing, the GDP-MNPs were preserved following the same conditions as for MNPs.

2.2. Magnetic nanoparticles conditioning

Prior to biocompatibility tests, the magnetic nanoparticles were sterilized by autoclavation (20 min, 120 °C) in distilled water. After cooling at room temperature, the magnetic suspension was transferred into Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovin serum (FBS) and 1% antibiotic (penicillin-streptomycin). Several hours later, due to the ionic strength change and surface modification induced by the culture medium, the magnetic particles agglomerated. Following magnetic separation, the particles were re-dispersed in complete DMEM at relatively high concentration, i.e. 7.5 mg/ml.

2.3. Cell culture preparation

To evaluate the cytotoxicity of MNPs and GDP-MNPs, human osteosarcoma cells were used. Thawing of the frozen cells was done at 37 °C through a thermostated water bath followed by washing with DMEM.

The cells were centrifugation at 300 × *g*, resuspended in 10 ml complete medium, and subcultivated in 25 cm^2 flasks. After 72 h, the cells were detached from the flask by trypsinization (trypsin-EDTA). Following addition of complete culture medium, the suspension was centrifugated 5 min at 300 × *g*, and the resulted pellet was resuspended in complete medium. The cell density was determined by using an automated cell counter (Bio-Rad, TC20™).

Finally, the cells were seeded at a density of 3×10^4 cells/mL in 24-well flat bottomed plates and incubated 48 h at 37 °C, 5% CO_2, and 95% humidity for test with magnetic particles.

2.4. Cytotoxicity evaluation

Cytotoxicity of the magnetic nanoparticles was evaluated through the 5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide (MTT) assay by using dimethyl sulfoxide (DMSO) as dissolving agent.

Thus, after 48 h incubation, the culture medium of the cells was replaced by magnetic particles in complete DMEM (1 ml/well). Magnetic nanoparticles were added to the cells in triplicate at concentrations of 7.5 mg/ml and incubated for other 24 h.

Next, the MTT solution was added to the cells and the MTT-cell reaction was allowed to proceed for 3 h at 37 °C in order to form insoluble formazan. Subsequently, DMSO was allowed to interact about 10 min at 37 °C with the synthesized formazan in each well.

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