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Magnetic solid lipid nanoparticles in hyperthermia against colon cancer

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ABSTRACT

A reproducible double emulsion/solvent evaporation procedure is developed to formulate magnetic solid lipid nanoparticles (average size ≈ 180 nm) made of iron oxide cores embedded within a glyceryl trimyristate solid matrix. The physicochemical characterization of the nanocomposites ascertained the efficacy of the preparation conditions in their production, i.e. surface properties (electrokinetic and thermodynamic data) were almost indistinguishable from those of the solid lipid nanomatrix, while electron microscopy characterizations and X-ray diffraction patterns confirmed the satisfactory coverage of the magnetite nuclei. Hemocompatibility of the particles was established *in vitro*. Hysteresis cycle determinations defined the appropriate magnetic responsiveness of the nanocomposites, and their heating characteristics were investigated in a high frequency alternating gradient of magnetic field: a constant maximum temperature of 46 °C was obtained within 40 min. Finally, *in vitro* tests performed on human HT29 colon adenocarcinoma cells demonstrated a promising decrease in cell viability after treatment with the nanocomposites and exposure to that alternating electromagnetic field. To the best of our knowledge, this is the first time that such type of nanoformulation with very promising hyperthermia characteristics has been developed for therapeutic aims.

1. Introduction

Cancer prognosis has taken advantage during recent decades on the development of nanoplatforms reporting an easier diagnosis (Howes et al., 2014; Srivatsan and Chen 2014) and/or therapy (Couvreur, 2013; Ediriwickrema and Saltzman, 2015). In fact, revolutionary engineering approaches on nanoparticle (NP) development have resulted in the conceptualization of cancer theranosis (the combined diagnosis and treatment of such a severe disease) (Arias, 2011; Charron et al., 2015; Chen et al., 2014). Focusing on cancer treatment, the development of nanotools not only has helped in the delivery of therapeutic agents (i.e. drugs and/or genes) to the malignant tissue/cells (Gozuacik et al., 2014; Yang et al., 2015), but also in the optimization of complementary or alternative treatment solutions to the disease, e.g. hyperthermia (Dutz and Hergt, 2014), photothermal therapy (Zhang et al., 2015), and photodynamic therapy (Pekkanen et al., 2014). In addition, the introduction of passive and/or active targeting approaches in nanoplatform development is contributing to the generation of more efficient nanomedicines (Arias, 2013; Mura et al., 2013; Pérez-Herrero and Fernández-Medarde, 2015).

Talking about hyperthermia treatment against cancer, nanoparticulate-based tools can help in definitively localizing the heating source (mainly iron oxide NPs) within the tumour tissue, while heating it by applying a high frequency alternating gradient of magnetic field (Hergt and Dutz, 2007). Magnetic hysteresis losses and oscillation of the magnetic moment of iron oxide NPs under the influence of an external alternating electromagnetic gradient have been postulated to be the main mechanism behind magnetic particle hyperthermia (Dutz and Hergt, 2014; Huber, 2005; Kettering et al., 2015). Despite hyperthermia can be further used in optimizing the accumulation of drug nanocarriers within the site of action (Frazier and Ghandehari, 2015) and/or to trigger drug release (Purushotham and Ramanujan, 2010), the majority of preclinical and clinical studies have been focused on the development of magnetic particle hyperthermia as a cancer treatment strategy (Dutz and Hergt, 2014; El-Hammadi and Arias, 2015; Reddy et al., 2012). More recently, it has been demonstrated

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that the inclusion of the iron oxide NPs within an adequate coating can report interesting benefits to the nanotool formulated for hyperthermia against malignancies (Dutz and Hergt, 2014; Fantechi et al., 2014; Khandhar et al., 2012; Kruse et al., 2014; Quinto et al., 2015; Sadhasivam et al., 2015), e.g. prevention of agglomeration/sedimentation *in vitro* and *in vivo*, guarantee of biocompatibility, and possibility for the introduction of further therapeutic functionalities (loading of therapeutic molecules, habitually drugs and genes, just to mention the most representative strategy). The matrix were the magnetic particles are embedded can also contain polymeric chains being hydrophilic in nature and/or targeting ligands for a passive and/or active targeting to cancer tissues/cells, respectively (Dutz and Hergt, 2014; Reddy et al., 2012). The biocompatible and biodegradable coating in these core/shell nanoparticulate systems is generally made of a polymeric material or a lipid-based structure (Reddy et al., 2012). In the latter case, the association of solid lipid structures onto magnetic NPs may make possible promising biomedical results (Puri et al., 2009; Rostami et al., 2014). Such matrices (solid lipid nanoparticles, SLNs) have been reported to be biocompatible, biodegradable, and well tolerated materials (Doktorovova et al., 2014), finding promising applications in the delivery of drugs and nucleic acids to the site of action (De Jesus and Zuhorn, 2015; Gastaldi et al., 2014; Rostami et al., 2014; Uner and Yener, 2007).

In brief, the use of the so-called magnetic solid lipid nanoparticles (MSLNs) have been postulated in drug delivery (Grillone et al., 2015; Rostami et al., 2014; Zhao et al., 2015) and as magnetic resonance imaging contrast agents (Peira et al., 2003), or even as theranostic agents despite a definitive proof of concept has not yet been provided (Albuquerque et al., 2015). Unfortunately, to the best of our knowledge it cannot be found a clear move toward the introduction of MSLNs in the hyperthermia arena. In point of fact, a recent investigation merely reported the formulation of MSLNs consisting of maghemite (γ-Fe_2O_3) cores embedded within a trilaurin solid matrix (Hsu and Su, 2008). The investigation demonstrated *in vitro* that the nanocomposites can be magnetically heated to control the release of encapsulated tetracaine molecules, while the heating capacity of the MSLNs (just as an aqueous dispersion) was exclusively analyzed *in vitro*.

In this work we describe a reproducible methodology to prepare spherical MSLNs as a versatile nanotool for hyperthermia against colon cancer. Magnetite (Fe_3O_4, mean diameter $\approx$ 15 nm, see Section 3.1.) was chosen as the magnetic core of the nanocomposites given its non-toxic and biodegradable character, and given its crystalline structure which enables a superparamagnetic behaviour (and thus the adequate responsiveness to magnetic fields) (Reddy et al., 2012). It can be further hypothesized a potential application of these nanocores in cancer diagnosis due to their excellent sensitivity in T_2^* and T_2 weighted magnetic resonance images (Reddy et al., 2012; Terreno et al., 2010). The quality of the solid lipid layer onto the Fe_3O_4 nanocores was analyzed by comparing the environmental scanning and transmission electron microphotographs, and the electrical and thermodynamic surface properties of the nanocomposite, to those of the pure iron oxide and the solid lipid colloids. Blood compatibility of the MSLNs was evaluated *in vitro* by determining their effect on platelet activation, complement system activation, haemolysis, and plasma clotting time. Finally, the magnetic responsiveness of these nanocomposites was analyzed by hysteresis cycle measurements, while their hyperthermia potentialities were evaluated under the influence of an oscillating electromagnetic gradient in HT29 cancer cells.

2. Materials and methods

2.1. Materials

All chemicals were of analytical quality from Panreac (Spain), except for formamide, glyceryl trimyristate, and pluronic® F-68 (Sigma-Aldrich Co., Spain). Water was deionized and filtered with a Milli-Q® System (Merck Millipore Co., Germany).

2.2. Methods

2.2.1. Formulation of Fe_3O_4, SLNs, and MSLNs

Colloidal Fe_3O_4 was prepared by chemical co-precipitation (Massart, 1981). In brief, a ferric chloride solution (4 mL, 1 M) and a ferrous chloride solution (1 mL, 2 M; in 2 M hydrochloric acid) were slowly added to an ammonia solution (50 mL, 0.7 M) at room temperature and under mechanical stirring (1000 rpm). Immediate upon mixing, Fe_3O_4 NPs were obtained. Long-term stability of these iron oxides was possible by keeping them in contact during 12 h with a perchloric acid solution (100 mL, 2 M). Cleaning of the particles was then accomplished by repeated cycles of centrifugation (1 h at 35000 rpm, Centrikon T-124 high-speed centrifuge, Kontron, France) and re-dispersion in water until the conductivity of the supernatant was $\leq$ 10 μS/cm.

SLNs were formulated by water-in-oil-in-water (w/o/w) double emulsion/solvent evaporation (DE/SEV) (Nabi-Meibodi et al., 2013; Qi et al., 2011). Concretely, 2 mL of water (aqueous phase) were emulsified in ice bath with the help of an ultrasonic probe (400 W, 30 cycles with a 1 s active − 2 s duration; Branson Sonifier 450, Emerson Electric Co., USA) in 5 mL of dichloromethane containing glyceryl trimyristate (0.4%, w/v) and the emulsifier soybean lecithin (0.75%, w/v) (oily phase). Then, 20 mL of an aqueous solution of the non-ionic surfactant pluronic® F-68 (0.5%, w/v) (outer aqueous phase) were added and sonicated for 1 min. Mechanical stirring (1000 rpm) of the w/o/w emulsion was continued for 30 min in ice bath, and the remaining organic solvent was evaporated in a rotary evaporator (Rotavapor® R II, Büchi, Switzerland) to obtain an aqueous dispersion of SLNs. The colloid was then cleaned by repeated cycles of centrifugation (1 h at 10700 rpm) and re-dispersion in water, until the conductivity of the supernatant was $\leq$ 10 μS/cm.

The preparation procedure of the MSLNs was equal to the one used in the formulation of pure SLNs, except that the inner aqueous phase of the double emulsion contained the Fe_3O_4 nanocores (0.5%, w/v). The aqueous dispersion of MSLNs being thus obtained was subjected to a magnetic cleaning procedure: repeated separation of the NPs from the liquid medium by using a permanent magnet (0.4 T), and re-dispersion in pure water until the conductivity of the supernatant was $\leq$ 10 μS/cm. Finally, in order to define the effect of the relative amounts of solid lipid matrix and Fe_3O_4 on the properties of the nanomaterial, the formulation of the MSLNs was repeated with glyceryl trimyristate:Fe_3O_4 proportions ranging from 1:4 to 4:1. The NP production performance (%) under all of these glyceryl trimyristate:Fe_3O_4 proportions was further determined [(amount of magnetic nanocomposites obtained (mg)/summation of materials used in the preparation of the magnetic nanocomposites (mg)) × 100].

2.2.2. Characterization methods

Mean particle size was measured in triplicate at 25.0 ± 0.5 °C by dynamic light scattering (DLS, Malvern Autosizer® 4700, Malvern Instruments Ltd., UK), while the stability of the formulations was evaluated by determining particle diameter after 1 month of storage at 4.0 ± 0.5 °C in water. To confirm the results, the NPs were observed by environmental scanning electron microscopy (ESEM, FEI Quanta™ 400 environmental scanning electron microscope,