

Mn-Zn Ferrite and Photosensitizer Co-loaded Cancer Theragnostic Agent for Magnetic Resonance Imaging and Photodynamic Therapy

Syu-Ming Lai*, Ming-Da Yang**, Mean-Jue Tung** and Ping-Shan Lai*

* Department of Chemistry, National Chung Hsing University, 250, Kuo Kuang Rd., Taichung 402, Taiwan

** Department of Electromagnetic Materials and Devices, Material and Chemical Research Laboratories, Industrial Technology Research Institute

ABSTRACT

In this report, a multifunctional cancer diagnostic system based on manganese-zinc ferrite (MZF) and chlorin e6 (Ce6) co-loaded nanoclusters were demonstrated for magnetic resonance imaging (MRI) and photodynamic therapy (PDT). The results showed that the MZF and Ce6 co-loaded nanoclusters with particle size around 100 nm exhibited almost non-toxic to the KB cells under 200 μg Fe/ml and had better T2 relaxivity and darker T2-weighted MR images comparing to the commercial Resovist®. Thus, this system may have highly potential diagnostic abilities for in nasopharyngeal cancer or other cancers.

Keywords: Manganese zinc ferrite; nano-clusters; magnetic resonance imaging; photodynamic therapy; theragnostic agents

1 INTRODUCTION

Magnetic resonance imaging (MRI) 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] Among the contrast agents used in MR imaging, 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. In addition, magnetic nanoparticles can be functionalized and concurrently respond to a magnetic field for use as potential theranostic tools.[2-5]

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.[6-10] It has been demonstrated that magnetic nanoparticle clusters have higher transverse relaxivity values than individual magnetic nanoparticle nanoparticles, and thus they can act as potential contrast agents for T_2 -weighted MR imaging.[6] This phenomenon can also be utilized to develop an ultrasensitive medical MR sensor.[11] 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.[12-13] For example, the CTAB ionic surfactant can stabilise the SPIO cluster easily due to the interaction between specific functional groups and iron. However, these ionic surfactants are quite toxic to cells. 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.[14] Thus, a facile method of magnetic nanoparticle cluster development using biocompatible stabilizers is necessary for biomedical applications.

Photodynamic therapy (PDT) is a treatment that uses the association of photosensitizers and specific light sources apply in the various diseases treatment. The photosensitizer will activate by illumination, followed by producing the reactive oxygen species (ROS) from molecular oxygen and proceed to destroy cancer cells.[15] In cancer therapy, PDT has advantages of painless, controlled therapy area by lighting, and repeating treatment for patients.[16-17] Many reports have demonstrated that the photosensitizers combining with magnetic nanoparticles for further function such as MRI and magnetic fluid guide targeting.[18-19] The photosensitizers also can generate singlet oxygen and fluorescence at the same time under the specific wavelength light irradiation.[20] The combination of magnetic nanoparticle and photosensitizer can provide not only MRI/optical imaging but also the highly efficient cancer killing for cancer treatments.

In this study, we demonstrated a facile procedure to fabricate manganese zinc ferrite nanoparticles (MZF) clusters with photosensitizer loading using the nonionic polymers through the adjustment of the amount of solvent and its evaporation rate. In the conventional procedure for clustering of nanoparticles, 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 MZF clusters resulted in less cytotoxicity with

more biocompatibility for biomedical applications. The potential use of these clusters as MR contrast agents and cancer treatment was also investigated in vitro and in vivo.

2 EXPERIMENTAL FLOW

2.1 Synthesis of Manganese Zinc Ferrite Nanoparticles

MZF with diameters of approximately 10 nm were synthesized by a high-temperature thermal decomposition method in a nitrogen atmosphere inspired by previous reports.[3, 5] In brief, the Mn(acac)₂ (0.5 mmole), Zn(acac)₂ (0.5 mmole), Fe(acac)₃ (2 mmole) and 1,2-hexadecanediol (2 mmole) were mixed in dibenzyl ether (20 ml) at 60 °C; the oleic acid (1 mmole) and oleylamine (1 mmole) were then added as surfactants into the mixed solution. This reaction was heated under precise control at the rate of 5 °C/min until it reached 200 °C; the reaction was then maintained at this temperature for 10 minutes. Then, the reaction system was heated at a rate of 2 °C/min to 298 °C and refluxed for 30 minutes. Finally, the reaction was precipitated using excess ethanol, centrifuged at 6,000 rpm, vacuum dried and then characterized with an X-Ray Powder Diffractometer (XRD, PANalytical X'Pert Pro MRD, Almelo, The Netherlands).

2.2 Preparation of MZF Clusters

To prepare the multifunctional MZF clusters, the emulsion and solvent-evaporation method was employed. Briefly, the dried MZF (5 mg) was redispersed in hexane (0.02 - 2 ml) with oleic acid at room temperature, and then the PEG-400 and Pluronic L81 in 10 ml water were added to this mixture during ultrasonic treatment for 10 minutes to form an O/W emulsion. Followed by added the chlorin e6 and PEG-PCL-PC in tetrahydrofuran (THF) under stirred vigorously. The solvent of hexane and THF were then removed from the emulsified solution, which had a different evaporation rate, by controlling the temperature of the hotplate under constant stirring at 900 rpm. After cooling down to the room temperature, the products were placed under external magnetic field and washed to remove free polymer and unloaded chlorin e6.

2.3 Characterization of MZF Clusters

The particle sizes of the multifunctional MZF clusters were analysed by dynamic light scattering (DLS, ZS 90, Malvern Instruments Ltd.) at 25 °C, and their morphologies were observed by a JEOL 1400 transmission electron microscope (TEM, JEM 1400, JEOL Ltd., Japan) with an accelerated voltage of 120 kV. The iron concentrations of the MZF clusters and Resovist® were determined quantitatively using an atomic absorbance spectrophotometer (GBC 932, USA). To evaluate the magnetic properties of MZF clusters 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 MZF clusters and Resovist® were serially diluted into several concentrations and placed into microcentrifuge tubes before imaging. T2-weighted magnetic resonance images were obtained on a 3 Tesla clinical magnetic resonance imaging system (Signa Excite 3 T, GE Healthcare, USA). The samples were placed in a homemade water tank positioned in the 8 channel head coil. A two-dimensional T2-weighted fast-spin echo pulse sequence was applied (TR/TE=3017/98.9). The matrix size was 320×192, the field of view was 14×7 cm, the slice thickness was 1 mm with a 0.5 mm gap, and the total scan time was 2 minutes and 43 seconds at a NEX of two. The images were further analysed at the image workstation provided by GE Healthcare (Advantage Workstation 4.207).

3 RESULTS AND DISCUSSIONS

The MZF was synthesized by a high-temperature thermal decomposition method, which used an oleic acid/oleylamine stabilizer; the MZF morphology observed with TEM showed a spherical/cubic shape with sizes of 10.97±1.51 nm using the SigmaScan Pro statistic software (Figure 1A). The XRD measurement demonstrated that the crystalline structure was consistent with the six characteristic peaks of MZF (Figure 1B).[5, 21] To evaluate the magnetic properties of the MZF nanoparticles, samples were measured under an external magnetic field by VSM sweeping between -10,000 and 10,000 G at room temperature. Figure 1C shows the hysteresis loop of the as-prepared MZF. The saturation magnetisation (M_s) value of the MZF was 70.469 emu/g with very small coercivity was observed for the oleic acid/oleylamine-stabilised MZF (3.308 G), which indicates that these magnetic nanoparticles are superparamagnetic and have a high potential for MR applications.

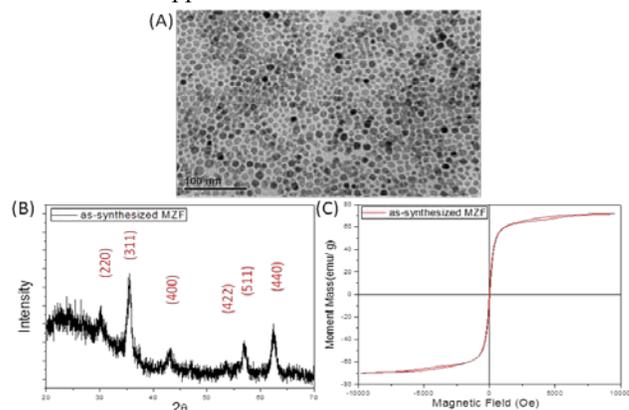


Figure 1: Characterizations of as-synthesized MZF, (A) TEM image, (B) XRD pattern, and (C) hysteresis loop.

The preparation of the MZF clusters with non-ionic, biocompatible polymers of pluronic L81, polyethylene

glycol, and PEG-PCL-PC was inspired by the nanocrystal stabilization and emulsion approaches that lead to the coexistence of two immiscible liquids as a well dispersion under the assistance of these polymers, which is utilized in this oil-in-water (O/W) system.[6, 22] The hydrodynamic size average and size distributions average of MZF clusters was 136.2 with PDI 0.191 evaluated by DLS, and the size observed by TEM was 53.83 ± 6.60 nm shown in Figure 2. MZF clusters were generated after the evaporation of the hexane/THF solvent with a digital-control hotplate at different temperatures. The particle sizes of clusters were measured by DLS which can directly reveal the real situation of MZF clusters in aqueous solution for further biomedical applications. In addition, the results from DLS can fast reflect the size change from the emulsified bodies to stable nanoclusters after solvent evaporation. According to our results, MZF solution can be emulsified and stabilized successfully using these polymers, which can align at the interface of oil and water to reduce the surface tension of the oil droplets in water.[23] In addition, the formulation of stable spheres was due to the strong interdigitated hydrophobic interactions between the alkyl chains of the oleic acid and the hydrophobic side of the pluronic L81 and PEG-PCL-PC.[24] Thus, hexane and THF with a low boiling point were utilised to create an oil phase that could easily be removed by evaporation. This phase-transfer process lead to the condensation of MZF and the formation of MZF clusters inside of the polymer micelles.

The optimized MZF clusters, their stability may be due to the full passivation of the surface of the clusters by these polymers. During this passivation process, the hydrophobic chains of polymers agglomerated with the oleic acid long carbon chain on the MZF particle surface and the hydrophilic poly ethylene glycol (PEG) chain was spread on the outer particle surface and directly contacted the water molecules to stabilize the MZF clusters without cluster-cluster agglomeration in aqueous solution.

To evaluate the contrast ability of the MZF clusters, T_1 - and T_2 -weighted MR images with different iron concentrations in aqueous dispersion were measured using a clinical MR scanner. Both Resovist[®] and the MZF clusters revealed a concentration-dependent signal intensity drop in the MR images, and concentration dependant contrast image was observed (Figure 2). In addition, MZF clusters exhibited remarkably darker images than Resovist[®] clusters of the same iron concentration did. Thus, it is suggested that MZF clusters exhibit better contrast capability in T_2 -weighted MR images than Resovist[®].

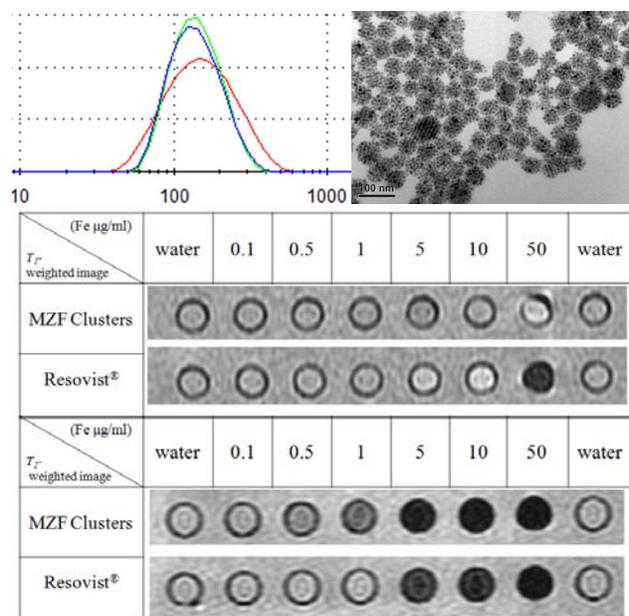


Figure 2: Size distribution, TEM and MRI images of MZF clusters.

4 CONCLUSION

In this study, highly biocompatible polymers were employed to stabilise MZF clusters and loaded with photosensitizers that can minimise the cytotoxicity from the conventional ionic surfactant. The as-prepared MZF clusters exhibited dual MRI imaging with T_1 and T_2 weighted contrast ability in both in vitro and in vivo studies. By loading the chlorin e6, the MZF clusters can be further functionalized with optical imaging and photodynamic treatment. The morphology and basic characterizations would not change obviously after photosensitizer loaded. Thus, the phase-transfer process by these polymers is a facile and prospective platform to form MZF clusters and further functionalize with photosensitizer that can serve as potential MR contrast agents and provide a multifunction platform for cancer therapy and diagnosis.

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*Corresponding author: Ping-Shan Lai, Department of Chemistry, National Chung Hsing University, No. 250, Kuo-Kuang Road, Taichung 402, Taiwan. , Tel: +886-4-22840411 ext. 428. Fax: +886-4-22862547. *E-mail address*: pslai@email.nchu.edu.tw (P.-S. Lai)