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Regular Article

Systematic magnetic fluid hyperthermia studies of carboxyl functionalized hydrophilic superparamagnetic iron oxide nanoparticles based ferrofluids

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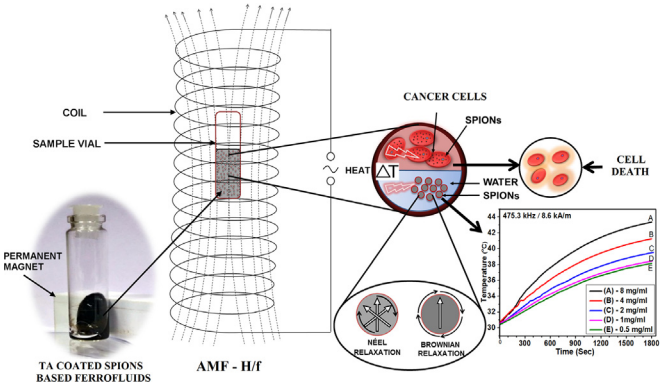
HIGHLIGHTS

- Carboxyl (terephthalic acid) functionalized SPIONs based ferrofluids are prepared.
- Heating efficacies of the SPIONs based ferrofluids are systematically investigated.
- Possible mechanisms for high heating efficacies of the ferrofluids are discussed.
- *In vitro* killing efficiency of the ferrofluids in MCF-7 cancer cells is determined.

GRAPHICAL ABSTRACT



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ABSTRACT

We have systematically studied heating efficiencies (via specific absorption rate–SAR/intrinsic loss power-ILP) of carboxyl (terephthalic acid-TA) functionalized hydrophilic SPIONs based ferrofluids (with good biocompatibility/high magnetization) and influence of following key factors in magnetic fluid hyperthermia (MFH): (i) alternating magnetic fields (AMFs - H)/frequencies (f) - chosen below/above Hergt's biological safety limit, (ii) concentrations (0.5–8 mg/ml) and (iii) dispersion media (water, a cell-culture medium and triethylene glycol (TEG)) for *in vitro* cancer therapy. In calorimetric MFH, aqueous ferrofluids have displayed excellent time-dependent temperature rise for the applied AMFs, which resulted in high SAR ranging from 23.4 to 160.7 W/g_{Fe}, attributed to the enhanced magnetic responses via π-conjugations of short-chained TA molecules on the surface of SPIONs. Moreover, ILP values up-to 2.5 nHm^2/kg (higher than the best commercial ferrofluids) are attained for the aqueous ferrofluids when excited below the recommended safety limit. Besides, the SPIONs dispersed in high viscous TEG have exhibited the highest SAR value (178.8 W/g_{Fe}) and reached therapeutic temperatures at faster rates for the lowest concentration due to prominent Neel relaxations. Moreover, these SPIONs have higher killing efficiency towards MCF-7 cancer cells in *in vitro* studies. Thus, the TA-based ferrofluids have great potential for *in vivo*/clinical MFH cancer therapies.

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1. Introduction

Ferrofluids (also called as magnetic fluids) are stable colloidal suspensions of single domain magnetic nanoparticles which are dispersed in a carrier liquid. Superparamagnetic iron oxide nanoparticles (SPIONs - particularly magnetite (Fe_3O_4)/meghamite (Fe_2O_3)) based ferrofluids are broadly investigated for the biomedical applications such as drug delivery [1–5], magnetic resonance imaging (MRI) [6–11], magnetic targeting [12,13], and magnetofection [14–16] due to their non-toxic nature, excellent chemical-stability and unique size-dependent superparamagnetic properties. Moreover, the SPIONs based ferrofluids are extensively investigated for magnetic fluid hyperthermia (MFH) therapy in cancer treatment via localized heating of tumors at therapeutic temperatures of 42–45 °C on the application of alternating magnetic fields (AMFs) with specific amplitudes (H in kA/m) and frequencies (f in kHz) [17–21]. The heating efficiency of the SPIONs based ferrofluids is generally measured through specific absorption rate (SAR i.e., heat generated per unit mass - Watts/gram$_{Fe}$ (W/g$_{Fe}$)) and deduced in terms of intrinsic loss power (ILP—a normalized SAR value - nHm^2/kg) to relate the heating efficacies of diverse ferrofluids under different magnetic fields via dissimilar MFH experimental set-ups. Over the last decade, numerous studies have been performed to enhance the SAR values by modifying the size and shape of the SPIONs [22–25]. For instance, SAR value has been reported to increase from 10 to 42 W/g (at 7.5 kA/m, 522 kHz) – by a factor of 4 - with the increment in the size (i.e., from 8 to 11 nm) of the SPIONs [22]. Recently, Bae et al. has reported a high SAR value of 2614 W/g for 30 nm sized chitosan oligosaccharide-coated iron oxide nanocubes [26]. However, the *in vitro/in vivo* toxicity of the SPIONs with different shapes (other than spherical) towards the normal cells or cancer cells is a major concern [27–30]. Besides, in most of these investigations, the MFH studies are performed at relatively high ferrofluid concentrations (not suitable for biological studies), and the *in vitro/in vivo* cancer therapeutic experimentations are also lacking.

Nevertheless, the SPIONs (with spherical-shape) coated with different biocompatible surfactants such as sodium oleate [31], pentenoic acid [32], glycyrrhizic acid [33], citric acid/albumin [34], and glycine [35] have exhibited relatively low SAR values of 14 W/g (at 15.9 kA/m, 62 kHz), 110.56 W/g (at 0.1 kW power, 142 kHz), 17.92 W/g (at 8.8 kA/m, 300 kHz), 16.72/10.66 W/g (at 3 kA/m, 215 kHz), and 77.6 W/g (at 0.385 kOe, 250 kHz) respectively, which could be due to high surface spin-canting effects from the σ-conjugation of the shorter chain coating molecules.

In addition, many of these reported literatures lack in detailed investigation of the heating responses of the SPIONs with respect to the key influencing factors - magnetic fields/concentrations/dispersion media, which make them inappropriate for further *in vitro/in vivo* MFH cancer treatments. Furthermore, the biological safety limit (known as $C = H*f - 5*10^9$ Am^{-1} s^{-1} - suggested by Hergt's et al. to prevent the non-localized heating in the tissues) has not been considered. Hence, there is a need for developing biocompatible SPIONs with suitable surface coatings to attain enhanced heating efficacies at their minimal concentrations in an appropriate medium under the applied magnetic fields - near to the biological safety limits.

Recently, we have developed hydrophilic SPIONs (spherical-shaped) that are *in-situ* functionalized with benzene dicarboxylic acid/terephthalic acid (TA with shorter chain length) via co-precipitation method, which exhibited high saturation magnetization (Ms) and better cytocompatibility in normal fibroblast (NIH3T3) cells with no toxicity effects up to 125 μg$_{Fe}$/ml [36,37]. Moreover, these TA-coated SPIONs have exhibited very high MRI relaxivity value of 735.3 mM^{-1} s^{-1} due to their enhanced magnetic response to the applied magnetic fields via π-conjugation paths of the TA molecules on the surface of these SPIONs [17,36]. Based on these promising results, in this work, we have prepared the TA-functionalized, spherical-shaped SPIONs via chemical co-precipitation/thermal decomposition methods and first-time investigated their heating responses/efficacies (via SAR/ILP) at different concentrations (0.5–8 mg/ml) by applying wide range of AMFs (with H = 8.7–15.4 kA/m and f = 166–1001 kHz) while using different dispersion media (aqueous medium, Dulbecco's modified eagle medium (DMEM – a biological medium) and triethylene glycol (TEG – a high viscous medium with density similar to human blood). Later, *in vitro* therapeutic studies on MCF-7 cancer cells are performed via MFH by using suitable TA-coated SPIONs based ferrofluids for further investigation in *in vivo*/clinical scenarios.

2. Experimental

2.1. Materials

Iron (III) chloride, Iron (II) chloride, Iron (III) acetylacetonate and TA are purchased from Sigma Aldrich. Ammonium hydroxide and TEG are obtained from Fisher Scientific.

2.2. Synthesis of SPIONs

SPIONs are synthesized via chemical co-precipitation/thermal decomposition methods and characterized as reported elsewhere [36,37]. Briefly in co-precipitation process, 8 mmol of iron (III) chloride, 4 mmol of iron (II) chloride, and appropriate amount of TA are mixed in distilled water (DW, Millipore). Then, the above mixture is heated to 80 °C under N_2 gas with magnetic stirring and then ammonium hydroxide is added and vigorously stirred for 1 h. Finally, the resultant black solution is cooled down to room temperature, magnetically-separated and washed with DW/ethanol for 2–3 times. The washed TA-coated SPIONs (marked as S1) are re-dispersed in three different solvents (aqueous medium (DW), DMEM and TEG) to get the respective ferrofluid samples (marked as F1, F2 and F3). In thermal decomposition process, 2 mmol of iron (III) acetylacetonate is dissolved in 20 ml of TEG along with 6 mmol of TA in a 100 ml round bottom flask. The flask is dehydrated at 120 °C for 60 min in the presence of nitrogen. Then, the flask is quickly heated and maintained at a refluxing temperature of 280 °C for another 60 min to prepare SPIONs. Similar washing step as in co-precipitation process is followed and the obtained TA-coated SPIONs (marked as S2) are re-dispersed in DW to get the aqueous ferrofluid sample (marked as F4). Later, the ferrofluid samples are analyzed for the concentration of the magnetic element (iron - Fe) by using potassium thiocyanate based standard protocol [38].

2.3. Characterizations of SPIONs

Size and morphology of the as-prepared TA-coated SPIONs are determined using transmission electron microscopy (TEM; JEOL 2010), while the hydrodynamic diameters and zeta potentials of the TA-coated SPIONs based ferrofluids (F1 - F4) are determined via dynamic light scattering (DLS, Horiba nanoPartica SZ-100-Z) technique.

2.4. Calorimetric magnetic fluid hyperthermia

Calorimetric magnetic fluid hyperthermia (MFH) studies are performed via sophisticated hyperthermia equipment (magneTherm – nanoTherics, UK). Herein, 1 ml of the aqueous ferrofluids (F1 and F4 samples) with five different concentrations ranging from 0.5 to 8 mg$_{Fe}$/ml (hereafter marked as mg/ml) are initially