Design of Maghemite/Poly(D,L-lactide-*co*-glycolide) Nanoparticles for Magnetic Fluid Hyperthermia

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Introduction

The interest in using superparamagnetic iron oxide (i.e.., maghemite, γ-Fe_2O_3) nanoparticles (NPs) to formulate multifunctional nanoplatforms for biomedical purposes mainly relies in their non-toxic and biodegradable character, and crystalline structure [1]. Crystallinity assures the superparamagnetic behaviour of the nanomaterial, and it will further determine the excellent response of γ-Fe_2O_3 towards magnetic gradients which could be used to control the *in vivo* fate of the nanocomposite (by magnetic guidance), allowing the accumulation of the drug/gene dose into the targeted site. Interestingly, the oscillation of the magnetic moment of such NPs under an alternating electromagnetic gradient will transform them into heaters that could induce a hyperthermia effect against cancer cells [2, 3].

The present investigation is focused on the development of a reproducible technique for the synthesis of maghemite/poly(D,L-lactide-*co*-glycolide) (core/shell) NPs. Poly(D,L-lactide-*co*-glycolide) (PLGA) was used as the biocompatible and biodegradable matrix where the γ-Fe_2O_3 nanocores were embedded. This FDA-approved polymer will be responsible for the vehiculization of the drug/gene dose. Thus, the therapeutic activity of drug molecules and genes would be significantly enhanced by their incorporation to such magnetic NPs. The coating efficiency of the copolymer around the magnetic cores was analyzed by electron microscopy, infrared absorption spectra, and electrical and thermodynamic surface characterizations of the core/shell NPs, as compared to those of the pure nanomaterials. The internal structure of γ-Fe_2O_3/PLGA NPs was characterized by X-ray diffractometry. Finally, the magnetic properties of the nanocomposites were investigated to define its magnetic responsiveness, and their heating property (hyperthermia effect) was also analyzed under the influence of an oscillating electromagnetic gradient.

Materials and Methods

Superparamagnetic γ-Fe_2O_3 NPs (size ≈ 7 nm) were prepared by oxidation of ultrasmall magnetite cores [4]. PLGA NPs were formulated by following a water-in-oil-in-water (w/o/w) double emulsion/solvent evaporation methodology [5]. The synthesis procedure of γ-Fe_2O_3/PLGA NPs was equal to the one followed for the preparation of the pure copolymeric NPs, except that the aqueous solution of surfactant agent also contained γ-Fe_2O_3 nanocores (6.25 %, w/v).

Mean particle diameter was determined in triplicate by PCS. To confirm the size measurements, the nanocomposites were checked by HRTEM and by SEM. FTIR spectrometry was used for the chemical characterization of the NPs. The characterization of the internal structure of γ-Fe_2O_3/PLGA NPs was achieved by X-ray diffractometry. Surface electrical properties of the NPs were analyzed by electrophoretic measurements as a function of both pH and KNO_3. A surface thermodynamic analysis of the NPs was also carried out using a well-known model [6]. The magnetic properties of the NPs were determined by using a vibrating magnetometer. Finally, the *in vitro* heating behaviour of the core/shell NPs in a high frequency alternating electromagnetic gradient (frequency and intensity: 250 kHz and 4 kA/m, respectively) was investigated in triplicate at 25.0 ± 0.5 ºC.

Results and Discussion

γ-Fe_2O_3/PLGA nanocomposites were found to be spherical NPs with an average diameter of ≈ 135 nm (polydispersity index: 0.283) (figure 1a). It was clear from these pictures that γ-Fe_2O_3 cores were satisfactorily embedded into a PLGA matrix. The coating efficiency was further analyzed using FTIR spectrometry, and electrical and thermodynamic surface characterization. For instance, the electrokinetics of the PLGA and γ-Fe_2O_3/PLGA NPs were almost indistinguishable, and clearly different from that of γ-Fe_2O_3 nanocores (figure 1b). Regarding the thermodynamic analysis, as it is observed in the figure 1c, the hydrophilic nature of γ-Fe_2O_3 was modified and the nanocores become hydrophobic (just like the copolymer) when embedded into the PLGA matrix. The comparison of the diffractogram of the core/shell NPs with that of the γ-Fe_2O_3 cores confirmed the mineralogical purity of the iron oxide and their high crystallinity, even upon complete coating by the copolymer. This is an important property to assure the superparamagnetic character of the nanocores.

Regarding the mechanisms through which the iron oxide nanocores are embedded into the copolymeric network, some arguments could be given if we keep in mind all the information described about the surface characteristics of the NPs. Under the synthesis conditions, we may speak of an attractive electrostatic interaction between the positively charged γ-Fe_2O_3 NPs and the negatively charged PLGA matrix. This attraction will tend to concentrate the copolymer in the vicinity of the iron oxide surface.

Thermodynamic arguments could also be given: it was determined that the van der Waals and acid-base interactions between γ-Fe_2O_3 and the copolymer were neatly attractive. Thus, it would be thermodynamically favorable for the PLGA matrix to remain in contact with the nanocores rather than as isolated entities in water.

The magnetic responsiveness and soft magnetic character of the core/shell NPs were determined by the hysteresis cycle. From the linear portions (low field) of the curve we could estimate the initial susceptibility ($\chi_i \approx 2.5$) and the saturation magnetization ($\approx$ 206 kA/m). Figure 1d shows the *in vitro* heating behaviour of a core/shell aqueous magnetofluid in a high frequency alternating electromagnetic gradient. Under the experimental conditions, the oscillation of the magnetic moment of the γ-Fe_2O_3/PLGA NPs transformed them into heaters. As a result, the temperature of the magnetofluid rose from room temperature to the minimum hyperthermia temperature ($\approx$ 41 ºC) in $\approx$ 25 min. Interestingly, it has been described that locally heating at this temperature a tumor mass for $\approx$ 30 min is enough to destroy it [3]. Thereafter, the maximum temperature reached 47 ºC after $\approx$ 45 min, being then stabilized until the end of the experiment. Hence, the data proves a good control of the temperature and heat flux, a basic requirement for hyperthermia taking into account that when the temperature rises > 48 ºC, healthy cells surrounding the tumor tissue are expected to be burn and damaged [2].

Conclusions

It has been described a reproducible method for preparing magnetically responsive nanocomposites consisting of maghemite nanocores embedded into a poly(D,L-lactide-*co*-glycolide) matrix. The efficiency of the synthesis procedure is demonstrated by electron microscope analysis, physical chemistry data, and by comparing the thermodynamic and electrophoretic surface properties of the core/shell NPs with those of their components. These nanocomposites may constitute a potential candidate for therapeutic applications, e.g., cancer treatment: they could be tailored to deliver appropriate amounts of a chemotherapy agent specifically into the tumor cells, in combination with a selective hyperthermia effect into the malignant tissue.

Acknowledgment

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References

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Figures

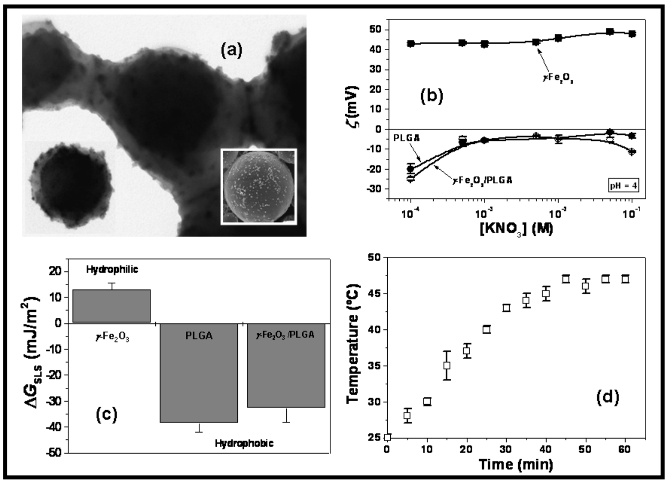

Figure 1. (a) HRTEM picture of γ-Fe_2O_3/PLGA NPs (Inset: SEM photograph of the NPs). (b) Zeta potential (ζ, mV) of γ-Fe_2O_3 (■), PLGA (●), and γ-Fe_2O_3/PLGA (○) NPs as a function of KNO_3 concentration at pH 4. (c) Solid-liquid interfacial energy of interaction (ΔG_{SLS}, mJ/m^2) and hydrophobic/hydrophilic character of γ-Fe_2O_3, PLGA, and γ-Fe_2O_3/PLGA NPs. (d) Heating curve of a γ-Fe_2O_3/PLGA magnetofluid (10 mg/mL) exposed to an oscillating electromagnetic gradient.