Thermally Responsive Lipid Coated Superparamagnetic Nanoparticles for Frequency Triggered Drug Delivery

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ABSTRACT

Herein we report a fabrication method of core-shell type silica coated superparamagnetic iron oxide nanoparticles (SPIONs) with thermally responsive lipids for controlled and targeted drug delivery using Alternative Current (AC) magnetic field for potential applications in cancer therapy. Core-shell SPIONs, loaded with anticancer drug, Doxorubicin (DOX) were coated with lipids such as dipalmitoylphosphatidylcholine (DPPC) and cholesterol so as to cap the mesopores. The particle size of the bare and coated SPIONs was measured using the dynamic light scattering technique, scanning and transmission electron microscopy (SEM and TEM). The change in surface morphology of bare and coated SPIONs was evidenced from SEM. The *in vitro* drug loading and release studies were carried in phosphate buffered saline (PBS) under the AC magnetic field as well as under thermal incubation condition at 37°C. The concentration of DOX in solution was determined by measuring the absorbance at 484 nm by UV-Visible spectrophotometer. The preliminary results indicate that the drug release behaviour under the AC magnetic field is relatively controlled compared to that of normal thermal incubation condition at 37°C.

Keywords: superparamagnetic iron oxide nanoparticles, lipid nanostructures, magnetoliposomes, drug delivery, hyperthermia cancer therapy

1. INTRODUCTION

Superparamagnetic iron oxide nanoparticles (SPIONs) have gathered a lot of attention in recent years because of their various potential biomedical applications, including contrast agent for magnetic resonance imaging, separation of biomolecules and drug delivery [1-5]. Due to their superparamagnetic properties, SPIONs are becoming increasingly popular in hyperthermia cancer therapy [6]. The nanoparticles can be targeted specifically to the cancer cell by applying an external magnetic field which can then release the drug to the target cell by inducing local hyperthermia. The size, morphology, surface charge and stability in suspension are the most important parameters which need to be cleverly controlled while considering the drug delivery application of nanoparticles. In order to have a good magnetic response the size of SPIONs need to be extremely small (< 10 nm). The small size of nanoparticles, however, lead to agglomeration of the particles which in turn causes poor stability and limited drug loading. Another major problem that can occur while using SPIONs as drug delivery vehicle is the leakage of drug molecules from the surface of the nanoparticles during the transport process in the blood stream which can reduce the drug efficacy. In order to overcome these problems, various materials such as silica [1, 2, 7-9], lipids [10] or commercial dispersing agents [11] have been used to modify the surface properties of SPIONs.

Amphiphilic molecules such as lipids possess a unique property of self-assembling in aqueous medium to give rise to different types of nanostructures [12]. Although bilayered lamellar is the most common nanostructure formed by lipid molecules in the presence of water, other structures such as hexagonal and cubic are also commonly seen. The type of nanostructure formed depends upon the lipid's structure and shape, its composition in water as well as on the physico-chemical conditions employed such as temperature and pressure. These self-assembled nanostructures have been extensively studied as model membrane systems in order to understand the various biological processes such as signal transduction, cell migration, cell proliferation [13]. Moreover, lipid based nanostructures have been employed in numerous disciplines including food, cosmetic and the pharmaceutical industry as carriers of nutrients, flavours, perfumes, drugs and other bioactive molecules [14, 15]. The nanoscale size, excellent biocompatibility, non-toxicity and their ability to solubilise both hydrophobic and hydrophilic molecules make lipid nanostructures a great component for drug delivery systems. These nanostructures not only protect the functional molecules from chemical and enzymatic degradation but also release bioactive molecules to the specific target and in a controlled manner [16]. However, in order to improve the applicability, the non-fluid viscous lipid phases, such as hexagonal and cubic, are dispersed into excess water to form oil-in-water type emulsions which are kinetically stabilized using stabilizers such as carbon nanotubes [17] or silica nanoparticles [18].

Multifunctional hybrid nanoparticle such as magnetoliposomes which consist of lipids and SPIONs (Figure 1), are emerging as a new class of nanomaterials for cancer therapy. These hybrid assemblies exhibit superior properties compared to the individual systems. Moreover, they provide an alternative method to the traditional chemo and radiation therapy to treat cancer as the latter two methods lack specificity and hence reportedly have harmful side effects [19].

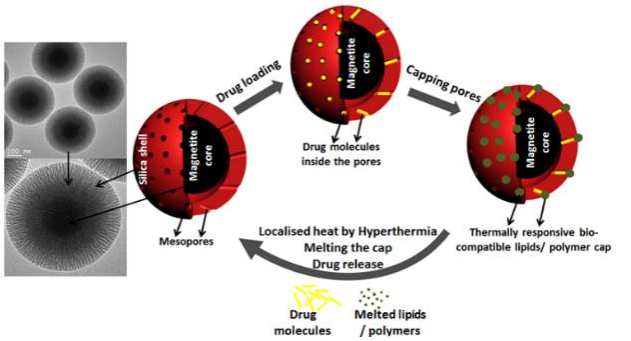

Figure 1 Schematic representation of multi-functional hybrid nanoparticles containing lipids and SPIONs for drug delivery applications.

2. EXPERIMENTAL

2.1 Synthesis of SPIONs

Bare SPIONs (Fe_3O_4) were prepared following a previously reported method [11].

2.2. Synthesis of mesoporous silica coated SPIONs

The core-shell type mesoporous silica coated SPIONs (Fe_3O_4@$nSiO_2$) were synthesized using a modified Stober system. 20 mg of Fe_3O_4 nanoparticles were dispersed into the solution containing 25 ml de-ionized water, 15 ml ethanol, 75 mg cetyl trimethyl ammonium bromide (CTAB) and 0.275 ml concentrated ammonia solution (28%). 0.125 ml tetraethoxy silane (TEOS) was added after vigorous stirring for 0.5 h and the reaction continued for 16 h. The product was separated and collected with a magnet, followed by washing with deionized water and ethanol for 3 times, respectively.

2.3 Preparation of lipid coated Doxorubicin loaded core-shell SPIONs

20 mg of a pure lipid dipalmitoylphosphatidylcholine, DPPC or a 3:2 (w/w) mixture of DPPC to cholesterol was dissolved in 2 ml chloroform in a 50 ml round bottom flask. The flask containing lipid solution was attached to a rotary evaporator and immersed in a water bath at 45 ºC and the solvent was evaporated under reduced pressure (Buchi Rotavapor R-114, Bauchi, Switzerland) to form a thin lipid film.

100 µg/ml Doxorubicin (DOX) solution was prepared by dissolving 1 mg DOX in 10 ml dimethyl sulphoxide (DMSO)/water (1:1).

30 mg of silica coated SPIONs were taken in 50 ml centrifuge tube and separated and washed with ethanol (x3, 5ml) followed by deionized water (x3, 5ml). 7.5 ml DOX solution was then added to the tube containing 30 mg of silica coated SPIONs and the mixture was vortexed for 5 minutes.

The solution of core-shell SPIONs with DOX was added to the flask containing the lipid film in which another 2.5 ml deionized water was added. The mixture was vigorously shaken manually for 5 minutes and then sonicated for 10 minutes in a continuous pulse mode at a maximum power of 40% using Vibra Cell Sonicator (Sonics and Materials Inc., USA) with a 13-mm diameter titanium probe.

2.4 Drug loading and release

The lipid coated DOX loaded core-shell SPIONs were centrifuged at a speed of 10,000 rpm for 10 min to separate the debris formed after sonication and non-entrapped DOX and core-shell SPIONs. The concentration of DOX in the supernatant was determined by measuring the UV absorbance at λ= 484 nm and comparing it with the pre-established calibration curve of known DOX concentrations in DMSO/water. The amount of DOX incorporated into the lipid coated core-shell SPIONs was obtained by subtracting the amount of free drug in the supernatant from the total amount of drug initially added.

Release of DOX from lipid coated nanomaterials was studied in PBS buffer under the AC magnetic field as well as under thermal incubation condition at 37°C. 4 mg of washed nanoparticles were treated with 1 mL of PBS buffer (pH = 7.1) at 37 °C under end-over-end rotation for up to 48 h. The nanoparticles were separated at different time intervals from the solution by magnetic separation and the absorbance was measured at 484 nm. The concentrations of DOX in the solution were determined by comparing the absorption value with a pre-established standard curve of known DOX concentrations in PBS buffer. Similarly, 4 mg of DOX loaded lipid coated nanoparticles were treated with 1 mL of PBS buffer and were placed under alternative AC magnetic field.

2.5 Characterization of SPIONs, core-shell SPIONs and lipid coated core-shell SPIONs

The particle size of SPIONs, core-shell SPIONs and lipid coated core-shell SPIONs was measured using the dynamic light scattering techniques (Zetasizer Nano, Malvern Instruments, UK and Mastersizer 2000, Malvern Instruments, UK), scanning electron microscopy (SEM) (FEI Quanta 200ESEM) and transmission electron microscopy (TEM) (JEOL JEM-2000 EX electron microscope Operated at 200 kV, images processed using Gatan Digital Microscope software). The surface morphology of the nanoparticles was observed using SEM. The heating ability of bare SPIONs and coated SPIONs was evaluated by DM100Series Nanoheating

Instrument (nanoScale Biomagnetics, Spain) using the supplied software "Maniac".

3. RESULTS AND DISCUSSION

The average particle size for the DPPC coated core-shell SPIONs with DOX reduced significantly after sonication. The particle diameter was found to be ~100 µm before the ultra-sonication and ~4 µm for sonicated particles (Figure 2). This suggested that the lipid coated core-shell SPIONs are highly aggregated even after sonication.

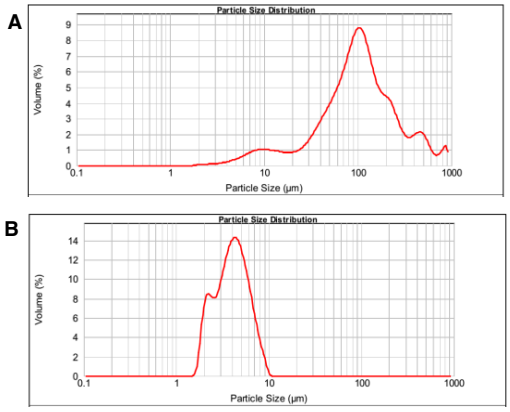

Figure 2 Particle size distribution of DPPC coated core-shell SPIONs with DOX (A) before sonication, (B) after ultra-sonication for 10 minutes using titanium probe.

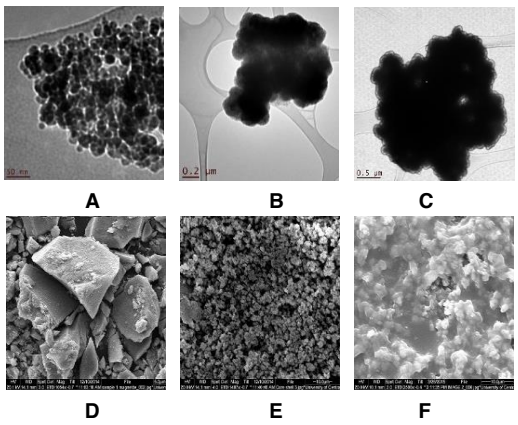

Figure 3 TEM images (A) bare SPIONs, (B) core-shell SPIONs, (C) DPPC coated core-shell SPIONs with DOX sonicated. (D), (E) and (F) represent their respective SEM images.

The observation was supported by TEM and SEM as shown in Figure 3. TEM shows that the bare SPIONs have particle size ~10 nm, which increased to 20 nm after coating them with silica. A thin film around the aggregated SPIONs was clearly seen which confirmed the lipid coating (Figure 3C). The change in surface morphology of the bare SPIONs after coating with silica and DPPC was also clearly observed by SEM images as shown in Figure 3 D, E and F.

The heating ability of SPIONs and core-shell SPIONs was tested for hyperthermia which indicated good heating response (Figure 4) and hence their suitability for drug delivery applications in cancer therapy.

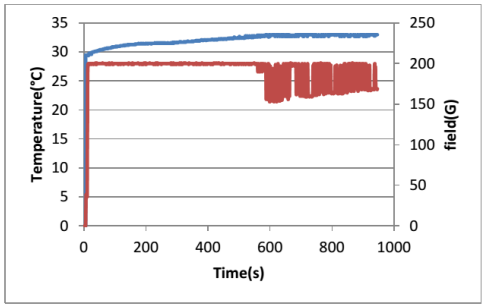

Figure 4 The magnetic heating profile of bare SPIONs (blue), silica coated SPIONs (red) at field frequency of 406 KHz.

Preliminary data suggest that drug release behaviour was different under hyperthermia compared to that for thermal heating using incubation (data not shown).

REFERENCES

1. Mahmoudi, M., et al., Advanced Drug Delivery Reviews, 2011. **63**(1-2): p. 24-46.
2. Sen, T., A. Sebastianelli, and I.J. Bruce, Journal of the American Chemical Society, 2006. **128**(22): p. 7130-7131.
3. Pankhurst, Q.A., et al., Journal of physics D: Applied physics, 2003. **36**(13): p. R167.
4. Langer, R. and D.A. Tirrell, Nature, 2004. **428**(6982): p. 487-492.
5. Perez, J.M., Nature nanotechnology, 2007. **2**(9): p. 535-536.
6. Hergt, R., et al., Journal of Physics-Condensed Matter, 2006. **18**(38): p. S2919-S2934.
7. Philipse, A.P., M.P. Van Bruggen, and C. Pathmamanoharan, Langmuir, 1994. **10**(1): p. 92-99.
8. Bruce, I.J., et al., Journal of Magnetism and Magnetic Materials, 2004. **284**: p. 145-160.
9. Sen, T. and I.J. Bruce, Microporous and Mesoporous Materials, 2009. **120**(3): p. 246-251.
10. Sen, T., et al., Rsc Advances, 2012. **2**(12): p. 5221-5228.
11. Sen, T., et al., Micro & Nano Letters, 2006. **1**(1): p. 39-42.
12. Mashaghi, S., et al., International Journal of Molecular Sciences, 2013. **14**(2): p. 4242-4282.
13. Patil-Sen, Y., et al., Physical Chemistry Chemical Physics, 2004. **6**(7): p. 1562-1565.
14. Mezzenga, R., et al., Nature materials, 2005. **4**(10): p. 729-740.

15. Pardeike, J., A. Hommoss, and R.H. Müller, International Journal of Pharmaceutics, 2009. **366**(1): p. 170-184.
16. Kulkarni, C.V., et al., International Journal of Pharmaceutics, 2015. **479**(2): p. 416-21.
17. Gaunt, N.P., et al., Nanoscale, 2015. **7**(3): p. 1090-1095.
18. Salonen, A., F.o. Muller, and O. Glatter, Langmuir, 2010. **26**(11): p. 7981-7987.
19. Harris, F., et al., Medicinal Research Reviews, 2013. **33**(1): p. 190-234.