Clusters of Iron Oxide Nanoparticles for Efficient Magnetic Resonance Imaging

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ABSTRACT

Clusters of iron oxide nanoparticles (IONP), which were stabilised by the nonionic surfactant d-alpha-tocopheryl poly(ethylene glycol 1000) succinate (TPGS), were easily and successfully prepared in an oil-in-water emulsion system. Notably, well-constructed clusters can be obtained by changing the amount of solvent and its evaporation rate. The optimized clusters were spherical in shape, had a diameter of approximately 97 nm, and were internalized by KB cells via energy-dependent endocytic pathway. In addition, these clusters had higher saturation magnetisation and r2 relaxation (253.85 s-1 mM-1) values and better T2-weighted contrast performance (r2/r1 = 20.5) than commercial Resovist®. TPGS-stabilised IONP clusters were also shown to be an efficient contrast agent for in vivo magnetic resonance imaging, especially in the liver and tumor regions. Iron staining of both tissues confirmed the accumulation of the nanoparticles in both areas. Thus, these clusters, which were prepared with the use of nonionic polymer surfactant, can potentially serve as efficient contrast agents for magnetic resonance applications.

Keywords: Superparamagnetic iron oxide nanoparticles; nanoclusters; magnetic resonance imaging

1 INTRODUCTION

Magnetic resonance (MR) imaging has been regarded as a noninvasive and powerful imaging tool that yields excellent soft-tissue contrast, has a high spatial resolution, and possesses tomographic capabilities without the hazard of ionizing radiation [1]. Recently, self-assembling clusters of metal nanoparticles have been extensively investigated in various fields. To prepare these clusters, various ionic surfactants, such as cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate, and polyethyleneimine, have been employed as stabilizers or emulsifiers to form water-dispersed spherical clusters [2-6]. It has been demonstrated that SPIO clusters have higher transverse relaxivity values than individual SPIO nanoparticles, and thus they can act as potential contrast agents for T2-weighted MR imaging [2]. This phenomenon can also be utilised to develop an ultrasensitive medical MR sensor [7]. To develop these magnetic nanoparticles for clinical applications, the surface chemistry of the nanoparticles/clusters needs to be carefully considered because this characteristic greatly influences the particles' fate within a biological system due to the mechanisms of cell recognition, biodistribution, immune response and nanotoxicity [8, 9]. Qiu and Chen et al. demonstrated that the CTAB coating on Au nanorod surfaces can cause cell apoptosis by altering the mitochondrial membrane potential and increasing the intracellular reactive oxygen species, thereby limiting the biomedical use of these nanorods [10]. Thus, a facile method of SPIO cluster development using biocompatible stabilizers is necessary for biomedical applications.

In this study, we demonstrated a facile and greener synthesis to fabricate iron oxide nanoparticles (IONP) cluster formation using the nonionic surfactant TPGS (d-alpha-tocopheryl poly(ethylene glycol 1000) succinate) through the adjustment of the amount of solvent and its evaporation rate. In the conventional procedure for cluster formation, size selection has to be carried out via centrifugation and/or filtration process which usually required time-consuming and low yield production. In comparison with the previous process of cluster preparation using ionic surfactants, non-ionic surfactant-assisted IONP clusters resulted in less cytotoxicity with more biocompatibility for biomedical applications. The potential use of these clusters as MR contrast agents was also investigated in vitro and in vivo.

2 EXPERIMENTAL FLOW

2.1 Preparation of IONP@TPGS Clusters

IONP with diameters of approximately 6 nm were synthesised by a high-temperature thermal decomposition method in a nitrogen atmosphere, as previously reported [11]. To prepare the TPGS-assisted IONP (IONP@TPGS) clusters, the emulsion and solvent-evaporation method was employed. Briefly, the dried IONP (5 mg) and TPGS (20 mg) were mixed in hexane (0.02 - 2 ml) at room temperature, and then 10 ml water was added to this mixture during ultrasonic treatment for 10 minutes to form an O/W emulsion. The hexane was then removed from the emulsified solution, which had a different evaporation rate, by controlling the temperature of the hotplate under constant stirring at 600 rpm (CO-PC420D, Corning, USA). After cooling down to the room temperature, the products were dialysed (MWCO: 3500) against water to remove free surfactant TPGS. The particle sizes of the IONP@TPGS formulations were analysed by dynamic light scattering at 25 °C, and their morphologies were observed by a JEOL 1400 transmission electron microscope with an accelerated voltage of 120 kV. The iron concentrations of the IONP, IONP@TPGS and Resovist® clusters were determined

quantitatively using an atomic absorbance spectrophotometer (GBC 932, USA). To evaluate the magnetic properties of IONP, IONP@TPGS and Resovist®, samples were examined with a vibration sample magnetometer (VSM, LakeShore) and Pulse NMR (Bruker, Minispec 20 MHz) at room temperature and at 37 °C. The IONP@TPGS clusters and Resovist® were serially diluted into several concentrations and placed into microcentrifuge tubes before imaging. T_2-weighted magnetic resonance images were obtained on a 3 Tesla clinical magnetic resonance imaging system (Signa Excite 3 T, GE Healthcare, USA).

2.2 In Vivo MRI

The in vivo experimental protocols were approved by the Institutional Animal Care and Use Committee of National Chung Hsing University (IACUC of NCHU). Female BALB/cAnN.Cg-Foxn1nu/CrlNarl nude mice (4-5 weeks old, 20±2 g) were obtained from the National Laboratory Animal Center (Taiwan). All mice were kept in an air-conditioned facility fitted with an artificial light–dark cycle and were provided with standard food and filtered water. The mice were acclimated to this environment for at least three days prior to subcutaneous injection in the right hindquarter with 1 × 107 KB cells suspended in serum-free Minimum Essential Medium. The tumor volume was calculated using the formula 1/2(4π/3)(L/2)(W/2)H, where L is the length, W is the width, and H is the height of the tumor. Treatments were initiated when the tumors reached a volume of 100 mm3. The animals were injected with 0.1 ml of PBS (control group) or IONP@TPGS clusters (4 mg Fe/kg) via a lateral tail vein. The animals that received PBS (vehicle) were used as controls. MR imaging of mice was conducted using a 7 Tesla MRI (Bruker, USA) under halothane gas anaesthesia before treatment and 24 hours postinjection. The images were analysed by Image J provided by the NIH (http://rsbweb.nih.gov/ij/).

2.3 H&E and Prussian Blue Staining.

After imaging at 24 hours postinjection, the mice were harvested, and the tumors and liver tissues were excised, weighed, fixed in formalin, embedded in paraffin and sectioned at 2 μm for histological analysis. The sections were deparaffinised, dehydrated and reacted with hematoxylin and eosin (H&E) [12]. To visualize the IONP by PB staining, the sections were reacted with equal volumes of hydrochloric acid (20%) and potassium ferrocyanide (10%) for 20 minutes and were observed using a light microscope (BX 50, Olympus, Japan) equipped with a digital camera (DP 20, Olympus, Japan).

3 RESULTS AND DISCUSSIONS

According to our results, IONP solution can be emulsified and stabilised successfully using TPGS, which can align at the interface of oil and water to reduce the surface tension of the oil droplets in water [13]. The particle size is one of the important concerns for in vivo applications, and the size-dependent enhanced permeability and retention (EPR) effects of the nano-clusters due to the leaky vasculature of the tumor microenvironment can be well observed with particle sizes smaller than 200 nm [14]. In this study, cluster with a particle size of approximately 96.9 nm and a PDI of 0.281 was speculated to be a potential particle with good passive targeting ability and thus employed for the following biological tests.

In general, superparamagnetic particles can be used as negative MR contrast agents based on their ability to shorten the spin-spin (T2) proton relaxation time that results in signal reduction and darkness on T2-weighted images. To evaluate the potential use of IONP@TPGS clusters as an innovative T2 MR contrast agent, the relaxation times (T1 and T2) were measured by 20 MHz (0.47 T) Minispec at 37 °C. The r2 and r1 relaxivity constants were calculated from the slopes of the linear plots of the relaxation rates of 1/T2 and 1/T1 against the iron concentration; these values are summarised in Table 1. The longitudinal relaxivity (r1) values of IONP@TPGS clusters and Resovist® were 12.37 s-1 mM-1 and 25.40 s-1 mM-1, respectively. The transverse relaxivity (r2) of IONP@TPGS clusters was found to be 253.85 s-1 mM-1, whereas the r2 of Resovist® was 151.95 s-1 mM-1. The IONP@TPGS clusters exhibited a higher r2 value and a three times higher r2/r1 ratio (20.52) than commercial Resovist® did (r2/r1 = 5.98), which indicates that the TPGS-stabilised IONP clusters can act as potential T2 contrast agents for MRI. These results proved that the enhancement of the transverse relaxivities r2, relaxivity ratios r2/r1, relaxivity and magnetisations can be observed as a clustering effect of IONP [15-18].

	Size	PDI	Iron concentration	Ms*	r 1 value	r 2 value	r 2/ r 1
	(nm)		(wt %)	(emu/g Fe)	(1/S · mM)	(1/S · mM)	
IONP@TPGS	96.9	0.281	10.350	110.033	12.37	253.85	20.52
Resovist	54.3	0.187	24.440	95.485	25.40	151.95	5.98

*Ms: saturation magnetization.

Table 1. Size and magnetic properties of IONP@TPGS clusters and Resovist® in aqueous solution.

To evaluate the cytotoxicity of the IONP@TPGS clusters, cells were incubated with various concentrations of IONP@TPGS clusters for 24 hours and then quantitatively analysed by MTT assay. As shown in Figure 1, IONP@TPGS clusters obviously revealed the viability over 100% as the iron concentration below 12.5 μg/ml. Similar results were also observed as our previous work using Pluronic F127-encapsulated IONP [18]. The increased metabolic activity may be due to the availability of additional free iron released from the IONP in the cells, which can be used to increase cellular metabolic activity in lower concentration [19]. The cell viability with IONP@TPGS clusters treatments can be maintained over

90% or 84% as the iron concentration increased to 25 or 50 µg/ml, respectively. Thus, IONP@TPGS clusters were revealed to be almost non-toxic to the KB cells. For the cells treated with Resovist®, the cell viability over 100% was clearly observed at certain iron concentration, especially at high concentration. The difference in viability between IONP@TPGS clusters and Resovist® treated cells may be due to the cell responses to different coating materials on the particle surface and different particle sizes.

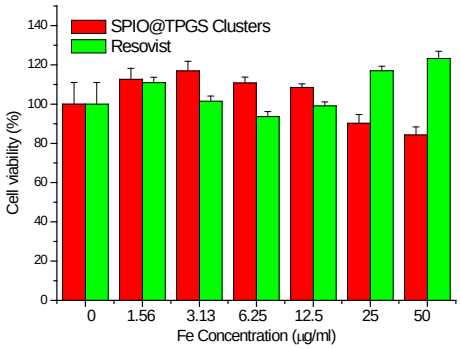

Figure 1: Cell viability of the cells treated with IONP@TPGS clusters and Resovist® as evaluated by the MTT assay in KB cells. Data are presented as means ± standard deviation.

For further evaluation of the contrast imaging capability of IONP@TPGS clusters in vivo, animals with KB tumors were scanned before administration or at 24 h post intravenous injection. As shown in Figure 2A, under T2-weighted imaging, the signal intensity of the tumor at the right flank (red circle) decreased markedly 24 h post injection, compared with the image before intravenous injection, which indicates that the IONP@TPGS clusters have an efficient tumor affinity and cause an strong T2 enhancement, which results in a darker image based on the EPR effect. It is known that Resovist® can be used to image liver tumors because it accumulates in the reticuloendothelial system (RES) of the normal liver to make a positive contrast in liver tumors. In this study, we also noticed a signal intensity decrease in the liver after IONP@TPGS administration (Figure. 2A, yellow circle). This finding reflects the phagocytic activity of macrophages in the liver, which is a mechanism that has been used for detecting liver tumors with Resovist®. Thus, the clusters caused a substantial negative enhancement of the signal intensity in both the tumor region and in the liver.

To verify the accumulation of IONP in the liver and tumor region, PB staining was employed to detect iron oxide in the tissue sections. As shown in Figure 2B, positive staining was observed in both the liver and tumor region. However, the liver revealed more PB stained area than the tumor did, which indicates that the accumulation of IONP in the liver is higher than that in the tumor. This result is consistent with our finding from MRI experiments.

The sections of liver and tumor tissue stained by H&E are shown in Figure 3. Glycogen infiltration was observed in the normal liver (Figure 3A) which is due to in vivo experimental procedure without starvation. Clearly, no noticeable morphological differences were observed in the liver before or after IONP@TPGS cluster administration (Figure 3A and 3B). However, the tumor region revealed scathe phenomena after injection of the IONP@TPGS clusters (Figure 3D; Figure 3C is control group). As our results, the IONP@TPGS clsuters did not influence the morphology of liver tissue but may cause cell death in tumor region. Thus, IONP@TPGS clusters can not only act as an improved MRI contrast agent but can also be a therapeutic agent for theranostic development. Further investigations are ongoing now in our lab.

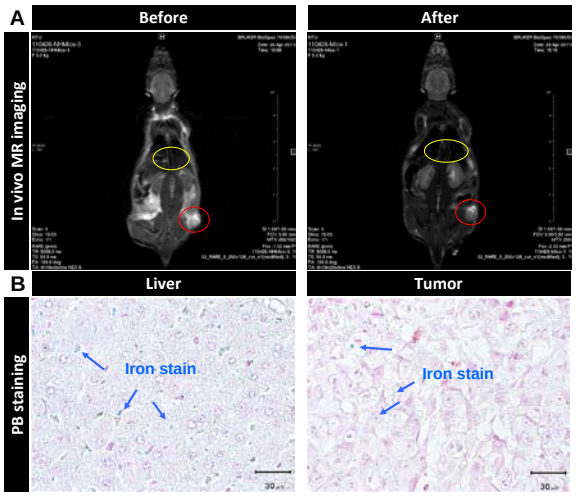

Figure 2: *In vivo* studies of KB-bearing mice before and after the 24-hour administration of IONP@TPGS clusters.

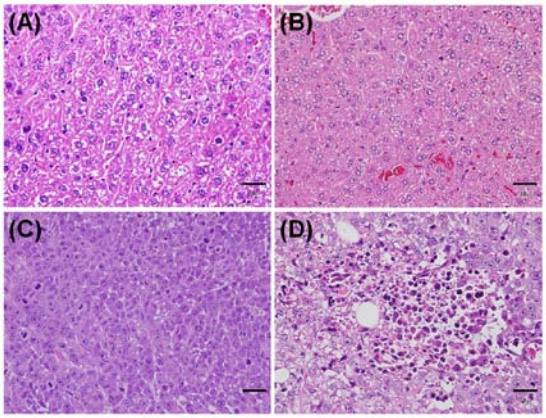

Figure 3: H&E staining of liver and tumor sections before and after the 24-hour administration of IONP@TPGS clusters in KB-bearing mice. (A) Normal liver; (B) liver treated with IONP@TPGS clusters; (C) control tumor and (D) tumor treated with IONP@TPGS clusters.

4 CONCLUSION

In this study, nonionic surfactant TPGS was employed to stabilise IONP clusters that can minimise the cytotoxicity from the conventional ionic surfactant. The as-prepared IONP@TPGS clusters exhibited a three times higher r2/r1 ratio than commercial Resovist® did and the in vivo MRI images were obviously darker after the administration of the IONP@TPGS clusters, especially in the liver and tumor areas. Thus, the phase-transfer process by TPGS is a facile and prospective platform to form IONP clusters that can serve as potential MR contrast agents for biomedical applications.

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