Magnetite/Chitosan Nanocomposite for Magnetic Gemcitabine Targeting to Cancer

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Introduction

Recent drug delivery strategies have attempted to maximize the concentration of chemotherapeutic molecules into the tumors, while minimizing their systemic distribution. Active targeting of anticancer drugs can be achieved employing advanced strategies that allow the selective transport of nanoparticles (NPs) to the target site by stimuli, or through a specific recognition mechanism (ligand- or receptor-mediated targeting). The former approach relies on nanoplatforms that can experience a change in their physicochemical properties under exposure to an external stimulus (e.g., acidic pH, magnetic gradients, etc.), leading to a specific concentration of the drug into the tumor interstitium. Magnetically responsive NPs must possess properties such as biocompatibility, absence of toxicity and immunogenicity, appropriate drug vehiculization capabilities and significant responsiveness to the magnetic gradients [1].

In this contribution, we describe a reproducible technique for the preparation of magnetic core/shell NPs consisting of a magnetite (Fe_3O_4) nucleus and a chitosan shell, and loaded with the anticancer drug gemcitabine. The coating efficiency of chitosan around the magnetic core has been analyzed using electron microscopy, Fourier transform infrared (FTIR) spectrometry, and electrical and thermodynamic surface characterization. The amount of gemcitabine loaded to the core/shell NPs has been investigated by spectrophotometry and electrophoresis. *In vitro* drug release profiles were characterized according to the drug loading procedure. The magnetic properties of the nanocomposites were evaluated to analyze NPs magnetic responsiveness. Finally, an *in vivo* proof of concept of the magnetically enhanced nanomedicine accumulation into the tumor tissue has been established using Prussian blue staining technique.

Materials and Methods

Superparamagnetic Fe_3O_4 NPs (size $\approx$ 10 nm) were prepared by chemical co-precipitation [2]. The formulation of Fe_3O_4/chitosan nanocomposites was based on the coacervation method for the synthesis of chitosan NPs [3]. Mean particle size was determined in triplicate by PCS. To confirm the size measurements, the nanocomposites were checked by HRTEM. FTIR spectrometry was used for the chemical characterization of the NPs. Surface electrical properties of the NPs were analyzed by electrophoretic measurements as a function of pH. A surface thermodynamic analysis of the NPs was also done using the model developed by van Oss et al. [4]. The magnetic properties of the Fe_3O_4/chitosan NPs were determined by using a vibrating magnetometer.
Gemcitabine loading onto the magnetic nanocomposites was performed, using two distinct procedures. The first one (entrapment method) was similar to that followed for the preparation of the Fe_3O_4/chitosan NPs, except that the aqueous phase also included the anticancer agent. The second procedure (adsorption method) involved the single adsorption of gemcitabine onto the preformed nanocomposites. Drug release experiments were performed in triplicate at 37.0 ± 0.5 ºC with core/shell NPs with the higher gemcitabine loadings. It was followed the dialysis bag method, and the release media was phosphate buffered saline (PBS, pH = 7.4 $\pm$ 0.1).
The proof of concept of the magnetically enhanced nanomedicine accumulation into the tumor tissue was done by developing a L1210 *wt* murine subcutaneous tumor model. When palpable tumors were obtained, the mice were randomly divided into 3 groups of 6 each, i.e. untreated, treated with magnetic NPs, and treated with magnetic NPs under the influence of a 400 mT extracorporeal magnetic gradient. The accumulation of the NPs was qualitatively evaluated in terms of iron content using Prussian blue staining technique [5].

Results and Discussion

The coacervation method allowed the formation of well-stabilized Fe_3O_4/chitosan NPs with a narrow size distribution (average diameter $\approx$ 190 nm, polydispersity index: 0.071). HRTEM microphotographs of the nanocomposites proved that the iron oxide nuclei were covered by a polymeric shell (figure 1a). The coating efficiency of chitosan has been further analyzed using FTIR spectrometry, and electrical and thermodynamic surface characterization.
The entrapment efficiency (EE, %) of gemcitabine increased significantly with the drug concentration in the incubation medium (i.e., from $\approx$ 1.9 to $\approx$ 8.1 for the gemcitabine concentrations 10^{-4} – 10^{-2} M) when the drug was loaded through the adsorption procedure. Maximum drug loading (DL, %) was low (i.e., $\approx$ 1.4) which could be explained by the unfavorable thermodynamic interaction between the hydrophobic

polymeric matrix and the hydrophilic gemcitabine. The electrokinetic analysis of the drug adsorption process qualitatively confirmed these findings.

Because of the previously discussed unfavorable thermodynamic interaction between gemcitabine and chitosan, we considered the possibility to improve gemcitabine loading by introducing the drug before the coacervation process (the entrapment procedure), in order to provoke the mechanical trapping of gemcitabine. Compared to the surface adsorption procedure, both the EE and the DL were dramatically increased whatever the initial gemcitabine concentration fixed. For instance, when the initial drug concentration in the adsorption/absorption medium was 10^{-2} M, these parameters rose from ≈ 8.1 % and ≈ 1.4 % (after gemcitabine adsorption) to ≈ 44.6 % and ≈ 13.4 %, respectively (when the antitumor molecule was entrapped into the chitosan matrix). A positive effect of the gemcitabine concentration on the loading efficiency into the core/shell NPs could also be observed.

The release of gemcitabine adsorbed onto Fe_3O_4/chitosan NPs was complete within 3 h, as a result of a rapid desorption. On the contrary, when entrapped within nanocomposites, gemcitabine leakage occurred through a biphasic process, with an initial fast (burst) release (≈ 35 % in 3 h), the remaining being released in a sustained manner over a period of 117 h (≈ 5 days) (Figure 1b).

The magnetic responsiveness and soft magnetic character of the gemcitabine-loaded NPs were determined by the hysteresis cycle (Figure 1c). From the linear portions (low field) of the curve we could estimate the initial susceptibility ($\chi_i \approx 1.6$) and the saturation magnetization (≈ 167 kA/m).

Regarding the proof of concept, tumor sections of the mice injected with the nanocomposites in the absence of an extracorporeal magnetic gradient revealed a negligible presence of the iron content as indicated by a weak staining. On the contrary, tumor sections of the mice injected with the magnetically-guided core/shell NPs showed significant accumulation of iron (and thus of drug), mainly deposited at the tumor periphery, where the external magnet was placed (Figure 1d).

Conclusions

We have designed a new magnetically responsive nanodevice suitable for i.v. administration in which Fe_3O_4 nuclei were successfully encapsulated into chitosan. The entrapment procedure of gemcitabine into these Fe_3O_4/chitosan NPs has resulted in a greater drug loading and slower drug release properties. These core/shell NPs may constitute a potential candidate for cancer treatment, as they are tailored to deliver appropriate amounts of gemcitabine specifically into the tumor.

Acknowledgment

Financial support from project GREIB-PYR 2011-1 (Granada Research of Excellence Initiative on BioHealth, Spain) is acknowledged.

References

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Figures

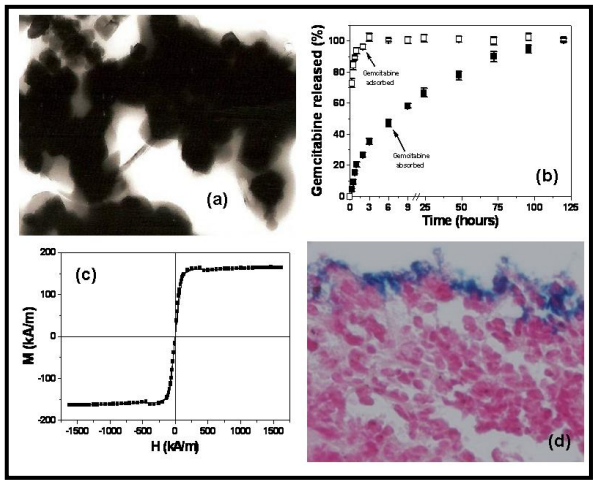

Figure 1. (a) HRTEM images of Fe_3O_4/chitosan NPs. (b) Release of gemcitabine (%), adsorbed (open symbols: □) or entrapped (full symbols: ■) from nanocomposites as a function of the incubation time. (c) Hysteresis cycle of the gemcitabine-loaded core/shell NPs. (d) Prussian blue staining test of tumors obtained from mice treated with Fe_3O_4/chitosan NPs with an extracorporeal magnetic gradient (400 mT for 2 h after injection) (5 mg/Kg equivalent of gemcitabine).