DEVELOPMENT OF THERANOSTIC AGENTS BASED ON IRON OXIDE-GADOLINIUM-CHITOSAN FOR CONTROLLED RELEASE OF DOXORUBICIN

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Abstract

Herein we report a theranostic system based on iron oxide-gadolinium nanoparticles coated by chitosan as a dual contrast agent for magnetic resonance imaging and controlled delivery application. Iron oxide nanoparticles were prepared by reduction of iron (III) chloride followed by surface modification using arenediazonium tosylate and diethylenetriaminepentaacetic acid for Gd(III) complexation. Nanoparticles were loaded with the anticancer drug doxorubicin and coated by low molecular weight chitosan to improve stability in solution and control the release of the drug. Dynamic light scattering, ζ-potential, thermogravimetric analysis, attenuated total refraction infrared spectroscopy and magnetic hysteresis curves reveal the success of surface modification and coating process. The amount of Gd(III) complexed and doxorubicin loaded were assessed by UV-Vis. Release studies were carried out in simulated physiological conditions. Results indicate that the obtained iron oxide-Gd(III) nanoparticles coated by chitosan are stable up to one month in physiological conditions and magnetic response is slightly decreased. The MRI analysis, doxorubicin high encapsulation efficiency and sustained release trend suggests that the presented system represents an interesting platform for the development of future theranostic agents.

Keywords: Iron oxide nanoparticles, gadolinium, chitosan, theranostic, controlled release, doxorubicin

1. INTRODUCTION

Magnetic resonance imaging (MRI) has been recognized as a powerful non-invasive diagnostic technique to visualize in high spatial resolution tissue morphology and anatomical details [1]. To obtain contrast enhancement and signal amplification different contrast agents have been developed and are divided into T_1 and T_2. T_1 agents alter the longitudinal relaxation times of water protons to produce bright positive signal intensity while T_2 alter the transverse relaxation times providing dark negative signal intensity [2, 3]. Gd(III) based complexes demonstrate high T_1 relaxivity while superparamagnetic iron oxide nanoparticles (NPs) present strong T_2 shortening effect [4]. The development of a dual contrast agent that can reduce T_1 and T_2, and simultaneously carry bioactive molecules for therapeutic applications has been gained a great interest in the last years.

Magnetic iron oxide NPs with a prolonged blood retention time, biodegradability and low toxicity have emerged as one of the prominent materials for contemporary diagnostic and therapeutic applications. Despite the advantages of iron oxide NPs two main drawbacks have to be solved. The first is related to the presence of dark areas in MRI which can be attributed to low signal causing artefacts, while the second is related to the low stability of iron oxide NPs in physiological solution, which causes formation of clusters that can become dangerous in case of IV administration. To overcome the first one, several studies have described an improvement of the sensitivity by combining iron oxide NPs and Gd(III) chelate making a dual contrast agent [2], while to avoid the formation of cluster and improve the stability in physiological solution, iron NPs are surface modified or coated, in particular by polymers.

The aim of this study was to develop a system, which could be used simultaneously for diagnostics and therapy. The presence of iron oxide and Gd(III) allows having a MRI contrast agent which could act simultaneously on T_1 and T_2 relaxation time while the coating enables the drug load and control the release. Iron oxide NPs were prepared by reduction of iron (III) chloride aqueous solution followed by surface modification using arenediazonium tosylate chemistry. The surface modification allowed the conjugation of diethylenetriaminepentaacetic acid (DTPA) as complexed agents for Gd(III) chloride. Obtained product was loaded with doxorubicin (DOX) and coated by chitosan (CS).

2. MATERIALS AND METHODS

2.1. Materials

Low molecular weight chitosan (CS), (M_w < 10^4 g/mol, D.D 75-85%); N,N-Diethylformamide 99%, *tert*-Butyl nitrite, 4-toluenesulfonic acid (p-TsOH),4-nitroaniline, iron trichloride ($FeCl_3$), sodium borohydride, diethylenetriaminepentaacetic acid (DTPA), Gadolinium (III)chloride, doxorubicin hydrochloride (DOX), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide and N-hydroxysuccinimide were supplied by Sigma Aldrich.

2.2. Preparation of iron oxide-Gd based NPs

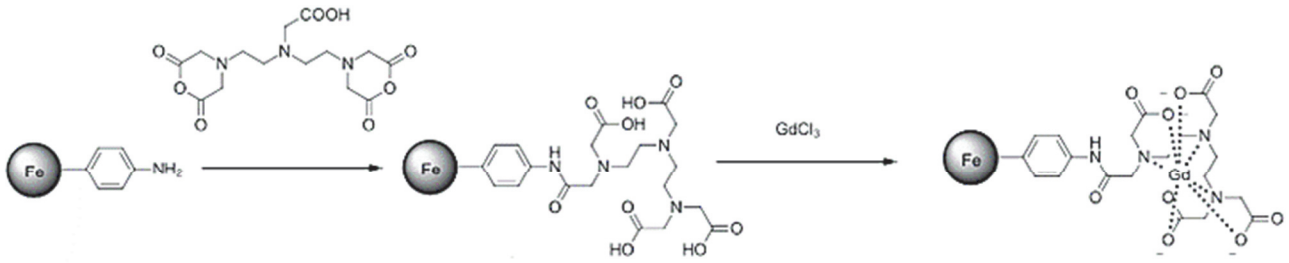

Figure 1 Schematic representation of iron-Gd(III) based NPs

As reported in **Figure 1** iron oxide-Gd based NPs were prepared in three main steps. In the first one, $FeCl_3$ was reduced by $NaBH_4$ under vigorous mechanical stirring. The shift from yellow to dark colour indicated the NPs formation. In the second phase an aqueous solution containing 4-aminobenzenediazonium tosylate was added and stirred for 30 minutes. The completion of the reaction was detected by the β-naphthol test. The NPs were recovered by magnet, washed several times by water, ethanol and acetone and dried at 40 °C [5]. In the last step DTPA was conjugated by reaction between amino groups on the iron oxide NPs surface and commercially available DTPA anhydride. Afterwards, the NPs were separated by magnet, washed and dried. The obtained product was dissolved in water and mixed with $GdCl_3$ solution for 24 h. The weight ratio between Gd(III) and iron was set to 15. NPs were recovered by centrifugation and dried under vacuum. FTIR-ATR TGA, UV-Vis analysis revealed the presence of surface organic layer due to modification and the Gd(III) complexation.

2.3. CS coating, DOX encapsulation and release

Surface modified iron oxide-Gd NPs was coated by CS to improve the stability in solution and avoid cluster formation. Briefly, CS was dissolved at 5mg/ml in aqueous solution containing 1 % v/w acetic acid. Afterwards, iron oxide-Gd NPs were added to CS solution (CS to iron weight ratio range from 1/5 to 5), stirred for 24 hours under slight warming and treated in ultrasound bath for 30 minutes at room temperature. Final product was recovered by magnet, accurately washed and freeze dried.

Dynamic light scattering (DLS) and ζ-potential analysis (Nano ZS Malvern Instruments) were carried out on coated and uncoated iron oxide and iron oxide Gd NPs in preparation media to evaluate stability, dimension and surface properties.

DOX was encapsulated during the coating process and the encapsulation efficiency evaluated by UV-Vis analysis.

In-vitro DOX release trend was investigated in simulated physiological media at 7.4 in thermostable condition (37° C) under orbital shake. The amount of drug released (*DR*) was determined by UV-Vis analysis as reported in our previous work [6].

3. RESULTS AND DISCUSSIONS

3.1. Nanoparticles characterization

The presence of the surface organic functional groups (OFGs) and coating were demonstrated by FTIR-ATR and TGA analysis. DTPA surface modified NPs showed peaks at 3350 cm^{-1} (O-H stretch of carboxylic groups), 1573 cm^{-1} (C-H stretch in aromatic ring) and at 686 cm^{-1} corresponding to Fe-O. In the spectra relative to iron NPs coated with CS the following peaks were observed: 664 cm^{-1} (Fe-O), 1018 cm^{-1} (C-O-C), 1400 cm^{-1} (C=C stretch in aromatic ring), 1578 cm^{-1} (C-H stretch in aromatic ring), 1707 cm^{-1} (C=O stretch in carboxylic acid) and a wide band at 3191cm^{-1} related to O-H stretch which indicates the presence of hydrogen bond.

Thermogram of uncoated NPs presents an initial weight loss due to the presence of volatile substances residual from the preparation, moisture and functionalization group. As the combustion takes place, Fe_3O_4 is transformed to γ-Fe_2O_3; although the associated weight gain was observed in the range 380-800°C. In case of CS coated NPs, CS began to degrade at near 250°C and the final decomposition temperature is close to 750 °C. In the obtained thermogram CS presents an 8% initial weight loss within 150 °C associated with the loss of the water molecules. The general trend of CS and CS-Fe is comparable with thermograms reported in the published work [7].

The average diameter of the NPs in solution after each modification is reported in **Figure 2A.** In contrast to DTPA, which does not affect the NPs dimension, the CS coating noticeably increases the diameter shifting from range 10-25 nm (uncoated) up to 180 nm (coated). Both formulations are in agreement with the previous studies [5, 6] indicating the reproducibility of the process. The presence of DOX also caused an increase in the average diameter, but less than -10% in presence of coating while over 50 % in the uncoated system. This is related to the different DOX allocation in the system and the forces involved [8]. In case of surface modified NPs, DOX is adsorbed on the surface by physical and electrostatic interactions with -COOH groups of DTPA while in presence of CS is entrapped inside the polymeric network by electrostatic and H-bond.

In **Figure 2B** the effect of CS coating on the magnetic response is reported. Different iron to CS weight ratio (range 15 to 0.05) have been tried to find the optimal compromise between efficacy of coating enhancement of stability, DOX loading and acceptable magnetic response. Results indicate that at iron to CS weight ratio of 1 and2, NPs possess good stability in preparation media up to 1 month with a reduction of magnetic response of 15%.

At iron to CS weight ratio 2 over 30 % of the initial amount of DOX has been loaded. In case of uncoated system, less than 10 % of DOX was adsorbed on the surface and directly exposed to external environment.

MRI analysis reveals that in iron-Gd NPs the presence of Gd reduce the T_1 relaxation time resulting in the increased signal intensity due to the seven unpaired electrons of Gd. Moreover, the presence of Gd(III) reduced the T_2 relaxation time related to the iron oxide core [3].

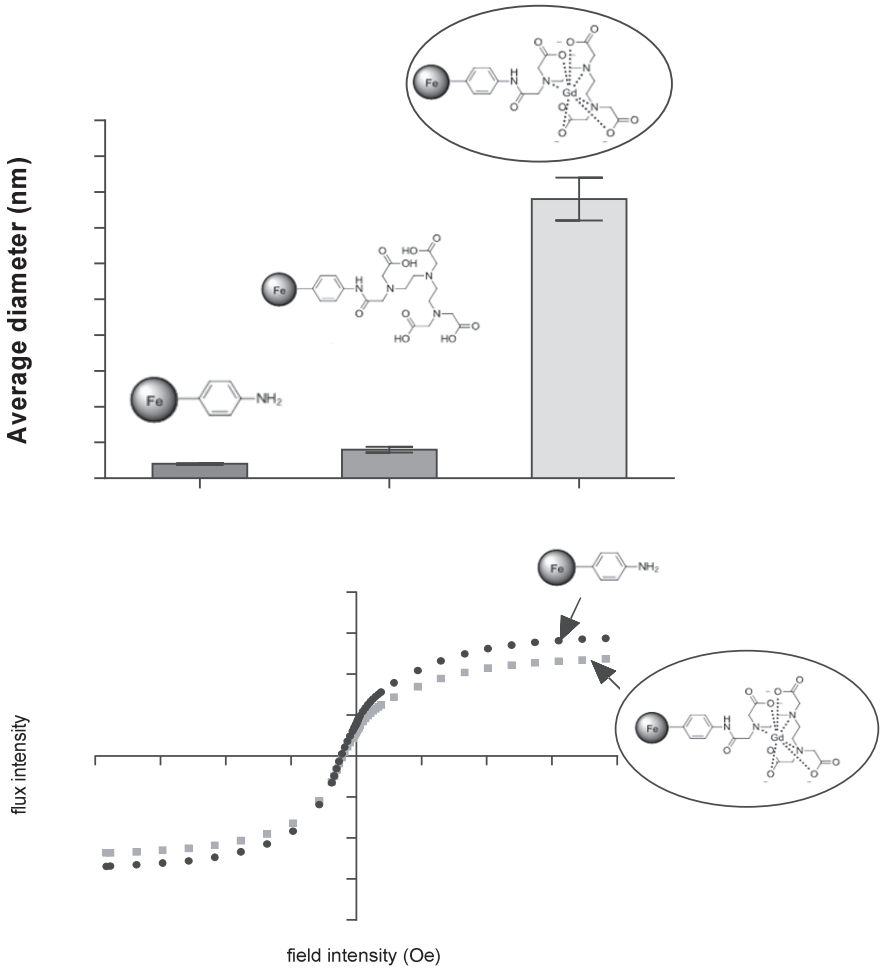

Figure 2 A) Average dimension of iron oxide NPs surface modified and coated by CS; B) magnetic hysteresis curve of the initial and final formulation of iron oxide based NPs

3.2. Release kinetics

The release rate of DOX from the system was monitored by collecting aliquots of the release media (pH 7.4; 37°C) and measuring the amount of DOX by UV-Vis analysis at 480 nm. Results demonstrate a light initial burst where up to 9% of the total loaded amount of drug is released within 1 h after contact with the media. This initial phase is followed by a sustained trend, which stand for one week and where 90% of the drug is released. Gd(III) release was monitored as well by reaction with arsezano (III) and UV-vis detection at 660 nm. Less than 30% of the loaded Gd(III) is quickly removed after contact with the media representing the not complexed fraction of Gd. Afterwards, a stationary phase, where 50% of the Gd(III) loaded was released after one month, took place. It suggests indicating the high stability of DTPA-Gd complex. As reported in the previous work [9], in contrast to DOX the slower release rate of Gd(III) could be related to the hydrogen bond between Gd(III) and DTPA -COOH groups. Moreover, the presence of CS coating could also play a certain role in improving the stability of Gd(III) inside the system.

Comparing the release trend to our previous studies [6, 9] the pH of the media (pH 7.4) clearly affects the trend, in particular in the first hours after immersion. It could be explained by the swelling properties of CS, which are strongly influenced by the external pH, as reported [9].

CONCLUSION

In summary, we report a system based on iron oxide-Gd NPs coated by CS as platform for future development of theranostic agents. Simultaneously, the presence of iron oxide and Gd(III) demonstrates T_1 and T_2 relaxation time reduction in MRI while the presence of CS coat allows encapsulation and controlled release of the anticancer drug DOX. Obtained system showed dimension in the range 150-180 nm, positive ζ-potential and long-term stability in physiological solution, up to one month at room temperature. CS coating at the optimal iron to CS weight ratio improves the NPs stability and DOX loading efficacy causing only a slight reduction in magnetic response. Presented results point out to the opportunity to develop inorganic-organic nanoparticles, which can deliver both contrast medium and drug, allowing monitoring and therapeutic activity simultaneously.

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