Biomaterials 32 (2011) 5663–5672

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Vitamin E (D-alpha-tocopheryl-co-poly(ethylene glycol) 1000 succinate) micelles-superparamagnetic iron oxide nanoparticles for enhanced thermotherapy and MRI

Prashant Chandrasekharan [a], Dipak Maity [b], Cai Xian Yong [c], Kai-Hsiang Chuang [c], Jun Ding [b], Si-Shen Feng [a,d,*]

[a] Department of Chemical and Biomolecular Engineering, Faculty of Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore 117576, Singapore
[b] Department of Materials Science and Engineering, Faculty of Engineering, National University of Singapore, 7 Engineering Drive 1, Singapore 117574, Singapore
[c] Laboratory of Molecular Imaging, Singapore Bioimaging Consortium, Agency for Science, Technology and Research Singapore, 11 Biopolis Way, #02-02 Helios, Singapore 138667, Singapore
[d] NUS Nanoscience & Nanotechnology Initiative (NUSNNI), National University of Singapore, Blk E3-05-29, 2 Engineering Drive 3, Singapore 117581, Singapore

ARTICLE INFO

Article history:
Received 2 April 2011
Accepted 18 April 2011
Available online 8 May 2011

Keywords:
Cancer nanotechnology
Colloidal stability
Magnetic micelles
Molecular imaging
Nanothermotherapy
Xenograft tumor model

ABSTRACT

We synthesized vitamin E TPGS (D-α-Tocopheryl-co-poly(ethylene glycol) 1000 succinate) micelles for superparamagnetic iron oxides formulation for nanothermotherapy and magnetic resonance imaging (MRI), which showed better thermal and magnetic properties, and *in vitro* cellular uptake and lower cytotoxicity as well as better *in vivo* therapeutic and imaging effects in comparison with the commercial Resovist® and the Pluronic®F127 micelles reported in the recent literature. The superparamagnetic iron oxides originally coated with oleic acid and oleylamine were formulated in the core of the TPGS micelles using a simple solvent-exchange method. The IOs-loaded TPGS showed greatest colloidal stability due to the critical micelle concentration (CMC) of vitamin E TPGS. Highly monodisperse and water soluble suspension was obtained which were stable in 0.9% normal saline for a period of 12 days. The micelles were characterized for their size and size distribution. Their morphology was examined through transmission electron microscopy (TEM). The enhanced thermal and superparamagnetic properties of the IOs-loaded TPGS micelles were assessed. Cellular uptake and cytotoxicity were investigated *in vitro* with MCF-7 cancer cells. Relaxivity study showed that the IOs-loaded TPGS micelles can have better effects for T2-weighted imaging using MRI. T2 mapped images of xenograft grown on SCID mice showed that the TPGS micelle formulation of IOs had ~1.7 times and ~1.05 times T2 decrease at the tumor site compared to Resovist® and the F127 micelle formulation, respectively.

1. Introduction

The surface coating material strongly affect the colloidal stability of nano-sized iron oxides (IOs) suspensions, which also plays key role in determining the adsorption, distribution, metabolism and excretion (ADME) process of the iron oxides after administration. Various macromolecules for IOs coating include simple sugars such as polysaccharide (Dextran) using epichlohydrin (CLIO, Cross linked iron oxide nanoparticles) [1,2], hydrophilic compounds such as poly ethylene glycols (PEG) [3], and high molecular weight amphiphilic polymers such as PLGA [4] and PLA-TPGS [5], which are currently under intensive investigation for biomedical applications such as nanothermotherapy and magnetic resonance imaging (MRI).

Amphiphilic macromolecules have the tendency to self-assemble to form nano-sized colloidal micelles in water at a concentration greater than the critical micelle concentration (CMC). These self-assemblies are oriented in such a way that the hydrophobic part of the amphiphile is kept in the core and the hydrophilic part is in contact with the water. A main concern in the various commercial micellar formulations of imaging and therapeutic agents is their stability. They would disassemble in diluted solution below the CMC. Micelles formed from amphiphilic copolymer may have better resistance to disassembly due to the enhanced interaction among the polymer chains in the micelle core [6–11]. Micelles can be prepared by simply adding the amphiphilic polymer at a concentration above its CMC in water while having higher encapsulation efficiency of the imaging or therapeutic agent in the core.

* Corresponding author. Tel.: +65 6516 3835; fax: +65 6779 1936.
E-mail address: chefss@nus.edu.sg (S.-S. Feng).

0142-9612/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2011.04.037

Miles *et al*. emphasized the importance and effect of the coating materials on particle formulation in physiological buffer, where the phosphate groups of the buffer solution can interact with the coating, replace them and thus cause instability of the iron oxides [12]. Colloidal stability of iron oxides is a great concern for their biomedical applications. Micellar aggregates can cause blood vessel blockage after administration, which may result in localized hypoxia, necrosis and hypersensitive reactions. Moreover, unstable micelles can be easily recognized by the macrophages, resulting in opsonization. As a result the micelles are readily taken up by the reticuloendothelial system (RES). A possible solution is to make use of surfactant polymers, which can be formulated into nanocapsules, nanospheres and micelles [13]. Nanocapsules contain a hydrophobic core carrying the imaging or therapeutic agent to be encapsulated. The hydrophobic core consists of solvent of high boiling point, which impedes its application in drug delivery due to the toxicity associated with it. Biodegradable nanoparticles, on the other hand, provide an excellent choice for excellent colloidal stability. However, nanoparticles synthesis requires careful optimization and proper selection of solvent and stabilizers. Preparation of micelle is less tedious if the stability problem can be addressed. Diluting the micelle suspension below the CMC, may cause disassembly of the micelle. In many cases, the micelles are cross linked using linker molecules. Unfortunately, most of the linker molecules are not acceptable to be used for biomedical applications [14].

Pluronic®F127 macromolecule surfactant (Poloxamer 407, HLB of 18–23, CMC of 0.05 wt.%) is a block copolymer, which is intensively used for biological application such as for encapsulation of cells [15], for membrane and membrane protein solubilization as well as for biomedical applications for designing drug delivery vehicle [16] and multifunctional nanoparticles [17–19]. D-α-Tocopheryl-co-poly (ethylene glycol) 1000 succinate (TPGS, HLB ~13, CMC 0.02 wt.%), on the other hand, has been an effective surfactant. TPGS is used in combination with chemotherapeutic drug in order to inhibit P-glycoprotein (P-gp) [20–23] and increase the chemotherapeutic efficacy for cancer treatment. P-gp protein is a class of multi-drug resistance proteins that are present on the cell membrane, which cause increased efflux of drugs, thereby reducing the efficacy of the drug. TPGS has also been used efficiently as an emulsifier for synthesis of nanoparticles of biodegradable polymers, providing a high encapsulation efficiency and cellular uptake of the drug *in vivo* [5,24,25]. TPGS has also been used for pro-drug design for enhanced chemotherapy [26,27].

In this research, F127 and TPGS are employed to develop micellar formulation of iron oxides for their potential application for nanothermotherapy and magnetic resonance imaging (MRI) in close comparison with the commercial Resovist®. The magnetic micelles of the two macromolecular surfactants of their various molecular weights and the hydrophilic-lipophilic balance (HLB) ratio are investigated. The polymeric surfactants are assigned a HLB, which determines the surfactant characteristics. For instance a lower HLB value means more hydrophobic and higher HLB value indicates more hydrophilic. The colloidal stability of the two magnetic micelles was attributed to the surfactant HLB. The two micellar formulations of iron oxides were investigated in close comparison for their hyperthermia and magnetic properties, *in vitro* cellular uptake and cytotoxicity, *in vivo* biodistribution and MRI imaging on SCID mice of xenograft tumor model.

2. Materials and methods

2.1. Materials

D-α-tocopherol poly ethylene glycol 1000 succinate (TPGS, $C_{33}O_5H_{54}(CH_2CH_2O)_{23}$) was from Eastman chemical company (USA). Pluronic®F127 (Poloxamer 407, molecular weight of 12,500) was brought from BASF (Ludwigshafen, Germany). Surfactants were freeze-dried for two days before use. Millipore water was prepared by a Milli-Q Plus system (Millipore Corporation, Bedford, USA). All chemicals including absolute ethanol, dimethylformamide (DMF) and tetrahydrofuran (THF) were of HPLC grade. They were used without further purification. Dialysis membrane was brought from Spectra/Por® MWCO of 1000. Hydrophobic iron oxides coated with oleic acid and oleylamine were synthesized by decomposition method reported elsewhere [5,28].

2.2. Synthesis and characterization of IOs-loaded micelles

Iron oxides-loaded micelles of TPGS and F127 were synthesized by a dialysis method. 150 mg of TPGS or 200 mg of F127 surfactant was taken in a 10% ethanol solution. 50 mg of iron oxide in THF was added into the 10% ethanol solution and sonicated by a probe sonicator at 25W for 2 min. The solution was transferred into a dialysis bag with an MWCO of 1000 and dialyzed against 5% ethanol for 2 h with change of solvent every 1 h and then with Milli-Q water for 6 h with changes of water every 1 h. Dialyzed solution was concentrated using a rotary evaporator system under reduced pressure. A certain amount of the sample was freeze-dried. The liquid sample was then analyzed for micelle size by Zetasizer (Nano ZS, Malvern Instruments Ltd, UK), morphology by transmission electron microscopy (TEM, JEM 2010F, JEOL, Japan). The amount of coating was calculated by thermogravimetric analysis (TGA) using dry sample. The iron content of the magnetic micelles was determined using inductively coupled plasma mass spectroscopy (ICP-MS).

2.3. ICP-MS analysis

A known volume of IOs-loaded micelle suspension or Resovists® were taken in glass test tube, 2 ml of concentrated nitric acid was added and heated to 90 °C for 45 min and the samples were analyzed using ICP-MS (Agilent ICP-MS 7500 Series) after sufficient dilution with Milli-Q water. The analysis of sample was done in comparison with the ICP-MS standard (Sigma).

2.4. Hysteresis

The hysteresis curve and the magnetic saturation (σ_s) of the dry IOs-loaded micelles were determined using a vibrating sample magnetometer (VSM, Lakeshore 7300 Series, USA). Dried sample of known mass was taken in non-magnetic aluminum sheet. The sample was subjected to varying magnetic field at room temperature and the magnetization was measured.

2.5. Cell viability assay

MCF-7 cancer cells were seeded in 96 well plates (Costar, IL, USA) at the density of 20,000 viable cells/well using Dulbecco's Modified Eagle Medium (DMEM), containing 10% fetal bovine serum and 1% antibiotics, and incubated for 24 h to allow cell attachment. The media was removed and then replaced with fresh media with IOs-loaded micelle suspension or Resovists® at the various concentrations. Untreated wells were used as control. Five hours prior the time point, 10 μl of MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole, 5 mg/ml in PBS) was added into the 96 well plates. The cells were incubated till the designated time point. After incubation, 100 μl of a stop mix solution composing of 20% SDS in 50% DMF was added into each well. The plate was incubated for an hour to dissolve the formazan crystals that are formed and the absorbance in each well was measured using a microplate reader (GENios, Tecan, Switzerland) at 550 nm and 630 nm as reference wavelength. The cell viability was then calculated as:

$$\%\ \text{Cell viability} = \frac{\text{Absorbance of sample well}}{\text{Absorbance of control well}} \times 100$$

2.6. Determination of SAR value and cell hyperthermia

Determination of the specific absorption rate (SAR) and cell hyperthermia was performed as described elsewhere in our earlier publication [29]. In brief, heating ability of the magnetic micelles was determined from the time-dependent calorimetric measurements using a RF generator (EASYHEAT-5060, Ameritherm) operating at 240 kHz frequency. One milliliter of aqueous suspension of magnetic micelles with the Fe concentration of 5 mg/ml were subjected to 89 kA/m AC field and time-dependent temperature rise was monitored for various designated times using an optical fiber based temperature probe (FLUOTEMP Series, FTP-LN2). The SAR was calculated using the following equation [29]:

$$\text{SAR} = C\frac{\Delta T}{\Delta t}\frac{1}{m_{Fe}}$$

where C is the specific heat of the solvent (here $C_{water} = 4.18$ J/g°C), $\Delta T/\Delta t$ is the initial slope of the time-dependent temperature curve and m_{Fe} is weight fraction of magnetic element (*i.e.* Fe) in the sample.

For *in vitro* cellular hyperthermia, 10^6 exponentially growing MCF-7 cancer cells in DMEM cell culture medium containing 10% FBS and 1% antibiotic-antimycotic solution were taken in 15 ml sterile polypropylene tubes and centrifuged at