Biodegradable Magnetic Nanomedicine Based on the Antitumor Molecule Tegafur

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Introduction

In drug delivery, magnetically responsive nanoparticles (NPs) must be biocompatible, non-toxic, and non-immunogenic, along with appropriate drug vehiculization capabilities (high loading values, and little burst release) and significant responsiveness to magnetic gradients. Magnetic NPs has been described to further induce the death of malignant tissues by magnetic fluid hyperthermia (a property by which such NPs under exposure to high frequency alternating magnetic gradients generate heat due to magnetic hysteresis loss). Hence, such multifunctional NPs (enhanced antitumor effect + hyperthermia) are expected to make possible a more selective, effective, and safety cancer treatment [1].
Here, we describe the formulation of magnetite/poly(hexylcyanoacrylate) (Fe_3O_4/PHCA) nanostructures loaded with tegafur for combined hyperthermia and chemotherapy against cancer. The anticancer activity of this chemotherapy molecule must be significantly improved by its loading to the core/shell nanomaterial, thanks to the expected minimization of the toxicity coming from an extensive biodistribution. The coating efficiency of the polymer around the magnetic core was analyzed using Fourier transform infrared (FTIR) spectrometry, and by electrical and thermodynamic surface characterizations. The amount of tegafur loaded to the magnetic NPs, and the corresponding *in vitro* drug release profiles was investigated. Spectrophotometry was validated and used successfully, as the analytical technique in the quantitative determination of both tegafur loading and release profiles. Tegafur adsorption was further qualitatively analyzed by electrophoresis. Magnetic properties and the heating characteristics (hyperthermia effect) of the Fe_3O_4/PHCA nanomaterial were also evaluated.

Materials and Methods

A chemical co-precipitation method was used to prepare Fe_3O_4 nuclei (size ≈ 10 nm) [2]. PHCA NPs were prepared by emulsion/polymerization of the corresponding monomer in an aqueous solution [3]. The synthesis procedure of core/shell NPs was equal to the one followed for the preparation of the pure polymer, except that the aqueous phase contained Fe_3O_4 nuclei. Mean particle diameter was determined by PCS. FTIR spectrometry was used for the chemical characterization of the NPs. Surface electrical properties of the NPs were analyzed by electrophoresis. A surface thermodynamic analysis of the NPs was carried out by following the model of van Oss [4]. The magnetic properties and the *in vitro* heating behaviour of the Fe_3O_4/PHCA NPs were also analyzed.
Tegafur loading to magnetic nanostructures was investigated by two procedures. The first one (entrapment method) followed for drug absorption into the core/shell NPs was similar to that above described for the preparation of the magnetic nanomaterial, except that the aqueous phase also included the chemotherapy agent. The second procedure (adsorption method) involved single drug surface adsorption onto the preformed magnetic polymer. A qualitative follow-up of the adsorption process was also done by electrophoresis. Drug release experiments were performed in triplicate at 37.0 ºC using the dialysis bag method. To that aim, magnetic nanomaterials with the higher tegafur entrapment efficiencies were investigated. The release media was phosphate buffered saline (PBS, pH 7.4) set at 37.0 ºC. UV-Vis absorption measurements were done to determine tegafur concentration in all the systems investigated at 271 nm. Good linearity was observed at this wavelength and the method was validated and verified for accuracy, precision, and linearity in all conditions tested.

Results and Discussion

The core/shell nanostructures were well-stabilized NPs with an average diameter of 120 ± 15 nm (polydispersity index: 0.103). The excellent coating efficiency of PHCA around the magnetic core was established using electron microscopy, FTIR spectrometry, and electrical and thermodynamic surface characterizations. The magnetic responsiveness of the core/shell NPs was defined by the hysteresis cycle (figure 1a). The initial susceptibility (χ_i) and the saturation magnetization were calculated to be ≈ 1.2 and ≈ 118 kA/m, respectively. Figure 1b shows the *in vitro* heating behavior of a Fe_3O_4/PHCA magnetofluid in a high frequency oscillating electromagnetic gradient. Due to this exposure, the oscillation of the magnetic moment of the nanostructures transforms them into heaters that can produce heat up to ≈ 46 ºC. Under the experimental conditions, this maximum temperature was stabilized until the end of the experiment. This proves a good control of the temperature and heat flux, a basic requirement for hyperthermia application [5].
With respect to tegafur incorporation to the nanocomposites, it was determined that the entrapment efficiency (EE, %) increased significantly with the drug concentration in the incubation medium (i.e., from ≈ 1.6 to ≈ 9.5 % for tegafur concentrations ranging from 10^{-5} to 10^{-2} M). The electrokinetic analysis

of the drug adsorption process qualitatively confirmed the very low drug loading onto the NP surface. Compared to the surface adsorption procedure, tegafur EE (%) and the drug loading (DL, %) were significantly increased whatever the drug concentration fixed. For example, when tegafur concentration in the adsorption/absorption medium was 10^{-2} M, these parameters rise from ≈ 9.5 % and ≈ 0.63 % (after drug adsorption) to ≈ 31.8 % and ≈ 2.6 % (when the antitumor molecule was absorbed into the core/shell matrix), respectively. A positive effect of tegafur concentration on the absorption efficiency into the Fe_3O_4/PHCA NPs was described when following both methodologies.

Finally, tegafur release profile from the nanocomposites was investigated at pH 7.4 (figure 1c). The release of drug adsorbed onto the core/shell NPs (adsorption procedure) was complete within ≈ 3 h, as a result of rapid desorption. On the opposite, a biphasic process occurs when the drug molecules were entrapped into the NP matrix: first, an early rapid release up to ≈ 35 % took place within 1 h, while the remaining tegafur absorbed was slowly liberated during the next 23 h. The rapid release probably represents the loss of surface-associated and poorly entrapped (adsorbed on the surface pores) drug. Finally, tegafur release during the slower release phase may result from NP disintegration by surface erosion, from drug diffusion through the NP matrix, or both. Such a biphasic profile, typical of these polymeric matrices, suggests that the major fraction of drug was entrapped into the PHCA shell.

Conclusions

We have defined the optimal formulation conditions to obtain a magnetically responsive colloid suitable for intravenous administration in which Fe_3O_4 nanocores are very efficiently coated by PHCA. Compared to the surface adsorption procedure, the entrapment of tegafur into the core/shell NPs during the synthesis process has resulted in greater drug loading and slower drug release properties. These nanocomposites may constitute a potential candidate for combined cancer treatment: they are tailored to deliver appropriate amounts of tegafur specifically into the tumor site, in combination with a selective hyperthermia effect into the cancer mass. This nanostructure opens promising possibilities to improve cancer treatment.

Acknowledgment

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Figures

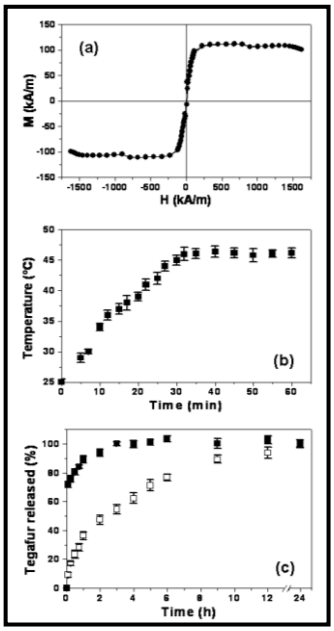

Figure 1. (a) Hysteresis cycle of the core/shell NPs. (b) Heating curve of a Fe_3O_4/PHCA magnetofluid (10 mg/mL) exposed to a high frequency alternating electromagnetic gradient (frequency: 250 kHz, and intensity: 4 kA/m). (c) Release of tegafur (%), adsorbed (full symbols: ■) or entrapped (open symbols: □) from the nanocomposites as a function of the incubation time in PBS (pH 7.4) at 37 ºC.