Superparamagnetic Iron Oxide Clusters for Magnetic Resonance Imaging

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ABSTRACT

Nano-cluseter of superparamagnetic iron oxide (SPIOs) is an attactive materials with highly relaxivity for magnetic resonance imaging, The stabilizers of clusters were commonly used in ionic form. However, these surfactant may reveal cytotoxicity for biomedical applications. In this study, a non-ionic polymeric surfactant TPGS formulated superparamagnetic iron oxides were prepared for magnetic resonance imaging and the structure and composition of the SPIO@TPGS clusters were characterized by transmission electron microscope and magnetic measurements. Our results show that the SPIO@TPGS clusters with a hydrodynamic diameter around 100 nm led to a significant advantage in terms of *T*2 relaxation and dose-dependent darkening of MR images as compared with a commercial SPIO contrast agent (Resovist®). TEM images reveal that the SPIOs aggregated and stabilized by TPGS in the water solution. This nonionic surfactant stabilized SPIO cluster may have a great potential for further biomedical applications.

Keywords: Superparamagneic iron oxide, nano-clusters, magnetic resonanc imaging

1 INTRODUCTION

Magnetic resonance imaging (MRI) has been regarded as a noninvasive powerful imaging tool that yields excellent soft-tissue contrast, has a high spatial resolution, and possesses tomographic capabilities. Among the contrast agents used in MRI, the practicability of employing superparamagnetic iron oxide (SPIO) nanoparticles as MRI *T*2-shortening agents for noninvasive cell-labeling or tumor detection in clinical practice has been demonstrated.[1-2]

The propertis of magnetic nanoparticles functionalized and concurrently respond to a magnetic field has made them as a useful tool for theragnostics-the combination of therapeutic and diagnostic technologies.[3] However, the surface chemistry is a critical determinant that regulates physiochemical characteristics of magnetic nanoparticles (MNPs), including their size, solubility, state of dispersion and magnetization values for further development. It is known that the surface chemistry greatly influences MNP fate in the biological system, including the mechanisms of their cell recognition, biodistribution and immune response[4-5] it presents a specific focus for advancing engineering strategies to minimize potential nanotoxicity.[6] In most caces, the ionic surfactant would alter the fluidy and integrity of cell membrane that cause cell damages. TPGS 1000 (d-alpha-tocopheryl poly(ethylene glycol 1000) succinate) was developed in the 1950s as a water-soluble form of vitamin E. TPGS 1000 is comprised of a hydrophilic polar (water-soluble) head and a lipophilic (water-insoluble) alkyl tai that can be used as a solubilizer and an emulsifier for nanoformulations. In this study, the TPGS-formed SPIO clusters were developed as MRI contrast agents for further clinical applications..

2 MATERIALS AND METHODS

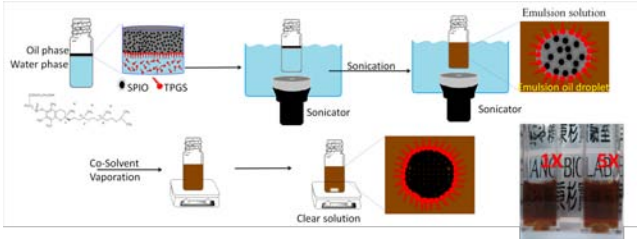

Figure 1 The preparation of SPIO@TPGS.

2.1. Materials

Iron (III) acetylacetonate ($Fe(acac)_3$), oleylamine, were purchased from Sigma Aldrich. Oleicaicd were purchase from SHOWA. And the Vitamin E TPGS 1500 were purchase from Eastman. The solvents were all dehydrated prior to use. The double distilled water was used as all solution solvent. The in vitro studies used the modified Eagles's medium (MEM), penicillin, streptomycin, and fetal bovine serum (FBS) were all purchased from Invitrogen.

2.2. Synthesis of superparamagnetic iron oxide

High-temperature organometallic synthesis of SPIO in nitrogen atmosphere with a diameter of around 6nm was carried out following the procedure detailed by Sun and Zeng.[7] Collecting the product by added excess ethanol and centrifuged at 6000 rpm. The stock of SPIO was dispersed in 25ml hexane with 0.5 ml oleic acid and 0.5 ml oleyl amine in room temperature and air dried before use.

2.3. Preparation of SPIO@TPGS formulations

SPIO encapsulated in TPGS was prepared using emulsion and co-solvent evaporation methods. The air dried SPIO solids (5 mg) and TPGS (20 mg) mixed in hexane in

room temperature, following which the mixture was added 10 ml of water. The mixture was given ultrasonic treatment for 10 minutes to create O/W micro emulsion. The preparation idea was inspired by Yadong Li's et al.[8] The hexane in the emulsion solution was allowed to evaporate at around 75℃ with constant stirring 600 rpm for 60 minutes. After preparation, the products were dialysis against water over 96 hours (MWCO: 3500) and freeze-dried then stock in -20℃ refrigerator before use.

2.4. Characterization

The freeze-dried final product of SPIO@TPGS was stocked in sample vial or dispersed in water or PBS. The solution samples of SPIO@TPGS was placed in a plastic cuvette and determinate the size distribution by dynamic light scattering (DLS, ZS 90, Malvern Instruments Ltd) at 25℃.

The morphology of SPIO and SPIO@TPGS were checked by a JEOL 1400 transmission electron microscope (TEM) with an accelerated voltage of 100 kV. Iron concentration of the SPIO, SPIO@TPGS and Resovist® was determined using an atomic absorbance spectrophotometer (Dionex ICS-90, USA). The samples were prepared from the diluted solutions or freeze-dried stocks, and added 5ml 10% HNO_3 solution for dilution and dissolve the freeze-dried stocks.

The magnetic properties of SPIO, SPIO@TPGS and Resovist® were examined with a 1.5T vibration sample magnetometer (VSM) and Pulse NMR at the room temperature. The sample preparations as follow: the freeze-dried of SPIO, SPIO@TPGS, Resovist® were weighted and package in the small piece of paper and putted the samples on the VSM holder. And the magnetic curves and relaxation rates were recorded in a field of 1.5T T_1T_2 enhancing relaxivity analysis. *T2*-weighted magnetic resonance images were obtained on a 1.5T clinical magnetic resonance scanner.

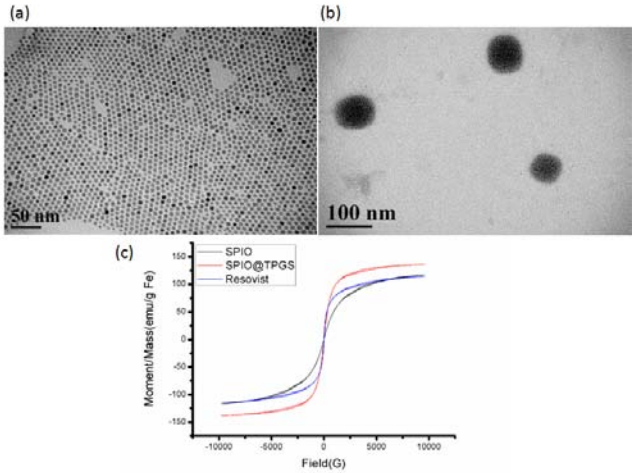

Figure 2. The TEM images of (a) SPIO (b) PIO@TPGS. The hysterysis loops(c) of SPIO, SPIO@TPGS, and Resovist®.

3 RESULTS AND DISCUSSIONS

3.1 Preparation of SPIO@TPGS nano-clusters

The experimental design is shown in Figure. 1. The idea to make the combination with SPIO and the non-ionic surfactant TPGS was inspired by the concept of the method of the stabilization of nano crystal (SNC) and the concept of an emulsion.[9-10] In this work, the non-ionic surfactant TPGS will line up at the interface of oil and water to reduce the surface tension of the tiny oil droplets, which would otherwise assemble themselves into large drops. The SPIO nanoparticles were initially stabilized by oleic acid and oleyl amine to disperse in the oil phase solvent due to their production. In the preparation of emulsion, there were some solvents like tetrahydrofuran, chloroform, and hexane have been tested in this study. Like our previous work, we used the tetrahydrofuran as an oil phase solvent in micelle preparation.[11] In this study, the chosen solvent was hexane because of low toxicity and easy availability than chloroform. In the conditional trial, the SPIO and TPGS were first dissolved in hexane (in a glass vial) with different ratio (data not shown in here) and different volume of hexane and following evaporation of the hexane with different temperature (controlled by hot plate). Similar results was reported by Yang Liu et. al.[9]

Mauny ionic surfactants have been used to prepare the cluster as previous reports.[8, 12] Feng et al.[13] reported the SPIO encapsulated in poly (lactic acid)-D-alpha-tocopherol polyethylene glycol 1000 succinate copolymer micelles as a side effect less drug carriers. And Shi et al. reported the preparation of superparamagnetic nanoparticles in fluorescent nanogels as a MRI contrast agents by used the polyvinyl alcohol as the clusters stabilizer. In this manner the non-ionic pharmaceutical surfactant TPGS was seldom to use. The surface of the clusters was fully covered by the surfactant molecules, with the polyetheleneglycol (PEG) side chain in directly contacted with water, and the vitamin E group aggregated with the oleic acid long carbon chain in the SPIO outer surface, the surfactant would stabilized and provided a highly dispersion in water solution.[10] Compare with conditional trail preparation, the large amount preparation would produce a little bit smaller of size and higher of polydisperse index (PDI) due to the difference of preparation system. The large preparation system were prepared in the round bottle flask and evaporated by programmed heater followed by the temperature increase curve measured in the conditional trial. But there had similar result between the conditional trial and large amount preparation, the size, PDI, and shape of the clusters were no obviously change.

Table 1. Characteristics of SPIO@TPGS and Resovist.

	Size	PDI	Iron concentration	Ms*	Mr*	r 1 value	r 2 value	r 2/ r 1
	(nm)		(wt %)	(emu/g Fe)	(emu/g Fe)	(1/S · mM)	(1/S · mM)	
SPIO@TPGS	107.3	0.398	11.09±0.01	137.04	0.61	11.39	68.94	6.05
Resovist	54.3	0.187	20.38±0.42	114.58	2.51	9.71	56.35	5.8

*Ms: saturation magnetization.
*Mr: remnant magnetization.

3.2 Characterization of SPIO@TPGS

High-temperature organometallic synthesis of SPIO was detailed by Sun and Zeng.[7] The synthesized SPIO nanoparticles size were in all cases lower than 10nm and the standard deviation is in the monodispersed range shown by TEM images in Figure 2(a-b). After the vitamin-E TPGS coating, the mean hydrodynamic diameter of SPIO@TPGS measured by the DLS was 107.3 nm, with the polydispersity (PDI= 0.398), and the iron concentration (in wt %) measured by the atomic absorbance spectrophotomete was 11.09± 0.01. Figure 1 had showed the SPIO@TPGS in the plastic cuvee after preparation in room temperature. We examined the SPIO@TPGS by using the TEM and verified the conformation. The TEM images showed that the SPIO@TPGS and assembled with narrow polydisperse in the size around 100 nm. Most of the particles were formed as a cluster of SPIOs while some agglomerated due to magneto-dipole interactions between particles[14] and the preparation of co-solvent evaporation in emulsion solutions.[15] The freeze-dried of SPIO@TPGS were dissolved in the water solution and other buffer solutions like PBS were also stable and well disperse.

The hysteresis loops shown in Figure 2 (c) of the SPIO, SPIO@TPGS and Resovist ® showed the superparamagnetic properties low magnetic coercivity, and high saturation magnetization, and the curve were all calculated with the iron content of their own. The magnetic resonance images of SPIO@TPGS and Resovist ® were shown in Figure 3 took by the clinical 1.5-TMRI scanner showed the contrast ability increased with the increase of iron concentration and a very powerful contrast was observed. The characterizations summery of SPIO@TPGS and Resovist® are shown in Table 1.

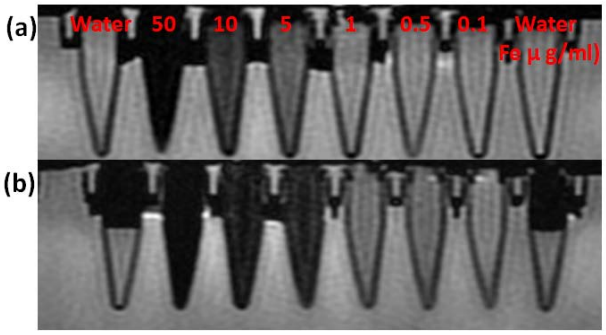

Figure 3. MR Images of (a) SPIO@TPGS and (b) Resovist in took by 1.5 T clinical MR scanner.

4 CONCLUSION

For the purpose of achieving the dual functions of MRI function, and reducing the multidrug resistances, a strategy was developed to prepare TPGS coated the superparamagnetic iron oxide nano-clusters with small size. This development of TPGS formulation nano-clusters for reducing the multidrug resistances and use in MRI, and showed that these efficient dual functions could be successfully employed in multidrug resistance cancer treatment and imaging. In comparison with traditional molecular-based contrast agents, a dual functional nano-clusters of a highly-integrated design can incorporate multiple functions, such as cell targeting, imaging, anti-multidrug resistances and therapy, within a single system. It may combine more interesting function into this system in the future.

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