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Organic-inorganic hybrid nanoparticles controlled delivery system for anticancer drugs

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ABSTRACT

The use of organic-inorganic hybrid nanocarriers for controlled release of anticancer drugs has been gained a great interest, in particular, to improve the selectivity and efficacy of the drugs. In this study, iron oxide nanoparticles were prepared then surface modified via diazonium chemistry and coated with chitosan, and its derivative chitosan-grafted polylactic acid. The purpose was to increase the stability of the nanoparticles in physiological solution, heighten drug-loading capacity, prolong the release, reduce the initial burst effect and improve *in vitro* cytotoxicity of the model drug doxorubicin. The materials were characterized by DLS, ζ-potential, SEM, TGA, magnetization curves and release kinetics studies. Results confirmed the spherical shape, the presence of the coat and the advantages of using chitosan, particularly its amphiphilic derivative, as a coating agent, thereby surpassing the qualities of simple iron oxide nanoparticles. The coated nanoparticles exhibited great stability and high encapsulation efficiency for doxorubicin, at over 500 μg per mg of carrier. Moreover, the intensity of the initial burst was clearly diminished after coating, hence represents an advantage of using the hybrid system over simple iron oxide nanoparticles. Cytotoxicity studies demonstrate the increase in cytotoxicity of doxorubicin when loaded in nanoparticles, indirectly proving the role played by the carrier and its surface properties in cell uptake.

1. Introduction

Nanomaterials hold great promise for improving diagnoses and therapies and designing novel approaches and formulations in treating a wide range of human illnesses (Unsoy et al., 2012) Some applications of such nanotechnology have been transferred into clinical settings, while others have been demonstrated *in vitro*. The success of nanomaterials in biomedical applications is down to the unique physical and chemical properties they possess. Among the various organic and inorganic materials available, superparamagnetic nanoparticles have proven to be of a great interest in recent decades in terms of biomedical utilisation (Allen and Cullis, 2004; Prabaharan and Mano, 2004; Babes et al., 1999). As a consequence of their physical, chemical, thermal and mechanical properties, superparamagnetic nanoparticles offer great potential for several biomedical applications; for example, in the following: i) cell labelling, and targeting; ii) drug delivery; iii) magnetic resonance imaging (MRI); iv) hyperthermia; and v) magnetofection (Goya et al., 2003; Pankhurst et al., 2003; Gupta and Gupta, 2005).

Depending on their hydrodynamic diameters, superparamagnetic nanoparticles are classified as: i) standard superparamagnetic iron oxide nanoparticles (SPIONs) (50–180 nm); ii) ultrasmall superparamagnetic iron oxide nanoparticles (USPIONs) (10–50 nm); and iii) very small superparamagnetic iron oxide nanoparticles (VSPIONs) (<10 nm) (Corot et al., 2006).

However, despite the mentioned before advantages of superparamagnetic nanoparticles, two main drawbacks exist, as follows: i) the tendency to form aggregates and agglomerates, and ii) low drug loading and an intensive burst effect in drug delivery applications (Prijic and Sersa, 2011). To overcome these disadvantages, various approaches have been developed, including surface functionalization and coatings.

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It has been widely demonstrated that a polymeric coating provides a steric barrier to prevent nanoparticle agglomeration, enhances drug loading capacity and reduces the initial burst (Yuan et al., 2013; Yiyun et al., 2007). However, some aspects have to be considered pertaining to the chemical structure (e.g. hydrophilicity/hydrophobicity and biodegradation characteristics), the molecular weight of the coating agent and the manner in which it is anchored or attached (e.g. electrostatic or covalent bonding), and the conformation and degree of particle surface coverage, (Berry and Curtis, 2003) as these may affect the performance of the iron core (Tartaj et al., 2005)

Both natural and synthetic polymers have been used to coat magnetic nanoparticles (Ali et al., 2016), among which chitosan has drawn considerable attention (McNeil, 2005).

Chitosan, which is obtained by alkaline deacetylation of chitin, is a biodegradable, biocompatible polysaccharide with different reactive sites, two hydroxyl groups in C_3 and C_6 and one amino group in D-glucosamine residues, thereby serving as an anchor for the conjugation of therapeutics, targeting ligands and imaging agents. It is well-known that the amine groups displaced along chitosan backbone chitosan molecules interact with Fe_3O_4 (Kumar, 2000; Chopra et al., 2007; Do Kim et al., 2004).

Chitosan-coated magnetic nanoparticles ("NPs") are generally synthesized by alkaline co-precipitation of Fe (II) and Fe(III) precursors in aqueous solutions containing the given polymer (Ducry and Stump, 2009). The polymer limits the core growth of iron oxide during such preparation increases stability via steric and electrostatic repulsions in aqueous media boosts drug loading and enhances control of the release (Mornet et al., 2004).

Herein, the iron oxide NPs underwent surface modification *in situ*, followed by the polymeric coating, the aim being to bolster the stability of the iron oxide NPs in simulated physiological solutions, as well as to raise loading capacity and heighten release properties, in addition to diminishing the initial burst effect, without losing the magnetic properties of the iron core.

So as to demonstrate any improvements related to modifying the surface and applying the polymeric coating, doxorubicin ("DOX") was chosen as the model drug.

DOX is an anthracycline antibiotic and, since being approved in the 1970s by the FDA, it has proven successfully at treating various cancers, including leukemia, breast cancer, ovarian cancer and lymphomas (Abaan et al., 2009; Goebel et al., 1996). However, it exhibits severe side effects, such as cardiotoxicity, which remain a major problem (Zou et al., 2003). In addition, the state of multidrug resistance usually develops, preventing further therapy with DOX. Therefore, a great deal of attention has been paid to developing a suitable nanocarrier-mediated drug delivery system to improve the efficacy of DOX, which would maximize therapeutic value while minimizing any side effects (Di Martino et al., 2015; Akiyoshi et al., 1996).

NPs were prepared via the co-precipitation method, followed by surface modification using arenediazonium tosylate; the subsequent coating and DOX loading process were carried simultaneously. Evaluation centered on both chitosan (CS) as the coating agent, which is hydrophilic, and its amphiphilic derivative, obtained by grafting low-molecular-weight polylactic acid (CS-g-PLA). The effects on the iron oxide NPs exerted by the surface modification and polymeric coating was investigated, namely in terms of stability, dimension, surface charge magnetic response and DOX encapsulation efficiency under different conditions. Release kinetics studies were carried out in simulated physiological fluids so as to discern the overall trend for release and reduction in the initial burst. The biocompatibility of the prepared formulations was evaluated *in vitro* by MTT assay using HeLa cell line. The resultant findings clearly demonstrate the benefits gained by combining such surface modification via arenediazonium tosylate with the addition of the polymeric coating in terms of NPs stability, DOX release profile and improving DOX cytotoxicity over time.

2. Materials and methods

2.1. Materials

The following were supplied by Sigma Aldrich: low-molecular-weight chitosan (CS), ($M_w < 10^4$ g/mol, D.D 75–85%); *N*-HydroxySuccinimide (NHS); *N*-(3-Dimethylaminopropyl)-*N*′-Ethylcarbodiimide hydrochloride (EDC), commercial grade, powder; methane sulphonic acid (MSA), *N*,*N*-Diethylformamide 99%; *tert*-Butyl nitrite, 4-toluenesulphonic acid (p-TsOH); 4-nitroaniline, iron trichloride ($FeCl_3$); sodium borohydride; and doxorubicin hydrochloride. $C_3H_6O_3$ L-Lactic acid, 80% water solution, was purchased from Lachner Neratovice, Czech Republic. Sodium chloride, potassium dihydrogen phosphate, sodium carbonate and sodium hydroxide were acquired from Penta, Prague, Czech Republic. The C_3H_6O solvent acetone, sodium hydroxide, sodium chloride, sodium phosphate and potassium phosphate were bought from IPL Lukes, Uhresky Brod, Czech Republic. Chloroform $CHCl_3$ (HPLC grade), acetic acid CH_3CO_2H (HPLC grade) and hydrochloric acid were purchased from Chromservis, Prague, Czech Republic.

2.2. Preparation of iron oxide NPs

The NPs were prepared in accordance with the procedure reported in a previous work (Guselnikova et al., 2015). The process can be divided into two steps, the first being creation of the iron oxide NPs, while the other is to carry out the surface modification. An aqueous solution (15 ml) of $FeCl_3{\cdot}6H_2O$ (0.406 g, 1.5 mmol) was slowly added to an aqueous solution (10 ml) of $NaBH_4$ (0.171 g, 4.5 mmol) under vigorous mechanical stirring. The colour of the solution changed immediately from yellow to dark, indicating the formation of nanoparticles. After 10 min, the aqueous solution (15 ml) of 4-carboxybenzenediazonium tosylate (0.68 g, 2.25 mmol) was added directly to the reaction vessel. The mixture was stirred for 30 min, and completion of the reaction was detected via conversion of 4-carboxybenzenediazonium tosylate by the test for β-naphthol. The obtained suspension was washed by 9 cycles of magnetic separation/redispersion in water (3×), ethanol (3×) and acetone (3×) and dried at 40 °C.

The presence of an organic layer on the iron oxide NPs surface was determined by FTIR-ATR (using a Nicolet iS5 FTIR Spectrometer equipped with an iD5 ATR accessory and ZnSe crystal, at resolution 4 cm^{-1}, for 64 scans) in addition to total organic carbon analysis (on an SSM-5000A, with a solid sample module, by Shimadzu).

2.3. CS-g-PLA synthesis and characterization

The amphiphilic polymer CS-g-PLA was prepared in accordance with a description given in a previous work (Di Martino and Sedlarik, 2014). In brief, 0.5 g of CS was soaked in dimethylformamide overnight and after dissolved by the addiction of 1% v/v acetic acid aqueous solution to obtain the final concentration of 2 mg/ml Afterwards, 0.5 g of PLA (Mw 10,000 g/mol), EDC and *N*-hydroxysuccinimide (NHS) (at the molar ratio of PLA: EDC: NHS = 1:1.5:3) was dissolved in 50 ml of chloroform, then the solution was added to the CS and kept under vigorous stirring for 48 h at room temperature. The reaction was stopped and the final product precipitated by adding NaOH 0.1 M, with subsequent centrifugation at 14,000 rpm for 20 min. The resultant pellet was accurately washed with chloroform, water (containing 1% v/v of acetic acid) to