Magnetosomes for Anticancer Therapies based on 5-Fluorouracil

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Introduction

Regardless of the long history in the use of antitumor molecules against cancer and the design of new multiple drug regimens to improve clinical success, chemotherapy failure habitually occurs even in malignancies that are more sensitive to such cytotoxic agents (i.e., breast cancer). It could be stated that the clinical use and efficacy of current anticancer drugs is seriously limited by [1, 2]: *i*) a non-specific systemic distribution and the difficulty of obtaining the adequate drug concentration into the tumor mass; *ii*) the rapid drug metabolism and clearance; and, *iii*) the development of resistances by the tumor. To beat this challenge stimuli-sensitive materials are under development to enhance the concentration of the chemotherapy agent into the cancer tissue, thus optimizing the therapeutic response while minimizing the associated toxic effects. These colloidal medicines are engineered to be used as a means to administer the drug into the body in a targeted way (controlling the biodistribution and specific release), and to overcome the resistance mechanisms [3, 4].
The present work is focussed on the formulation of magnetosomes loaded with the antimetabolite 5-fluorouracil (5-FU) against cancer. This molecule presents a broad spectrum of activity against solid tumors, alone or in combination chemotherapy regimes. The loading of this hydrophilic drug to a nanocarrier may increase its antitumor efficacy. This is so because the controlled delivery to the targeted malignant mass significantly reduces the side effects, including gastrointestinal, haematological, neural and dermatological ones, and myelosupression. Drug localization into the site of action should optimize the 5-FU pharmacokinetic profile. Another advantage is the decreased cardiotoxicity of the degradation compounds generated in the basic medium of the injected vials [5].

Materials and Methods

A chemical co-precipitation method was used to prepare magnetite (Fe_3O_4) nuclei [6]. The technique developed to formulate the magnetosomas has been based on the widely followed thin film hydration method for the preparation of liposomes (also synthesized for this work) [7]. Geometry of the core/shell structures was characterized by high resolution transmission electron microscopy (HRTEM), scanning electron microscopy (SEM), and photon correlation spectroscopy (PCS). The coating efficiency of the lipid vesicle around the magnetic core was analyzed by Fourier transform infrared (FTIR) spectrometry, and by electrical surface characterizations (based on electrophoretic determinations).
The amount of 5-FU loaded to the magnetosomes was then investigated. Spectrophotometry was validated and used successfully, as the analytical technique in the quantitative determination of drug loading (at 266 nm). The magnetic properties of the magnetosome were also evaluated. Drug entrapment efficiency (EE, %) was determined after purification of the vesicles from untrapped 5-FU by dialysis in bidistilled water using a cellulose membrane (cut-off: 2000 Da). Additionally, two samples of the 5-FU-loaded magnetosome formulations were investigated. The first one was maintained at 25.0 $\pm$ 0.5 ºC, and the second one was kept at 4.0 $\pm$ 0.5 ºC. Both series were protected from light. Drug release from the nanoformulations was analyzed at 266 nm by spectrophotometric determinations during 30 days. All the experiments were carried out in triplicate.

Results and Discussion

The magnetosomes were found to be well-stabilized composite nanostructures with an average size of 105 $\pm$ 15 nm, and moderately monodisperse (figure 1a). Superparamagnetic magnetite and liposomes were also used in this investigation (mean diameter: 11 ± 3 nm and 1.5 ± 0.3 µm, respectively).
Regarding the FTIR analysis, all the infrared bands of the liposomal structure were present in the spectrum of the magnetosomes, a clear indication that the shell observed in figure 1a was indeed a lipid coating. The efficiency of this coating was further demonstrated by comparing the zeta potential of the magnetosomes with those of the iron oxide nuclei and of pure liposomes. Figure 1b show that the zeta potential (ζ) values of the composite nanoparticles are similar to the ones for the pure vesicular microstructures and clearly different to the ones of the magnetic cores, this suggesting the efficiency of the coating.
The magnetic responsiveness of the magnetoliposomes was defined by their hysteresis cycle (figure 1c). Such lipid-based core/shell nanoparticles show a soft magnetic behavior, and in fact the increasing and decreasing branches of the hysteresis cycles are hardly distinguishable, considering the sensitivity of our magnetometer. The initial susceptibility (χ_i) and the saturation magnetization were determined to be 2.5 ± 0.2 and 206 ± 12 kA/m, respectively.

With respect to the 5-FU incorporation to the magnetic nanocomposites, it was determined that the EE (%) was greater compared to the equivalent liposome formulation. In detail, when the 5-FU concentration was 1 mg/mL in the aqueous phase of the synthesis procedure, this parameter rise from 39.4 ± 0.1 % (liposome formulation) to 47.6 ± 0.1 % (when the anticancer agent was incorporated into the magnetosomes). Thus, 5-FU loading to the magnetosomal formulation could be considered high in comparison with previously reported results in liposomes [8]. Finally, the stability studies pointed out the possibility of storage for 5-FU-loaded magnetosomes, both at 25.0 $\pm$ 0.5 ºC and at 4.0 $\pm$ 0.5 ºC. In fact, the amount of drug retained after 30 days did not importantly varied.

Conclusions

In this work we have shown that it is possible to embed superparamagnetic magnetite nuclei within a lipid-based vesicle. A reproducible method is described for the loading of magnetosomes with 5-fluorouracil. The synthesis conditions enabled the formulation of composite nanoparticles with a well-defined geometry (acceptable for drug delivery to cancer), adequate physicochemical stability, controllable surface charge, and relatively high 5-FU loading values. Current investigations are focused on the analysis of the *in vitro* and *in vivo* antitumor activities of the formulation.

Acknowledgment

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Figures

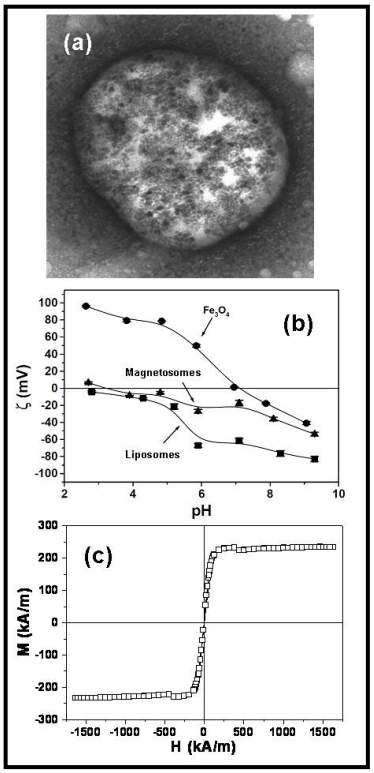

Figure 1. (a) HRTEM image of a magnetosome. (b) Zeta potential (ζ, mV) of Fe_3O_4 (●), liposomes (■), and magnetosomes (▲) NPs as a function of pH in the presence of 10^{-3} M KNO_3. (c) Hysteresis cycle of the core/shell NPs.