Globulin Conjugated Fe_3O_4 Nanoparticles for Magnetic Hyperthermia

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Abstract. Magnetic hyperthermia is a non-invasive cancer treatment used synergistically with the existing cancer treatments. The challenges of an efficient magnetic hyperthermia are improved biocompatibility and enhanced heating characteristics. We have addressed both the challenges by fabricating globulin conjugated iron oxide (Fe_3O_4) nanoparticles. The haemolysis experiments show that the globulin- Fe_3O_4 nanoparticles are non-haemolytic with 1.3 % haemolysis. It is observed from the magnetic hyperthermia experiments that, the Specific Absorption Rate value of the globulin- Fe_3O_4 nanoparticles is almost 1800 W/g, when compared to 1100 W/g of the Fe_3O_4 nanoparticles without globulin . This is because of the improved colloidal stability. Thus we report here that the globulin- Fe_3O_4 nanoparticles enhance both the biocompatibility and the SAR value for magnetic hyperthermia, thus addressing both the challenges in clinical magnetic hyperthermia.

Introduction

Magnetic hyperthermia is a non-invasive strategy which ensures localized heating of the tumor site [1, 2]. Magnetic nanoparticles are usually injected into the body or the tumor site under an alternating current (AC) magnetic field. This raises the temperature of the tumor region upto 42-46℃, thus causing tumor cell death due to necrosis [3, 4] due to the enhanced permeation and retention characteristic of the tumor site. Good biocompatibility and heating efficacy is the basic requirement for an efficient magnetic hyperthermia system.

Previous attempts to combinatorially improve the biocompatibility and SAR value include, using noble metal-iron oxide core-shell nanoparticles [5, 6], surface functionalization by biomacromolecules [7, 8] etc., Keshavarz et al [9], and Samanta et al [10] have also reported that albumin conjugation enhances the colloidal stability and thereby the SAR value of magnetic nanoparticles. Our previous work also shows that serum albumin conjugation improves SAR value of 30 nm magnetite nanoparticles [11, 12]. This paper attempts to extend the work to Fe_3O_4 nanoparticles conjugated with other serum protein, globulin. Globulins have a higher molecular weight than albumin and are abundantly found in blood plasma [13].

We have fabricated 20 nm Fe_3O_4 nanoparticles and converted to biocompatible phase by globulin conjugation. The haemolytic studies show that globulin-Fe_3O_4 nanoparticles are non-haemolytic with a % haemolysis of 1.3 %. The SAR value of globulin- Fe_3O_4 nanoparticles is almost 1800 W/g. Thus inline with our previous

results, globulin conjugation over Fe_3O_4 nanoparticles also improves both the biocompatibility and heating characteristics of the magnetic hyperthermia system. This also probed us to look deeper into the contribution of blood plasma proteins and ions towards heating efficiency of a magnetic hyperthermia system.

Materials and Methods

The Fe_3O_4 nanoparticles were synthesized by the thermal decomposition method as described elsewhere as it yields uniform, monodisperse nanoparticles [14]. The hydrophobic as-synthesized Fe_3O_4 nanoparticles were converted to hydrophilic phase by APTMS. Briefly, to 1 mg Fe_3O_4 nanoparticles in 10 ml toluene, 90µ L of APTMS was added and maintained in the shaker at room temperature for 72 hours. The reaction mixture was later washed with ethanol and deionised water. Globulin was conjugated over the APTMS-Fe_3O_4 nanoparticles. Briefly, 200µ L of EDC mixture containing 26mM EDC and 10mM NHS in MES (2-(N-morpholino) ethane sulfonic acid) buffer was added to 1 mL of 2mg/mL globulin and 2ml of APTMS- Fe_3O_4 nanoparticles in 1X PBS (0.1mg/mL) at a pH of 7.2-7.4 was mixed with the activated globulin pre-mix and left in the shaker overnight at room temperature. The reaction was terminated and the product was washed.

Blood sample retrieved from 5 weeks old male Wistar rat was centrifuged at 700 rpm and erythrocytes were collected. The pellet was re-suspended in 0.1 M NaCl at a ratio of 1:4. The test samples- Fe_3O_4 nanoparticles, APTMS- Fe_3O_4 nanoparticles and globulin- Fe_3O_4 nanoparticles, each of ferric ion concentration 0.5 mg/mL were added to the erythrocytes and incubated at 37℃ for 2 hours. The absorbance of the leaked haemoglobin (Hb) was measured at 540 nm. The haemolytic index was also calculated according to ASTM F756-00 standards- 0-2% is non-haemolytic; 2-5% is mildly haemolytic and >5% is haemolytic [35]. % Haemolysis was calculated using the equation 1,

$$\%Haemolysis = [A_t - A_n / A_c - A_n]100 \quad (1)$$

A_{t-} absorbance of test sample at 540 nm; A_{n-} absorbance of negative control (0.1M NaCl) at 540 nm; A_{c-} absorbance of positive control (distilled water) at 540 nm.

The Specific Absorption Rate (SAR) value was calculated from the magnetic hyperthermia studies. The sample was placed inside a copper coil generating an applied AC magnetic field at an amplitude of 32.4 kAm^{-1}, frequency of 360 kHz and a magnetic field of 600 Oe was studied. SAR is expressed as the heat released by the magnetic nanoparticles under an applied AC magnetic field. The SAR value is calculated from equation 2,

$$SAR = C_{wat} \frac{\Delta T}{\Delta T}\Big/_{\Delta t} * \frac{1}{C_{Fe}} \ (W g^{-1}) \quad (2)$$

C_{wat-} Specific heat of the medium (distilled water), 4.18 J /g/ ºC; $\Delta T/\Delta t$- The initial slope of the time-dependent temperature curve; C_{Fe-} Concentration of ferric ions in the medium, 0.1mg/mL.

Results and Discussion

Synthesis of Fe_3O_4 Nanoparticles

The size of the monodisperse, closed packed iron Fe_3O_4 nanoparticles was confirmed using Transmission Electron Microscope micrographs from fig. 1(a). The magnetization (Ms) of Fe_3O_4 nanoparticles was found to be 70 emu/g as shown in fig. 1(b). The XRD plot in fig 1(c) shows that the particles were mono crystalline. The diffraction peaks can be indexed as cubic spinel Fe3O4 (JCPDS no.19-0629), corresponding to (220), (311), (400), (422), (511) and (440).

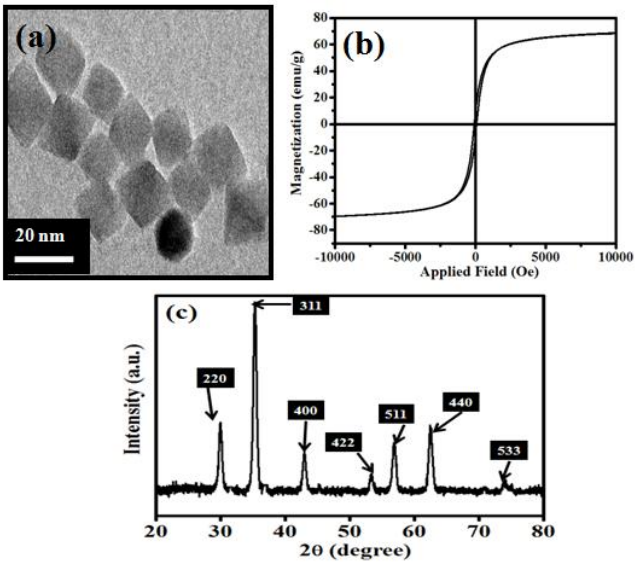

Figure. 1. a) 20 nm Fe_3O_4 nanoparticles b)Magnetic saturation is 70 emu/g c) crystallinity and purity of 20 nm Fe_3O_4 nanoparticles

Conversion to Biocompatible Phase by Globulin Conjugation

Fig. 2 shows that the average hydrodynamic radius of APTMS- Fe_3O_4 nanoparticles is 25±2 nm and globulin- Fe_3O_4 nanoparticles is around 40±4 nm. From the inset TEM image it is evident that Fe_3O_4 nanoparticles are individually conjugated with globulin.

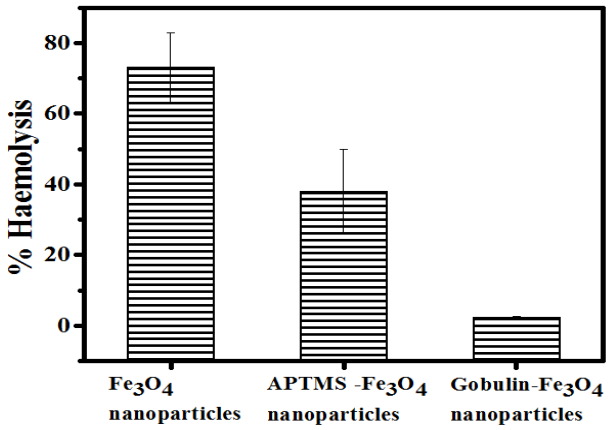

Figure.2. a) Average hydrodynamic radius of APTMS- Fe_3O_4 nanoparticles and globulin- Fe_3O_4 nanoparticles. Inset fig. individually conjugated globulin- Fe_3O_4 nanoparticles

Haemolytic Studies- Improved Biocompatibility

The oxidative stress due to Fe_3O_4 nanoparticles ruptures the erythrocytes causing the Hb leakage. The absorbance of leaked haemoglobin under 540 nm was noted to calculate % haemolysis. From fig. 3 it is evident that the % haemolysis of Fe_3O_4

nanoparticles is 72% and that of APTMS- Fe_3O_4 nanoparticles is 35% showing that they are highly haemolytic in nature. The % haemolysis of globulin- Fe_3O_4 nanoparticles is only 1.3% and hence is non-haemolytic in nature.

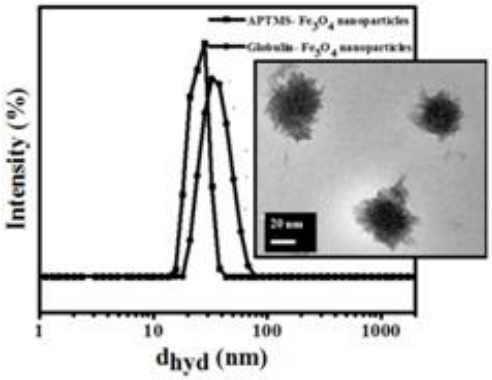

Figure. 3. % Haemolysis plot

Magnetic Hyperthermia Studies- Enhanced Heating Efficiency

From Figure 4 globulin- Fe_3O_4 nanoparticles show a higher ΔT of 7°C in 3 minutes when compared to 4.8℃ of APTMS- Fe_3O_4 nanoparticles. This substantiates that within a short exposure to the magnetic field, there is a higher ΔT which can destroy tumor. A high SAR value of 1800 W/g for globulin- Fe_3O_4 nanoparticles when compared to 1100 W/g for APTMS- Fe_3O_4 nanoparticles also proves the same. This is shown in the inset figure. This is because of improved colloidal stability of globulin-Fe_3O_4 nanoparticles.

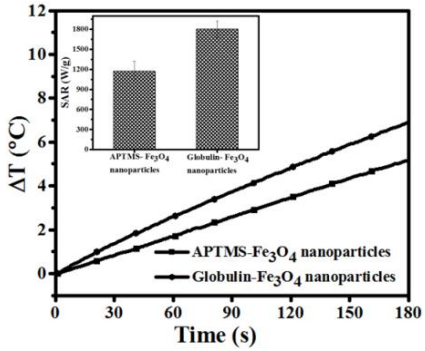

Figure.4. Temperature raise plot. Inset SAR value comparison plot

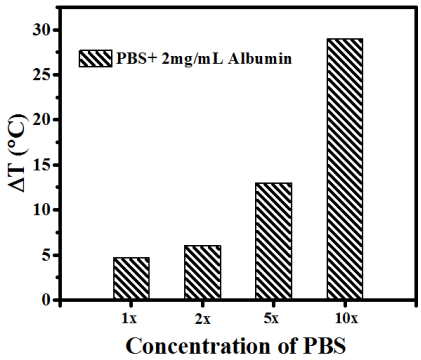

Figure. 5 Physiological components like proteins and ions cause ΔT under magnetic field.

This result and our previous results show that physiological proteins and ions have some contribution to magnetic hyperthermia under magnetic field. Fig. 5 shows that 2mg/mL albumin (abundant physiological protein) in various concentration of PBS cause ΔT even without Fe_3O_4 nanoparticles. The reason for this phenomenon is

beyond the scope of this paper, though it throws insights into the effect of physiological components to magnetic hyperthermia.

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